Chapter 5
Bleaching and Oxidation of Human Hair

Abstract The physical chemistry of both chemical bleaching and sunlight effects on human hair are described. Recently we have become more aware of the critical involvement of free radical chemistry on both chemical and sunlight oxidative processes for human hair, therefore these effects are included. The beta layers of the cortical lipids with their high density of double bonds with allylic hydrogen atoms are very sensitive to free radical propagation reactions which can degrade the lipids themselves and also lead to protein degradation. Over the past decade our understanding of the biosynthesis and the structures of the melanin pigments has improved greatly; the most current biosynthetic pathway has been added to this Chapter. Initial oxidation reactions remove 18-MEA and free lipids from the surface and between cuticle cells. When metals like iron or copper are present free radical chemistry is increased leading to degradation of lipids and enhanced protein degradation not only at disulfide bonds but even at peptide bonds. Oxidative cleavage of disulfide bonds inside cuticle cells also occurs. Degradation of disulfide bonds inside cortical cells occurs next as well as degradation of hair pigments. Other amino acid functional groups are attacked and oxidatively degraded.

5.1 Introduction

Since the 4th Edition, we have added to our learning about photochemical effects on hair, photoprotection of hair, the surface chemistry of photobleached and chemically bleached hair and the properties of and the biosynthesis of different hair pigments. We have discovered that the beta layers of the cuticle are more sensitive to nucleophilic attack by species such as the hydroperoxide anion and mercaptans, but beta layers of the cortical lipids with their multiplicity of double bonds (oleic plus palmitoleic acids, plus cholesterol and cholesterol sulfate) and tertiary hydrogen atoms (cholesterol and cholesterol sulfate) are more sensitive to free radical chemistry. On the other hand, the proteinaceous membranes of the CMC are resistant to non-radical oxidizing and reducing agents. An expanded

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section in this Chapter deals with free radical chemistry of the important groups in hair and other types of damaging reactions of hair bleaches, oxidation dyes and sunlight degradation. The effects of these reactions on adhesion failure or crack formation in the hair fiber are also described.

A new section entitled *Hair pigmentation and genetics* summarizing the genes and SNP's (single nucleotide polymorphisms) involved in hair pigmentation that control the natural color of human hair has been added in Chap. 3. This current Chapter describes the response of the red hair pigments to photodegradation which differs from that of the brown-black eumelanin. Additional evidence is presented confirming that both photochemical and chemical oxidation of 18-methyl eicosanoic acid (covalently bound lipid on and in the surface) and the disulfide bonds at or near the fiber surface lead to increased levels of sulfur acids primarily as sulfonic acid. The resultant effect creates an acidic, hydrophilic hair surface from a neutral, hydrophobic virgin surface.

Hair pigment size and type are highly important to hair color with the largest pigment particles in black hair and the smallest in blonde hair. Age and geo-racial effects on hair pigmentation are described. Hair pigments, hair dyes, antioxidants and specialty silicones have all been shown to exhibit some effects on photoprotection. These findings are described in this Chapter.

The composition of amino acid residues in bleached hair and in hydrolysates of oxidized keratin fibers is described in Chap. 2 and also in publications by Zahn [1, 2], Robbins et al. [3–6], Maclaren et al. [7, 8], and Alter and Bit-Alkis [9]. Although, several questions remain unanswered with regard to the structures and reactions of hair pigments [10–14], general features of the chemical structure of hair and its reactions with bleach products are best described by the language of physical-organic chemistry.

5.2 Hair-Bleaching Compositions

Cook [15] described hair bleaching compositions several years ago. Complete formulations were listed by Wall in the book edited by Sagarin [16]. The most reliable up-to-date qualitative information on bleaching compositions is found on product ingredient labels. From ingredient labels and the information in this Chapter, hair bleaching compositions can be formulated. Hydrogen peroxide is the principal oxidizing agent used in bleaching compositions, and salts of persulfate are often added as "accelerators" [15]. The pH of these products is generally from 9 to 11. Stabilizers (e.g., sequestrants) and separate containers are often used to reduce the rate of decomposition of the peroxide and to provide satisfactory shelf life.

A maximum hair lightening product for either stripping or frosting hair will generally consist of three different parts, the hair lightener base (alkalinity), the lotion developer (containing the peroxide) and the booster powder or accelerator containing salts of persulfate. The solution applied to the hair will be prepared just prior to use by mixing approximately 50 g of the lightener base with 100 g of the

Table 5.1 Hair lightener base

Ingredient	Percentage
Cocodiethanol amide (standamide KD)	9
Oleic acid	8
Dodecyl benzene sulfonate	7
Neodol 91-2.5	6
Concentrated ammonium hydroxide	6
Sodium sulfate	1
Deionized water	q.s.

Table 5.2 Lotion developer

Ingredient	
Hydrogen peroxide (30%)	17
Dodecyl benzene sulfonate (50%)	16
Nonoxynol-9	6
Cetyl alcohol	3
Stearyl alcohol	2
Phosphoric acid	1
Water	55

Table 5.3 Booster powder (accelerator)

Ingredient	Percentage
Potassium persulfate	27
Sodium silicate	26
Ammonium persulfate	25
Silica	20
Sodium lauryl sulfate	1.8
Disodium EDTA	0.2

lotion developer and two to three packets of the booster powder (approximately 10–12 g in each packet).

To formulate the hair lightener base of Table 5.1, add the amide, the sulfonate and neodol to the water while stirring at room temperature. Add the oleic acid with stirring and then slowly add the alkalinity followed by sodium sulfate.

For the lotion developer of Table 5.2, dissolve the dodecyl benzene sulfonate and nonoxynol-9 in water. Heat to 60°C and add the melted cetyl and stearyl alcohols while stirring. Cool and add the phosphoric acid and hydrogen peroxide (Table 5.3).

For permanent hair dyes even where small shade changes to a lighter color are required, bleaching is also involved. For these systems of permanent dyes, extra peroxide is formulated into the creme developer for the necessary bleaching action. For formulas of this type see the section of Chap. 6 that describes the formulation of permanent hair dyes.

For the spray in the hair lightener of Table 5.4, stir and dissolve the hydroxyethyl cetyldimonium phosphate in water followed by polysorbate-20. Then add the quaternium-80, benzoic acid and disodium EDTA followed by the fragrance.

Table 5.4 Spray in hair lightener

Ingredient	Percentage
Water	q.s.
Hydrogen peroxide (30%)	10
Hydroxyethyl cetyldimonium phosphate	1
Polysorbate-20	1
Quaternium-80	0.5
Benzoic acid	0.3
Disodium EDTA	0.2
Fragrance	0.2

5.3 Reactions of the Proteins of Human Hair with Bleaches

5.3.1 Chemical Oxidation of the Disulfide Bond

The primary purpose in bleaching human hair is to lighten the hair. This goal is most readily accomplished by oxidation. However, because of the severe reaction conditions required for destruction of the chromophoric groups of hair pigments, side reactions with the hair proteins occur simultaneously. Wolfram [12] provided evidence that hydrogen peroxide, the principal component of hair bleach systems, reacts faster with melanin than with hair proteins. However, since hair is primarily proteinaceous it contains a large percentage of oxidizable groups. For example hair contains thioester bonds at the surface and between cuticle cells. Hair also contains disulfide bonds of the cortical matrix and of the cuticle proteins. Because these groups are in the structural proteins of hair, degradation of these proteins also occurs during bleaching.

Chemical bleaching with either alkaline peroxide or alkaline peroxide-persulfate first attacks the thioester groups that bind 18-methyl eicosanoic acid to the surface proteins. This reaction partially removes the hydrophobic surface barrier and it creates sulfur acids (primarily sulfonate groups) on and in the fiber surface. These actions provide an acidic, hydrophilic hair surface with a lower isoelectric point [6, 17–19]. Beard et al. [19] have shown that peroxide alone doesn't oxidize the hair surface, however peroxide with alkali (as in hair bleach compositions) does, but with an induction period. This induction period is reduced or even eliminated by inclusion of small amounts of surfactant in the oxidizing medium. The surfactant most likely removes interfering free lipids from the surface providing access for the nucleophilic hydroperoxide anion to the thioester and disulfide linkages for reaction. When certain metals such as iron or copper or even persulfate are present free radical degradation can also occur. These reactions will be described later in this Chapter.

Chemical bleaches weaken the cell membrane complex by oxidizing thioester between cuticle cells. Bleaches also oxidize cystine residues of the matrix in the cortex and other hair regions rich in cystine such as the A-layer and the exocuticle inside cuticle cells. These reactions result in breakdown of the cell membrane

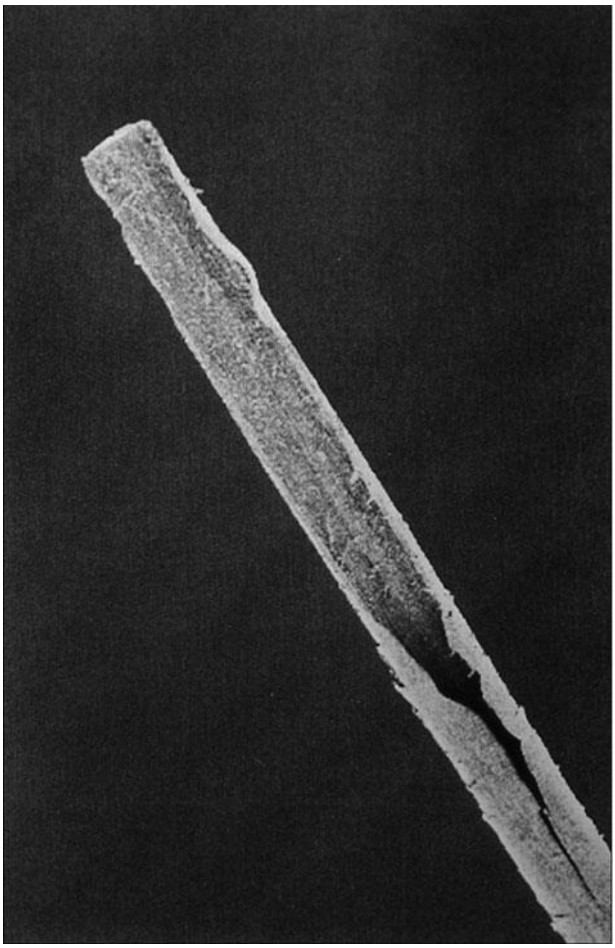


Fig. 5.1 Hair fiber oxidized with alkaline peroxide for a few hours and extended to fracture dry. Much of the cortex has been broken from the interior of the fiber leaving a hollow sleeve of essentially cuticle layers remaining (SEM kindly provided by Sigrid Ruetsch)

complex, the cuticle and cortex components and ultimately dissolves proteins in these regions. The electron micrographs of Figs. 5.1 and 5.2 illustrate the effects of fracturing hydrogen peroxide oxidized hair (extensively oxidized) by extending the treated fibers to break. The cell membrane complex has been weakened by the chemical oxidation as illustrated by the relatively clean breaks between cuticle layers of Fig. 5.2. This effect is illustrated further by the appearance of a hollow cuticular tube with the cortex largely removed (Fig. 5.1). This consequence suggests a relatively clean separation at the cuticle–cortex junction in the cuticle–cortex cell membrane complex. After chemical bleaching there is also a greater tendency for cuticle scale lifting via cell membrane complex failure as illustrated by several electron micrographs in Chap. 6.

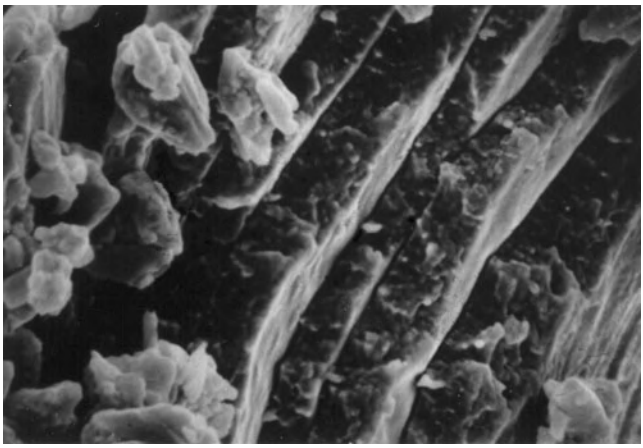


Fig. 5.2 Close up of the fiber in Fig. 5.1 at the main fracture site. Note the clean breaks between the scales at the cell membrane complex (SEM kindly provided by Sigrid Ruetsch)

Zahn [1] first demonstrated that the primary reaction of oxidizing agents with the proteins of human hair occurs at the disulfide bond of cystine. Small amounts of degradation also occur to the amino acid residues of tyrosine, threonine, and methionine during severe bleaching [5]. The main site of attack, however, is at the disulfide bonds of the cystyl residues in the fibers. Robbins and Kelly [5] have shown that 15–25% of the disulfide bonds in human hair are degraded during "normal" bleaching, however, as much as 45% of the cystine bonds may be broken during severe "in practice" bleaching. This latter amount of damage may occur while frosting hair, or while bleaching hair from black or brown-black to light blond.

The kinetics of the oxidation of cystyl residues in hair by hydrogen peroxide has not been reported, although there is evidence to suggest that this reaction is a diffusion-controlled process. Harris and Brown [20] reduced and methylated keratin fibers and demonstrated that the wet tensile properties decrease almost linearly with the disulfide content. Alexander et al. [21] arrived at this same conclusion after oxidizing wool fiber with peracetic acid. Robbins has observed a similar phenomenon for hair oxidized with alkaline hydrogen peroxide. A portion of these data is described in Chap. 9. Therefore, one may conclude that the percentage loss of the wet tensile properties that occurs during bleaching such as the decrease in the 20% index [21] is an estimate of the percentage of cystine linkages that are broken. For a more complete discussion of the effects of bleaching on the tensile properties of hair, see Chap. 9.

Edman and Marti [22] described the change in the 20% index of hair fibers as a function of treatment time in 6% hydrogen peroxide, at 32°C using a 25:1 solution-to-hair ratio at a pH of 9.5. Their data are plotted in Fig. 5.3 vs. the

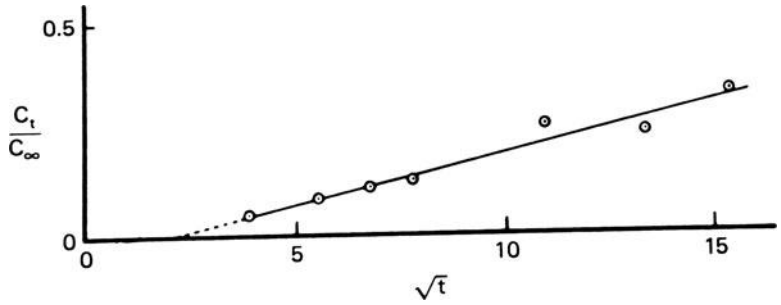


Fig. 5.3 Rate of cleavage of cystine cross links estimated from tensile properties [24] (Reprinted with permission of the Journal of the Society of Cosmetic Chemists)

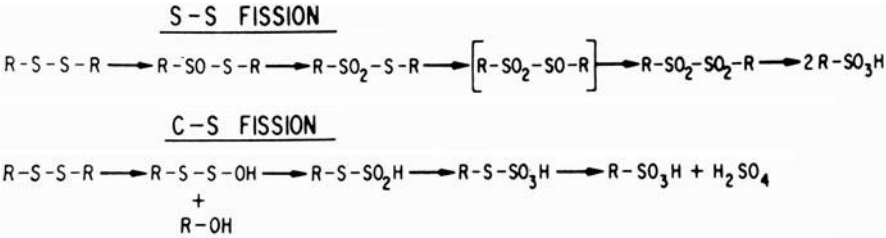


Fig. 5.4 Schemes for disulfide bond fission [24] (Reprinted with permission of the Journal of the Society of Cosmetic Chemists)

square root of time providing a straight line indicative of a diffusion-controlled process. These data have been applied to an equation developed by Crank [23] describing diffusion from a stirred solution of limited volume into a cylinder of infinite length:

$$C_t/C_\infty = 2\left[2/\sqrt{\Pi}(Dt/a^2)^{1/2} + \ldots.\right]$$

The term C_t is the 20% index at time t and represents the amount of cleaved disulfide at time t; C_∞ is the 20% index at time zero representing the total amount of disulfide before oxidation; and a represents the fiber radius, assumed to be 40 μm. Considering these assumptions, one obtains an approximate diffusion coefficient of 1.8×10^{-9} cm^2/min. This diffusion coefficient is of the anticipated magnitude, suggesting that the oxidation of the disulfide bond in hair by alkaline hydrogen peroxide is a diffusion-controlled reaction.

Two schemes have been proposed for the oxidative degradation of disulfide bonds [24, 25]: a sulfur to sulfur (S–S) fission process, and a carbon to sulfur (C–S) fission process see Fig. 5.4.

Table 5.5 Functional groups involved in the oxidation of disulfides and mercaptans

Disulfide oxides	
–S–S–	Disulfide
–SO–S–	Monoxide
$–SO_2–S–$	Dioxide
$–SO_2–SO–$	Trioxide
$–SO_2–SO_2–$	Tetroxide
Sulfur acids	
–SH	Mercaptan
–S–OH	Sulfenic acid
$–SO_2H$	Sulfinic acid
$–SO_3H$	Sulfonic acid

Table 5.5 defines the different functional groups involved in these two oxidative schemes. Figure 5.4 shows that if the oxidation of cystine in hair proceeds totally through S–S fission, then two moles of sulfonic acid should be produced per mole of reacted disulfide. However, if the reaction goes totally through C–S fission, then only 1 mole of sulfonic acid can be produced from each mole of disulfide that reacts. Nachtigal and Robbins [4] have shown that this ratio is greater than 1.6 for frosted hair, suggesting that this reaction occurs largely through the S–S fission route. Secondly, if this reaction occurs through the C–S fission route, the alcohol produced would be a seryl residue and on hydrolysis would produce significantly larger quantities of serine in bleached hair hydrolysates than in hydrolysates of unbleached hair. However, this is not the case because Robbins and Kelly [5] have shown that in samples of hair bleached on heads with commercial bleaching products, the amount of serine remaining is equal to or less than that of unbleached hair. Thus, the oxidative cleavage of the disulfide bond that occurs during the chemical bleaching of human hair by current bleach products is predominately an S–S fission process.

Since chemical bleaching of human hair is carried out in an aqueous alkaline oxidizing medium, hydrolysis of the cystine oxide intermediates (Fig. 5.4) should be competitive with their oxidation. In fact, disproportionation of the cystine oxides [22] may also occur, adding to the complexity of the total reaction scheme; however, the net highest oxidation state of a disulfide under S–S fission conditions is sulfonic acid. This effect is illustrated by the oxidation and hydrolysis reactions of the cystine oxides summarized in Fig. 5.5. Note that disulfide trioxides have never been isolated from oxidized hair. Nevertheless, these species are suggested as "possible intermediates" since both disulfide dioxides and disulfide tetroxides of pure compounds [25] have been isolated by oxidation in an acidic medium. Cystine monoxide and dioxide are sensitive to alkaline hydrolysis [26, 27] but have been isolated from aqueous acidic oxidations [27]. Both the trioxide and tetroxide should be even more sensitive to alkaline hydrolysis than the monoxide and dioxide [25]. Although the importance of hydrolysis relative to oxidation for each of the cystine oxides is not known, it is certain that hydrolysis will be increasingly important with increasing pH. At the pH of current bleach products (pH 9–11) the rate of hydrolysis of these species should be competitive with

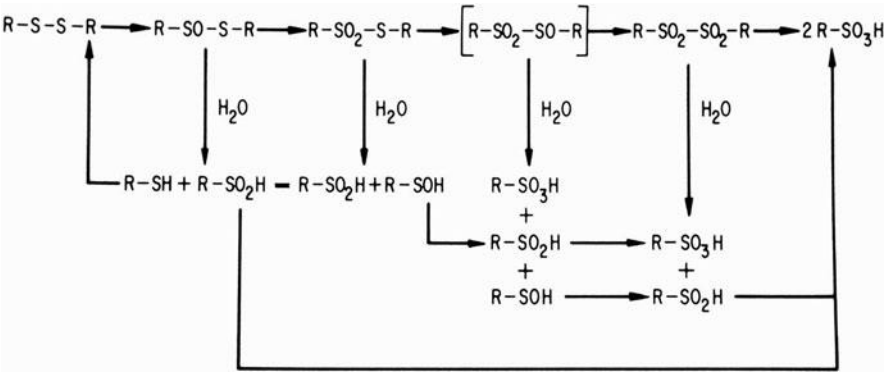


Fig. 5.5 S–S fission of disulfides in an aqueous alkaline oxidizing medium [24] (Reprinted with permission of the Journal of the Society of Cosmetic Chemists)

oxidation, thereby decreasing the probability of existence of these species in major quantities in bleached hair.

Intact hair from bleaching experiments using alkaline hydrogen peroxide and peroxide-persulfate has been examined by both infrared spectroscopy [3, 9] and electron spectroscopy for chemical analysis [6]. Evidence for intermediate oxidation products of cystine (the monoxide through tetroxide) could not be found. However, one cannot conclude that very small quantities of these species do not exist in bleached hair. The primary conclusions from these spectroscopic studies are: (1) the principal end product from the oxidation of cystine during chemical bleaching of hair with either alkaline peroxide or alkaline peroxide-persulfate is cysteic acid, and (2) the cleavage of cystine proceeds primarily through the S–S fission route.

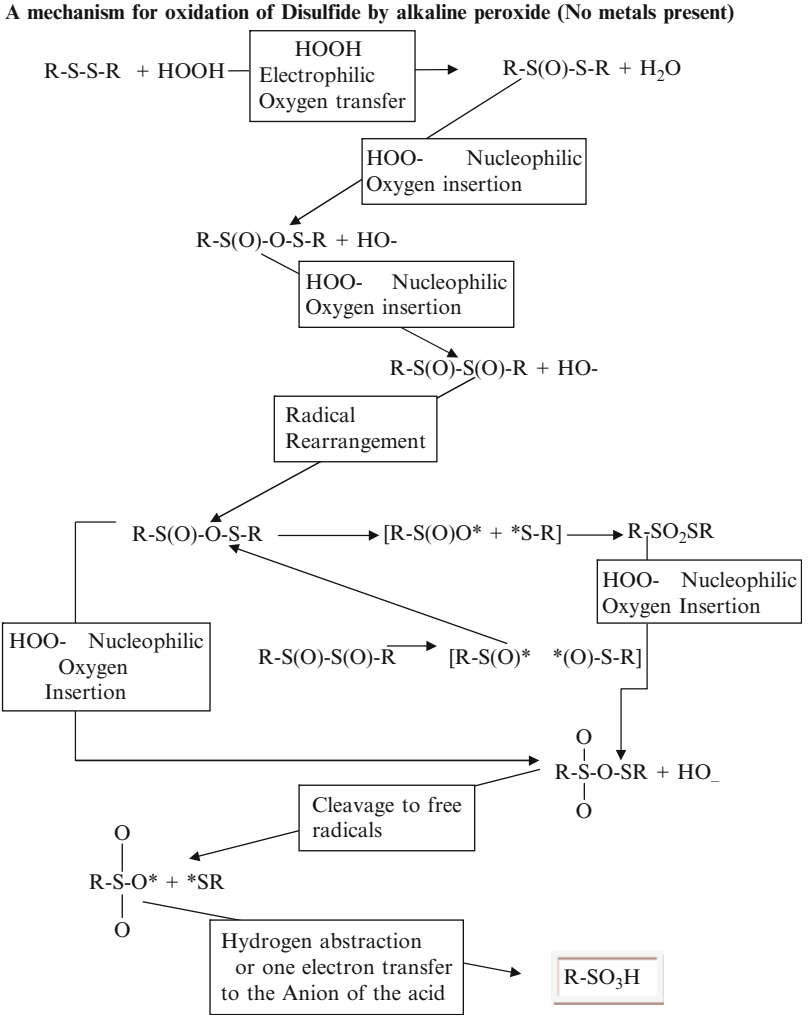
Zahn and co-workers [2], using two-dimensional gel electrophoresis, separated up to 62 isolated protein spots from human hair. From the fluorogram of bleached hair, these scientists identified cystine oxides (monoxide and dioxide). Although, the exact quantities of these intermediate oxidation products vs. cysteic acid were not reported, the quantities were indicated to be small relative to the cysteic acid content [2].

To summarize, sulfonic acid is the principal established end product of the oxidative cleavage of the disulfide bond from the chemical bleaching of human hair with current hair bleach products [3, 9]. The mercaptan content of bleached hair is lower than that of unbleached hair [4]. The intermediate oxidation products of cystine, that is the disulfide monoxide, dioxide, trioxide, and tetroxide do not exist as significant end products of hair bleaching using today's commercial bleach products [3, 6, 9]. Nevertheless, evidence has been presented demonstrating low levels of cystine oxides in bleached hair [2].

Considering all the species from the oxidation of disulfides described in Fig. 5.5, the sulfinic acid is the only species of even moderate stability [28] remaining to be examined. Sulfenic acids are notoriously unstable [29], and disulfide trioxides and disulfide tetroxides are even more sensitive to alkali than are the dioxides and the monoxides. Specific mechanisms for the oxidation of the disulfide bond are described in the next section of this Chapter.

5.3.2 *Proposed Mechanisms for Oxidation of Disulfide Bonds by Alkaline Peroxide*

When virtually no metals are present to generate free radicals, the primary mechanism for oxidation of the disulfide bonds in hair with alkaline hydrogen peroxide occurs through the monoxide and dioxide primarily via electrophilic oxygen transfer and nucleophilic oxygen insertion according to the following pathway through S–S fission. A large part of this mechanism was described to me in a private communication by Dr. Jennifer Marsh:



When metals like iron II or copper I are present hydrogen peroxide can react with these to form free radicals by Fenton's reaction (below) and the oxidation mechanism follows a different pathway but still lead to sulfonate via the S–S fission pathway as summarized below:

Mechanism for the oxidation of disulfide by alkaline peroxide with Metals (Fe++ or Cu+)

$$H_2O_2 + M = HO^{\cdot} + HO^{-} \quad \text{(Fenton Reaction)}$$

$$HO^{\cdot} + \text{R-S-S-R} \rightarrow \text{R-S}^{\cdot}\text{-S}^{+}\text{(OH)-R} \quad \text{(Cation radical)}$$

$$\text{R-S}^{\cdot}\text{-S}^{+}\text{(OH)-R} + O_2^{\cdot -} \rightarrow \text{R-S(O}_2\text{)-S-R} \rightarrow 2\ \text{R-S-O}^{\cdot}$$

$$\text{R-S-O}^{\cdot} + O_2^{\cdot -} \rightarrow \boxed{\text{R-SO}_3^{-}}$$

Marsh et al. [30] described the formation of the hydroxyl and perhydroxyl radicals and molecular oxygen that result in hair by the decomposition of alkaline hydrogen peroxide in the presence of transition metal ions like iron and copper. These free radicals induce formation of cysteic acid from disulfide in the F-layer, and other regions of the fiber during the oxidation dye process when transition metals like copper or iron are present. However, Marsh et al. [30] demonstrated that the inclusion of certain chelants into oxidation dye formulations can inhibit or reduce the formation of cysteic acid at or near the hair fiber surface. These effects are explained by the chelants binding low levels of copper known to be in some tap waters. This action by chelants (in alkaline peroxide) inhibits the known metal induced free radical formation and the resultant formation of sulfonate by the oxidation of disulfide at or near the fiber surface. See Chap. 2 for a description of trace metals found in human hair.

5.3.3 Oxidation of Other Amino Acid Residues

Robbins and Kelly [5] have examined bleached and unaltered hair by hydrolysis and amino acid analysis. Their results for severely bleached hair are summarized in Table 5.6 and suggest that methionine, tyrosine, lysine, and histidine, in addition to cystine, are degraded to the greatest extent (tryptophan could not be evaluated in this study).

These results are consistent with the relative sensitivities of these species to oxidation. Cystine and its reactions with oxidizing agents have already been described. Methionine is also sensitive to oxidation and is probably oxidized to

Table 5.6 The effect of bleaching on the amino acid residues in hair [5]

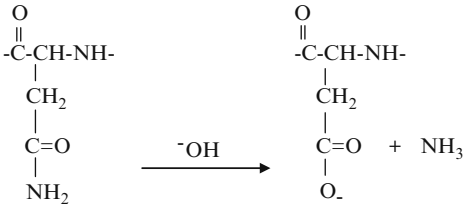
Amino acid	Micromoles/g dry hair		
	Nonfrosted	Frosted	% Difference[a]
Half-cystine	1,509	731	−50
Methionine	50	38	−24
Tyrosine	183	146	−20
Lysine	198	180	−10
Histidine	65	55	−15

[a]Only those amino acids found to be 10% or lower in bleached hair are included in this table

its sulfoxide and possibly to methionine sulfone. Tyrosine, with its electron-rich phenolic ring, is also sensitive to oxidation. The amine salts of lysine and histidine should be resistant to oxidation, although the free amines of these species may be slowly oxidized in the bleach medium.

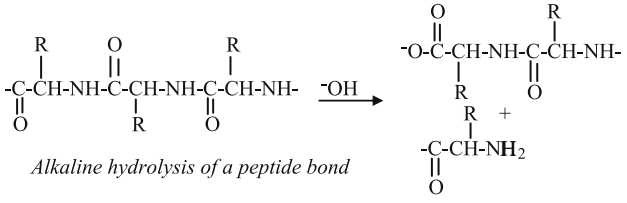
5.3.4 *Hydrolysis or the Action of Alkalinity*

Since bleaching compositions are usually formulated between pH 9 and 11, the hydrolysis of peptide and amide bonds and the formation of lanthionyl residues in hair are possible side reactions during bleaching. The hydrolysis of amide groups of the residues of aspartic and glutamic acids, in addition to the formation of cysteic acid residues, will increase the ratio of acidic to basic groups in the fibers; i.e., amide hydrolysis will decrease the isoelectric and isoionic points of the fibers.



Amide of aspartic acid residue

Peptide bonds are the major repeating structural unit of polypeptides and proteins. Hydrolysis of peptide bonds can occur at high pH and is most likely to occur during frosting or bleaching from black or brown-black to light blond, where long reaction times and higher concentrations of alkalinity and oxidizing agent are employed.



Alkaline hydrolysis of a peptide bond

Harris and Brown [20] have shown that the wet tensile properties of keratin fibers are related to the disulfide bonds, whereas the dry tensile properties are influenced more by peptide bond cleavage [21]. In an examination of frosted hair, Robbins found a 4–8% decrease in the dry tensile properties (see Chap. 9 for details). This suggests that some peptide bond hydrolysis occurs during severe bleaching conditions. Note that the frequency of peptide bonds is nearly an order of magnitude greater than that of the disulfide bonds in human hair.

The formation of lanthionyl residues in alkaline media is described in Chap. 4. Note that if lanthionine is formed during hair bleaching, its sulfoxide and sulfone are also possible oxidation products.

5.3.5 Summary of Chemical Bleaching of Hair Proteins by Peroxide

When hair is exposed to chemical bleaching, changes occur in the surface layers removing some of the 18-methyl eicosanoic acid. Free lipids at the surface and between scales are removed or oxidized. The reaction with 18-MEA results in the formation of acidic sulfur compounds such as mercaptan, sulfinate and sulfonate groups (predominately sulfonate for chemically bleached hair). A decrease in the free lipid content in the surface layers also results which with removal of 18-MEA provides a dry feel to the hair. These changes convert the virgin hair fiber and especially the surface from a hydrophobic, entity with little surface charge to a more hydrophilic, more polar and more negatively charged surface; see Chap. 6 for additional details. In addition, cystine degradation occurs in the cystine rich A-layer and the exocuticle of the cuticle. Tensile data clearly shows that cystine degradation occurs in the matrix and intermediate filaments of the cortex too. In addition, methionine, tyrosine, histidine and lysine are modified by oxidation and some of the peptide and amide groups are hydrolyzed by alkaline degradation.

5.4 Oxidation of Hair Proteins and the Cell Membrane Complex by Sun and UV Light

Light radiation attacks hair proteins of the cuticle and the cortex, in addition to the cell membrane complex lipids (attached to the cell membrane proteins) and the hair pigments. The emphasis in this section is on the photochemical degradation of the proteins and lipoproteins of hair with special emphasis on the cell membrane complex lipids and proteins. Later in this chapter, in the discussion on hair pigments, the effects of light radiation on melanins are considered.

Unfortunately, many of the published manuscripts dealing with photochemical degradation employ different units of radiation or widely varying exposures making

comparisons difficult. Signori [31] suggested that comparisons be made in terms of total irradiation energy using units of J m^{-2} $month^{-1}$ (Joules per meter squared per month). A typical Florida Month of exposure provides 295 $\times$ 10^6 J m^{-2} $month^{-1}$ of UV plus visible light and 25 $\times$ 10^6 J m^{-2} $month^{-1}$ of UV light. Many photochemical treatments of hair in the literature are well beyond this level of exposure. However, 1–4 Florida months of exposure appears to be a reasonable high level of exposure that might occur on the hair of Florida sunbathers.

The covalently bound lipids of the CMC of the cuticle are sensitive to oxidation, reduction and to alcoholic alkalinity while the lipid Beta layers of the cortex are affected more by lipid solvents and free radical chemistry. For example, the Beta layers of the cuticle are more sensitive to nucleophilic attack by species such as the hydroperoxide anion and mercaptans, but the Beta layers of the cortex with their multiplicity of double bonds (oleic plus palmitoleic acids, plus cholesterol and cholesterol sulfate) and tertiary hydrogen atoms (cholesterol and cholesterol sulfate) are more sensitive to free radical chemistry. On the other hand, the membranes of the CMC are resistant to oxidizing and reducing agents [32]. Several of these chemical actions make the CMC more vulnerable to fracture, to cuticle fragmentation and to the propagation of cracks through the cortex leading to split hairs.

There is evidence that an appreciable amount of free-lipid (not covalently bound to hair proteins) is in the Beta layers of the cuticle and likely in all lipid layers of keratin fibers [33]. Furthermore, about 50% of free-lipid in human hair is fatty acid and free lipid provides acidic groups to the hair surface and decreases the isoelectric point as shown by Capablanca and Watt [34] for wool fiber. As hair is exposed to repeated shampooing, blow drying, rubbing and to sunlight, changes occur on and in the surface layers. These changes involve removal of some free lipids by shampoos, photo-degradation of 18-MEA, disulfide and other functional groups. Consequently, the fiber so weakened can form fractures in or between layers from severe bending, stretching and abrasive actions during combing and/or brushing.

These actions expose “new” protein material and sulfur acids primarily sulfonate groups with an accompanying decrease in the free and bound lipid content of the hair surface. In that manner, the virgin hair surface is converted from a hydrophobic entity with little surface charge to a hydrophilic, polar and negatively charged surface. The more exposure of the hair to chemical and abrasive actions, such as the further from the root ends the more hydrophilic, more polar and more negatively charged the surface becomes.

5.4.1 Damage by Shampoos and Conditioners and Irradiation

The dissolution or the removal of structural lipids or proteinaceous matter from hair, primarily from the CMC or endocuticle, by shaking keratin fibers either in surfactant or shampoo solutions or even water has been demonstrated by several different scientists. For example, Marshall and Ley [35] demonstrated the extraction of proteinaceous components from the cuticle of wool fiber by shaking it in

sodium dodecyl sulfate solution, a common surfactant in many shampoos. Gould and Sneath [36] examined root and tip end sections of scalp hair by TEM and observed holes or vacancies in thin cross-sections of the hair. This hair had never been chemically treated. These holes were more frequent and larger in tip ends than in root ends. These scientists attributed these holes to damaging effects by shampooing and weathering involving the breakdown and removal of the non-keratin portions (CMC and endocuticle) of the hair leaving the intercellular regions more susceptible to fracture under stress. It is likely that sun exposure helped to make the hair more vulnerable to the actions of shampoos; however sunlight effects were not examined in this study.

Beta-delta failure [37] is one of the most common types of fractures in hair fibers. This fracture occurs in the cuticle–cuticle CMC between the upper Beta layer and the adjoining Delta layer (see Fig. 6.31) and it occurs most readily when the fiber is dry. Gamez-Garcia [38] noted that the lower the relative humidity or the moisture content of hair, the lower the strain level required to produce Beta-delta failure.

Beta-delta failure was observed by Negri et al. [37] on wool fiber who noted disruption of the cuticle–cuticle CMC along the upper Beta layer in TEM sections. With this type of fracture, the Delta layer and the lower Beta layer are both retained on the underside of the "old" outermost cuticle cell. As a result 18-MEA is left as the "new" hair surface once the "old" outermost cuticle cell is abraded away. This type of fragmentation has been described in detail by Feughelman and Willis [39] who proposed that the failure of adhesion between overlapping scales involves 18-MEA. Furthermore, the chain branch in 18-MEA provides mobility and a reduction in adhesion between scales leading to Beta-delta failure. Therefore, chemical degradation of 18-MEA, both on the surface and between scales leads to further weakening of this structure and more rapid Beta-delta failure leading to faster cuticle fragmentation and cuticle loss. Ruetsch and Kamath [17] have shown that 18-MEA is degraded by chemical and photochemical bleaching. Ruetsch et al. [40] has shown that it is degraded by alkalinity and Robbins [41] has shown that it is degraded by permanent wave treatments.

5.4.2 Wet Versus Dry State Failure and Oxidative Exposure

Deformations such as stretching as shown by Robbins [42] and by Kamath and Weigmann [43] (including extension cycling by Gamez-Garcia [38]), bending as in a snag or a knot [42, 44] or twisting in the wet state are very different than deformations in the dry state. This is because failure in the wet state generally involves fractures or breaking bonds in hydrophilic layers, such as the endocuticle or the central contact zone of the CMC. In contrast, failure in the dry state generally involves fractures in or between hydrophobic layers, e.g., Beta-delta failure [39, 42]. Failure in the wet state generally involves hydrophilic regions because when a layer or region is completely swollen less mechanical stress is required to distort that layer

and to produce a fracture. At low relative humidity or swelling condition of a hydrophilic layer, more mechanical stress is required to distort it relative to hydrophobic layers and therefore fractures are generally produced in hydrophobic layers.

Extension of undamaged hair to break generally produces smooth fractures [44]. However, as the hair becomes more damaged or as the relative humidity is decreased and especially at lower humidity more step fractures are produced [43]. Step fractures involve extensive fracturing in the cortex–cortex CMC, most likely in the lipid or Beta layers. Kamath and Weigmann [43] demonstrated at low moisture content, crack initiation occurs most often in the cortex. However, at high moisture content, fractures almost always initiate at or near the fiber surface because of the high pressure of the swollen cortex against the cuticle.

Step fractures involve the axial propagation of cracks either through the cortex–cortex CMC (see Fig. 1.45) or the medulla [43] and because these regions are hydrophobic they tend to occur more frequently in the dry state than when hair fibers are wet [43]. Kamath and Weigmann [43] also concluded that, the CMC seems to "play an important role in stress transfer and axial splitting" of human hair fibers.

The abrasion resistance of human hair is decreased by most chemical treatments including photo-oxidation as shown by the "Protein loss" test of Sandhu and Robbins [45] or by the release of labile and eluted proteins as described by Inoue et al. [46]. These tests are both wet state methods. The interior of cortical cells is degraded by alkaline peroxide, thereby weakening the cuticle and cortex cells internally. Alkaline peroxide degrades the cuticle–cuticle CMC weakening the cellular cohesion or the resistance of scales to break apart. Cuticle fragmentation in the dry state is caused primarily by the rupture between cuticle cells through Beta-delta failure [38, 39] and the resultant chipping of cuticle from the hair via abrasive actions. Cuticle loss in the wet state is primarily caused by rupturing of cuticle cells internally and is greater in chemically damaged hair such as alkaline peroxide treated, permanent waved or irradiated hair than in chemically untreated hair. This type of cuticle loss is due to the decrease in disulfide crosslinks and an increase in hydrophilic sulfonate groups [45].

Fatigue testing a method developed at TRI-Princeton by Ruetsch, Kamath and Weigmann (involves attaching a weight to a hair and dropping the weight multiple times to continuously shock or jar the fiber) shows that alkaline peroxide treatment of human hair fibers when fatigued produces numerous scale edge fractures with scale edge chipping. Ruetsch [47] fatigue tested peroxide treated hair in the dry state followed by stretching and found extensive fracturing in the CMC between the scales due to a weakened cuticle–cuticle CMC by oxidative treatments, chemical or simulated sunlight. This effect is most likely due to oxidative attack on thioester linkages that disrupts the Beta layers of the cuticle–cuticle CMC.

Takahashi et al. [48] provided evidence that wet cuticle wear in Asian hair is due more to CMC failure (possibly involving the central hydrophilic "contact zone" of the Delta layer) rather than failure inside cuticle cells as in Caucasian hair (most likely endocuticular failure). Takahashi et al. [48] showed that wet cuticle wear on Asian hair occurs at a faster rate than on Caucasian hair. This increased wet cuticle

wear is because of differences between Asian and Caucasian hair in the elasticity of the different layers inside cuticle scales. Takahashi et al. showed that the scales of Asian hair are removed faster by wet sonication after extension to 35% or by bleaching the hair followed by shampooing and wet combing the hair over a large number of cycles. In the latter case after 90 grooming strokes for four cycles fewer scales were found on Asian hair relative to Caucasian hair (1.3 vs. 3.2 scales respectively).

On further examination of the hair using an Atomic Force Microscopic probe these scientists found a greater difference in elasticity as a function of depth for the Caucasian vs. Asian hair (1.41 vs. 1.26). Therefore, Takahashi et al. concluded that the scales of Asian hair are removed more by fracturing in the cuticle–cuticle CMC (even in the wet state) while the scales of Caucasian hair fractures inside the scales most likely in the swollen endocuticle. It is interesting to note here that Nakamura [49] by staining reactions has concluded that the composition of the proteins of the Delta layer of the cuticle–cuticle CMC is very much like that of the very hydrophilic endocuticle.

5.4.3 CMC Lipids Degraded by Both UV and Visible Light

Hoting and Zimmerman [50] studied radiation damage to hair as a function of wavelength and determined that the CMC lipids of hair fibers are degraded most by visible light, but also by UV-A and UV-B light. These results help to explain the weakened CMC (of cuticle and cortex) and the multiple step fractures that result from the axial propagation of cracks through the cortex–cortex CMC in sunlight oxidized hair. Furthermore, Hoting and Zimmermann demonstrated that cystine, proline and valine are degraded more in light brown hair than in black hair confirming that the photo-protective effect by hair pigments is stronger in dark hair than in light hair.

Korner et al. concluded that one weak link to photo-chemical attack on lipid structures in the cuticle CMC are the tertiary hydrogen atoms of 18-MEA [51]. Cholesterol and cholesterol sulfate also contain tertiary hydrogen atoms and should provide sites for hydrogen abstraction in photo-chemical reactions. In addition, the allylic hydrogen atoms of oleic and palmitoleic acids and of cholesterol and cholesterol sulfate in the cortex–cortex CMC are especially vulnerable to photo-oxidative reactions as described later in this Chapter.

Long term irradiation does not provide for clean breaks between structural components of human hair as was observed for peroxide oxidized hair. However, long term radiation leads to cross-linking or fusion reactions similar to long term radiation on wool fiber as explained in the next section of this Chapter. For hair damaged by sunlight, in some cases, the lipids of the cuticle CMC appear altered to a greater extent than the more susceptible areas of the cortex CMC because the outer layers of the fiber receive higher intensities of radiation.

5.4.4 *Short Term Irradiation Attacks CMC Lipids Producing Internal Step Fractures*

Fracturing of wool fiber exposed to simulated sunlight has been studied microscopically by Zimmermann and Hocker [52]. Electron micrographs of human hair fibers exposed to simulated sunlight and then fractured were provided to this author by Sigrid Ruetsch showing that human hair provides similar effects to wool fiber. Zimmerman and Hocker demonstrated that stretching non-irradiated control wool fibers in water provided primarily smooth fractures, while short and intermediate times of simulated sunlight exposure caused the fibers to break mainly as step fractures. These scientists suggested that short and intermediate-term irradiation damages the lipids of the CMC (all three types of CMC) and thereby provides many internal step type fractures by axial propagation of cracks through the photochemically damaged cortex CMC.

5.4.5 *Long Term Irradiation Produces Fusion Reactions Across Structural Boundaries*

Longer term irradiation creates cross-links in the fibers fusing the hair across structural boundaries creating amorphous fractures. Cracks do not occur between structural boundaries as in less damaged hair, but clean smooth fractures across cuticle and outer cortex boundaries see Figs. 5.6, 5.7, 5.8 and 5.9. Figures 5.10, 5.11 and 5.12 show the effects of ultraviolet exposure followed by reaction with alkaline hydrogen peroxide for different times (15 min to 2 h). The effects of alkaline

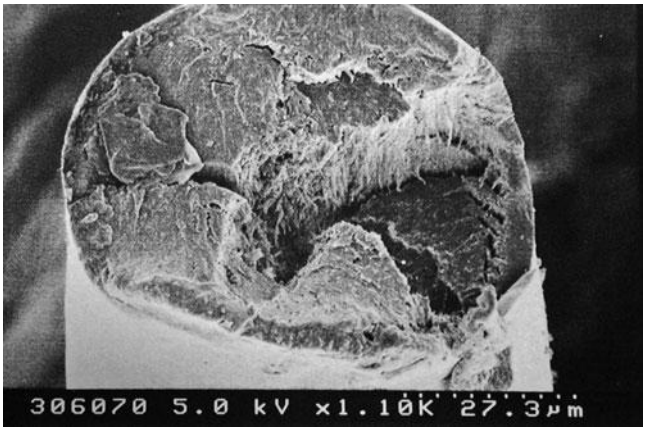


Fig. 5.6 Hair fiber exposed to ultraviolet radiation and extended to break dry (SEM was taken at the fracture site and kindly provided by Sigrid Ruetsch)

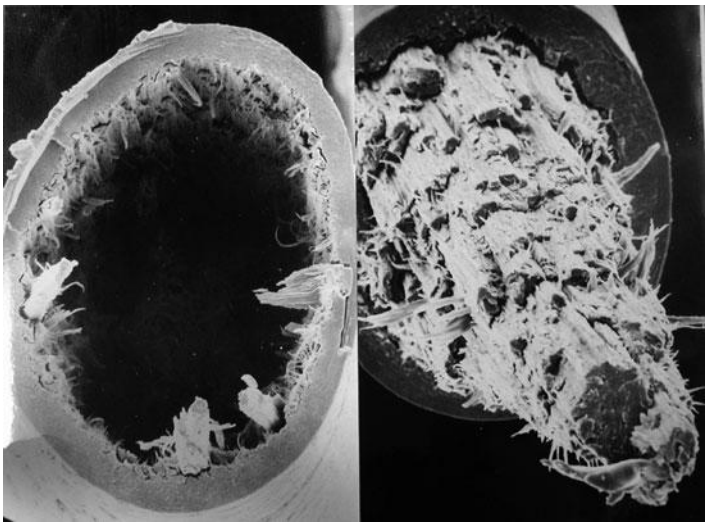


Fig. 5.7 Another hair fiber exposed to ultraviolet radiation and extended to break. SEM's were taken at the fracture site. *Top*: shows a sphire-like break containing multiple step fractures that broke away from the *Bottom*: leaving a hollow cavity in the hair (Micrographs kindly provided by Sigrid Ruetsch)

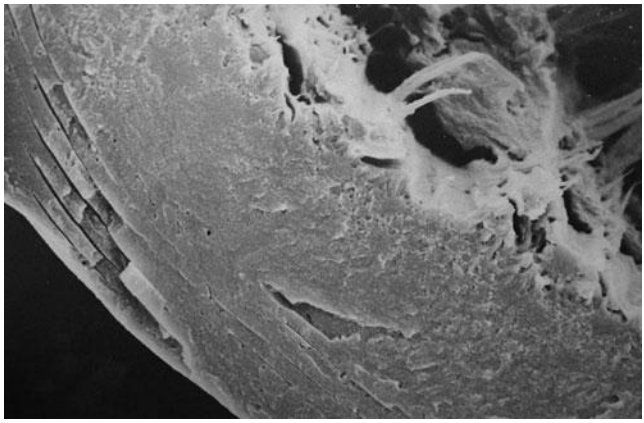


Fig. 5.8 High magnification of the periphery of the fiber in Fig. 5.7. Note the diminished definition of cuticle layers indicative of fusion reactions (SEM kindly provided by Sigrid Ruetsch)

peroxide on hair after long term exposure to ultraviolet are to fuse and then dissolve parts of the cuticle providing for even less structural differentiation. Part of the cuticular proteins are solubilized by these combined chemical treatments into gelatin-like glue that is re-deposited between the fibers, see Fig. 5.11. This effect was produced after only 15 min exposure to alkaline peroxide after long term photochemical degradation. The total lack of surface structural definition is seen

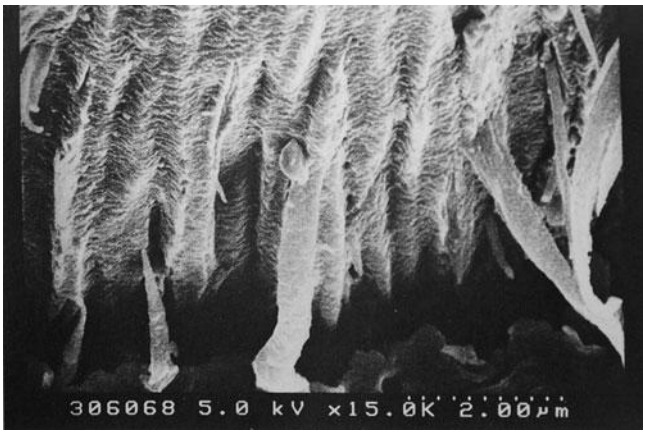


Fig. 5.9 High magnification view of the cortex of the UV exposed-fractured fiber of Fig. 5.6. Note the fusion occurring between cortical cells (SEM kindly provided by Sigrid Ruetsch)

Fig. 5.10 Hair fiber exposed to ultraviolet radiation and then bleached for 15 min with alkaline peroxide. Note the decreased scale definition (SEM kindly provided by Sigrid Ruetsch)

in the most extreme case in Fig. 5.12 where no cuticle scale definition exists after long time ultraviolet exposure and 2 h with alkaline peroxide. This lack of scale definition is probably due to the combined dissolution of the cuticle and the re-deposition of proteinaceous material onto the surface, thereby, masking the scales as occurs in the peracid treatment described later in this Chapter.

Zimmermann and Hocker [52] demonstrated that short and intermediate term radiation attacks CMC lipids providing for increased Beta-delta failure in the cuticle–cuticle CMC and multiple step fractures in the cortex CMC. Longer term irradiation produces amorphous fractures by fusion reactions through the creation of carbonyl groups that are cross-linked through lysine, analogous to the oxidative damage to proteins and mitochondrial decay associated with aging described by

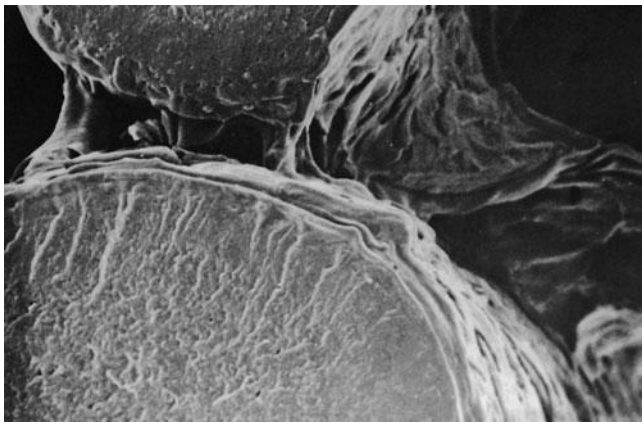


Fig. 5.11 Hair fibers exposed to ultraviolet light and then bleached with alkaline peroxide for 4 h. Note the decreased scale differentiation and the apparent "glueing" together of the fibers by the "gelatinized" hair proteins (SEM kindly provided by Sigrid Ruetsch)

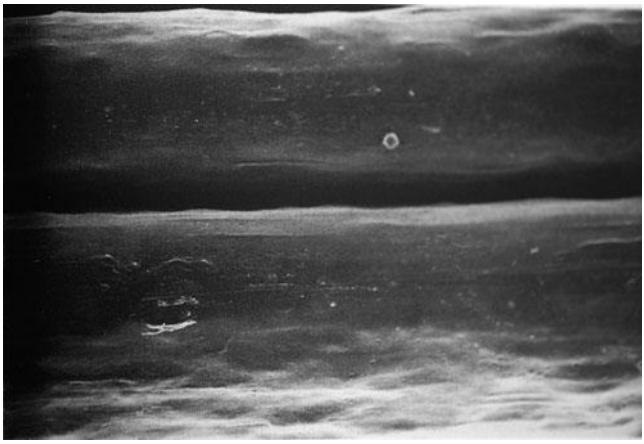
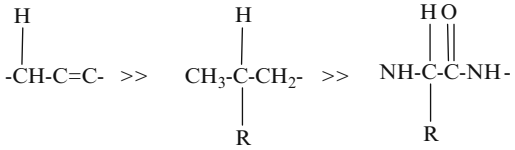


Fig. 5.12 Hair fibers exposed to ultraviolet radiation and bleached with alkaline peroxide for 2 h. Note the total lack of scale definition (SEM kindly provided by Sigrid Ruetsch)

Dean et al. [53]. These fusion reactions start in the periphery of the fiber where it receives higher intensities of radiation than the core, providing a smooth fracture at the periphery and multiple step fractures in the interior of the fibers (Figs. 5.7 and 5.8). If the fibers are exposed long enough to light radiation, amorphous fractures are produced across the entire fiber.

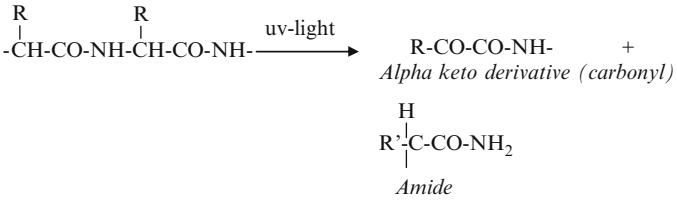
Short and intermediate term exposures to radiation are propagated by abstraction of hydrogen atoms from tertiary carbon atoms of 18-MEA [51] and of allylic hydrogen atoms of oleic and palmitoleic acids in lipids of the cuticle CMC. The cortex-CMC contains tertiary hydrogen atoms on cholesterol and cholesterol sulfate and allylic hydrogen atoms on oleic and palmitoleic acids, and on cholesterol and

cholesterol sulfate that react similarly. The abstraction of hydrogen atoms from tertiary carbon atoms on amino acid side chains (analogous to the tertiary carbon atoms on 18-MEA and cholesterols) has been shown by Goshe et al. [54] to predominate over the abstraction of hydrogen atoms at the alpha carbon atom of amino acids in poly-peptides. However, the abstraction of hydrogen atoms at allylic positions should be even faster.



Allylic Tertiary hydrogen atom Tertiary hydrogen at α-carbon

These facts help to explain why the Beta layers are degraded faster by photo-oxidation than the hair proteins. Photochemical reactions in the Beta layers on allylic groups are very fast and lead to axial failure including splitting. However, photochemical reactions in the proteins are slower leading to cross-linking (fusion reactions) and amorphous fractures.

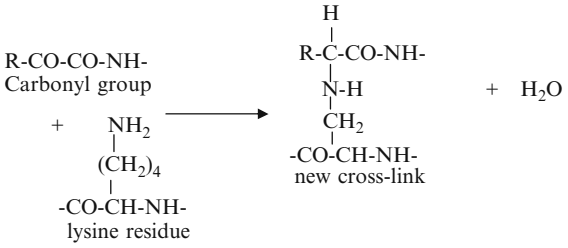


Rupture of peptide bonds by uv light

Oxidation at the peptide backbone carbon has been shown to occur from ultraviolet exposure both in wool [55] and in hair [6], producing carbonyl groups (alpha keto acid/amide) and amide groups. Ultimately these reactions can lead to new cross-links as described in the equations below. The formation of carbonyl groups is favored in the dry state reaction more than the wet state. This reaction has been documented using infrared spectroscopy by Robbins [3] and Dubief [18].

Figures 5.6, 5.7, 5.8 and 5.9 (described earlier) illustrate hair fibers exposed extensively to simulated sunlight and extended to break. These SEM's show that long term ultraviolet exposure causes severe chemical degradation to the hair proteins. As indicated above, the damage is so extensive that structural differentiation is diminished. This physicochemical degradation usually occurs at a higher level in the hair fiber periphery with a gradient to a lower level of oxidative damage deeper into the fiber. Such damage leads to unusual fracture patterns during extension, see Figs. 5.7, 5.8 and 5.9. The breakdown of disulfide bridges within structural units of the A-layer and the exocuticle and matrix and the establishment of new intra- and intermolecular cross-links via reaction of carbonyl groups with protein amino groups (see reactions described below) within and between

structural units decreases structural definition. These reactions collectively lead to a fusion of different structures and a gradual increase in brittleness accompanied by a loss of structural differentiation as shown in these photomicrographs (Figs. 5.7, 5.8 and 5.9).



Formation of cross-links from rupture of peptide bonds by uv light

Subsequent exposure to aqueous alkaline solution or to alkaline peroxide solutions leads to rapid dissolution of the affected areas (Figs. 5.10 and 5.11). Longer exposure of these ultraviolet radiated fibers with oxidizing solutions leads to dissolution/ elimination of scale differentiation and dissolution of the melanin granules, see Fig. 5.12.

5.4.6 *Fusion Reactions at Peptide Bonds from Free Radicals at Alpha Carbon Atoms*

The fusion reactions of wool and human hair (described above) are believed to be related to the oxidative damage of proteins and mitochondrial decay associated with aging described by Dean et al. [53] and are believed to involve the abstraction of a hydrogen atom from the alpha carbon of an amino acid residue in a protein chain in a keratin fiber [56] which can then either add oxygen to form a hydroperoxide or lose hydrogen to form a dehydropeptide. Meybeck and Meybeck [56] concluded that either route forms an alpha-keto acid (carbonyl group) after hydrolysis. This effect results by cleaving the protein chain to form the alpha-keto acid and a primary amide. The alpha-keto acid then reacts with a lysine residue to form a new amide cross-link in the fibers and thus the fusion reaction is completed.

Carbonyl groups have been shown to be formed by irradiation of wool with simulated sunlight and this reaction is related to photoyellowing of keratin fibers. Holt and Milligan [55] identified keto acids (carbonyl groups) in irradiated wool by reductive amination with sodium ^{3}H-borohydride in ammonia. This reaction converts keto acids to the corresponding ^{3}H-amino acids. By this type of labeling, Holt and Milligan demonstrated that by irradiating wool, carbonyl groups are formed from alanine, glycine, proline, serine, threonine, glutamic acid and tyrosine. Therefore, free radicals were formed from these amino acids by hydrogen atom abstraction at the alpha carbon atom.

However, as indicated earlier, the fusion reactions are slower than the other free radical reactions involving hydrogen atom abstraction, because the abstraction of hydrogen atoms from tertiary carbon atoms of side chains of amino acid residues has been shown to be prevalent over the abstraction of hydrogen atoms alpha to the peptide function [54] and the abstraction of a hydrogen atom from an allylic group is even faster. Furthermore, the consequences of the fusion reactions appear only after the most intense and longest radiation times on wool fiber as shown by Zimmermann and Hocker [52].

5.4.7 *Photoprotection by an Oxidation Dye*

Hoting and Zimmermann [50] demonstrated that the CMC lipids of the cortex of hair, previously bleached with peroxide-persulfate, are more readily degraded by radiation than the lipids of chemically unaltered hair or the lipids of hair dyed with a red oxidation dye. This conclusion was reached by analysis of the cholesterol containing lipids of hair which reside primarily in the cortex CMC. Peroxide-persulfate oxidation of hair is primarily a free radical oxidative process and it leaves hydroperoxide groups in the hair in the CMC and in other regions.

Thus the action of sunlight on peroxide-persulfate bleached hair (containing hydroperoxides) makes the hair more vulnerable to cuticle fragmentation and to splitting that the CMC plays a significant role in. In this same paper these scientists demonstrated that one red oxidation dye provides photo-protection to both UV-A and visible light but not to UV-B light. Hair when treated with this red dye when compared to chemically untreated hair retards the degradation of the CMC lipids most likely by the dye acting as a radical scavenger.

5.4.8 *Other Physical Effects from Photochemical Reactions with Hair*

Beyak [57] and Dubief [18] demonstrated that sunlight and ultraviolet light decrease the wet tensile properties of human hair. Beyak related these effects to the total radiation that the hair is exposed to, rather than to any specific wavelength. However, more recently, hair protein degradation by light radiation has been shown to occur primarily in the wavelength region of 254–400 nm. Hair proteins have been shown by Arnaud et al. [58] to absorb light primarily between 254 and 350 nm.

Dubief also determined that swelling of photochemically damaged hair is increased relative to undamaged control hair. This effect was demonstrated by both the alkali solubility test [18, 59] and by swelling in sodium hydroxide [18].

Several amino acids of hair absorb light in this region (254–350 nm). Therefore, these amino acids are the most subject to degradation by light. The following amino

acids have been shown to be degraded by weathering actions (primarily light radiation) on wool fiber by Launer [60] and by Inglis and Lennox [61]; cystine and methionine (two sulfur-containing amino acids); the aromatic and ring amino acids—phenylalanine and tryptophan (often associated with photo-yellowing of wool), histidine, and proline; and the aliphatic amino acid leucine.

Pande and Jachowicz [62] used fluorescence spectroscopy to monitor the decomposition of tryptophan in hair. These scientists demonstrated that photodegradation of tryptophan occurs in hair. In addition, they speculated that photodamage to tryptophan can increase the sensitivity of other amino acids to photodegradation as explained in the section of this Chapter entitled, *Mechanisms for photochemical reactions in human hair*.

5.4.9 Other Photochemical Reactions with Hair Fibers

Robbins and Kelly [63] analyzed amino acids of both proximal and distal ends of human hair and demonstrated significantly more cysteic acid in tip ends. They attribute this change to weathering actions, specifically to ultraviolet radiation attack on disulfide and thioester bonds. This same study also found significant decreases in tip ends for tyrosine and histidine similar to the weathering effects in wool fiber. Decreases were also reported in the lysine content in this study on hair weathering. This effect could be from the cross-linking reaction of lysine with carbonyl groups formed by ultraviolet attack on peptide bonds (described above).

Robbins and Bahl [6] examined the effects of sunlight and ultraviolet radiation on disulfide sulfur in hair via electron spectroscopy for chemical analysis (ESCA) [6]. Both UV-A (320–400 nm) and UV-B (290–320 nm) radiation were shown to oxidize sulfur in hair. The primary oxidation occurs closer to the hair fiber surface, probably from attack on thioester and disulfide, producing a steep gradient of oxidized to less oxidized hair from the outer circumference of the hair to the fiber core.

The ESCA binding energy spectra (S 2p sulfur) for weathered hair and hair exposed to an ultraviolet lamp in the laboratory are similar but differ from spectra of chemically bleached hair (alkaline hydrogen peroxide). Similar binding energies suggest similar end products and similar mechanisms of oxidation. As described earlier in this chapter, the mechanism for peroxide oxidation of pure disulfides and for disulfide residues in hair is believed to proceed through the S–S fission route. On the other hand, for irradiation of pure cystine, existing evidence by Savige and Maclaren [25] suggests the C–S fission route as the preferred route for photochemical degradation of cystine and for other pure disulfides (see Fig. 5.4). For the photochemical reaction, if the pH is neutral or alkaline, then the C–S fission route is the preferred one; however, if the pH is acidic, then the homolytic or S–S fission route is more likely to occur.

The evidence from ESCA suggests that both the chemical and the photochemical degradation of cystine in hair are similar to that of pure disulfides [6], that is, for chemical degradation, S–S fission occurs, while for photochemical degradation the,

C–S fission route is the preferred route. For the S–S fission route, the main end product is sulfonic acid. For the C–S fission route, the main products are the S-sulfonic and sulfonic acids [25]. However, ultimately, S-sulfonic acid is degraded by light to sulfonic acid [25]. The ESCA spectra suggest that cystine S-sulfonate and cysteic acid are both formed in weathered (tip) ends of hair and in hair exposed to ultraviolet light. But, cysteic acid is the primary end product formed from the oxidation of cystine in hair during chemical bleaching [6]. These results suggest that the mechanism for the radiation-induced degradation of cystine occurs through the C–S fission pathway and is different from the chemical oxidation of cystine that proceeds mainly via the S–S fission route.

Tolgyesi [64] and Ratnapandian et al. [65] proposed a homolytic scission of the disulfide bond by sunlight; however this mechanism ignores the resultant end products of the reaction. It is likely that the photochemical reaction is not as clean as that of the chemical route. Therefore, both pathways are possible and likely to occur when the reaction is photochemically induced. Nevertheless, the formation of cystine S-sulfonic acid cannot be explained by the homolytic scission of the disulfide bond alone. Mechanisms for both hemolytic scission and C–S scission will be described in the next sections of this Chapter.

5.4.10 Summary of Sunlight Oxidation of Hair Proteins

As hair is exposed to sunlight changes occur by removal of 18-MEA at the surface and between scales by the free radical oxidation of sulfur compounds forming mercaptan, sulfinate and sulfonate groups (primarily sulfonate) and a decrease in the free lipid content in the surface layers. These changes convert the virgin hair surface from a hydrophobic, entity with little surface charge to a more hydrophilic, more polar and more negatively charged surface; see Chap. 6 for additional details. Cystine degradation occurs inside cuticle cells in the cystine rich A-layer and the exocuticle of the cuticle and in cortical cells because tensile results show that cystine degradation occurs in the matrix of the cortex too and likely in the Intermediate Filaments too; however the strongest effects are in the uppermost surface layers [6]. Cystine and tryptophan, methionine, tyrosine, histidine and lysine are also modified by oxidation. With long term or extensive sunlight exposure, peptide bonds are degraded by sunlight forming new cross-links which first occurs in the cuticle layers and ultimately in the cortex.

5.5 Mechanisms for Free Radical Reactions in Human Hair

Kirschenbaum et al. [66] provided evidence from photo-irradiation of human hair under UVA and visible light for the formation of the hydroxyl free radical. Their results showed that bleached and red hair provide a greater yield of hydroxyl

radicals than brown hair. This latter effect is due to the fact that there is more eumelanin in brown than bleached and red hair and it is a more effective radical scavenger than pheomelanin.

Millington [67] in a review on photoyellowing of wool describes the formation of hydroxyl radicals, oxygen radicals, superoxide and hydroperoxides as being the species that drive photoyellowing reactions. Millington described the generation of hydroxyl radicals in several schemes as in the initial photo-chemical excitation of a photo-chemical absorber or radical initiator such as melanin [67–69] pheomelanin or tryptophan [66, 67].

$$\text{Initiator} + \text{hv} \rightarrow \text{Initiator}^{*}\{\text{Initiator}^{*} \text{ denotes excited state}\}$$

The second step is the formation of free radicals from the excited state of the initiator.

$$\text{Initiator}^{*} \rightarrow \text{B}^{\bullet} + \text{C}^{\bullet}$$

The next step involves abstraction of a hydrogen atom from the keratin fiber to form a free radical on the hair or wool keratin.

$$\text{B}^{\bullet} + \text{Keratin-H} \rightarrow \text{Keratin}^{\bullet} + \text{B-H}$$

Propagation then occurs by reaction with oxygen forming a hydroperoxide of the keratin.

$$\text{Keratin}^{\bullet} + \text{O}_2 \rightarrow \text{Keratin-OO}^{\bullet}$$

$$\text{Keratin-OO}^{\bullet} + \text{ Keratin-H } \rightarrow \text{Keratin-OOH} + \text{Keratin}^{\bullet}$$

The Keratin hydroperoxide can react with either a transition metal like Fe or Cu that is either in solution or in the fiber or with light to form a hydroxyl radical and a Keratin hydroxyl radical to continue the chain reaction.

$$\text{Keratin-OOH} + \text{hv} \rightarrow \text{Keratin-O}^{\bullet} + \text{HO}^{\bullet}$$

$$\text{Keratin-OOH} + \text{Metal } \rightarrow \text{Keratin-O}^{\bullet} + \text{HO}^{-} + \text{Metal}^{+1}$$

Another scheme to provide Hydroxyl radicals along with hydrogen peroxide and superoxide was described by Millington [67] in this same review. In this scheme, a chromophore such as melanin or Tryptophan can absorb light and be elevated to an excited state.

$$\text{Chr} + \text{hv } \rightarrow \text{Chr}^{*}$$

The excited chromophore can then react with molecular oxygen to form superoxide anion radical.

$$Chr^{*} + O_2 \rightarrow \ Chr^{+\bullet} + {O_2}^{-\bullet}$$

Dismutation of superoxide then occurs to hydrogen peroxide and molecular oxygen.

$$2{O_2}^{-\bullet} + 2H^{+} \rightarrow \ H_2O_2 + O_2$$

Hydrogen peroxide can then react with either a transition metal (Fenton reaction; primarily ferrous or cuprous ion) or by photolysis to form hydroxyl radicals. In the case of hydrogen peroxide oxidation of hair, H_2O_2 is already present, and if Cu or Fe is present, then the Fenton reaction (below) can occur to produce hydroxyl radicals.

$$H_2O_2 + hv \ \rightarrow 2HO^{\bullet}$$

$$H_2O_2 + \text{Metal} \ \rightarrow HO^{\bullet} + OH^{-} + \text{Metal}^{+1}$$

Millington proposed these schemes to provide hydroxyl radicals, hydroperoxides and oxygen radicals that he felt were important to the yellowing mechanism of wool fiber. Hydrogen peroxide can also be generated during the photo-degradation of Tryptophan which can then generate hydroxyl radicals by either reacting with trace metals or by photolysis. These reactions involving hydrogen peroxide or keratin hydroperoxides, especially with metals such as Cu or Fe, are very relevant to the reaction of alkaline hydrogen peroxide with human hair during bleaching or oxidative dyeing.

Millington discussed different photo-yellowing mechanisms and concluded that the evidence supports that the yellowing of wool fiber is caused by the photo-oxidation of several species and not just the photo-oxidation of tryptophan to N-formyl kynurenine to kynurenine and finally to 3-hydroxy kyneureine that has been shown to occur in wool fiber [70].

Superoxide anion radical has been shown by Bruskov et al. [71] to be generated by heating aqueous buffers containing oxygen and transition metal ion impurities. In this reaction, molecular oxygen is excited to singlet oxygen which is reduced to superoxide by the metal. Millington [67] pointed out that some dyes in the presence of an electron donor can generate superoxide radical and hydrogen peroxide by an electron transfer mechanism. In addition, Misra [72] pointed out that superoxide radicals are formed by autoxidation of a large number of compounds including simple thiols, some iron complexes and reduced flavins and quinones.

These reactions are all fundamental to the reactions that can occur in human hair particularly in those regions of the fiber where metals like Fe and Cu exist or where even low ppm levels of Cu or Fe are in the water supply.

5.5.1 The Formation of Sulfur Type Free Radicals in Keratin Fibers

The events occurring when human hair is exposed to sunlight involve photo-degradation of the disulfide bond. This reaction affects the wet tensile properties. In addition, the surface becomes more hydrophilic. Photo-degradation of the thioester groups occurs at the surface and in the Beta layers of the cuticle CMC. A yellowing reaction occurs that involves tryptophan, cystine and other amino acids in the cuticle, the cortex and other areas involving light or heat. Bleaching of hair pigments occurs in cortical cells (most but not all of pigment granules are inside cortical cells; a few are in between in the cortex CMC [73]), and hair fibers become more sensitive to cuticle fragmentation (involving disulfide bonds inside cuticle cells and the other bonds in the cuticle CMC) and to axial fracturing which involves events occurring in the cortex CMC.

The best description of the mechanism of photo-degradation of the disulfide bond that I could find has been described by Millington and Church [74]. Any mechanism to account for the changes occurring to cystine when keratin fibers are exposed to sunlight must account for an increase in three products, cysteic acid, cysteine S-sulfonate and cysteine (thiol) groups [74]. When keratin fibers are exposed to sunlight, UVB radiation in the range of 280–320 nm is absorbed by tyrosine and tryptophan residues and both of these species are excited to a higher energy level [74].

$$\mathrm{Tyr} + \mathrm{hv}(280-320\,\mathrm{nm}) \rightarrow {}^{1}\mathrm{Tyr}^{*}$$

$$\mathrm{Trp} + \mathrm{hv}\,(280-320\,\mathrm{nm}) \rightarrow {}^{1}\mathrm{Trp}^{*}$$

The energy absorbed by tyrosine is transferred to other groups including tryptophan and cystine.

$${}^{1}\mathrm{Try}^{*} + \mathrm{Trp} \rightarrow {}^{1}\mathrm{Trp}^{*} + \mathrm{Tyr}$$

It has been known for some time that cystine is an effective quenching agent for tyrosine and tryptophan in proteins and the quenching mechanism has been described as an electron transfer process forming this type of radical anion: $\mathrm{RSSR}^{\bullet-}$

Several papers discuss the quenching by cystine disulfide in proteins when tyrosine or tryptophan nearby or adjacent to cystine in a polypeptide chain [75]. It is possible that cystine quenching of tryptophan or tyrosine could be involved in the reaction of alkaline peroxide with human hair. However, the radical anion of cystine residues (above) can also form by one electron transfer involving metal ions. This reaction is highly likely in the oxidation of human hair [76] with hydrogen peroxide. Furthermore, radical anions of disulfides dissociate to form an equilibrium with a thiol anion and a thiyl radical as indicated below.

$$RSSR^{\bullet -} \rightleftharpoons RS^{-} + RS^{\bullet}$$

The thiyl radical can then add oxygen to form an SII oxidized state.

$$RS^{\bullet} + O_2 \rightarrow RSOO^{\bullet}$$

This SII radical can rearrange to form the SVI radical.

$$RSOO^{\bullet} \rightarrow RSO_2^{\bullet}$$

The SVI radical can add oxygen to form the hydropersulfate radical.

$$RSO_2^{\bullet} + O_2 \rightarrow RSO_2\text{-}OO^{\bullet}$$

The hydropersulfate radical can abstract a hydrogen atom to form the hydropersulfate.

$$RSO_2\text{-}OO^{\bullet} + R\text{-}H \rightarrow R^{\bullet} + RSO_2\text{-}OOH$$

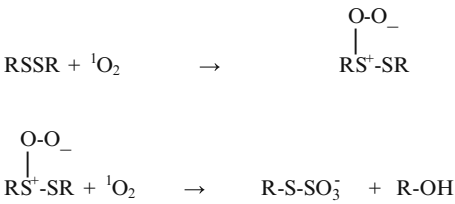
The hydropersulfate can similarly be reduced to form the sulfonate and water.

$$RSO_2\text{-}OOH \rightarrow RSO_3H + H_2O$$

In this reduction reaction, the hydropersulfate can generate hydroxyl and other radicals.

5.5.2 *Proposal for the Photochemical Mechanism for C–S Fission of Disulfides*

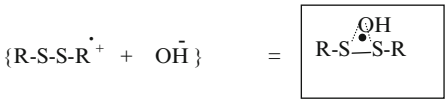
The mechanism for the formation of cysteine S-sulfonate has not been totally resolved. Millington and Church [74] proposed that since the S-sulfonate is formed at higher wavelengths (UVA range and the higher the wavelength of radiation the more cysteine S-sulfonate formed) singlet oxygen is most likely involved as a competing mechanism involving attack on the disulfide to form a zwitterionic species as proposed by Murray and Jindal [77]. Millington and Church [74] suggested this zwitterion could rearrange to produce the S-sulfonate (Bunte salt). However, Millington and Church did not explain exactly how the rearrangement would occur. This species could produce the S-sulfonate with additional oxidation as suggested below. This reaction could involve singlet oxygen or molecular oxygen.



Millington and Church cited a reference by Schmidt [78] showing that singlet oxygen is not produced at low UV wavelengths such as 265 nm but is present after radiation at 350 nm.

5.5.3 *Photochemical Reaction of Disulfide with Hydroxyl Radical in Aqueous Solution*

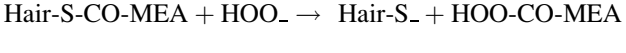
The above oxidation of the disulfide bond induced by photolysis involves the formation of singlet oxygen or the disulfide anion radical generated by various means. Bonifacic et al. [79] studied the primary steps in the reactions of organic disulfides with hydroxyl radicals in aqueous solution and identified the formation of a radical cation adduct as a key intermediate of this reaction:



The primary fate of this adduct radical depends on the pH of the medium. In basic solution, the primary initial products are thiols and R-S-O$^{\bullet}$ (sulfinyl radical) formed most likely by SN2 substitution of RS^{-} by OH^{-}. Ultimately these materials (thiols and sulfinyl radicals) can be oxidized to sulfonate.

5.5.4 *Photochemical Reactions of Thioesters in Hair*

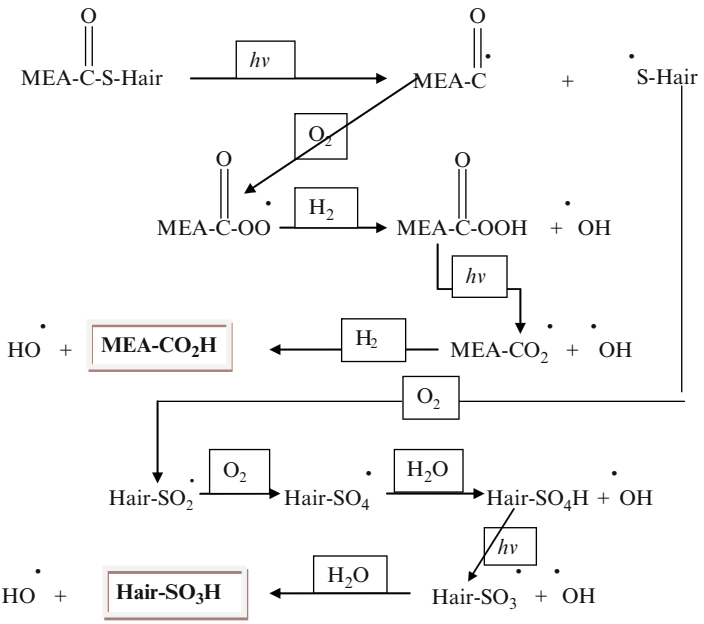
The thioester bond can be cleaved by the hydroperoxide anion or by the hydroxide anion by nucleophilic scission of the thioester link. For nucleophilic attack by the hydroperoxide anion one mole of the peroxy acid of MEA will be created, another source of hydroxyl free radicals.



The thiol group can then be oxidized by oxygen, by hydrogen peroxide or the peroxy acid to Sulfonate. In the case of oxygen the first step is the oxidation to the disulfide. With oxygen, this reaction can be catalyzed by Fe or Cu and the formation of a metallic ion-thiol complex is believed to be responsible for increasing the rate of this oxidation with oxygen [80, 81].

The reaction of sunlight on thioester is very different. A mechanism for this reaction is summarized below. The first step in the mechanism for the oxidation of thioester in sunlight involves the formation of an acyl and thiyl radical by the action of ultraviolet light acting on the thioester group. Takahashi et al. [82] and Chatgilialoglu et al. [83] have shown that the formation of acyl radicals from different acyl groups including thioesters occurs by the action of ultraviolet light. Takahashi [82] confirms by the following quote that this is a well accepted reaction, "it is well accepted that upon irradiation, thioesters undergo homolytic cleavage to form an acyl and a thiyl radical pair with subsequent reaction." The paper by Brown et al. [84] describes several free radicals formed in hexane or di-t-butyl peroxide solvent at room temperature using 308 nm laser flash photolysis on aldehydes or ketones. Among the acyl radicals generated were $CH_3C^{\bullet}{=}O$; $CH_3{-}CH_2{-}C^{\bullet}{=}O$; $(CH_3)_3CC^{\bullet}{=}O$; and $C_6H_5C^{\bullet}{=}O$ and these acyl radicals are analogs to the acyl radical that would be formed from 18-MEA attached to the hair surface. In addition, some of the corresponding acyl peroxyl radicals were formed in the work by Brown et al. by reaction of the acyl radicals with oxygen similar to what is proposed in the mechanism described below.

Proposed Mechanism for the Oxidation of Thioester in Hair by Sunlight:



The above oxidation of thiol may not proceed through the disulfide bond because of "Proximity", that is, if the thiol groups bound to hair proteins are not close enough to another thiol or sulfur group to form a disulfide bond then that reaction cannot occur. Therefore, this oxidation could bypass the disulfide stage and occur via mobile oxidizing species such as molecular oxygen (superoxide, singlet oxygen, hydrogen peroxide or hydroperoxide anion, etc).

Ruetsch et al. [40] state that for both the chemical (alkaline peroxide) and photochemical oxidation of human hair thioester groups on the surface are converted to sulfonate. However, they do not describe mechanistically how this happens. In describing the chemical oxidation they state, "A side reaction of bleaching is the hydrolysis of the thioester linkages". It is true that some hydrolysis could occur. However since the hydroperoxide anion is a stronger nucleophile than hydroxide anion I would expect more cleavage by the hydroperoxide anion and also by free radical degradation. Nevertheless as indicated, I could find nothing in the hair or wool literature describing this mechanism.

5.5.5 *Carbon Based Free Radicals from Tryptophan and Phenylalanine*

Evidence for the free radical decomposition of tryptophan has been presented by Domingues [85]. Tolgyesi [64] suggested that tryptophan, tyrosine and phenylalanine are involved in free radical formation. The hydroxylation of phenylalanine to tyrosine has been observed by Bringans et al. [70] (confirming the presence of hydroxyl radicals in the oxidation of phenylanine). These references implicate several carbon based free radicals in the chemistry of human hair.

5.5.6 *Free Radicals from Allylic and Tertiary Versus Alpha Hydrogens*

Another important reaction involves formation of carbon based free radicals that produce hydroperoxides and thus are chain propagation reactions. This reaction and the subsequent reactions of its products have already been explained as well as the relative stability of different allylic, tertiary and alpha hydrogen atoms in this Chapter in the section entitled, *Long Term Irradiation Produces Fusion Reactions Across Structural Boundaries*. Furthermore, the preference for allylic free radical formation can be found in most organic chemistry texts such as Ege [86] who explains that allylic free radicals will be formed preferentially over even those at tertiary carbon positions because allylic free radicals are more stable.

5.5.7 *Chlorine Oxidation of Human Hair*

Allworden [87] was the first to treat hair with chlorine and bromine water. Allworden noted that bubbles or sacs form at the surface of the fibers during this type of treatment. This oxidizing system diffuses across the epicuticle membrane

and degrades the proteins beneath the membrane producing smaller, water-soluble proteins and polypeptides too large to migrate out of the hair. At the same time it degrades and weakens the epicuticle. As a result, swelling occurs beneath the epicuticle, due to osmotic forces, producing the characteristic Allworden sacs (see Fig. 1.29).

The qualitative observations of the Allworden reaction are produced by relatively large concentrations of chlorine or bromine water. Fair and Gupta [88] were the first to investigate the effect of chlorine water on hair, at the parts-per-million level, in an attempt to assess the effects of chlorine in swimming pools on hair.

In this study, hair effects were measured by following changes in hair fiber friction. In general, the effect of chlorine was to increase the coefficient of fiber friction and to decrease the differential friction effect. Changes in hair friction were observed even at parts-per-million levels of chlorine. Effects increased with the number of treatments and with decreasing pH from 8 to 2.

The actual oxidizing species present in this system depends on pH and is either chlorine or hypochlorous acid (HOCl). Apparently, hypochlorous acid is the more active species on hair, since degradation is greater at lower pH. Although the chemical changes of these interactions were not examined, one would expect thioester and disulfide bond cleavage and peptide bond fission similar to the effects shown for the reaction of chlorine and wool fiber [89]. For a more complete discussion of these effects see the section in Chap. 1 entitled *Epicuticle and Hair Fiber Structure* and in Chap. 2 entitled *Composition and Components of the Epicuticle*.

5.5.8 Peracid Oxidation of Human Hair

Peracetic acid was the first peracid studied extensively in keratin fiber research. This highly reactive species ultimately became the vehicle used in the well-known keratose method by Alexander and Earland. This method is used to isolate keratose fractions from keratin fibers and is described in Chap. 1 of this manuscript.

Large higher molecular weight peracids such as m-diperisophthalic acid have been studied. Such larger peracids tend to focus the oxidative degradation to the outer regions or the periphery of the fibers producing a ring oxidation effect analogous to ring dying, see Fig. 5.13. This figure contains a cross section of a hair fiber (in water) after reaction with m-diperisophthalic acid for nine treatments. Note the extreme swelling at the periphery of the fiber leaving an intact non-swollen or non-reacted fiber at the core. Figure 5.14 represents an SEM of the surface of an untreated control fiber (dry) in this study. Figures 5.15, 5.16 and 5.17 depict the surface of human hair fibers in the dry state after 3, 7 and 20 treatments for 15-min reaction times with m-diperisophthalic acid compared to the control (Fig. 5.14). Note the folds in the scales, indicating dissolution of scale material. These folds increase as the number of treatments increases producing a matrix-type appearance

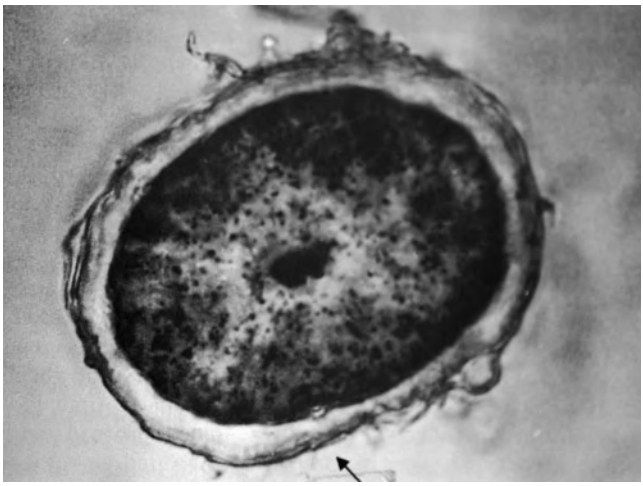


Fig. 5.13 Light micrograph of a cross-section of a hair fiber reacted nine times for 15 min with m-diperisophthalic acid. Note the extensive swelling and cuticle damage in the periphery

Fig. 5.14 SEM of a control fiber prior to treatment with m-diperisophthalic acid. Treatment was on the head of a panelist

after seven treatments. After 20 treatments with this peracid, the complete loss of cuticle scales has occurred, see Fig. 5.17.

Figures 5.18, 5.19 and 5.20 illustrate another perspective of this treatment by viewing the fibers in the wet state in water after different reaction times. Figure 5.18 depicts the effects after three treatments and Fig. 5.19 after six peracid treatments. At this stage, after six treatments, the fibers begin to display an Allworden type reaction in water. After nine treatments, scale material is still present, but the Allworden reaction is only transitory. Apparently, the proteins inside the cell membranes are so degraded that they cause such a large uptake of water that the

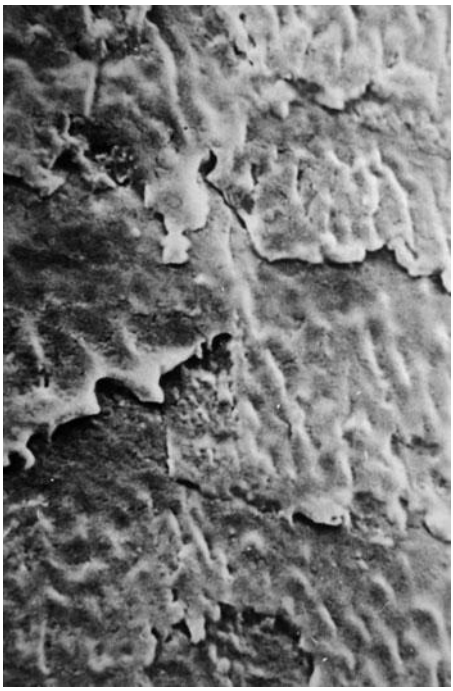


Fig. 5.15 SEM illustrating the hair surface dry after three treatments with m-diperisophthalic acid. Note the axial folds in the scales compared to the control in Fig. 5.14. These folds are created by the loss of cuticular proteins from oxidation

weakened membranes rupture after long water exposures. This effect is analogous to the effects of the long-term exposure of hair fibers to sunlight followed by treatment with alkaline peroxide, described to this author in a private communication by Sigrid Ruetsch.

The light micrograph of the hair fiber in Fig. 5.21 was obtained from a fiber taken directly from the scalp of an individual in a forensic study. However, the treatment was unknown. John T. Wilson a forensic scientist provided this micrograph to me. From the above micrographs, I conclude that this hair was exposed extensively to sunlight and peroxide bleaching. It is interesting that such cuticle scale degradation can be produced on hair on live heads. The hair fiber of Fig. 5.22 shows what appears to be a classical Allworden reaction; however, this effect was obtained after 5 thirty-minute exposures to alkaline hydrogen peroxide that bleached the hair from dark brown to golden brown followed by a single 15-min treatment with m-diperisophthalic acid.

After multiple treatments with m-diperisophthalic acid when bundles of fibers or tresses are allowed to dry the fibers actually appear glued together. These glued fibers are reminiscent of the combined photochemical plus peroxide bleach treatment of Fig. 5.11. Examination of hair fibers treated 12 times with this peracid and dried provided evidence that the fibers are actually glued together (Fig. 5.23). Apparently the proteins of the cuticle are sufficiently solubilized so that some of the proteinaceous glue-like matter migrated out of the scales leaving a gelatin-like

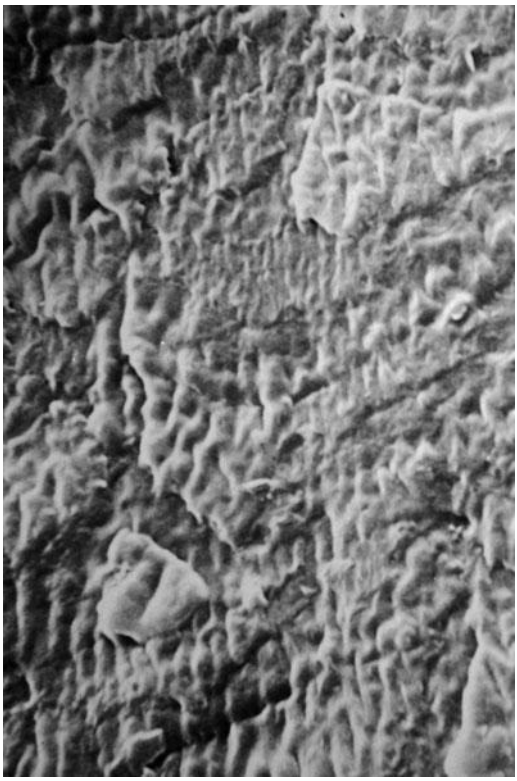


Fig. 5.16 SEM of the hair surface after seven treatments with m-diperisophthalic acid. Note the greater number of folds in the scales compared to Fig. 5.15

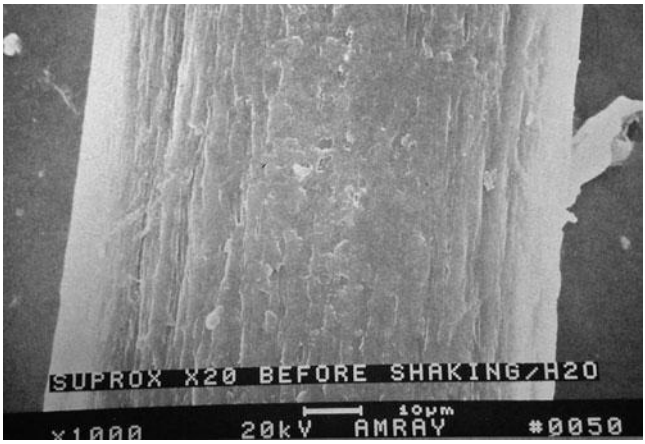


Fig. 5.17 SEM of the hair surface after 20 treatments with m-diperisophthalic acid. Note the complete absence of cuticle scales (Reprinted with permission of the Journal of the Society of Cosmetic Chemists)

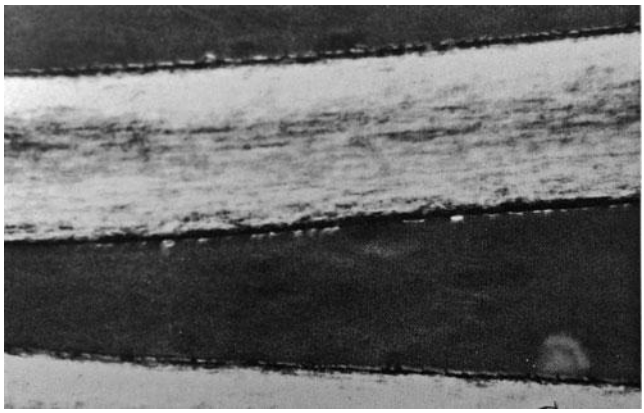


Fig. 5.18 Light micrograph (optical section) illustrating the hair surface in water after three treatments with m-diperisophthalic acid. Note the swelling in the cuticle layers

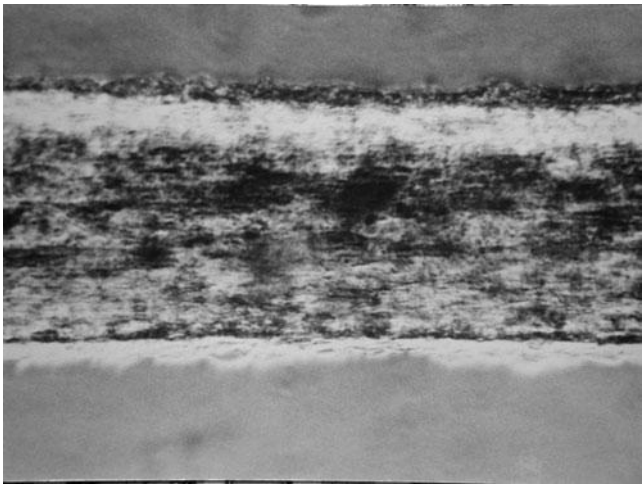


Fig. 5.19 Light micrograph (optical section) illustrating the hair surface in water after six treatments with m-diperisophthalic acid. Note the extensive swelling of the cuticle scales

deposit on drying that actually glued the fibers together. The cuticle scales from fibers of this treatment, Fig. 5.23, appear to be gone over most of the fiber surface, but are actually covered by the proteinaceous deposit. The scales appear only where "glue" has been separated from the underlying scales. After 20 treatments with m-diperisophthalic acid, the scales are totally removed. Fracturing of hair fibers after this peracid treatment has not been examined, but would likely reveal some interesting new findings.

Fig. 5.20 Light micrograph (optical section) of the hair surface in water after nine treatments with m-diperisophthalic acid. Note the extensive swelling and lifting of cuticle scales

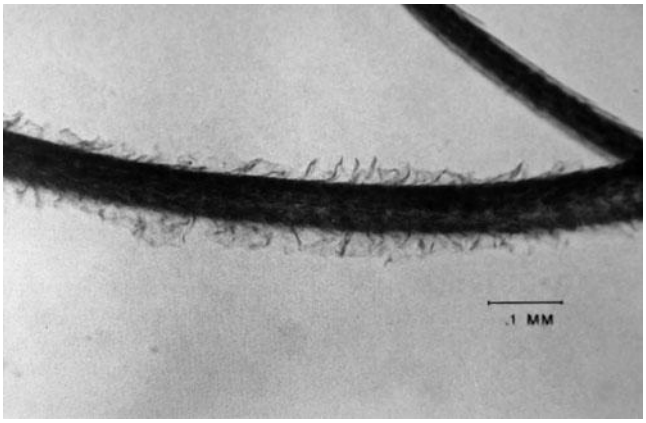


Fig. 5.21 Light micrograph of a damaged hair fiber taken from the head of a forensic study. Treatment unknown, but probably ultraviolet exposed and chemically bleached (Light micrograph kindly provided by John T. Wilson)

5.6 Hair Pigment Structure and Chemical Oxidation

5.6.1 Hair Pigment Production and Pigment in Different Hair Types

The principal pigments of human hair are the brown-black melanins (eumelanins) and the less prevalent red pigments, the pheomelanins. These latter pigments at one time were called trichosiderins. The genes involved in the formation of the

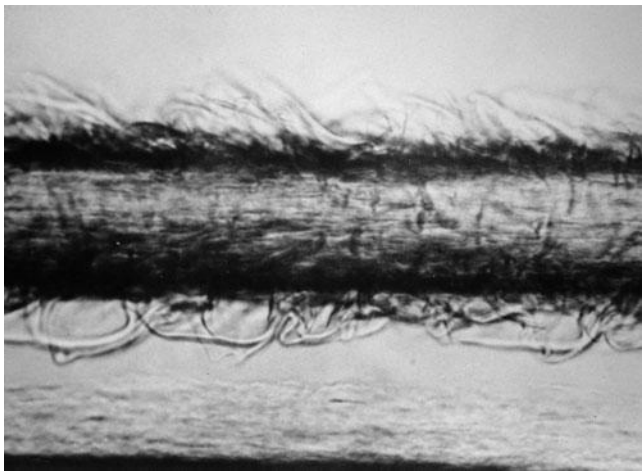


Fig. 5.22 Hair fiber bleached five times for 30 min with alkaline peroxide and then once with m-diperisophthalic acid. Note the large "Allworden" sacs at the surface

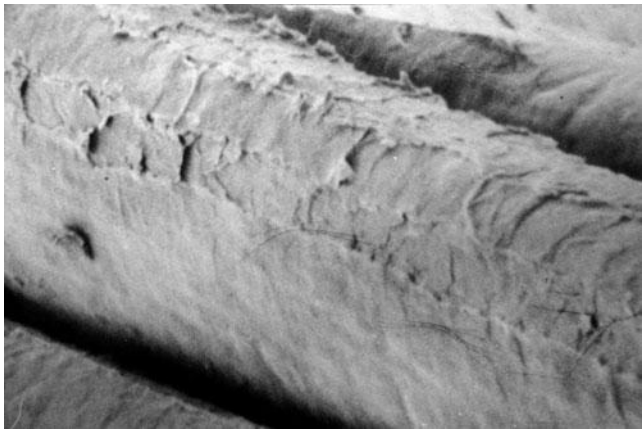


Fig. 5.23 Hair fibers from a tress treated nine times with m-diperisophthalic acid and dried. Note the "apparent" absence of scales on part of the fibers and the presence of scales where the fibers have been pulled apart. After treatment, to the naked eye, the fibers appeared to be glued together until broken apart

melanosomes and different hair colors are summarized in Chap. 3 in the section entitled, *Hair Pigmentation and Genetics*. For the discussion, in this Chapter the brown-black pigments of hair will be referred to as melanins and the yellow–red pigments will be referred to as pheomelanins.

Birbeck and Mercer [90] determined that the pigments in scalp hair reside within the cortex and medulla as ovoid or spherical granules. Barnicot et al. [91] concluded that the pigment granules generally range in size from about 0.4–1.0 μm along their major axis; see Figs. 5.24 and 5.25. These SEMs show some partially dissolved

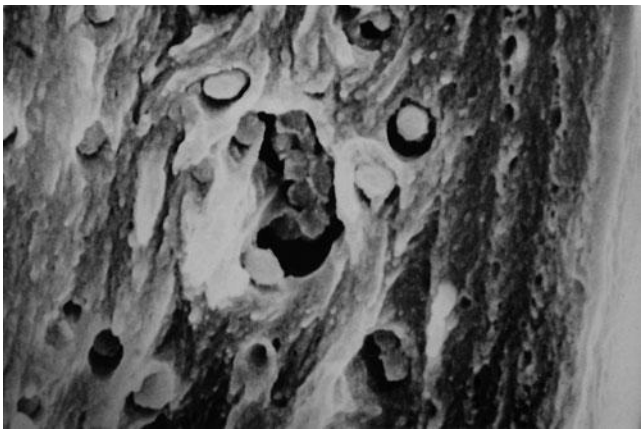


Fig. 5.24 Hair fibers exposed to ultraviolet radiation and then fractured exposing melanin granules (SEM kindly provided by Sigrid Ruetsch)

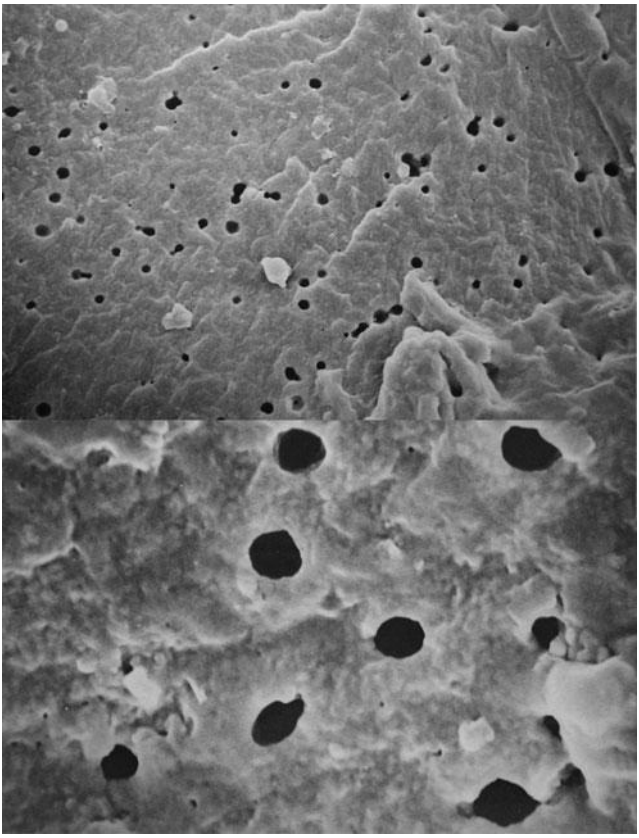


Fig. 5.25 Hair fibers exposed to ultraviolet radiation followed by bleaching with alkaline peroxide and then fractured. Note the holes or vacancies where melanin granules once were SEM kindly provided by Sigrid Ruetsch

melanin granules and some holes or vacancies where melanin granules have been dissolved from the fiber. Hair pigments are produced by the melanocytes (melanin producing cells) and are packed into the melanosomes which are pigment containing granules. The pigment granules are then transferred into the keratinocytes (hair fiber cells). Tobin and Paus [92] suggested that with age there is a deficiency in the melanosome transfer process. A relatively small number of melanocytes are necessary to produce an intensely pigmented hair fiber of 1 m or longer. These melanocytes function in 7–15 hair cycles to produce pigmented hairs for up to four decades or longer [92]. Tobin and Paus also suggested that each melanocyte has a "melanogenesis clock" or each melanocyte can produce a limited amount of melanin which determines a melanocyte lifetime. They concluded that epidermal pigmentation changes are more subtle than those in the graying of hair because the melanocytes in the hair bulb age faster due to the highly intense production of melanin required by hair cycles. Furthermore, the hair graying effect results from a decrease and the eventual termination of the activity of the enzyme tyrosinase in the lower hair bulb. This enzyme is involved in the reaction called Raper's scheme described in the next section in this Chapter.

Figure 5.24 shows pigment granules as they appear inside the fiber. This micrograph was obtained after fracturing photo-oxidized hair. Figure 5.25 also shows ovoid cavities where pigment granules have been dissolved and removed by photo-oxidation and subsequent treatment with alkaline peroxide. Hair pigments are found in the cortex and the medulla and not normally found in the cuticle of scalp hair. Most but not all of the pigment granules are inside cortical cells; some are in between these cells in the cortex–cortex CMC [73]. Menkart et al. [93] concluded that pigment granules generally comprise less than 3% of the total fiber mass, as estimated by the residue weight after acid hydrolysis.

Schwan-Jonczyk [94] suggested that the size of melanin granules, in addition to the total melanin content and type of melanin (eumelanin vs. pheomelanin) determine hair color. For example, she cites that Black African hair contains large eumelanin granules about 0.8 μm along their major axis, while Japanese hair has smaller eumelanin granules about 0.5 μm and blonde European hair contains even smaller primarily pheomelanin granules about 0.3 μm. Schwan-Jonczyk suggested that as a general rule, black hair contains primarily large eumelanin granules, medium to light brown hair contains both eumelanin and pheomelanin granules and blonde hair contains primarily pheomelanin granules that are smaller.

These observations by Schwan-Jonczyk are consistent with those of Swift [95] who several years ago in his Ph.D. thesis reported, via measurements with the electron microscope, that melanin granules from hair of black Africans is larger than those from Caucasian hair. Bernicot and Birbeck [96] determined that the pigment granules from dark European hair are on average larger than those of blonde and red hair which is consistent with Schwan-Jonczyk's conclusions. Fitzpatrick et al. [97] confirmed that the pigment granules of hair of African descendants tend to be larger than those of dark European hair.

The pigment in hair is produced by melanocytes that are associated with each individual hair fiber. Thus, there is no transfer of pigment or melanosomes from one

hair fiber to another during biosynthesis. This fact accounts for hairs of very different pigmentation (colors) growing adjacent to each other. The melanocytes in the skin of Blacks appear similar to those of Caucasians. However, Barnicot and Birbeck [96] state that the pigment granules in the skin of Blacks are larger and more numerous than in the skin of Caucasians, similar to the pigment granules in the hair of Blacks and Caucasians.

This heavy pigmentation in African type hair is very useful in two distinctly different situations. First of all the pigments in hair protect it from many photochemical [98] and thermal reactions such as in straightening of hair with hot irons or presses. These protective actions become more important to Blacks as they age and the graying process begins. Because this protection decreases as the pigments are reduced in the hair. Secondly, hair pigments reduce the amount of light scattered from the hair. Therefore, these pigments help to improve hair luster as shown by Keis et al. [99]. Keis et al. determined that single hairs of African Americans are among the shiniest of hairs [99]. It is only because of the high coiling and poor alignment that an array of African type hair appears dull. Therefore, curly to highly coiled hair of Blacks that is often thought of as not being shiny would be even less shiny if it contained less pigment.

Fine hair tends to be lighter in color than coarse hair. The extreme case supporting this statement is that vellus hair, the finest of all hairs does not contain pigment, whereas most permanent hairs that is the coarsest of hairs generally contain pigment. Caucasian hair on average is finer than Asian or African hair and it also tends to be lighter in color.

There are likely exceptions to this conclusion that fine hair tends to be lighter in color than coarse hair. Exceptions are likely because hair color is determined by several variables including the type of melanin pigment present, the size of the pigment granules and the density (frequency) of the pigment granules that are dispersed throughout the cortex of human scalp hair fibers. Nevertheless, there are several other references (below) supporting this conclusion.

Pecoraro et al. [100] examined hair from 26 infants within 76 h of birth considering hairs from 13 males and 13 females and found that the mean coarseness of dark hairs from dark complexioned newborns was 37 μm while the average diameter for light colored hairs from light complexioned newborns was 22 μm.

Trotter and Dawson [101, 102] examined hair from 310 children and adult Caucasians (French Canadians) and found that more coarse hair tends to be darker than finer hair [101, 102], see Table 5.7. In addition, Bogaty [103] has shown in his review of the anthropological literature that Caucasian children's hair is on average finer, rounder, less frequently medullated and lighter in color than adult's hair.

One possible exception to the above conclusion is gray hair. The graying process in terms of comparisons of gray to white and dark hair needs additional study, however, we do know there is less pigment in gray hair than in dark hair. Most likely the pigment granules of gray hair are also smaller in size, both actions a result of changes in the melanization process with ageing described above. Hollfelder et al. [104] have provided evidence from five Caucasians that gray hairs on the same

Table 5.7 Caucasian children's hair tends to be finer and lighter than adult's hair[a] [101]

Ages	N	Diameter (μ)	Brown–black	Blond–dark blond	Light blond
0–4	46	58	35	50	15
5–9	36	66	75	22	3
10–14	45	69	96	4	0
15–19	56	74	98	2	0
20–29	52	73	98	2	0
30+	75	70	97	3	0
	310				

[a]Data from Anthropological study of French Canadian hair by Trotter and Dawson [102]

person are coarser and wavier than highly pigmented hairs. This observation by Hollfelder et al. is consistent with observations by Yin et al. [105] that fine Caucasian hair is straighter than coarse Caucasian hair.

Van Neste [106] examined approximately 60 hairs from each of three different scalp sites (left and right top of head and occipital) from 24 women. Twelve of these women were menopausal with an average age of 59.6 and 12 were premenopausal and younger but the average age was not given. A total of 3,343 hairs were examined after classification as pigmented (P) and non-pigmented (W). The average diameter of W hairs exceeded the P hairs by 10.27 μm, $p = 0.0001$. The medulla of W hairs was more developed than in the P hairs, $p = 0.0001$ and the growth rate of the W hairs was about 10% faster than the P hairs. This study is in agreement with the one by Hollfelder et al. suggesting that gray-white hairs are coarser than pigmented hairs.

However, Gao and Bedell [107] studying gray hair and dark hairs from four persons plus one sample of pooled gray hair, measured cross-sectional parameters with a laser-scanning micrometer and found no significant differences in the maximum center diameter, center ellipticity and cross-sectional areas; however the center minimum diameter of the black fibers were slightly larger than for those of gray hairs. At this time, there is more evidence favoring that gray hairs are coarser than pigmented hairs; however, the evidence is not overwhelming.

Coarser gray hairs would reinforce the thought proposed by several that the medulla is involved in graying, because the medulla at one time appeared to be involved in the genetic abnormality of pili annulati or ringed hair. This abnormality appears as bands or rings of silver or gray and dark regions along the fiber axis. But, ringed hair has been shown to contain bands with and without holes in the cortex along the axis, and these bands correspond to the gray and dark bands. For a more complete description of ringed hair, see Chap. 1.

Nagase et al. [108] demonstrated that hair with a porous medulla gives a whitish appearance with lower luster. These scientists actually measured a decrease in color from hair with a porous medulla. This effect is attributed to an increase in the scattering of light by the medullary pores, part of which is due to a change in refractive index by the hair to air interfaces at medullary spaces. Therefore, gray hair can be made whiter by a porous medulla which adds to the primary effect of graying produced by less pigment.

As indicated, hair pigments function to provide photochemical protection to hair proteins and lipid structures especially at lower wavelengths where both the pigments and hair proteins absorb light (primarily between 254 and 350 nm). Hair pigments absorb light and dissipate the energy as heat. Thus, the pigments are slowly degraded or bleached and in that process they inhibit or minimize degradation to the structural proteins and lipids of hair, inhibiting hair damage which can be detected in the tensile properties [107].

Methods for pigment granule isolation usually involve dissolving the hair from the granules [13, 91, 109–115]. Laxer [112] described a non-hydrolytic method involving reflux for 24 h in a phenol hydrate-thioglycolic acid mixture. The general composition of melanin granules consists of pigment, protein, and minerals. Flesch [13] reported a similar general composition for the pheomelanin-containing granules. Schmidli et al. [113, 114], after acid or alkaline hydrolysis of hair, isolated melanin combined with protein and suggested that melanin exists in combination with protein in the granules, sometimes referred to as melanoprotein.

Since the pigment granules of human scalp hair are located primarily in the cortical cells and the medulla, it is reasonable to assume that pigment degradation by chemical means is a diffusion-controlled process. However, evidence supporting this contention is not available at this time. In fact, determining the rate-controlling step in this process is a large-order task, since it is difficult to quantitatively follow the loss of pigment in hair. Furthermore, two important side reactions consume oxidizing agent: the previously described oxidation of amino acid residues [5] and, in addition, dibasic amino acid residues of hair associate with many oxidizing agents, including hydrogen peroxide and persulfate [115, 116].

5.6.2 Eumelanins and Pheomelanins: Their Biosynthesis and Proposed Structures

As indicated, melanins are synthesized in melanocytes (melanin producing cells) in structures called melanosomes; eumelanin from the amino acid tyrosine and/or phenylalanine and pheomelanin from tyrosine and cysteine.

Raposo et al. [117] described the development of melanosomes in four stages. Five stages are presented here. In early development, melanosomes appear as round amorphous vesicles. Yasumoto and Hearing [118] demonstrated that the gp100 protein (Pmel17) generates structural changes in melanosomes producing fibrillar elliptical melanosomes from the amorphous vesicles. After these structural changes other proteins including melanogenic enzymes, pH regulators and transport proteins are targeted to the melanosomes which begin synthesis of the melanin pigments. When the melanosomes are filled with pigments the melanosome granules are transferred to keratinocytes the cells that form the shaft of hair fibers.

Donatien and Orlow [119] suggested that melanin is deposited in melanosomes on a protein matrix inside the melanosomes. Donatien and Orlow [119] identified the si locus and membrane-bound p locus proteins as melanosomal matrix proteins.

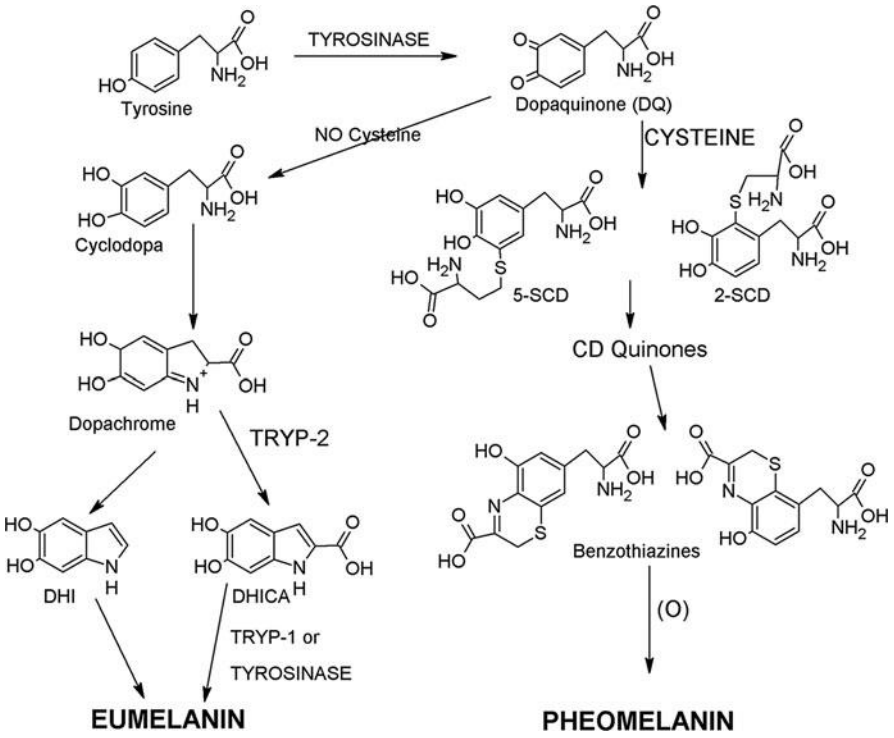


Fig. 5.26 Biosynthetic pathway for the formation of eumelanin, pheomelanin and mixed melanins proposed by Ito and Wakamatsu [131]

Melanin containing melanosomes ultimately become melanin granules after being transferred into keratinocytes.

The intensity or depth of hair color is related to both the size of the melanin granules and the total melanin content or the melanin granule density. In addition, the proportion of eumelanin to pheomelanin is also important, but not the only factor in determining the shade of hair color. Orlow et al. [120] prepared dihydroxyindole-2-carboxylic acid (DHICA) (see Fig. 5.26) enzymatically and via chemical synthesis. Orlow et al. then polymerized DHICA to form brown melanin type polymers that were soluble above pH 5. They also formed black, insoluble melanin precipitates from dihydroxyindole (DHI), dopa or dopachrome (see Fig. 5.26).

When DHICA was in molar excess, mixtures of these two monomers (DHI and DHICA) under the same reaction conditions formed brown melanins. However, black melanins were formed when DHI was in excess. A similar color effect likely exists for natural melanins in human hair. It is also possible that an analogous situation occurs for pheomelanin for intermediates in this process (see Fig. 5.26) causing shifts from yellow to red or perhaps even from brown to red to yellow. At this stage 5-cysteinylDOPA is one of the preferred intermediates in the pheomelanin pathway.

Schwan-Jonczyk [94] suggested that Black-African hair contains ovoid or spherical eumelanin granules about 0.8 μm along their major axis existing as single granules or aggregates. Asian hair contains single melanin granules about 0.5 μm while dark blond European hair contains agglomerates of pheomelanin granules about 0.3 μm along their major axis [94]. As a general rule the darker the hair the higher proportion of eumelanin to pheomelanin in the granules and dark hair generally contains very little pheomelanin.

From cross-sections of African hair vs. dark-brown Caucasian hair the melanin granule density clearly appears higher in African hair. Publications by Kita et al. [121, 122] on melanin granule size and density in East Asian hair reported a higher melanin density in the outer cortex vs. the inner cortex which is typical of Caucasian and African hair too. These scientists found no difference in melanin granule size and density in infant hair compared to 20–30 year olds. However, the minor axis of the granules was significantly smaller at age 60–70 than for the other age groups. The density of the melanin granules was also lower at the advanced age than for the other two age groups [121, 122].

5.6.3 Degradation Products of Melanins

Several years ago, R.A. Nicolaus, G. Prota and others [123–125] isolated a few pyrrole carboxylic acids and indole derivatives from degradation studies of melanins. Two of the pyrrole derivatives were pyrrole-2,3-dicarboxylic acid (PDCA) and pyrrole-2,3,5-tricarboxylic acid (PTCA). PDCA has been suggested by Ito [126] and Borges et al. [127] as a marker for 5,6-Dihydroxyindole (DHI) and PTCA a marker for 5,6-dihydroxyindole-2-carboxylic acid two important monomeric units suggested by Ito and Wakamatsu [128] to create the oligomers that comprise the eumelanin polymer in hair and skin (see Fig. 5.27).

Pyrole tricarboxylic acid (PTCA) and pyrole dicarboxylic acid (PDCA) are degradation products of eumelanins formed from oxidation with either permanganate or alkaline peroxide [123–125, 127]. Other degradation products have been isolated from pheomelanins and used for analysis or for proposing structures [129], see Fig. 5.27. Napolitano et al. [129] have shown that AHPs, 3-AT, BTCA and TTCA are degradation products of pheomelanins. The former two from hydroiodic acid hydrolysis and the four from alkaline peroxide oxidation.

Several degradation products of eumelanins and pheomelanins are depicted in Fig. 5.27. Analytical schemes have been described using these degradation products as markers for eumelanin and pheomelanin. Oxidation with acidic permanganate and alkaline peroxide has been used with both eumelanins and pheomelanins. Oxidation of DHICA melanins provides PTCA as a marker and DHI melanins provide PDCA, however, the yields of PDCA are so small that a large multiplying factor must be used to approximate the amount of DHI melanin. This multiplier provides a potentially large error especially in eumelanin plus pheomelanin

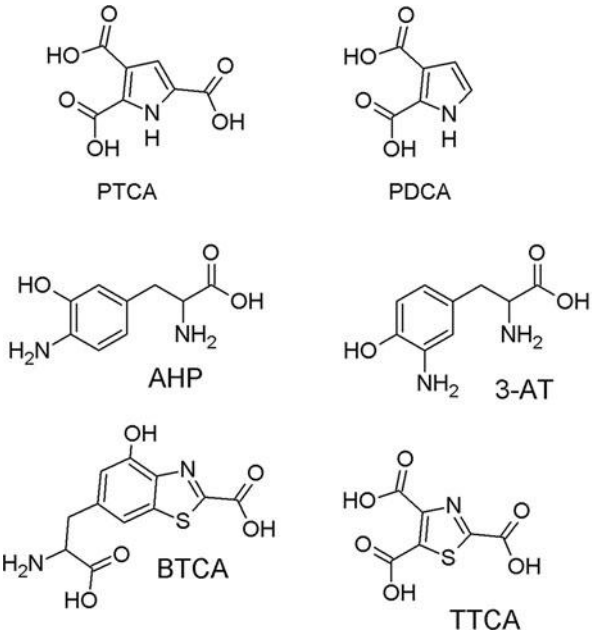


Fig. 5.27 Degradation products of eumelanins and pheomelanins. PTCA and PDCA are produced by either acidic permanganate or alkaline peroxide oxidation of eumelanins. PTCA is considered a marker for DHICA eumelanins and PDCA a marker for DHI eumelanins. AHPs (AHP and 3-AT) are produced by HI hydrolysis of pheomelanins. AHP is considered a marker for pheomelanin, while 3-AT its isomer is considered to interfere in the determination and should be separated from AHP

(smaller multiplying factor) comparisons. In some instances PTCA is used as an indicator of total eumelanin.

Alkaline peroxide oxidation of pheomelanins provides TTCA in low yield; however much higher yields of AHPs are provided by HI hydrolysis of pheomelanins. For the two amino hydroxyl phenylalanines (AHPs) depicted in Fig. 5.27 the 4-amino-3-hydroxy phenylalanine has been called specific AHP by Ito, Wakamatsu and Rees [130]. This term, specific AHP, is used because that isomer is provided in higher yield from pheomelanin and because the other isomer, 3-AT of Fig. 5.27, can be produced by HI hydrolysis of certain proteins which interferes in the AHP analysis for pheomelanin [130].

5.6.4 Biosynthetic Pathway for Mixed Melanogenesis

Several papers have shown that in the formation of most natural melanins, for human hair or skin, mixed melanogenesis occurs rather than exclusively forming eumelanin or pheomelanin [127, 131]. Ito and Wakamatsu [131] in an important paper proposed the biosynthetic pathway for formation of mixed melanins summarized in Fig. 5.26. In this pathway, tyrosine is oxidized by the enzyme tyrosinase to dopaquinone (DQ). This enzymatic reaction occurs faster near neutral than at acidic pH. When cysteine is present above a concentration of about 0.13 μmolar then S-cysteinylDOPAs are formed; however 5-ScysteinylDOPA is the preferred intermediate. 5-ScysteinylDOPA rapidly cyclizes internally to the

corresponding benzothiazine or other intermediates as suggested by Napolitano et al. [132]. These are in turn oxidized to pheomelanin oligomeric units and then to red-yellow pheomelanin polymers.

Chintala et al. [133] proposed via experiments with mice that the SNP Slc7a11 forms a protein that transports either cystine or glutathione (a tripeptide containing cysteine) to the melanosomes. A reducing agent such as βME is necessary to reduce the disulfide bonds of cystine to form cysteine for pheomelanin production. A related process must also occur with cystine or glutamate transport/reduction for pheomelanin formation in human hair. However, this process has not been described prior to this writing.

If cysteine is not present or below the critical concentration of 0.13 μmolar then the eumelanin pathway is followed and cyclodopa is formed from dopaquinone. Cyclodopa then cyclizes internally to dopachrome; pH is also important to this reaction which occurs faster near neutral than at acid pH. In the next step, the enzyme TRYP-2 prevents the decarboxylation of dopachrome allowing the formation of DHICA. If TRYP-2 is absent decarboxylation occurs forming DHI. Both DHI and DHICA are capable of polymerizing to form eumelanin type polymers. However, when the DHICA concentration is above that of DHI, brown melanins are formed. But, when the DHI level is higher, black melanins are formed.

Ito and Wakamatsu [131] concluded that when tyrosine concentration is high and cysteine is low and the pH is near neutral, the eumelanin pathway is preferred. Therefore, higher ratios of eumelanin/pheomelanin are formed. However, when tyrosine concentration is low and cysteine concentration is high and the pH is acidic the pheomelanin pathway is preferred. In that case lower ratios of eumelanin/pheomelanin are formed.

5.6.5 Casing Model for Mixed Melanogenesis

Ito and Wakamatsu [131] proposed a casing model for mixed melanogenesis which appears to be the predominant pathway for formation of the pigments in human hair fibers and in skin. This model is becoming more widely accepted because most natural pigments of hair and skin contain both eumelanin and pheomelanin [130]. For example, Thody et al. [134] found eumelanin and pheomelanin in samples of epidermis from 13 Caucasian subjects with different types of skin. In addition they found that the relative proportions of eumelanin to pheomelanin in the hair of these same subjects correlated with the relative proportions in skin.

In the casing model, DQ is first formed by oxidation of tyrosine by tyrosinase. Then cysteine reacts with DQ to form cysteinylDOPAs which cyclize and oxidize to form pheomelanin molecules. These reactions occur in the membrane of melanosomes. Pheomelanin molecules are apparently released to the interior of the melanosome where they aggregate or cluster to form a core of pheomelanin. A switch then occurs to begin the production of eumelanin as described in the biosynthetic scheme summarized in Fig. 5.26. Eumelanin polymers are then

released to the interior of the melanosome where they deposit on top of the core of pheomelanin. Thus, the final hair color, its intensity and shade are influenced by the thickness and the uniformity of the eumelanin coating as well as by the size of the core of the pheomelanin. These factors are probably more relevant to the shade of the final hair color than the ratio of eumelanin to pheomelanin.

5.6.6 pH and Melanogenesis

Fuller et al. [135] examined melanocytes from both Blacks and Caucasians. The number of melanocytes and the tyrosinase levels were found to be virtually the same. However, the activity of tyrosinase was nearly tenfold higher in the melanocytes of Blacks. Fuller et al. [135] treated the Caucasian melanocytes with ammonium chloride and with the ionophores nigericin and monensin. These ingredients increased the pH and rapidly increased tyrosinase activity. However, when Smith et al. [136] added sodium hydrogen exchangers (NHEs), which add protons, the activity decreased in Black melanocytes. But, these same NHEs had virtually no effect on the activity of Caucasian melanocytes.

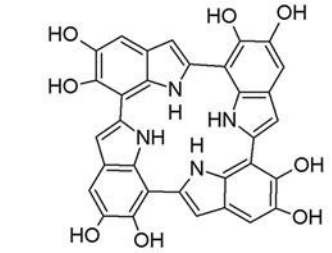
Fuller et al. [135] also found that treatment of Caucasian melanocytes with the weak base acridine orange (a fluorescent staining agent) stains Caucasian melanosomes but not Black melanosomes. This staining reaction suggests that Caucasian melanosomes are acidic and those from Blacks are neutral. Fuller pointed out that it is well known that tyrosinase acitivity is low in acid and higher at neutral pH. Therefore, pH is important to the rate of reactivity of tyrosinase. And higher pH near neutral, produces more eumelanin in melanocytes in the skin of Blacks vs. Caucasians.

Ancans and Tobin et al. [137] examined several different skin types and determined that melanosomal pH determines the rate of melanogenesis, the ratio of eumelanin to pheomelanin and the maturation and transfer of melanosomes to keratinocytes. These scientists also suggested that the P protein is involved in providing the effective pH for these effects. Cheli et al. [138] more recently confirmed the critical role of melanosomal pH in pigmentation and identified cyclic-adenosine monophosphate (c-AMP) and the α-melanocyte stimulating hormone (α-MSH) as important factors for pH control.

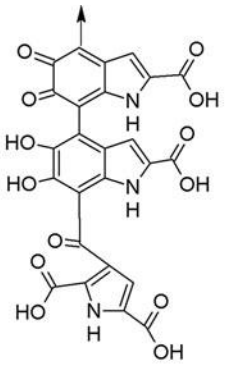
5.6.7 Proposed Structures for Eumelanin and Pheomelanin

5.6.7.1 Proposed Structures for Eumelanin

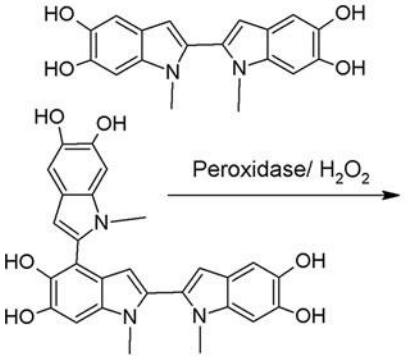
The exact structures for eumelanin or pheomelanin are not known. More than 50 years ago, Mason [139] proposed that melanin consisted of a homopolymer formed from dihydroxyindole. Nicolaus [140] then proposed that melanin is a complex random polymer formed from several intermediates of the Raper scheme,

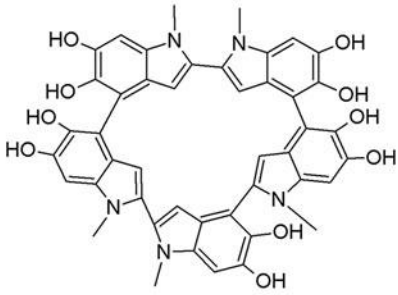


Proposed structure for tetramer of Eumelanin of DHI by Kaxiras et al



Partial structure for Eumelanin proposed by Ito & Wakamatsu. Carboxyls are attached to H or COOH groups and the arrow indicates site for attachment to other units.





Cyclic DHI type Macrocycle isolated by Arzillo et al (2010)

Fig. 5.28 Suggested partial structures for Eumelanin by Kaxiras et al. [143] and by Ito and Wakamatsu [131]. The cyclic structure shown in the *bottom right hand corner* is a structure isolated by Arzillo et al. [144] formed from the dimer and trimer of the dihydroxyindoles shown on the *left* from reaction with peroxidase/hydrogen peroxide under biomimetic conditions

which is essentially the eumelanin pathway described in Fig. 5.26. The Nicolaus proposal is clearly closer to our current point-of-view.

More recently, Napolitano et al. [141] provided evidence that the oligomeric units of synthetic DHICA eumelanins are of low molecular weights (in the range of 500–1500 Daltons) similar to the units suggested by Ito and Wakamatsu [131] in Fig. 5.28. Estimates of the molecular weights of the polymers are higher but these estimates are in question because of the poor solubility and irreversible binding to the chromatographic columns used in the molecular weight analysis.

Ito and Simon [142] described the current view in 2004 of the structure of eumelanins in a concise letter to the editor. Ito provided additional details in

these papers [126, 128, 131, 142] with representative structures for oligomeric units for both eumelanin and pheomelanin [131]. Two suggested representative structures for the oligomeric units of eumelanins are depicted in Fig. 5.28. The structure suggested by Ito and Wakamatsu is based primarily on the degradation products formed from the oxidation of eumelanin and its chemical properties. Actually this structure with a methylene group in place of the carbonyl joining the rings was proposed a few years earlier by Napolitano.

A theoretical structural model for Eumelanin containing only DHI has been suggested by Kaxiras et al. [143] (see Fig. 5.28) and is an interesting one in that it addresses many of the physical and chemical requirements of eumelanin. This structure would theoretically be formed from DHI or its hydroquinone and/or tautomers. These tautomers contain 4 or 5 DHI units to form the basic oligomer which is a porphyrin type ring structure. This type of structure is capable of capturing and releasing a variety of metal ions, an important property of eumelanin pigments. Smaller or larger ring formations are unstable.

Kaxiras et al. [143] proposed that the more stable ring systems which are these tetramer and/or pentamer oligomers would stack in "planar graphite-like arrangements" to form the polymeric structure. This model is consistent with X-ray scattering data of melanin structures. The calculated absorption spectrum for this model suggests it is dark black and thus consistent with DHI-rich eumelanin. However, introducing DHICA and other monomers into this type of model has not been addressed at the time of this writing.

In 2010, Arzillo and Napolitano et al. [144] isolated the macrocyclic structure formed from the methylated dihydroxyindole depicted in Fig. 5.28. This structure was formed by reaction with peroxidase and hydrogen peroxide under biomimetic conditions and provides evidence that structures analogous to the one proposed by Kaxiras et al. [143] can be formed by polymerization of dihydroxyindoles.

5.6.7.2 Proposed Structures for Pheomelanins

A general chemical structure for natural pheomelanins recently proposed by Ito and Wakamatsu [131] is depicted in Fig. 5.29. This structure consists of benzothiazine monomeric units that are combined. This structure is consistent with degradation products and the biosynthetic scheme summarized in Fig. 5.26. However, Napolitano et al. [132] recently monitored the oxidative formation of pheomelanin type products from 5-cysteinylDOPA by liquid chromatography/UV and mass spectrometry and suggested that such structures need reassessment concluding that species such as those summarized in Fig. 5.30, based on absorption properties and reduction behavior, are likely involved in the formation of pheomelanin. Napolitano et al. [132] suggested that "beyond the involvement of 3-oxo-3,4-dihydrobenzothiazine and benzothiazole units it is not possible to go" at this time.

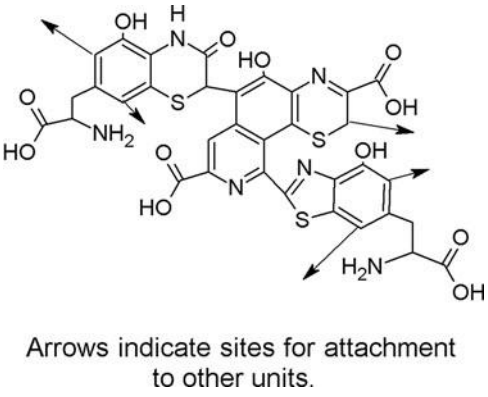


Fig. 5.29 Structure proposed for pheomelanins by Ito and Wakamatsu [131]

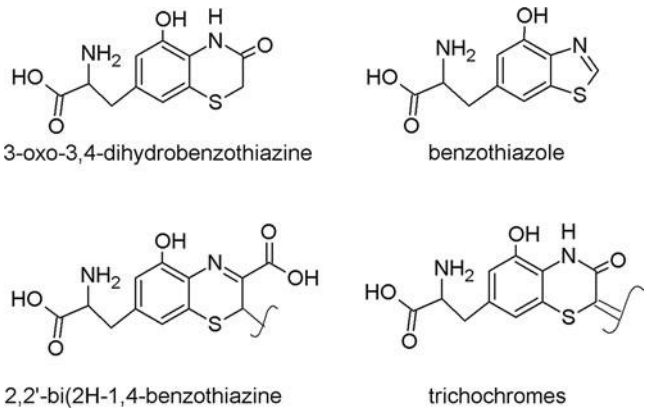


Fig. 5.30 Units suggested for pheomelanins by Napolitano et al. [132]

5.6.8 Degradation Products of Hair Pigments and Different Hair Colors

Use of eumelanin and pheomelanin degradation products (in the section entitled, *Degradation Products of Melanins*) for analysis of human hair of different colors has been provided in several papers. Borges et al. [127] collected and analyzed human hair from 44 subjects: 3 African Americans with black hair, 1 Amerindian with black hair, 6 Asians with black hair, 6 Caucasians with black hair, 2 Hispanics with black hair, 12 people with brown hair, 8 people with blonde hair and 6 people with red hair. Borges et al. determined multiplication factors that they used to approximate the relative amounts of eumelanin and pheomelanin in these hair samples. The data of Table 5.8 shows that black hair contains more total eumelanin than other hair types and red hair has the most pheomelanin or the most CystDOPA (see the biosynthetic scheme of Fig. 5.26 for CystDOPA). Differences of total melanin as

Table 5.8 Analysis of eumelanins in human hair by Borges et al. [127]

Hair color/hair type	μg/eumelanin/mg hair			μg pheomelanin type/mg hair		
	DHI	DHICA	Total eumelanin	2-CystDOPA[a]	5-CystDOPA[b]	Total pheomelanin
Black (African Am.)	8.5	6.0	14.5			
Black (Amerindian)	9.5	5	14.5			
Black (Asian)	7.5	5	12.5			
Black (Caucasian)	4	3	7[c,d]			
Black (Hispanic)	6	4	10			
Brown hair	2	2	4	75	15	90[e,f]
Blonde hair	1.5	1	2.5	75	25	100[e,f,g]
Red hair	1.5	0.5	2.0	650	300	950[e,g]
Black hair				85	15	100[f]

[a]3-AT was used as a marker for 2-CystDOPA (in spite of possible interference [20]) and for these types of distinctions seems to be OK
[b]4-amino-3-hydroxyphenylalanine (AHP) was used as a marker for 5-CystDOPA
[c]Indicates significant difference from African Am. Black hair at $p < 0.01$ level for eumelanin
[d]Indicates significant difference from Asian Black hair for Total eumelanin and DHI only
[e]Indicates significant difference from all Black hair types for eumelanin at $p < 0.0001$ level
[f]Significantly different for pheomelanin for all four hair types analyzed at $p < 0.01$ level
[g]Indicates significant difference from Brown hair at $p < 0.05$ level

large as a factor of 2 were found in the different black hair samples. Borges et al. also found both eumelanin and pheomelanin in all hair samples. However, only low levels of pheomelanin were found in black hair samples.

Some of the conclusions of Borges et al. are summarized below:

- Average black human hair contains about 1% pheomelanin and 99% eumelanin
- Brown and blonde hair contains about 5% pheomelanin and 95% eumelanin and differs primarily in the amount of total melanin present.
- Red hair contains about one third pheomelanin and two third eumelanin. No attempts were made to differentiate between different shades of red hair in this work.

With regard to hair color, Rees [145] suggested that black hair has a high ratio of eumelanin to pheomelanin, whereas red hair has a low ratio of eumelanin to pheomelanin, and blonde hair contains little of either eumelanin or pheomelanin. The data of Borges et al. [127] in Table 5.8 are consistent with these suggestions. The data by Borges et al. suggest that brown hair has slightly higher amounts of eumelanin than blonde hair and nearly equal amounts of DHI and DHICA whereas black hair has more eumelanin and a higher ratio of DHI to DHICA than brown hair.

Napolitano et al. [129] examined 16 different colors of human hair for three pheomelanin markers AHPs, TTCA, BTCA and one eumelanin marker PTCA. Among these 16 different hair colors were Black, Dark Brown, Brown, Blonde, Light Blonde and Albino and 10 different colors of red hair. She also provided the

yield of each marker in ng per mg of hair, but did not attempt to convert the data to amounts of eumelanin or pheomelanin. I analyzed these data by various regression models and found no significant difference using all her raw data and various ratios and sums. However, when I analyzed these data for the ten red hair plus the albino hair sample for the ratio of PTCA/AHPs vs. the sum of PTCA plus AHPs I found a highly significant quadratic fit with an R^2 of 0.85 using the natural log of the ratio vs. the sum of PTCA plus AHP's.

I then looked at the six non-red hair samples and arbitrarily assigned a color factor of 1–6 for hair darkness with black as 6, dark brown as 5, brown as 4, Blonde as 3, light blonde as 2, and albino as 1 and found a highly significant relationship between this arbitrary color factor and the sum of AHPs plus PTCA (linear model $p = 0.0007$; $R^2 = 0.958$; RMSE = 23.94 and quadratic model $p = 0.0018$; $R^2 = 0.985$; RMSE = 16.339).

These statistical analyses suggests that for regression models for natural hair color based on the degradation products for eumelanin and pheomelanin it is better to separate black-brown-blonde hair from red hair. One reason is that the multiplying factors for the different markers for eumelanin and pheomelanin are too different and interfere with comparisons between red and non-red hair samples.

Napolitano et al. [129] suggested four basic types of pigmentation for human hair based on degradation criteria:

Eumelanic Type I (PTCA 100–300 ng/mg)
Eumelanic Type II (PTCA 50–100 ng/mg)
Pheomelanic Type I (BTCA 1,000–2,500 ng/mg and TTCA 200–250 ng/mg)
Pheomelanic Type II (TTCA 100–300 ng/mg)

From their data, the eumelanic type I hair covers black, dark brown and brown and distinguishes that group from the blondes and the many different reds. Pheomelanic type I cover the deep and dark reds and is distinguished from the other types primarily by BTCA providing values from 1,000 to 2,500 ng/mg of pheomelanins. The other two types do not appear to be as clearly distinguished.

Panaella and Napolitano et al. [146] published a newer method suggesting simultaneous determination of PTCA and BTCA as markers for eumelanin and pheomelanin which is worth exploring. See Chap. 3 for a discussion on the Genetics of hair pigmentation in the section entitled, *Hair Pigmentation and Genetics* which helps to explain some of the color differences in hair.

5.6.9 Chemical Oxidation of Hair Pigments

Wolfram and co-workers [12, 147] studied the oxidation of human hair with and without pigment. They also studied the oxidation of melanin granules isolated from human hair. These scientists found that hair with pigment degrades hydrogen peroxide at a measurably faster rate than hair without pigment. Since melanin

represents about 2% of the hair, this result suggests a faster rate of reaction of peroxide with hair pigment than with hair proteins.

For the reaction of peroxide with hair containing no pigment vs. hair containing pigment, the initial reaction rates are similar (through 10 min). However, longer reaction times (30–90 min) produce markedly different reaction rates. The initial rates, due to reaction with the surface and cuticle layers are expected to be similar, since pigment is not in the cuticle. However, as the reaction continues and the pigment becomes involved, peroxide is degraded faster by the pigment-containing hair.

Treatment of isolated melanin granules (from hair) with a large number of reagents (at different pH values) including thioglycolic acid, persulfate, permanganate, or perchlorate failed to provide detectable physical changes in the granules. However, treatment of the granules with alkaline hydrogen peroxide produces disintegration and dissolution of the granules. Wolfram also found that the pH of dissolution is at a maximum near the pK of hydrogen peroxide (pH 11.75). Furthermore, the dissolved pigment produces an intensely colored solution that fades on further reaction.

Wolfram [147] examined the effects of different oxidizing agents on their ability to decolorize soluble melanin and found the following order of efficiency: permanganate > hypochlorite = peracid > peroxide. This finding suggests that the melanin pigments within the granules are not accessible to most oxidizing agents. Furthermore, the granules must be degraded perhaps even solubilized before extensive decolorization of the pigment chromophore can occur. The first step (dissolution of the pigment granules) is a relatively specific reaction requiring oxidation at specific sites. Hydrogen peroxide is not as strong an oxidizing agent as permanganate or peracetic acid, but it is actually more effective for dissolving the granules than either of these other two oxidizing species. Once the granules are dissolved, reactions to degrade the chromophoric units of melanin can proceed more readily. Since the melanin chromophoric units contain many different sites susceptible to oxidation, the rate of the second step (degradation of the pigment chromophore) proceeds faster with the stronger oxidizing agents—e.g., permanganate > hypochlorite = peracid > peroxide—which is the order for decolorization of the solubilized melanin.

Although persulfate is not a stronger oxidant than hydrogen peroxide, mixtures of persulfate and peroxide provide a more effective bleaching system than peroxide alone. Martin [148] studied the persulfate oxidation of fungal melanins. He determined that persulfate is a selective oxidizing agent, releasing only those portions of melanins containing primarily fatty acids and phenolic compounds. Persulfate and peroxide are both somewhat selective in their attack on melanins. Presumably, peroxide attacks different portions or sites on the melanin macromolecules that facilitates solubilization of melanin so that the more potent persulfate can degrade it in solution. Thus, one might conclude that persulfate and peroxide complement each other in terms of their ability to bleach melanin pigment and therefore to bleach human hair.

Wolfram and Hall [147] also isolated several products from the reaction of alkaline hydrogen peroxide with melanin pigments, including proteinaceous species up to 15,000 daltons. These scientists further developed a procedure for the isolation of melanoprotein and determined the amino acid composition of melanoprotein from East Asian hair. They found fewer cystine linkages in melanoprotein than in whole fiber and a larger percentage of ionizable groups—i.e., approximately 35% more dibasic amino acid residues and 15% more diacidic groups.

Wolfram and Albrecht [149] concluded that hue differences in hair not only result from chemically different pigments, but also from differences in the degree of aggregation and dispersion of the eumelanin pigment. Both eumelanin and pheomelanin pigments contain polypeptide chains with similar amino acids as shown by Arakindakshan Menon et al. [150]. The red hair melanin contains more sulfur (as 1,4-benzothiazine units) than the brown-black melanins.

There are undoubtedly several similarities with regard to the chemical bleaching of eumelanins and pheomelanins. Wolfram and Albrecht [149] suggested that pheomelanin in hair is more resistant to photodegradation than the brown-black eumelanins. The more recent study by Hoting and Zimmermann [50] demonstrated that light-brown hair containing a mixture of pheo- and eumelanins is affected by all segments of light including visible light, whereas eumelanins are more photostable. These scientist examined degradation to the granules gravimetrically and by examining the polymers by infrared spectroscopy. The aromatic rings of both melanic structures are of high electron density, and consequently are both sensitive to attack by oxidizing agents, as demonstrated for the brown-black melanins. The eumelanin in addition provided greater protection to the proteins of hair than pheomelanin. Takahashi and Nakamura [151] studied the photolightening of red and blonde hair in both UV and visible light and their results are described below.

5.6.10 Photochemical Degradation of Melanins

Photochemical degradation of hair proteins occurs primarily near 254–350 nm, the primary absorbance region of un-pigmented hair as shown by Arnaud [58] and by Hoting, Zimmerman and Hocker [152]. Although several amino acids are degraded by light, the primary degradation occurs at cystine and thioester. Launer [60] and Inglis and Lennox [61] have provided evidence for photochemical degradation to other amino acids including methionine, histidine, tryptophan, phenylalanine, and leucine.

The mechanism for photochemical degradation of cystine is believed to involve both C–S and S–S fission mechanisms (see the section on photochemical degradation of hair proteins described earlier). Hair pigments function to provide some photochemical protection to hair proteins, especially at lower wavelengths, where both the pigments and the proteins absorb light (254–350 nm). Hair pigments

accomplish this protection by absorbing and filtering the impinging radiation and dissipating this energy as heat wherein virtually no damage occurs to the pigments. But above some energy level of excitation the ability of melanin to convert all of its absorbed energy to heat fails resulting in damage to its structure and chemical degradation.

Eumelanin ring opening may result from either ionic (chemical degradation) or free-radical reaction (photochemical degradation). Slawinska and Slawinski [153] suggested that these two mechanistic schemes may have some common intermediates. The ionic pathway probably begins by nucleophilic attack of the peroxide anion on the o-quinone group. Slawinska and Slawinski suggest that photochemical degradation of melanin occurs through a similar peroxide intermediate.

The first steps in the photochemical degradation of the eumelanin chromophore probably involves excitation to a radical anion and then attack by the oxygen radical anion on the o-quinone group, see Fig. 5.31. Ring opening of the six-membered ring indolequinone species then follows.

A related scheme may be involved for the photochemical degradation of pheomelanins.

Sarna et al. [154] concluded that pheomelanins are very similar to eumelanins with regard to their susceptibility to photooxidation. However, Arakindakshan Menon et al. [150] suggested that pheomelanins are more easily induced to an excited state than eumelanins. But, Wolfram and Albrecht [149] presented evidence that eumelanins are more sensitive to photochemical or chemical degradation than pheomelanins. And Hoting et al. [152] showed that the pigment of light-brown hair is affected by UV-A, UV-B and visible light, but eumelanins are more stable to light and provide a greater photoprotective effect.

Takahashi and Nakamura [151] clarified these seemingly disparate views on the relative photochemical degradation of pheomelanin and eumelanin by comparing the relative degradation of the pure pigments and the relative degradation of the pigments in hair. Both visible and UV light degrade pheomelanin in red hair, but

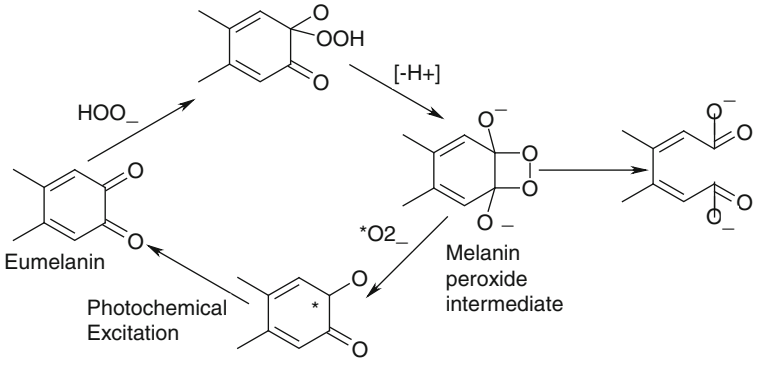


Fig. 5.31 Proposed mechanisms for degradation of melanins [153]

eumelanin in the hair fiber has been described as being more sensitive [149]. On the other hand, studies with isolated pheomelanin vs. eumelanin show that for the pure pigments pheomelanin is more sensitive to UV light than eumelanin [151]. This difference was proposed by Takahashi and Nakamura who observed the consequences of light acting directly on the pigments whereas in the fiber the light is attenuated by absorption and reaction with hair proteins producing radicals that react with the pigments.

Takahashi and Nakamura [151] also observed that blonde hair contains more eumelanin than pheomelanin and unlike red hair when exposed to UV light does not lighten until it is washed with water after irradiation. These scientists suggested that the washing or water effect may involve the hydroxyl free radical which may be required to be above a certain concentration to decompose eumelanin.

5.6.11 Photoprotection of Hair

Pande et al. [155] demonstrated that hair dyes (both oxidation and semi-permanent) grant a photoprotective effect to hair proteins providing a significant decrease in cortical damage. This protection shows up in tensile testing and the effect is greater the darker the dyes used. Meinert et al. [156] examined several commercial antioxidants for their sun protection properties in both pre-sun and after-sun hair products. White tea extract and Provitamin A when applied to hair in a pre-sun base formulation (at 0.05%) and then exposed to UV plus Visible light offered a higher tensile breaking stress than the same pre-sun base formulation without antioxidants. The other antioxidants produced either no significant difference or a lower tensile value. Less color change and lightening of non-dyed hair was observed with the pre-sun base plus White Tea Extract. The damage to the hair proteins was greater at higher radiant flux or lower relative humidity.

Schlosser [157] concluded that certain silicones, namely resins like trimethylsiloxysilicate or propylphenylsilsesquioxane are able to protect dyed hair (permanent dyed hair) from color change induced by UV radiation. However, silicones like dimethiconol/dimethicone did not show such an effect. Schlosser claimed that it is possible to reduce color facing both by wash-out and from UV radiation by adding silicones to the formulation of permanent and semi-permanent hair dyes.

5.6.12 Summary of Some Physical Properties of Bleached Hair

The gross or bulk chemical changes produced in hair by oxidation reactions including bleaching have been described in the previous sections of this Chapter. Details of changes in the breakage, stretching, bending, swelling and other physical properties are described in Chaps. 9 and 10.

5.7 Safety Considerations for Hair Bleaches

The primary safety concerns with hair bleaches, as with most hair care products, arise from misuse or failure to comply with the usage instructions. Skin irritation, hair breakage, oral toxicity, sensitization and scarring alopecia either have been reported from use (misuse) of hair bleaches or are mentioned on the warning labels of these products.

Bergfeld [158] has reviewed adverse effects of hair cosmetics recorded at the Cleveland Clinic Dermatology Department over a 10-year period. Effects attributed to hair bleaches were simple skin irritation and hair breakage. However, Bergfeld reported neither sensitization reactions nor complex toxic symptoms for hair bleaches.

Bourgeois-Spinasse [159] indicates a few incidents of allergic manifestations caused by ammonium persulfate powder; however, most hair bleaches today use potassium persulfate as the primary bleach accelerator. Bergfeld [158] reported permanent hair loss following misuse of hair bleaches and attributed it to scarring alopecia, although Bergfeld did not specify the extent of hair loss observed. Bergfeld concludes that side effects from hair bleaches are minimal, if the consumer is aware of damaged hair, any inherent skin disease and complies with the product usage instructions.

Treatment combinations are oftentimes more damaging to hair than one might expect. For example, extensive sunlight exposure in combination with chemical bleaching or chemical bleaching plus permanent waving must be done very carefully because of the compound damage provided by these combined treatments.

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