This student paper was written as an assignment in the graduate course

Free Radicals in Biology and Medicine

(77:222, Spring 2001)

offered by the

Free Radical and Radiation Biology Program
B-180 Med Labs
The University of Iowa
Iowa City, IA 52242-1181
Spring 2001 Term

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MELANIN:

WHAT MICHAEL JACKSON DIDN'T KNOW

by

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For: 77:222, Spring 2001

8. March 2001

Abbreviations:

ACTH- adrenocorticotropin, $Asc^{? -}$- ascorbate radical, ß-LPH- beta-lipotropin, CDM- cysteinyldopa-melanin, DHI- 5,6-dihydroxyindole, DHICA- 5,6-dihydroxyindole-2-carboxylic acid, DM- dopa-melanin, $DQ^{?+}$- diquat, MSH- melanocyte-stimulating hormone, POMC- propiomelanocortin, $PQ^{?+}$- paraquat, $RB^{?2+}$- rose bengal, $RFH^{?-}$- riboflavin radical, ROS- reactive oxygen species, $TetraQ^{?+}$- tetraquat, $TriQ^{?+}$- triquat, TRP1- tyrosinase related protein 1, TRP2- tyrosinase related protein 2, UV- ultraviolet

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1. Abstract

Melanin is a light-absorbing pigment present in the skin, eyes, hair, feathers, scales and certain internal structures of both homeothermic and heterothermic animals. Melanin is a complex biological polymer. Several types of melanins have been described. All melanins are insoluble salts and amorphous natural semiconductors. In the structure of melanin, free radicals exist in equilibrium with nonradical groups. Its physical properties offer both photoprotection and vision enhancement, decreasing the risk of catharacts in the lens of the eye and the risk of macular degeneration in the retina. Its chemical properties enable it to function as a free radical scavenger, alone or in association with zinc which functions as an antioxidant. Melanin-related abnormalities of human pigmentation may be genetic (*e.g.* albinism), autoimmune (*e.g*. vitiligo) or endocrine (*e.g.* Addison's disease).

2. Introduction

Melanin is the pigment that is responsible for the color of skin, eyes, hair, feathers, and scales. It is one of two pigments found in human skin and hair and adds brown to skin color; the other pigment is carotene, which contributes to yellow coloring. In the eyes, melanin is located in the iris and the pigment layer of the retina [1]. Other locations of melanin are the midbrain (*substantia nigra*), some internal membranes, the cephalopoda's ink *etc*. [2]. Melanin is formed in specialized organelles in the skin called melanosomes. In humans, it is formed under the action of MSH, a hormone synthesized along with ACTH, ß-LPH and ß-endorphin from POMC, a preprohormone secreted by the anterior lobe of the pituitary gland [1]. Melanins from natural sources fall into two general classes: 1) eumelanins- black to dark brown; 2) pheomelanins- reddish-brown. Figure 1 (from [3]) is a graphic outline of the beneficial functions of eumelanins, the predominant epidermal melanins:

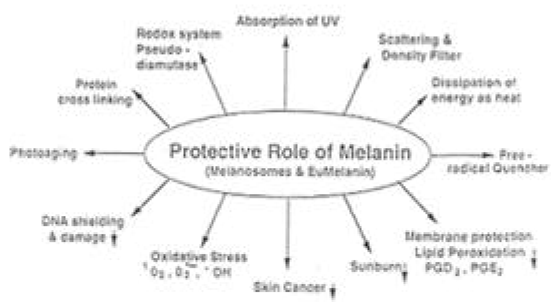


Figure 1. The photoprotective role of eumelanins in human skin

3. Biosynthesis of melanin

The melanins are considered to be polymers of the basic building blocks shown in Fig.2 (from [4]), where the curly lines indicate sites of attachment to the extended polymer and possibly to proteins. However, the details of the polymerization and the role of protein linkages in the natural melanin complex are not known.

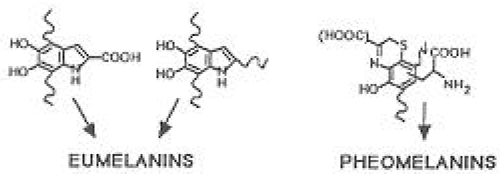


Fig.2. The building blocks of eumelanins and pheomelanins (note the presence of S in the structure of pheomelanins - that are derived from cysteinyldopas *vs*. the absence of S in eumelanins -that are derived from DOPA)

Melanin can be synthesized from tyrosine or DOPA as shown in Fig. 3(from [5]):

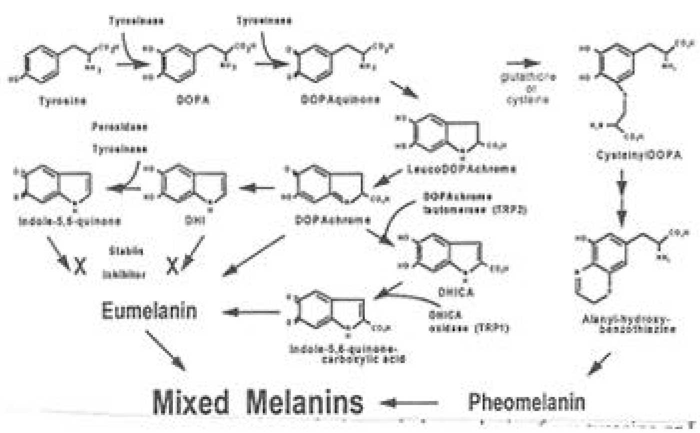


Fig. 3. The synthesis of eumelanins and pheomelanins from tyrosine or DOPA

4. The physical properties of melanin

All the melanins are insoluble salts. For example, *Sepia officinalis* ink melanin is a Ca and Mg salt. Melanin conductivity, surface properties, unpaired electrons, hydration, interstitial activity change reversibly by applying an electric voltage. Such properties render these amorphous natural semiconductors of interest for biological research [6].

The average epidermal absorption coefficient depends on both the melanosomal absorption coefficient (μ_a) and the volume fraction(f_v) of melanosomes in the epidermis. The latter is estimated to vary as follows:1) for light-skinned Caucasians, f_v = 1-3%; 2) for well-tanned Caucasians and Mediterraneans, f_v = 11-16%; 3) for darkly pigmented

Africans, f_v = 18- 43% [4]. Also, the general shape of the melanosome absorption spectrum is to be approximated to $\mu_a = 1.70 \times 10^{12}\ nm^{-3.48}$ /cm for skin and $\mu_a = 6.49 \times 10^{12}\ nm^{-3.48}$/cm for retina, where nm refers to the wavelength expressed in nanometers. Figure 3 (from [4]) is a representation of the extinction coefficient versus wavelength for eumelanin and pheomelanin:

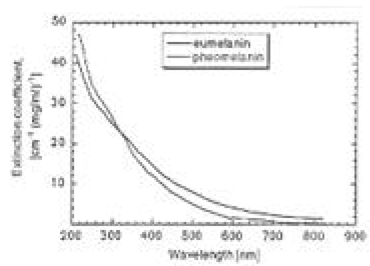


Fig. 4. Extinction coefficient vs.wavelength for eumelanin and pheomelanin

Melanin absorbs light smoothly over a broad spectral range: it absorbs the higher energy light more strongly than the lower energy light; therefore, melanin absorbs UV more than blue, the blue more than the green, *etc.* and provides protection to the lens of the eye against UV- thereby decreasing the risk of catharacts; it also filters the different colors in proportion to their ability to damage the tissue of the retina, thereby reducing the risk of macular degeneration [7].

5. Melanin as a free radical scavenger

EPR measurements showed that melanin free radicals are generated by the electron exchange between electron donors and acceptors produced in situ during melanin synthesis [8]. The melanin polymer exhibits stable free radical properties because of semiquinones, which seem to have a protective action in cells probably by acting as a sink for diffusible free radical species. It has been proposed that the majority of melanin free radicals exist in equilibrium with nonradical groups. For instance, in the eumelanin polymer the semiquinone radicals ($QH^{?}/Q^{?-}$) exist in a thermodynamic equilibrium with hydroquinones (QH_2) and quinones (Q) ([9]):

$$QH_2 + Q \ ? \ 2Q^{?-} + 2H \quad (1)$$

These equilibria are depicted in Figure 5 (from [8]) for both types of melanins:

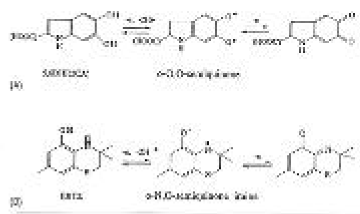


Figure 5. The structural equilibria for eumelanin (A) and pheomelanin (B) subunits

Melanins, and eumelanins in particular, act as free radical scavengers. As shown in Figure 4 (from [10]), eumelanins are redox polymers which exhibit both oxidizing and reducing abilities:

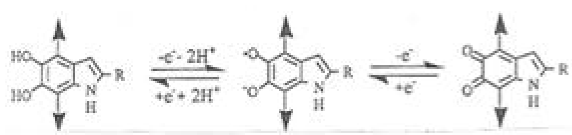
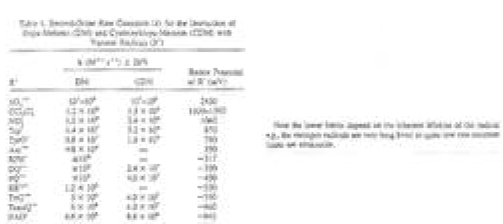


Figure 6. Redox reactions of eumelanins

Eumelanins participate in the inhibition of lipid peroxidation (*via* chelation of metal cations capable of initiating oxidative reactions in unsaturated lipids), the inhibition of free radical damage to enzymes and the scavenging of ROS such as superoxide radicals, hydrogen peroxide, hydroxyl radicals and singlet oxygen (Table 1, from [11]). The presence of melanin in the pigment layer of the retina reduces the risk of macular degeneration due to its antioxidant ability (neutralization of free radicals such as $O_2^{?-}$).



As shown in Table 1(where DM and CDM are synthetic analogues of eumelanins and pheomelanins, respectively), the ability of melanins to act as free radical scavengers for both oxidizing and reducing radicals depends on the redox driving force, the charge on the radical and the lifetime of the radical.

As mentioned above, one of the mechanisms by which melanin inhibits peroxidative processes is through scavenging the pro-oxidant superoxide radical. Because melanin pigments contain both reducing (QH_2) and oxidizing (Q) groups, it was postulated that superoxide can interact with the pigment along two possible pathways ([8]):

$$QH_2 + O_2^{?\,-} \;?\; Q^{?\,-} + H_2O_2 \qquad (2)$$

$$Q + O_2^{?\,-} \;?\; Q^{?\,-} + O_2 \qquad (3)$$

It was found that melanin competes with EDTA for metal ions; using 1,10 – phenantroline to chelate ferrous iron, it was shown that melanin is capable of reducing Fe^{3+} to Fe^{2+} [8]. Further evidence for the occurrence of the Fenton cycle was obtained by the spin trapping method, which showed the formation of hydroxyl radicals, possibly in the following steps (from [8]):

$$Fe^{3+}\text{-EDTA} + DM \;?\; Fe^{3+}\text{-DM} + EDTA \qquad (4)$$

$$Fe^{3+}\text{-DM} + e^{-} \;?\; Fe^{2+}\text{-DM} \qquad (5)$$

$$Fe^{2+}\text{-DM} + EDTA \;?\; Fe^{2+}\text{-EDTA} + DM \qquad (6)$$

$$Fe^{2+}\text{-DM} + H_2O_2 \;?\; Fe^{3+}\text{- DM} + HO^{?} + OH^{-} \qquad (7)$$

$$Fe^{2+}\text{-EDTA} + H_2O_2 \;?\; Fe^{3+}\text{-EDTA} + HO^{?} + OH^{-} \qquad (8)$$

The superoxide radical was reported to mediate hydroxyl radical formation in the presence of ferric iron during melanin photo-oxidation; in the presence of the Fe^{3+}-EDTA complex, the illumination of melanin leads to hydroxyl radical formation, observed as DMPO/OH spin adducts (from [8]):

$$O_2^{?\ -} + Fe^{3+}\text{-EDTA} \ ? \ Fe^{2+}\text{-EDTA} + O_2 \qquad (9)$$

$$O_2^{?\ -} + O_2^{?\ -} + 2H \ ? \ H_2O_2 + O_2 \qquad (10)$$

$$Fe^{2+}\text{-EDTA} + H_2O_2 \ ? \ Fe^{3+}\text{-EDTA} + HO^{?} + OH^{-} \qquad (11)$$

Melanin is able to reduce nitroxide radicals [8]:

$$>N\text{-}O + Mel_{red} \ ? \ >N\text{-}OH + Mel_{ox} \qquad (12)$$

Nitroxides bearing a positive charge were reduced more easily than those bearing a negative charge; this reflected a negative charge of carboxylate groups at a neutral pH on the surface of melanin, which inhibited the interaction with any anionic species but facilitated interaction with cationic species. Melanin behaves as a natural cation exchange material and is thus able to incorporate various ions both *in vitro* and *in vivo*. Another argument pleading in favor of the role of melanin as a free radical scavenger is its association with zinc in melanosomes, where zinc is an abundant trace element. Two classes of independent binding sites participate in the interactions of cations with DM, with the association constants for Zn^{2+} being $K_1 = 5.87 \times 10^5$ mol^{-1} and $K_2 = 4.85 \times 10^3$ mol^{-1}[12]. An essential biochemical function of Zn^{2+} is to serve as an antioxidant. It was predicted that relatively high concentrations of Zn^{2+} might be present in those tissues vulnerable to oxidation, *e.g.* the hair, skin, eye and spermatozoa [12]. Zn^{2+} stabilizes semiquinone radicals in melanin and increases free radical activity in melanosomes.

.6. Aspects of melanic pigmentation: diseases, dreams and disasters

Several melanin- related pathological conditions are known, *e.g.* oculocutaneous albinism, an autosomal recessive disease characterized by skin and eye hypopigmentation due to a mutation in the gene coding for the enzyme tyrosinase(involved in the pathway of melanin synthesis) [13]. The main character in H.G. Wells' novel "The Invisible Man" succceds in synthesizing a magic substance that renders a cat invisible-with the exception of its eye melanin, persisting in the form of two haunting, mobile phosphorescent points. However, the Englishman -an albinos -acquires himself the invisibility that in time will lead to his own destruction. Abnormalities of hyperpigmentation of the skin and of the lining of the mouth are due to a variety of conditions, the most important being Addison's disease (adrenal insufficiency) [13]. A famous patient was former President John Fitzgerald Kennedy, whose flashing smile and "healthy tan" were long envied by electoral opponents. On the other hand, the phenomenon of racial prejudice and discrimination has often led to desperate attempts to adapt, endure and prevail. Pop singer Michael Jackson, a well-known plastic surgery addict, is said to have artificially whitened his skin, trying to dramatically reinvent his image on stage; Jackson's official version is that he suffers from vitiligo- an autoimmune disease characterized by patch-like discolorations of the skin. The main character in "The Bluest Eye", the most recent novel of Nobel Prize laureate Toni Morrison, is Pecola- a little black girl in rural Ohio who passionately wishes and prays for what she cannot have- blue eyes. What Pecola, Michael Jackson and The Invisible Man have in fact in common is an ardent desire to escape from their own body- how strange that they should all meet in melanin.

7. Summary

Melanin, the eye and skin pigment that has had a marked social and cultural impact throughout human history, is actually a light-absorbing redox polymer endowed with a photoprotective role and which also acts as a free radical scavenger.

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