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The Red and the Black

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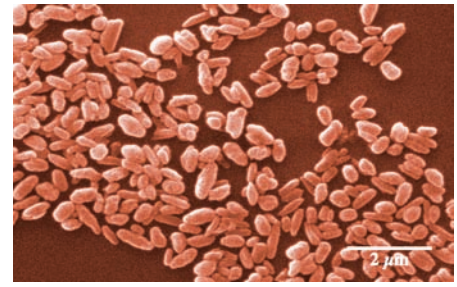
CONSPECTUS

"Pigmentation, which is primarily determined by the amount, the type, and the distribution of melanin, shows a remarkable diversity in human populations, and in this sense, it is an atypical trait."—E. J. Parra.



Melanin is found throughout the human body, skin, eye, brain, hair, and inner ear, yet its molecular structure remains elusive. Researchers have characterized the molecular building blocks of melanin but have not been able to describe how those components fit together in the overall architecture of the pigment. Melanin is categorized into two distinct classes, pheomelanin (red) and eumelanin (black). Although these classes share a common biosynthetic origin, specific molecular reactions occurring early in pigment production differentiate these two types. Pure eumelanin is found throughout nature, which has allowed researchers to characterize and quantify its chemical properties. However, pure pheomelanin is not observed in nature and rarely makes up more than ~25% of the total melanin present. In this Account, we explore our current understanding of the structure and reactivity of the red and black pigments.

Epidemiological studies of skin and ocular cancers suggest that increasing relative proportions of pheomelanin correlate with increased risk factors for these diseases. Therefore, understanding the factors that control the relative abundance of the two pigments has become increasingly important. Consequently, researchers have worked to elucidate the chemistry of pheomelanin to determine whether the pigment could cause these cancers and, if so, by what mechanisms. The photoactivation of oxygen by pheomelanin in the UV-A range could contribute to the development of UV-induced cancers: recent measurement of the surface photoionization threshold of intact melanosomes reveals a lower photoionization potential for pheomelanin than eumelanin. A complementary study of intact human melanosomes isolated from different colored irides reveals that the absorption coefficient of the melanosome decreases with increasing pheomelanin content. These results suggest that the epidemiological data may simply result from an increased exposure of the underlying tissues to UV light.

A Journey

"Melanin pigmentation has aroused the curiosity and attention of man since the beginning of recorded history."[1]

The study of pigmentation began over four centuries ago. One of the first experimental studies, reported by Santorio Santorius (1561−1636), attributed the color of black skin to the presence of bile.[2] In the early 18th century, studies failed to find bile in skin and Alexis Littre (1658−1726) reported the presence of an insoluble black pigment adhered to the reticular membrane.[3] Melanin had been discovered, but progress on its origin, synthesis, and chemical properties needed to await the advances in analytic and synthetic techniques now taken for granted in the field of chemistry.

Otto v. Fürth and Hugo Schneider made the innovative proposal that melanin (derived from the Greek word *melas*, black) resulted from the reaction of an intercellular oxidase with aromatic groups in certain proteins.[4] Bruno Bloch was inspired by this hypothesis and sought experimental evidence, finding that cells carried out a catalytic reaction by which they oxidize dopa to melanin; he named the participating enzyme, dopa-oxidase.[5] In 1895, Emile Borquelot and George Betrand discovered tyrosinase in fungi, and subsequently, Betrand established that tyrosinase converted tyrosine into a black pigment

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similar to mammalian melanin.[6] In 1927, Henry Stanley Raper isolated dopa as the primary product of the reaction of tyrosinase with tyrosine and through careful chemical studies established the primary chemical steps for the enzymatic oxidation of tyrosine to melanin.[7] In doing so, Raper isolated derivatives of 5,6-dihydroxyindole (DHI) and 5,6-dihydroxyindole-2-carboxylic acid (DHICA); these two indoles are now considered to be the fundamental building blocks of the black pigment eumelanin. In 1948, Howard Mason extended Raper's molecular scheme and introduced a "polymer" model of melanin, involving self-condensation of indole quinones.[8] During the 1960s, studies of various natural pigments results in the classification of melanin into two major types: eumelanin (eu, a Greek prefix for "good, well") and phaeomelanin (phaeo, a Greek prefix meaning "dark", now commonly written pheomelanin). An important chemical difference between these classes was revealed in 1966 when Guiseppe Prota, Mario Piattelli, and Rodolfo Nicolaus proposed that pheomelanin resulted from the specific reaction of cysteine with the quinones produced by tyrosine oxidation, a reaction that does not occur in the synthesis of eumelanin.[9]

Throughout these four centuries of work, the precise molecular structure of melanins has remained elusive. Fortunately, there are powerful and informative approaches for probing molecular composition. Most are based on oxidative and reductive degradation of the pigments, followed by identification and quantification of the molecular markers produced, which in turn can be quantitatively related to the presence of specific chemical moieties present in the original pigment. In 1952, Luigi Panizzi and Rodolfo Nicolaus identified pyrrole 2-3-5-tricarboxylic acid (PTCA) as the most significant fragment from the degradation of melanin granules isolated from the ink sac of the cuttlefish *Sepia officinalis*.[10] Between 1967 and 1968, Guiseppe Prota, Rodolfo Nicolaus, and collaborators isolated melanin from red feathers and established the centrality of 5-*S*-cysteinyldopa as a precursor to pheomelanin.[11] 5-*S*-Cysteinyldopa was then first detected in melanoma tissue and urine by Hans Rorsman and co-workers in 1972.[12] In 1968, Ernesto Fattorusso and Luigi Minale and their associates identified degradation fragments from pheomelanin, including 4-amino-3-hydroxyphenylalanine (4-AHP).[13] Subsequently, the late 1960s through the 1980s witnessed great advances in the development of quantitative approaches based on oxidative degradation and reductive hydrolysis of natural pigments to generate meaningful insight into aspects of the molecular structures of melanins.[14,15]

Shosuke Ito refined many of these analytical approaches, and his methods are currently the best approaches available for deriving molecular information about melanins.[16] Specifically, the yields of PTCA, pyrrole-2,3-dicarboxylic acid (PDCA), and 4-AHP produced by degradation techniques are routinely used to infer the relative contributions of specific molecular monomers, but not their chemical connectivity, to the overall architecture of the pigment.

First Steps

"Eumelanin and pheomelanin both derive from the common precursor dopaquinone, which is formed following the oxidation of tyrosine by tyrosinase."[17]

Figure 1 illustrates the current model for the biosynthetic pathways of eumelanin and pheomelanin, commonly called the Raper-Mason scheme. There remains a significant gap in knowledge between the structures of the initially formed molecules and that of the oligomers or polymers that make up the pigment. However, it is clear that eumelanin is assembled from DHI and DHICA and that pheomelanin is derived from benzothiazine units. Ito and co-workers provided standard approaches for quantifying mixtures of pigments.[17−19] They developed protocols for quantifying (a) the relative amounts of eumelanin and pheomelanin present in a sample of pigment and (b) the relative amounts of DHI and DHICA present in eumelanin. The ability to determine such structural information from this type of analysis plays an important role in developing structure/function models for melanins.

Figure 1 shows that the synthetic pathways for eumelanin and pheomelanin branch from different reactions involving dopaquinone. Applying data from pulse radiolysis studies reported by Tad Land and Patrick Riley, Ito and workers were able to quantify kinetics for several steps of the Raper−Mason scheme.[20] The data indicate that one can think of the synthesis of melanins in terms of a 3-step process. Initially cysteinyldopa formation will occur to the exclusion of eumelanin synthesis, as long as the concentration of cysteine is greater than 0.13 μM. Second, oxidation of cysteinyldopa to pheomelanin occurs for concentrations of cysteinyldopa greater than 9 μM. Finally, eumelanin is generated, occurring after most of the cysteinyldopa and cysteine levels are depleted. While there are no studies that provide a real time view of melanin formation within the melanosome, electron microscopy of melanosomes at differing degrees of melanization indicate that pigment granules gradually grow to diameters of ~30 nm within the melanosome, and once they reach this size, no free space can be detected.[21,22] Given this morphology, the 3-step process characteristic of samples containing both pheomelanin and eumelanin would predict a structural motif where the ~30 nm granule would have

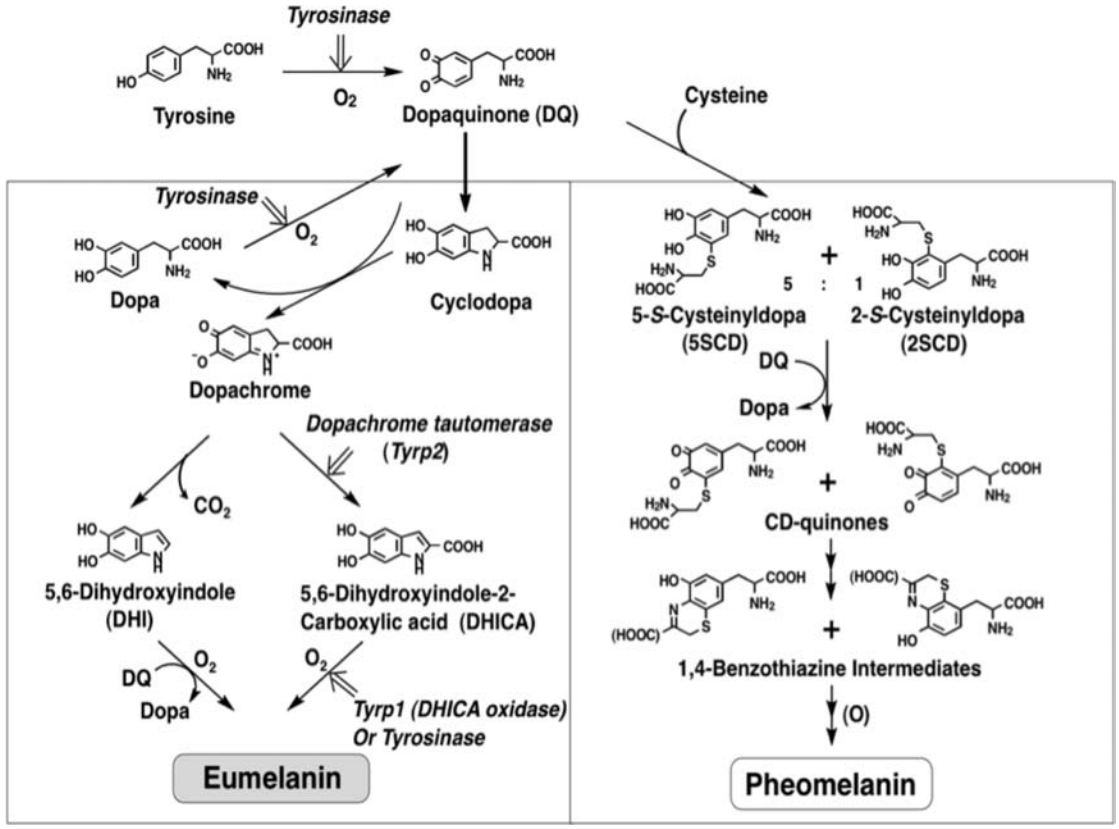

FIGURE 1. Biosynthetic pathways leading to eumelanin and pheomelanin production. Note that the activities of tyrosinase, Tyrp1 and Tyrp2, are involved in the production of eumelanin, while only tyrosinase (and the precursor amino acid cysteine) is necessary for the production of pheomelanin Reproduced with permission from from ref 20. Copyright 2008 American Society for Photobiology.

pheomelanin at their core and eumelanin at the surface. We will discuss data in support of such a model later in this Account, but first, we need to identify a spectroscopic signature that enables the definitive identification of the type of melanin present on or near the surface of the melanosome.

Modes of Behavior

"Oxygen clearly plays a role in melanin photoreactions: it is known that during irradiation oxygen in consumed and melanin free radicals are produced."[23]

Motivated, in part, by the hypothesis that the increased susceptibility of fair skin individuals to skin cancers is linked to greater phototoxicity of pheomelanin,[24,25] there has been extensive interest in comparing the reactivity of eumelanin and pheomelanin, especially in their ability to generate reactive oxygen species. Because pure pheomelanin is not found in nature, the majority of what is known about this pigment comes from the study of synthetic models. Ito has reported on the optimized conditions for preparing the synthetic pigment,[26] which has enabled laboratories to follow a common protocol. Tadeusz Sarna and co-workers reported the first action spectra for photoinduced oxygen consumption for a variety of synthetic and natural melanins, and revealed that the action spectra differ significantly from the absorption spectrum of the pigment.[23,27] In 1999, we put forth an explanation for this disparity in the case of eumelanin by demonstrating that the action spectrum matches the absorption spectra of oligomeric constituents of the natural melanins.[28] Furthermore, aggregation of these constituents affects their aerobic reactivity. Miles Chedekel and co-workers were the first to report detailed studies on the photodynamic properties of pheomelanin, finding that excitation through the UV region resulted in the formation of superoxide radical anions.[29,30]

In efforts to understand the potential link between pheomelanin and cancer, Prota and co-workers reported that UV sensitivity is associated with high pheomelanin and low eumelanin levels.[31] In contrast, Jonathan Rees and co-workers examined the concentrations of eumelanin and pheomelanin present in human skin before and after exposure to UV radiation, concluding that factors other than the amount of pheomelanin may be important in determining cancer susceptibility.[32] Douglas Brash and co-workers examined the induction of DNA lesions and apoptosis upon UV exposure of

congenic mice of with black, yellow, and albino coats: these mice vary in the amount of pheomelanin present.[33] Their data provide support for the hypothesis that melanin-induced apoptosis could be a contributing factor to the observed epidemiology of skin cancer for black-, blond-, and red-haired populations. A suggestion in support of a causative role of pigments is also provided by experiments on UV-A-induced DNA single-strand breaks in human melanocytes differing only in the type of pigment produced; such reactivity occurs in cells expressing pheomelanin, arguing that the origin is an intrinsic chromophore, most likely pheomelanin itself or a molecule produced along the pigment's synthetic route.[25]

Building on the mechanistic work published by Chedekel, we used photoemission electron microscopy (PEEM) to quantify the photoionization threshold for natural eumelanin and pheomelanin using melanosomes isolated from human black and red hair, respectively.[34] This system was chosen as melanosomes from red-hair tend to fall apart upon isolation, thereby exposing not only the pigment present on their surface, but that throughout the melanosome. Given the morphological consequences of the 3-step synthetic process described above, it was important to choose a system containing pheomelanin that would expose the core of the organelle. The PEEM data show that both pigments are characterized by an ionization threshold at 282 nm, reflecting that eumelanin is present in both samples. Pheomelanosomes, however, exhibit a unique second ionization threshold at 326 nm, which was attributed to the benzothiazine structural motif present in pheomelanin.

To provide compelling evidence in support of this assignment, the threshold ionization potentials for synthetic pheomelanin were probed by two complementary techniques, time-resolved spectroscopic detection of solvated electrons and EPR measurements of the photoconsumption of oxygen, following excitation of dissolved synthetic pheomelanin in water.[35] Figure 2 presents the collective results. All studies are consistent with the photoionization threshold determined for the melanin isolated from human red hair.

What Decoration Confers Distinction?

"In general, persons with the greatest constitutive pigment show the lowest rates of non-melanoma and melanoma skin cancer, and the degree of skin pigmentation is inversely proportional to, and predicts, the amount of erythema that follows acute ultraviolet radiation exposure."[32]

The fact that pheomelanin has a lower ionization potential than eumelanin is supportive of the view that pheomelanin could be an active contributor to the greater incidence rate

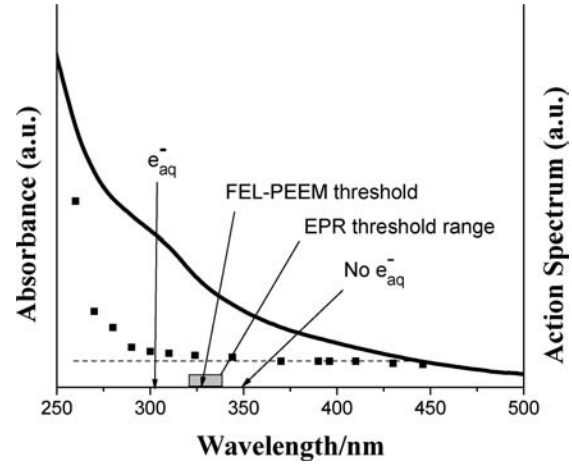

FIGURE 2. Absorption (solid line) and action (squares) spectra reported by Chedekel and co-workers for the photogeneration of superoxide radical anion by synthetic pheomelanin is reproduced. Also indicated are the thresholds for photoionization determined by PEEM measurements on human pheomelanosomes, femtosecond absorption spectroscopic detection of solvated electrons, and EPR-oximetry on synthetic pigment. The measurements present a consistent view that the photoionization threshold of pheomelanin is around ~325 nm. Reproduced with permission from ref 36. Copyright 1999 Colorado State University.

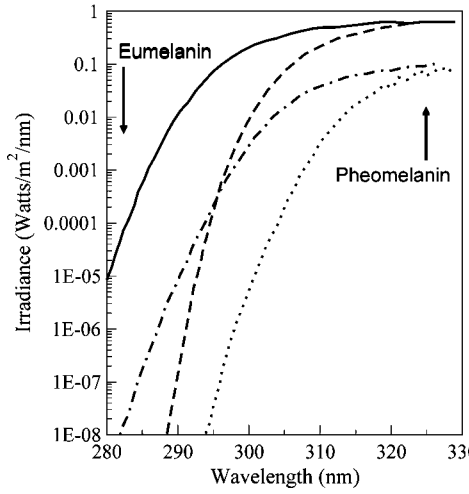

FIGURE 3. Incident solar radiation at the surface of the Earth is plotted as a function of wavelength, solar zenith angles (SZA) and ozone concentration (in Dobson units, DU). The four curves correspond to (—) SZA = 0, 100 DU, (- - -) SZA = 0, 400 DU, (- · -) SZA = 75, 100 DU, and (···) SZA = 75, 400 DU. Also indicated on the graph are the threshold ionization potentials for human eumelanosomes and pheomelanosomes. Reproduced with permission from from ref 20. Copyright 2008 American Society for Photobiology.

of UV-induced cancers observed for red-haired vs black-haired individuals. Figure 3 shows the solar irradiance at the Earth's surface for several different solar zenith angles.[36] The threshold ionization energies of the human hair melanosomes obtained from PEEM experiments are also indicated. These data show that one is exposed to the wavelengths of light suf-

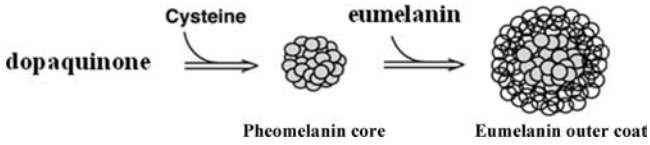

FIGURE 4. Casing model for mixed melanogenesis. Note that in the process of mixed melanogenesis, pheomelanic pigment is produced first, followed by the deposit of eumelanic pigment. Eumelanin is believed to act as a photoprotective antioxidant, while eumelanin acts as a phototoxic pro-oxidant.

ficient to ionize pheomelanin but that the atmosphere nearly completely blocks the light required to photoionize eumelanosomes.

However, for pheomelanin to be active in inducing such oxidative stress in natural systems, it must be present on or near the surface of the melanosomes. If the pheomelanin is present in the core of melanosome, then it is highly unlikely that photoionization will lead to electrons that can escape the organelle. This becomes even more improbable upon consideration of the excellent scavenging properties of eumelanin. Thus, it is now important to determine whether the morphology of mixed melanins is such that pheomelanin lies at the core surrounded by a eumelanin coat or if the pigments are mixed throughout the volume occupied by the pigment granules. A "casing" model (pheomelanin encased by eumelanin, Figure 4) was originally proposed in 1982 by Rorsman based on biochemical evidence[37] and has since received indirect support by several other studies,[38–40] in addition to the recent kinetic analyses by Ito discussed above.[20] Because different photoionization thresholds characterize the two pigments, PEEM imaging of the melanosome surface can uniquely determine whether or not both pigments comprise its surface. Melanosomes isolated from the stroma of human irides are an optimal system for such a study. First, the size and general morphology of the melanosomes are constant for different colored irides.[41] Second, different colored irides contain melanosomes of varying eumelanin/pheomelanin ratios; in particular, this ratio is 14.8 and 1.3 (over an order of magnitude change) for melanosomes isolated from dark brown and blue-green irides, respectively.[42] Thus, a complete study could be achieved using a single type of human melanosomes. Wavelength-dependent PEEM imaging of these melanosomes establish that only eumelanin is present on or near their surfaces.[41] These data provide direct evidence for a casing model. This model is not unique to the iris melanosomes; neuromelanin granules isolated from various regions of the human brain also have such a structure.[43] The finding that pheomelanin is encapsulated by an excellent electron scavenger (eumelanin) in natural systems would suggest that the structure of the melanosome mitigates the adverse photochemical properties of pheomelanin. Because the intact melanosome is composed of ~30 nm diameter pigment granules, changes in the relative amounts of eumelanin and pheomelanin would then be manifested by changes in the diameter of the core and the thickness of the outer eumelanin coat. Damage to this coating and or significant reduction in the amount of eumelanin present could compromise the protective ability of eumelanin, providing mechanisms for the exposure of pheomelanin and consequently contributing to oxidative stress.

A recent study on the eumelanin and pheomelanin content in uveal melanoma cells finds that melanoma cells have a very low eumelanin content and eumelanin/pheomelanin ratio, in fact, significantly lower than that from normal melanocytes.[44] These differences likely render melanoma cells more susceptible to mutagenic effects of UV radiation and oxidative stress and may enhance their proliferation thereby accelerating the progression of melanoma. Epidemiological studies indicate that the incidence of cutaneous melanoma in individuals with light colored skin is greater than that from individuals with dark colored skin. The relative risk of white/black varies from 12.6−17.1 in different reports.[45,46] Studies of eumelanin and pheomelanin content of epidermal melanocytes from different donors indicate that the eumelanin/pheomelanin ratio correlates with the color of the skin and the ethnic background of the donors. Melanocytes from dark-colored skin and African-American donors have a greater amount of eumelanin and a high ratio of eumelanin/pheomelanin as compared with lighter-colored skin and Caucasian.[47] All these results argue in support of the conclusion that increased pheomelanin content leads to an increased risk factor for cancer. If we are to attribute the origin of this risk factor to photoinduced oxidative stress by pheomelanin, then the pigment would need to become exposed on the surface. One mechanism by which this could occur is through oxidative damage to the melanosome over time, exposing or releasing pigment. Because the outer eumelanin layer becomes thinner with increasing pheomelanin content, such a model of "damage-induced" exposure of pheomelanin is consistent with the epidemiological observations. But other properties of the melanosomes may also change significantly with composition and correlate with the epidemiological data. We now specifically address this question through measurement of the UV absorption properties of intact melanosomes of varying pigment composition.

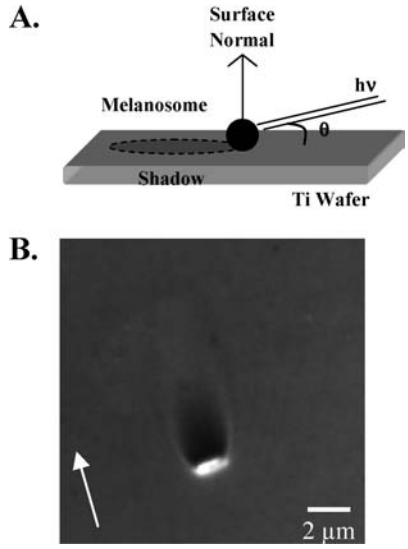

FIGURE 5. (A) Experimental geometry for photoemission electron microscopy of single melanosomes. (B) Photoemission electron microscopy image of a newborn bovine RPE melanosome. The white arrow indicates the direction of the incident light. The image reveals the shadow created by the absorption and diffraction of the incident light by the melanosome. Reproduced with permission from ref 49. Copyright 1985 American Chemical Society.

The Torments of Weakness

"It is now generally agreed that solar exposure is a major external factor in the causation of cutaneous melanoma in light skinned populations with red hair and a marked susceptibility to the acute effects of ultraviolet (UV) radiation."[31]

We recently demonstrated that PEEM could be used to directly quantify the absorption coefficient of single, intact melanosomes.[48] The approach is shown schematically in Figure 5A. Light enters the microscope at a small, but defined, angle of incidence relative to the plane of the surface. Experiments to date have been done with an intracavity frequency-doubled continuous wave argon ion laser, producing light at 244 nm (5.07 eV). Silicon coated with a thin film of Ti is chosen as the substrate because the work function of the film (4.33 eV) is lower in energy than the incident light. As a result, both the substrate and the melanosomes emit electrons and are imaged by the microscope. The melanosomes have a higher efficiency for ionization at this wavelength and are easily distinguished from the background emission of the substrate. Absorption of the incident light by the melanosome is manifested by a shadow on the surface, Figure 5B. Thus, similar to an absorption spectrometer, regions of the substrate correspond to I_o when the incident light arrives unobstructed by melanosomes, while the shadow regions correspond to I. Beer's Law ($A = \log_{10}(I_o/I) = \varepsilon cl$), then enables direct determination of the absorption coefficient, εc, for the selected melanosome.

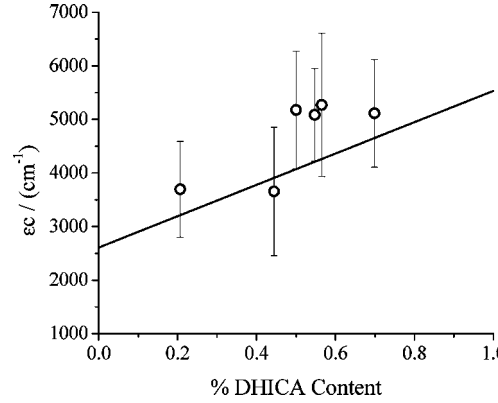

FIGURE 6. Absorption coefficients for eumelanosomes isolated from different biological sources are plotted as a function of the mole fraction of DHICA present in the pigment. The line represents the predicted absorption arising from the sum of the absorptions of contributing DHI and DHICA monomers. Reproduced with persmission from ref 55. Copyright 2010 American Chemical Society.

In the case of eumelanin, the molecular chromophores are generated from the monomeric dihydroxyindole building blocks DHI and DHICA. The literature values for the absorption cross sections of DHI and DHICA vary depending on the solvent or pH;[49−54] an average of the literature values at $\lambda =$ 244 nm gives 2610 and 5530 M^{-1} cm^{-1}, respectively. These results suggest that the absorption of the melanosome will vary with the relative content of DHI and DHICA. The PEEM technique was used to investigate the dependence of the absorption coefficient on the relative DHICA/DHI content and the results are shown in Figure 6.[55] The data reveal that the absorption coefficient varies with the DHICA/DHI ratio determined from the chemical degradation analyses; with increasing DHICA content, the absorption coefficient of the melanosome increases. The line shown in the plot represents the predicted absorption coefficients as determined from a linear combination of the average absorption cross-section values of the two monomers. The observed absorption coefficients of the melanosomes are consistent with the predicted trend, suggesting that the absorption properties of the constituent pigment can be quantified in terms of the concentrations of the contributing monomers and their respective absorption cross sections.

For melanosomes containing varying amounts of eumelanin and pheomelanin, one needs to consider that changes in both the DHICA/DHI and eumelanin/pheomelanin ratios can contribute to measured differences in absorption between samples. Wielgus and Sarna address the DHICA/DHI ratio of colored human irides; by chemical degradation analyses, they find a constant DHICA:DHI ratio for melanosomes isolated from different colored irides.[56] Thus changes in the observed

absorption coefficients of melanosomes isolated from different colored human irides would reflect how the relative contributions of the two different pigments, pheomelanin and eumelanin, affect the absorption properties of the intact organelle.

Comparing melanosomes from dark-brown and blue-green irides, a 25% *decrease* in the absorption coefficient at 244 nm is observed for a 7-fold *increase* in the relative concentration of pheomelanin.[57] At first glance, one might assume that this means pheomelanin has a smaller absorption cross section than eumelanin. Such a conclusion would be in stark contrast to studies on synthetic pigments, where comparable absorption coefficients are observed. We note that while the relative proportions of the two pigments are changing for the melanosomes from different colored irides, the overall dimensions, or volume, of the melanosomes remains constant. This raises the question as to whether the number of chromophores changes as a function of the pigment ratio, which raises the question as to the relative volume of the chromophore units associated with eumelanin and pheomelanin. While the exact molecular structure of the chromophores are not known, we do know that the UV chromophore in pheomelanin is built from molecules such as dihydro-1,4-benzothiazine-3-carboxylic acid (DHBTCA) and 6-(2-amino-2-carboxyethyl)-4-hydroxy-benzothiazole (BZ). Ito and co-workers characterized the absorption properties of the pheomelanin precursors DHBTCA and BZ and found that these molecules have absorption cross sections of 8570 and 7250 M^{-1} cm^{-1} in a 0.1 M HCl solution.[58]

We now consider the molecular volumes of these pigment building blocks, DHI, DHICA, BZ and DHBTCA have volumes of 0.129, 0.172, 0.195, and 0.268 nm^3, respectively (http://www.molinspiration.com/services/volume.html). The chemical degradation analyses used to determine the eumelanin and pheomelanin content is a measure of the number of these monomer building blocks present in the intact pigment, and for a constant total volume, an increase in the number of DHBTCA units must then result in a loss of a larger number of DHI/DHICA units because DHBTCA occupies a larger volume than a DHI/DHICA molecule. Therefore, with increasing pheomelanin content, the total number of monomeric units (eumelanin and pheomelanin combined) must decrease.

Combining information about the volume of the melanosome, the relative amounts of eumelanin and pheomelanin, and the volumes for the molecular building blocks, we estimate a 32% decrease in the number of chromophores in blue-green iridal stroma melanosomes compared to dark-brown iridal melanosomes. This is consistent with the measured absorption decrease (25%). We therefore suggest that the decreased absorption of the melanosome results from a decrease in the number of absorbing chromophores present as the relative content of pheomelanin increases, not that pheomelanin has a reduced UV absorption compared to the black pigment. Further analysis of these data suggests that pheomelanin absorbs slightly more at 244 nm than eumelanin (by a factor of 1.2), consistent with reports[59] of the absorption spectra for synthetic model pigments.

We recall that the relative amounts of pheomelanin and eumelanin correlate with the epidemiology of skin and ocular cancers; specifically, increasing relative proportions of pheomelanin correlates with an increased risk factor for these diseases. These epidemiological observations spurred a significant effort aimed at determining whether pheomelanin plays a causative role in these cancers. But these results on the UV-absorption properties of the melanosome offer a different perspective. Instead of focusing on an increased photoreactivity, oxidative stress, associated with increasing pheomelanin content, the absorption data indicates the photoprotective role of the melanosomes is compromised with increased pheomelanin content. The correlation between epidemiological data and the eumelanin/pheomelanin ratio may then simply reflect an increased exposure of the underlying tissues to UV light accompanying the increasing pheomelanin content in the melanosome.

Intrigue

"Melanosomes provide a unique and rich environment for research, with many significant and diverse implications for human health."[60]

There remain many unanswered questions about melanin and melanosomes and opportunities for the development of new approaches to advance our understanding of structure and function. There will clearly continue to be research efforts aimed at elucidating the molecular structure of different melanins found in nature, and that work promises to build the foundation upon which the functional role of the pigment in different tissues and species can be understood. Below, we briefly pose three questions that represent challenging research areas that expand on the concepts presented in this article.

While experimental evidence validates a casing model for mixed melanin systems, are the two pigments present in their pure form, or do they copolymerize? No study to date addresses this very fundamental question as to the nature of mixed melanins in nature, and it is not clear how to experimentally approach the issue.

Why is there such a great diversity of DHI/DHICA ratios in melanosomes that only contain eumelanin? This ratio affects the UV-absorptivity of the melanosomes but should also influence other functions such as metal-binding capacity. No systematic studies have been reported.

Is there a defined molecular morphology at the surface of the melanosome? Many properties of the melanosome begin with the interaction of its surface with the surrounding environment, yet whether the molecules on the surface are randomly arranged (as is commonly invoked in a polymer-based model for the pigment) or are organized and assembled in a specific manner to facilitate a variety of functions remains unaddressed. Once again, no study to date addresses this question, and it is not clear how to experimentally approach the issue.

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BIOGRAPHICAL INFORMATION

John D. Simon received a BA from Williams College and a Ph. D. from Harvard University. After a postdoctoral fellowship with Professor M. A. El-Sayed at UCLA, he joined the faculty at UCSD. In 1998, he moved to Duke as the George B. Geller Professor of Chemistry. He served as department chair from 1999−2004 and is currently Vice Provost for Academic Affairs. In addition to pigments, his group studies the binding of reactive metals to proteins and peptides associated with neurodegenerative diseases and the association of proteins with nanoparticles.

Dana Peles received her B.S. degree in Chemistry and Mathematics from Randolph-Macon College. She is currently a Ph.D. candidate at Duke University. Her research is focused on the applications of photoemission electron microscopy to pigments and nanomaterials.

FOOTNOTES

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