

Optical Absorption Spectra of Melanins - a Comparison of Theoretical and Experimental Results

Researchers at Innovene, the University of San Antonio, and the University of California, Irvine, and Accelrys have used MS Modeling's DMol³, VAMP, and Discover codes to study the effect of aggregation and oxidation on the optical absorption of eumelanin oligomeric sheets. The oligomeric hypothesis is supported by Atomic Force Microscopy (AFM) characterizations of synthetic eumelanins, formed by auto-oxidation or electrochemical oxidation of dihydroxyindole (DHI). Comparison of calculated absorption spectra to experimental spectra demonstrates a red shift in absorption with oxidation and stacking of the eumelanin, and validates the theoretical results. The work will lead to the development of better sun screen-based products, aiding the protection against, and the prevention of, skin cancer.

Melanin is the biopolymer that accounts for much of the coloration in nature.^{1,2} In humans, it is responsible for the color of the hair, skin, and eyes. Yet the optical absorption spectra corresponding to these colors have a common feature: a broadband, structure-less curve that is a monotonically increasing function of energy.³

The shape of the absorption spectrum of melanin also appears to relate to skin cancer in a particular way.⁴ People with red hair have a greater risk of skin cancer from exposure to sunlight. Thus the shape of the melanin absorption spectrum may be efficient with regard to protection against major diseases in humans.

Theoretical work on melanin and its possible proto-molecules is relatively sparse due to the size of the molecules involved and to the unknown nature of the overall melanin structure. In previous work, the researchers performed Density Functional Theory (DFT) calculations on the monomer models.⁵ From the optimized structures, absorption spectra were calculated. The results agreed well with earlier theoretical work⁶ as well with published experimental work.⁷

Industry Sectors

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DMol³
VAMP
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Then optical spectra of melanin using a particular structural model that assumes a poly-quinone layer were calculated.⁸ From this work it was proposed that the smooth optical absorption for melanin is obtained by superposition of all the constituent absorptions.

The structural models used in this previous work were limited to single sheet oligomers. In this work,⁹ the simulations include aggregates of the oligomeric sheets as well as changes in their oxidation states. These are important considerations for several reasons. A variety of experimental probes point to extensive stacking of oligomers in eumelanin.¹⁰

Changes in subunit oxidation state are relevant, as the darkening and bleaching of melanin has been correlated to oxidation/reduction reactivity, and is believed to be essential in its natural physiological functions.¹¹ It is therefore of great interest to evaluate the model for melanin by simulating its optical properties for oligomeric sheets in various oxidation states and to compare such spectra with the experimental data.

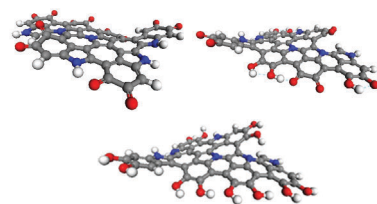
Computational Results

In previous work, an oligomeric model of eumelanin was developed using the fully oxidized form of melanin.⁸ Here, this chemical model is extended by including half-reduced and fully-reduced proto-molecules as well as the oxidized form. Shown in Fig. 1 are the resulting energy-optimized hexamers for the three oxidation states.

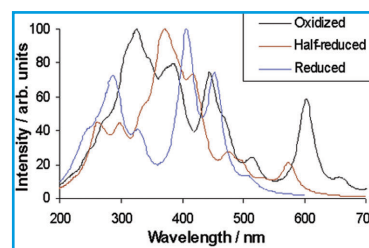
Calculated absorption spectra for the single sheet structures are shown in Fig. 2. The absorption maximum of the reduced form is red-shifted as compared to the oxidized form. The predicted absorption in the red part of the spectrum is also lower in the reduced form. The absorption spectrum of the half-reduced form exhibits intermediate behavior, with absorbance bands over a broad wavelength range.

The large number of possible hydrogen-bonding interactions precluded simple energy minimization of layered structures in the reduced and half-reduced forms; therefore, the molecular dynamics quenching technique was applied to generate a manifold of low-energy structures for the stacked oligomers. Only intra-sheet hydrogen bonds are expected for the oxidized structure, and consequently the quenching procedure led to well defined low-energy structures in which a stacked structure is built with the individual sheets somewhat shifted against each other. Optical spectra were calculated for the oxidized hexamer stacked in 1 through 3 sheets, the results being displayed in Fig. 3.

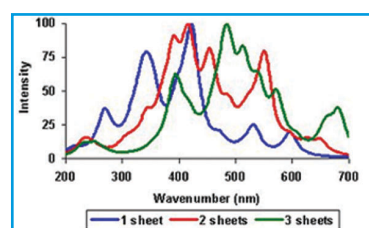
Overall the spectra become more red-shifted and smooth with increasing number of sheets.



▲ Figure 1 DFT optimized hexameric DHI melanin structures. (a) Oxidized form, (b) half-reduced form, (c) fully reduced form. Carbon atoms are displayed in gray, oxygen atoms in red, nitrogen atoms in blue, and hydrogen atoms in white.



▲ Figure 2 Calculated absorption spectra for single sheet hexamers of the oxidized, half-reduced and fully reduced melanin proto-molecules.



▲ Figure 3 Calculated absorption spectra for hexamers of the oxidized form. Displayed are the spectra of the non-stacked form (1 sheet) and stacked forms with 2 and 3 sheets.

Experimental Results

Melanin samples from DHI were prepared by both auto-oxidation and electrochemical deposition. AFM image and the particle size distribution of sub-monolayer eumelanin deposited by electrochemical oxidation of DHI were recorded. Analysis gives the average particle height as 27.93 Å. By comparison, AFM imaging of colloidal melanin prepared from DHI auto-oxidation identified particles ranging from 7 to 22 Å in height, with few larger particles up to 35 Å seen (Fig. 4).

The absorbance spectra obtained by electrolysis of a thick DHI melanin film at various potentials is shown in Fig. 5. As the potential of the film is altered from its fully reduced state at -300 mV, the absorbance increases above 400 nm with increasing potential. Between -300 and 50 mV, an isobestic point at ca. 417 nm is maintained, which denotes a simple interconversion of stable species. Under anaerobic conditions, this absorbance change is reversible and can be repeated several times. At potentials in the range from +50 to +300 mV, the characteristic increase at 500 nm continues, but the reversibility is lost, indicating a change in the chemical composition.

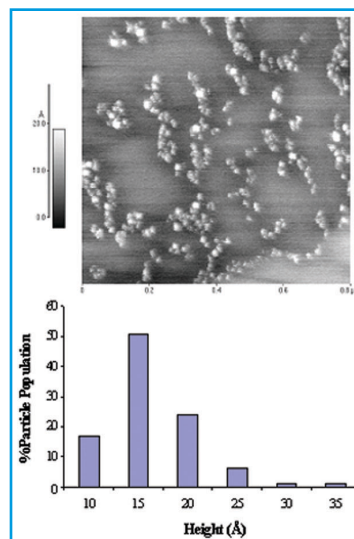
Discussion

AFM images of DHI melanin all show nanoscale particles; the colloidal samples have average particle heights of 13.8 Å.¹ The average height of electrochemically deposited particles is 27.9 Å which corresponds to 6-7 stacked sheets, which roughly agrees with the ~ 2 nanometer sized molecular structures calculated in this study. Formation of bigger particles by electrochemical deposition in comparison with auto-oxidation is probably due to difference in polymerization conditions.

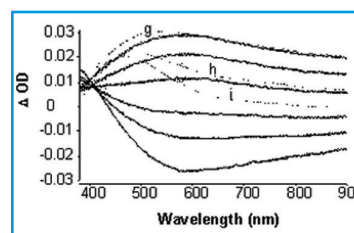
For the non-stacked oligomers (Fig. 2) the absorption in the UV part of the spectrum of the reduced form is red-shifted compared to the oxidized form accompanied by a reduction of the absorption in the infrared part of the spectrum. Absorbance spectrum of the non-stacked half-reduced form exhibits intermediate behavior and has peaks over the entire wavelength range.

Stacking increases the absorption in the red-end part of the spectrum and smoothes the absorption spectrum (Fig. 3). For the half-reduced form the stacked model exhibits less absorption in the UV spectral range accompanied by an increase of absorption on the red end of the spectrum. For the fully reduced form stacking narrows the spectral range in which the reduced form absorbs to a region of 300-500 nm.

The effect of oxidation on the absorbance of DHI-melanin was demonstrated by spectroelectrochemical measurements, which allows fine control over the redox state of the sample.



▲ Figure 4 AFM images of auto-oxidized DHI melanin deposited on HOPG from isopropanol/water solution. Bottom: histogram of % population vs. height for auto-oxidized DHI melanin.



▲ Figure 5 Changes in the optical absorption spectra of a thick DHI-melanin film during electrochemical oxidation from -300 mV; spectra taken at a) -300 mV b) -100 mV; c) -50; d) 0; e) 50; f) 100; g) 200; h) 300; and i) 400 mV.

The calculated optical absorption spectra generally indicate a trend of a stronger absorption in the red end of the spectrum with higher oxidation state of the melanin. So the calculations agree in general terms with the experimental observations. However, this does not prove that this particular molecular structure as utilized for these calculations is unique and that other structures would not likewise red shift with higher oxidation state. But it is important to note that the spectral region affected by the electrochemical oxidation of melanin is very similar to those theoretically calculated for these structures (300-700 nm). We take this to indicate that the molecular structures most likely involved are of similar size and degree of conjugation to those considered in this study.

The overall effect of stacking is similar to that of oxidation, red shifting the absorbance spectra of the hexameric eumelanin model systems. This may explain the broad absorbance of natural melanins that extends to the near IR; the lack of such stacking might explain the spectra of pheomelanin, such as is found in the skin of people with red or blonde hair. Pheomelanin contains a high proportion of cysteine incorporated during the oxidative polymerization of L-dopa, and X-ray scattering does not indicate the average distance between sheets of 3.4 Å as in eumelanin. Likewise, pheomelanin's absorbance drops off dramatically past 500 nm, and more closely resembles that of highly oxidized DHI melanins.

Such de-stacking effects are likely the cause of the significant bleaching at high wavelengths seen in Fig. 5. Within a specific range, the oxidation of DHI melanin is chemically reversible, but beyond 100 mV, the spectral changes are irreversible indicating oxidative degradation. Ring-opening decomposition of a DHI oligomer would decrease its effective conjugation and likewise decrease its propensity to stack due to increased sterics and solubility.

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