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Published on Web 10/06/2009

Disentangling Eumelanin “Black Chromophore”: Visible Absorption Changes As Signatures of Oxidation State- and Aggregation-Dependent Dynamic Interactions in a Model Water-Soluble 5,6-Dihydroxyindole Polymer

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Abstract: A fundamental unsettled issue concerning eumelanins, the functional biopolymers of human skin and hair, is why they are black. The experimental difficulty lies in the virtual insolubility of these pigments, causing marked scattering effects and hindering characterization of the intrinsic absorption properties of the heterogeneous species produced by oxidative polymerization of 5,6-dihydroxyindole (DHI) and related monomer precursors. The synthesis of spectrally robust, water-soluble DHI polymers is therefore an important goal in the prospects of disentangling intrinsic absorption properties of eumelanin components by circumventing scattering effects. Reported herein is the first water-soluble DHI polymer produced by oxidation of ad hoc designed 5,6-dihydroxy-3-indolyl-1-thio-β-D-galactopyranoside (**1**). The dark brown polymer exhibited a distinct band at 314 nm and a broad visible absorption, resembling that of natural eumelanins. Main isolable oligomer intermediates including 2,7′- and 2,4′-biindolyls **2** and **3**, attest the close resemblance to the mode of coupling of the parent DHI. Sodium borohydride reduction caused decoloration and a marked absorbance decrease in the visible region around 550 nm, but did not affect the UV band at 314 nm. Measurements of absorbance variations with dilution indicated a linear response at 314 nm, but a significant deviation from linearity in the visible region, with the largest decrease around 500 nm. It is argued that eumelanin black color is not only intrinsically defined by the overlap of π-electron conjugated chromophores within the individual polymer components, as commonly believed, but also by oxidation state- and aggregation-dependent interchromophoric interactions causing perturbations of the heterogeneous ensemble of π-electron systems and overall spectral broadening.

Introduction

Although black is a common property in the inorganic world, among living organisms it seems to be associated mainly with eumelanins, the peculiar class of biopolymers of human skin, hair, eyes, and brain.[1−4] Eumelanins can rightly be regarded among the most enigmatic pigments found in Nature because of their high molecular heterogeneity, amorphous and particulate character, and complete insolubility in all solvents, which have so far hindered detailed structural elucidation.[5,6] Current models point to a hierarchical architecture of eumelanin particles[7−9] consisting of π-stacked layered molecular systems building on oligomeric motifs derived from oxidation of two key precursors, 5,6-dihydroxyindole (DHI) and its 2-carboxylic acid (DHICA). These are highly oxidizable catechol-containing indole derivatives which are biosynthesized in epidermal pigment cells, the melanocytes, by the tyrosinase-catalyzed oxidation of tyrosine.[10] Model chemical studies have shown that DHI undergoes oxidative coupling mainly via 2,4′- and 2,7′-bondings to give dimers and complex mixtures of oligomers thorough different mechanisms.[11−15] How these processes and ensuing supramolecular interactions participate in eumelanin buildup remains obscure.

An unresolved and related topic to this issue concerns the black color of eumelanins, which is atypical of organic

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chromophores and is commonly associated to a broadband monotonic absorption throughout the entire UV/visible range.[6] Due to large amounts of proteins and other impurities present in natural pigments, studies of the optical properties of eumelanins have usually been carried out on commercially available synthetic pigments made from tyrosine and partially solubilized at pH 11.5.[16−18] The lack of information about the fundamental structural units of such synthetic pigments and the harsh alkaline conditions favoring extensive structural degradation might, however, affect interpretation of the experimental data and their relevance to natural eumelanins.

Although in the aggregated form both scattering and pure molecular absorption effects have been suggested to cause the black appearance of eumelanins,[19] a substantial body of evidence suggests that the monotonically decreasing spectrum is in fact due to “real absorption”, possibly by a multichromophoric heterogeneous ensemble of species of different chemical structures.[20]

In this context, the crucial gap concerns the absorption properties of the oligomer constituents, and how they vary with molecular size and redox state. While it is commonly agreed that the oxidized forms of DHI oligomers have red-shifted HOMO−LUMO gaps, no definite evidence for the existence of stable visible light-absorbing chromophores derived from DHI is so far available, apart from pulse radiolysis and computational investigations of labile transient species.[12] In fact, no stable colored DHI-based chromophore has been so far isolated or created, an issue of the utmost relevance to the key question of why eumelanin is black. Further progress in this area depends therefore on the availability of reliable, structurally defined, and water-soluble model polymers, which would not only yield useful information as to one of the supposed crucial functions of eumelanins, namely photoprotection, but may also inspire solid state physicists and materials scientists pursuing soft biocompatible functional materials.[21,22] The information gained in aqueous media may moreover effectively integrate recent data from organic-soluble eumelanin-like pigments from DOPA[23] or DHICA benzyl and octyl esters.[24]

Herein, we report the first water-soluble eumelanin-type polymer from DHI as a unique investigative tool to inquire into the origin of eumelanin black color and optical properties.

Materials and Methods

5,6-Diacetoxyindol-3-yl-1-thio-*β*-D-galactopyranoside (1-Ac). Synthesis of the thiogalactosyl donor *S*-GalDonor is reported in detail in SI. A mixture of *S*-GalDonor (1.31 g, 2.5 mmol), 5,6-diacetoxyindole (0.587 g, 2.5 mmol), and *N*-bromosuccinimide (448 mg, 2.7 mmol) was dissolved in acetonitrile (60 mL) under an argon atmosphere. The mixture was kept under stirring for 4 h at 60 °C, cooled to rt, and diluted with dichloromethane. The mixture was washed with water, and the aqueous phase re-extracted with dichloromethane. The collected organic phases were dried over anhydrous sodium sulfate and concentrated *in vacuo*. The residue was purified by silica gel flash-chromatography (eluant: petroleum ether/ethyl acetate mixtures) to yield pure **1** acetyl derivative (1.5 g, 49% yield) High resolution ESI+MS found 618.1264 $[M + Na]^+$, calcd for $C_{26}H_{29}NO_{13}SNa$ 618.1257. Spectral data are listed in Table S1, Supporting Information

Isolation of Dimers 2 and 3 (As Acetylated Derivatives). A solution of **1-Ac** (60 mg, 0.10 mmol) in MeOH (2 mL) was treated with sodium *t*-butoxide under a nitrogen atmosphere for 1 min to obtain complete deacetylation (MS evidence) and then diluted with 0.1 M phosphate buffer (pH 7.0, 10 mL) and treated with HRP (77 U/mL) and H_2O_2 (36 μL of a 30% solution, 0.35 mmol). After 15 s reaction time, the oxidation was halted by addition of sodium dithionite in water up to a 50 mM final concentration and lyophilized. The powder was acetylated and then extracted with 10% ammonium chloride. The ethyl acetate extractable fraction was fractionated by thin layer chromatography ($CHCl_3$ −AcOEt 65/35) to afford **2-Ac** (R_f =0.4, 5 mg, 10% yield, High resolution ESI(+)-MS found 1211.2468 $[M+Na]^+$, calcd for $C_{52}H_{56}N_2O_{26}S_2$ Na 1211.2460) and **3-Ac** (R_f 0.3, 4 mg, 8% yield, high resolution ESI(+)-MS found 1211.2455 $[M+Na]^+$, calcd for $C_{52}H_{56}N_2O_{26}S_2$ Na 1211.2460) and. Spectral data are reported in Tables S2 and S3, Supporting Information.

Spectrophotometric Studies. Oxidation of **1** in 0.1 M phosphate buffer pH 7.0 was carried out with the substrate at 12.5 mM using HRP (25 U/mL) and H_2O_2 (12.5 mM), and after 20 min the solution was diluted 25−100 fold with 0.1 M phosphate buffer pH 7.0; the spectrum was recorded in a thermostatted cell at 298 K with or without filtration through a membrane with 0.47 μm pores. When necessary, the mixture was treated with a solution of $NaBH_4$ in water (20 mM). DHI was also used at 12.5 mM, and after 20 min spectra were taken on the solution after separation of the pigment.

Results

Synthesis of Glycated 5,6-Dihydroxyindole Monomer. Glycosylation processes have been selected by Nature as a highly effective means of solubilizing organic compounds in water to allow for their excretion. Capitalizing on a recently reported procedure for installing sulfur-containing functionalities onto a 5,6-dihydroxyindole ring,[25] we have thus focused on the synthesis of 5,6-dihydroxy-3-indolyl-1-thio-*β*-D-galactopyranoside (**1**) as a candidate model system for preparation of a water-soluble eumelanin (Figure 1).

Thioglycosidation proved a viable route to indole functionalization, owing to the chemical versatility of the sulfur derivatives which can be subjected to a variety of transformations, thus opening several pathways for further postpolymerization manipulations. Additionally, thioglycosidation at the 3-position, which is negligibly involved in the polymerization process, was expected to cause lesser perturbations of the monomer, oligomer, and polymer chromophores.

Because of the marked facility of the 5,6-dihydroxyindole system to oxidation, synthetic strategies were targeted to the acetylated derivative, 5,6-diacetoxyindole. The choice of acetyl was favored over other catechol protecting groups because these groups can be conveniently removed in situ just prior to oxidative polymerization experiments.[12−14]

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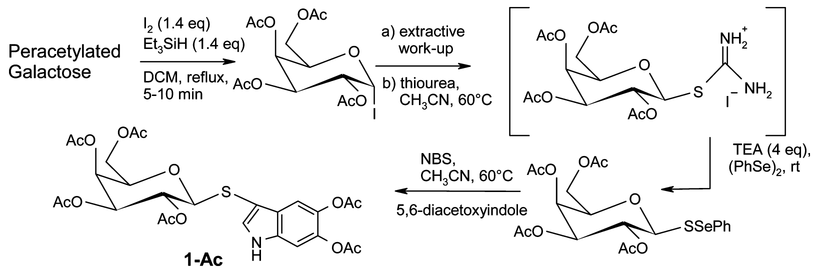


Figure 1. Synthetic strategy for preparation of **1** as the acetyl derivative.

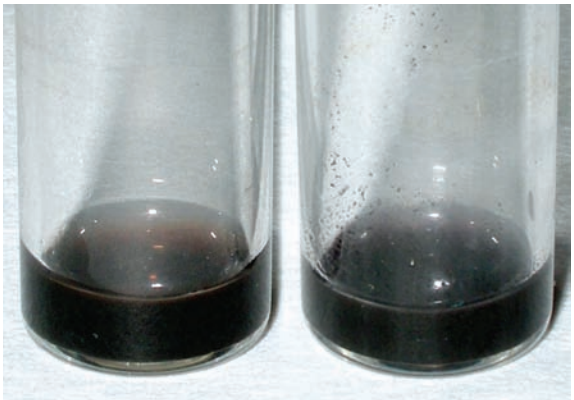
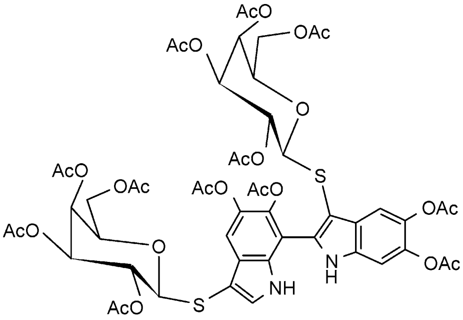


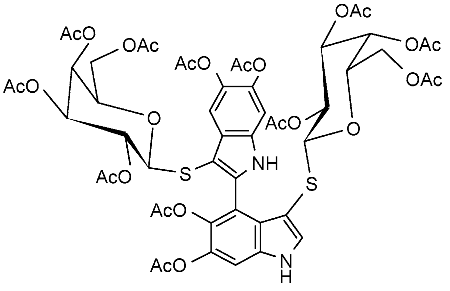
Figure 2. Eumelanin-like pigments produced by oxidation of **1** (left) and DHI (right).

Unfortunately, several attempts to achieve thioglycosidation of 5,6-diacetoxyindole at the 3-position by using a suitable soft electrophilic agent proved unfruitful. An expedient solution to this problem came, however, when the opportunity to generate soft thioglycosidation agents by synthesizing selenophenyl thioglycosides was considered.[26] These derivatives could be prepared by a straightforward sequence of three fast reactions requiring a single chromatographic purification. Whereas direct coupling of 5,6-diacetoxyindole with selenophenyl glycoside was unproductive, activation with stoichiometric amounts of *N*-bromosuccinimide (NBS) eventually resulted in the neat generation of the desired conjugate **1-Ac** in very satisfactory yields (Figure 1).

Preparation and Chemical Characterization of the Model Soluble Polymer. Treating the acetylated derivative of **1** with methanolic sodium *t*-butoxide allowed for acetate removal and the safe generation of the desired **1**. This latter was subjected to oxidation with the horseradish peroxidase (HRP)/H_2O_2 system, the most efficient oxidizing agent for 5,6-dihydroxyindole polymerization.[14] When visually observed, the reaction proceeded with the rapid development of a dark-brown coloration, whose intensity depended on substrate concentration. Notably, a completely soluble pigment was obtained from as much as 12.5 mM **1** (Figure 2), where the well-known formation of a precipitate is observed when the same treatment is conducted on DHI.



2-Ac



3-Ac

Figure 3. Structures of the biindolyl intermediates isolated from the oxidation of **1** after reduction and acetylation steps.

Dynamic light-scattering measurements showed a profile typical of a homogeneous solution devoid of scattering effects. This was deduced from the absence of the typical oscillating light intensity profile which appears when scattering units have non-Brownian motion. Elaboration of data indicated a hydrodynamic radius distribution centered at 100 nm suggestive of a monodisperse high-molecular weight polymer (See Supporting Information (SI)).

Direct mass spectrometric analysis on the soluble polymer was little informative under a variety of conditions. However, investigation of the mixture after reduction and acetylation (see SI) allowed clear identification of oligomers up to six units, thus ruling out significant side processes (e.g., oxidative breakdown, loss of glycosyl residues, etc.) during oxidative polymerization.

To gain insight into the mode of coupling of **1** during polymer buildup, which is important to match the basic structural features of the soluble and insoluble DHI-derived polymers and to validate the soluble eumelanin model, a milligram-scale reaction was stopped in the early stages by the addition of sodium dithionite to reduce oxidized species, and the crude oligomer-containing mixture was acetylated according to an established protocol.[14] Thin layer chromatography of the mixture thus obtained allowed the isolation of two main oligomer intermediates, which were identified as the acetylated 2,7′- and 2,4′-biindolyls **2** and **3** (Figure 3).

The similarity of the isolated dimers with the main species produced by oxidative coupling of DHI[11] demonstrated that the sulfur substituent on the 3-position of **1** did not affect the oxidation behavior and the mode of coupling of the 5,6-

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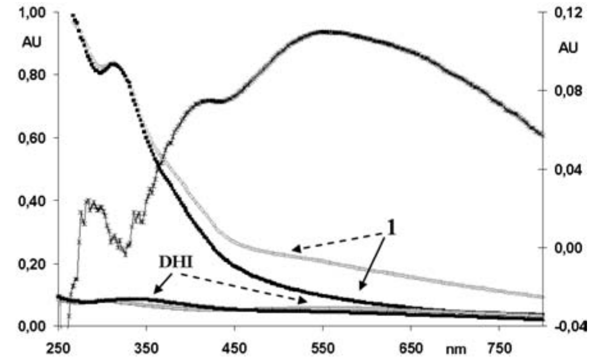

Figure 4. Absorption spectra of the polymers produced by oxidation of 12.5 mM **1** or DHI (20 min reaction time), prior to (gray lines) and after (black lines) reductive treatment with $NaBH_4$. Shown is also the difference spectrum (−×−) of the untreated and treated polymer from **1** (right axis).

dihydroxyindole system in the soluble polymer. This suggested that the oligomeric soluble pigment constituents displayed interunit bonding patterns that are similar to those involved in eumelanin buildup, thus supporting the validity of the new water-soluble model system for studies of eumelanin properties.

Absorption Properties of the Soluble Polymer. Figure 4 shows the UV/visible spectrum of the dark solution obtained by oxidation of 12.5 mM **1** with HRP/H_2O_2 in phosphate buffer, pH 7.4. The spectrum was taken 20 min after the oxidant addition, when no further spectrophotometric change was apparent even after additional H_2O_2 treatment, indicating that the melanization process was complete. Moreover, the absorption profile of oxidized mixtures from **1** did not show appreciable changes with time over at least 24 h.

Data show that after oxidation was complete the chromophore of **1** (λ_{max} at 290 nm) was replaced by a broadband spectrum with a well detectable maximum around 314 nm and an almost featureless absorption throughout the visible region (380−780 nm) approaching the baseline at the low-energy end of the spectrum. This absorption profile is not dissimilar from those of the insoluble DHI polymers and of intact natural eumelanin samples from Sepia[27] and hair,[19] in which however significant scattering contributions are present. Given the complete lack of scattering effects, the spectrum reported herein demonstrates for the first time the generation of stable, visible-light-absorbing oligomeric/polymeric chromophores by oxidation of a DHI derivative in aqueous medium.

Careful reductive treatment with sodium borohydride caused visual color discharge from dark brown to pale yellow. Spectrophotometric analysis consistently indicated a marked absorbance decrease in the visible region but no significant variation of the extinction coefficient at the 314 nm UV peak (Figure 4). For comparative purposes, the spectral changes occurring during the oxidation of DHI at similar concentrations under the same conditions were reported (Figure 4). The resulting black pigment suspension exhibited a relatively poor absorption in the UV and visible regions dominated by scattering contributions. A weak absorption maximum around 314 nm was apparent, resembling the spectrum of the soluble polymer. Modest changes in the absorption intensity in the visible region occurred upon treatment with sodium borohydride.

To assess whether oxidized quinonoid substructures contributed homogeneously to the various regions of the visible spectrum, the extent of the absorbance variations as a function of wavelength was determined. A plot of the absorbance differences measured in the visible region after reductive treatment (Figure 4) against wavelength gave a peak around 550 nm, suggesting a prevalent superposition of oxidized chromophores in that region.

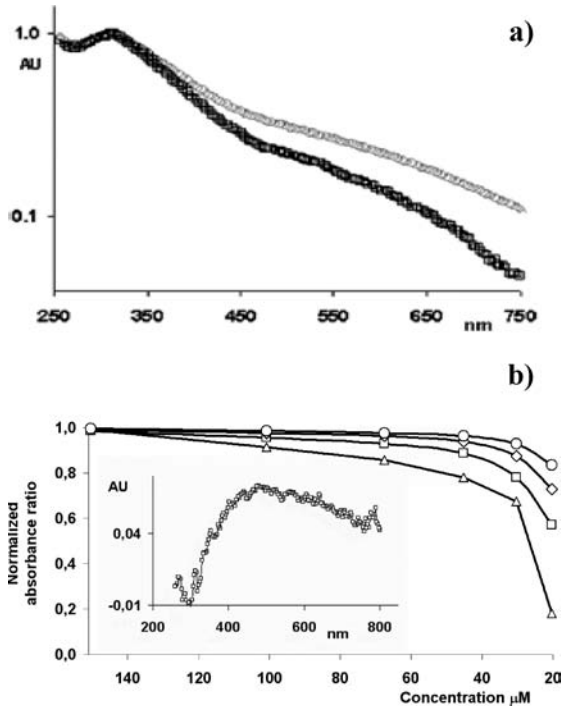

Figure 5. (a) Effect of dilution on the absorption properties of soluble eumelanin. Gray line: 500 μM; black line: 30 μM. Concentrations are based on starting monomer, plots are normalized against the absorbance at 314 nm. AU = (normalized) absorbance units. Note that absorbance is on logarithmic scale for the sake of clarity. (b) Normalized plots of the absorbance ratios A_x/A_{314} at selected wavelengths (○ 384 nm, ◇ 420 nm, □ 550 nm, △ 750 nm) as a function of dilution (for all wavelengths normalization was obtained by dividing all absorbance ratios by the relevant value at 150 μM concentration). (Inset) Plot of A_x/A_{314} ratio differences (slopes) determined at 70 μM and 30 μM concentrations as a function of wavelength. AU = adimensional units.

In further experiments the effects of dilution on the absorption properties of the soluble pigment were investigated. For this purpose, polymer concentrations were assumed as the starting monomer concentration under conditions of complete oxidation. Figure 5a compares the absorption spectra of polymer solutions taken at 500 μM and 30 μM concentrations after proper dilution. The graphs clearly show a decrease of the visible absorption contribution relative to the 314 nm band at the lower concentration. Consistent with this observation, a separate plot of the absorbance values at 314 nm (A_{314}) against dilution over the 500 μM →30 μM concentration range indicated a linear response, whereas a significant deviation from linearity was apparent for absorbance values (A_x) measured in the visible range.

This effect was better appreciated when absorbance intensity ratios A_x/A_{314} at selected wavelengths were plotted as a function of dilution (Figure 5b). Inspection of the curves revealed significant wavelength-dependent changes that are much more pronounced in the visible range. Noteworthy, comparison of the A_x/A_{314} ratio changes after dilution revealed a maximum variation around 500 nm (Figure 5b, inset). These results can be taken to suggest the occurrence of reversible aggregation

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processes in solution, reinforcing specifically the absorption of light above 500 nm.

Discussion

The origin of eumelanin black color has been the subject of a long-lasting debate. While early views[28] favored the presence of low-lying conduction bands and/or charge-transfer interactions resembling those in quinhydrones, subsequent concepts were based on an amorphous semiconductor model[29] or emphasized the importance of scattering effects. These latter comprise both wavelength-dependent Rayleigh scattering, causing the steep rise in the spectrum below 300 nm,[30] and wavelength-independent Mie scattering, superimposing a broad background on the absorption spectrum.[6]

The design and realization of the first water-soluble DHI-based eumelanin-like polymer was aimed to provide a means of obviating the notorious impossibility to solubilize oligomer/polymer eumelanin components without affecting basic structural units, and thus to gain a direct insight into the visible chromophore generated during DHI polymerization. The results reported herein indicate that these expectations have been met.

A first relevant point for discussion concerns the impact of the bulky (and water-soluble) galactosyl moiety and the thioether bridge at the 3-position on the mode and degree of polymerization of the 5,6-dihydroxyindole unit. Although this was in principle a possible limitation of this study, chemical analysis showed in fact that **1** retains the typical mode of polymerization of DHI via 2,4′- and 2,7′-bonds, indicating that the sulfur substituent at C-3 exerts little or no chemical influence on the adjacent reactive 2-position. As an additional remark, although studies of DHI polymerization in vitro have shown the involvement of the 3-position during the oxidative coupling of dimers to tetramers,[13] this is expected to be only one of the many possible modes of chain elongation in the complex process of polymer buildup, so that the use of a 3-substituted monomer would not detract too much from the value of the soluble polymer from **1** as a tool for structural investigations. It is relevant to note, in this connection, that all known trimers from DHI feature only 2,4′- and 2,7′-bondings,[11] suggesting that chain elongation pathways via sequential addition of the monomer to dimers and higher oligomers should not involve the 3-position to any significant extent. Whether **1**-related dimers and oligomers can give rise to soluble dark polymers that can be used as model eumelanins is yet an important goal for future work and requires further synthetic efforts aimed at preparing *S*-thiogalactosyl dimers in sufficient amounts for chemical polymerization studies.

The above arguments would also imply that the marked difference between the polymers from DHI and **1** is specifically due to the sugar group ensuring solubilization of the growing polymer and is not simply the consequence of inhibited reactivity at the 3-position. This is also supported by literature reports showing that 3-alkyl-5,6-dihydroxyindoles behave on oxidation like other 5,6-dihydroxyindoles giving rise to 2-linked oligomers and eventually to dark, insoluble eumelanin-like pigments.[31,32]

It is difficult, based on present data, to draw any conclusion about the average molecular weight of the polymer. Since each molecular entity has apparently a globular shape in solution with a diameter of ~100 nm, and considering that the average dimension of a single indole unit may be approximated to about 1 nm, it can be speculated that the polymer consists of chains of at least 100 units. Indeed, mass spectrometry was not conclusive in this regard, and thus, it is possible that the 100-nm species are in fact molecular aggregates. Verification of this latter point by dilution experiments and dynamic light-scattering measurements proved to be difficult because of the high extinction coefficient of the pigment preventing meaningful scattering determination in a sufficiently broad concentration range. However, the low monomer concentration used for polymer analysis would entail an unusually high aggregation constant for a disordered heterogeneous polymer, whereby formation of 100 nm aggregates seems unlikely.

Several important observations regarding the chromophoric features of the soluble polymer deserve a comment. A first critical point is that even when melanization is complete, soluble DHI oligomers/polymers show a well detectable and stable maximum at 314 nm, resembling that of reduced DHI oligomers.[30,33] This spectral feature can be reasonably attributed to oligomeric structural components in the reduced state, based also on its insensitivity to reductive treatments. On the other hand, the lack of effect of additional oxidant equivalents on the absorption profile provides unambiguous demonstration that reduced (catecholic) and oxidized (quinonoid) substructures coexist in the soluble pigment polymer and that oxidation cannot be forced beyond a certain threshold. This is a most important conclusion that would confute models of eumelanin structure based on hypothetical fully oxidized polyquinonoid architectures.[34]

A second, most relevant observation, stems from the $NaBH_4$ reduction experiments. These demonstrated that the visible light absorption and, hence, the dark color of DHI polymers depend on the presence of oxidized subunits. It was recently shown that no visible chromophore can be generated from reduced (catecholic) oligomers however extended the molecular size may be.[30] The reason lies in the peculiar mode of coupling of the indole units which is often incompatible with linear or rod-shaped oligomeric structures allowing fully extended π-electron delocalization. Thus, significantly red-shifted HOMO−LUMO gaps and visible light absorption can be achieved only by oxidation. This concept was addressed in a number of previous papers[6,34−36] but could never be unambiguously demonstrated in aqueous medium, since oxidized quinonoid oligomers are too elusive to be identified as stable entities in solution. Our data would provide the first direct experimental verification of the existence in solution of stable, visible-light-absorbing chromophores derived from DHI-related units.

The third and perhaps most significant outcome of this study relates to the nonlinear decrease of the visible components of the chromophore with dilution. This points to the occurrence of reversible aggregation phenomena in solution that may

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crucially affect eumelanin absorption features. Charge-transfer or redox processes would be responsible for the marked enhancement and broadening of the chromophores around and above 500 nm with increasing polymer concentration. Aggregation has been reported to affect the optical properties of natural and synthetic eumelanins by extending the spectrum to lower energies.[37] The spectral changes reported in this paper are in agreement with this conclusion and would yield for the first time some information on the basic features of the process in solution under conditions independent of scattering effects.

In light of the new information, it is now possible to address the key question of why eumelanin is black on the basis of the chemical disorder model proposed by Meredith and co-workers.[20,38] This model envisages the broadband monotonic absorption of eumelanin-type polymers as the consequence of the superposition of a large number of inhomogeneously broadened Gaussian transitions associated with each of the components of the eumelanin ensemble. There has been much debate in the past about the effective length of eumelanin oligomer/polymeric chains and hence about the spatial distance over which electronic delocalization may occur. Pulse radiolysis investigation of quinonoid species from DHI-derived biindolyls[12] showed that intense visible chromophores can be generated even within the small boundaries of dimeric scaffolds. Thus, overlapping individual chromophores may in principle account for coverage of the entire UV−visible range even at a low degree of polymerization. In this connection, it has been calculated that as few as 11 individual species are needed to cover the UV, visible, and near-infrared with realistic inhomogeneous line broadening.[6]

On the basis of the results of this paper, the chemical disorder paradigm can now be integrated and elaborated into an improved dynamic model emphasizing the importance of interchromophoric interactions. The basic underpinning of the improved model is that it is the *coexistence* of oxidized and reduced substructures that is essential for the broadband visible-light absorption of eumelanin-like polymers. In particular, our data suggest that in solution eumelanin components can be thought of as chains of extended π-electron systems interrupted at irregular intervals, e.g. by hindered rotation around interunit bonds forcing deviation from coplanarity,[39] to form more or less discrete chromophoric units. Furthermore, within each oligomer/polymer chain the chromophoric entities may be dynamically equilibrated, e.g. by tautomerism, thus giving rise to chromophore mixing.

Conclusions

We have synthesized the first water-soluble 5,6-dihydroxyindole polymer to address the origin of the peculiar black color of eumelanin-type polymers. The data support the notion that eumelanin chromophores cannot be defined exclusively on an intrinsic basis by the conjugation length of the isolated absorbing species but can be defined also by the external perturbations of conjugated chromophoric units caused by interactions between the oxidized and reduced polymer chain domains. This would entail a dynamic composition of intrinsically and extrinsically defined light-absorbing species rather than a mixture of static entities with absorption features intrinsically defined by the degree of conjugation.

Although the relevance of these model studies to the actual optical properties of natural eumelanins remains to be assessed, it is likely that aggregation phenomena akin to those reported in solution occur as the early events in the multistep process of eumelanin particle buildup. When extrapolated to the solid state, this concept would lead to an extreme scenario of structure−property relationships dominated by emerging chromophoric components largely extrinsic in nature.

Acknowledgment. This work was supported by a grant from Italian Ministry of University (PRIN 2006 project). L.P. thanks "L'Oreal Italia Per le Donne e la Scienza" for a research fellowship.

Supporting Information Available: Material and methods and experimental procedures; mono- and bidimensional NMR spectra for compounds **1-Ac**, **2-Ac**, **3-Ac**; MALDI MS spectrum of the oxidation mixture of **1** (after reduction and acetylation treatment); complete ref 4. This material is available free of charge via the Internet at http://pubs.acs.org.

JA905162S

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