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# Convergent Proton-Transfer Photocycles Violate Mirror-Image Symmetry in a Key Melanin Monomer

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We present evidence for mirror-image rule[1] violation in spectra of 5,6-dihydroxyindole-2-carboxylic acid (DHICA), a key melanin monomer, and propose that this phenomenon is due to convergent adiabatic and nonadiabatic excited-state intramolecular proton-transfer (ESIPT) processes. Specifically, excitation into the $S_1$ or $S_2$ states of a catecholate anion of DHICA, represented by dual bands in the absorption spectrum, leads to emission from the $S_1$ state of its proton-transfer conjugate. This suggests that intramonomer ESIPT may function as an energy dissipation mechanism in the macromolecular pigment.

Eumelanin, a subclass of melanin, is a major component of the human pigmentary system.[2,3] It displays intriguing photophysics, including a broad, monotonically decreasing absorbance spanning the near UV, visible, and IR ranges, and efficient radiationless deactivation leading to ground-state regeneration with yield approaching unity.[4,5]

Eumelanins are brown-black macromolecular pigments derived from 5,6-dihydroxyindole (DHI) and DHICA.[3] The insolubility and heterogeneity of eumelanin make it a difficult material for experimental study. As a result, little is known about its structure−property relationships.[6,7] DHICA is stable in solution, making it an important model compound for the indirect inference of these relationships in eumelanin.

Figure 1 displays the scaled absorption and emission spectra of DHICA in aqueous solution at pH 9.0. Descriptions of DHICA synthesis, purification, and spectroscopy are included in the Supporting Information. There is an absorption peak near 3.8 eV and a distinct shoulder near 4.2 eV; excitation into either of these bands leads to emission which peaks at 3.0 eV in the scaled emission. Mirror-image rule violation is manifest in the lack of a shoulder in the emission spectrum and a broadened width of the emission relative to the main absorption peak.

Acid−base dissociations in DHICA occur with p$K_a$'s at 4.25, 9.76, and 13.2.[8] Comparison with DHI and catechol suggests that the second and third of these correspond to successive titration of the catecholic moiety. Strict identification with these sites is problematic because macroscopic p$K_a$'s of multiprotic acids with interacting sites do not correspond to individual site p$K_a$'s.[9] Instead, they represent averages over all pathways connecting the ensembles with $n$ and $n - 1$ protons. The spectra which we report were collected below the second macroscopic p$K_a$ of DHICA, where the ratio of dianions to monoanions should be small. However, the proximity to the catechol-like p$K_a$ mandates consideration of all possible DHICA monoanions in our analysis. These are displayed in Figure 2.

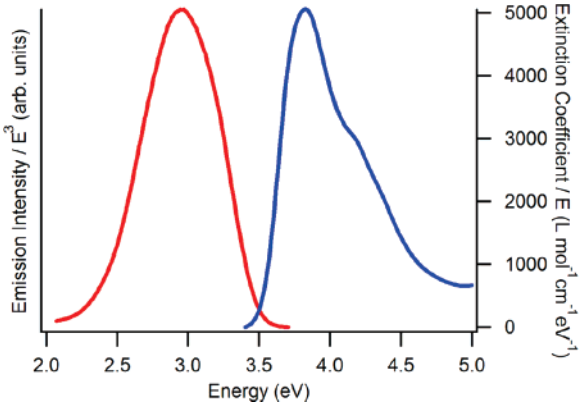


***Figure 1.*** Scaled absorption (blue) and emission (red) spectra of DHICA in aqueous solution (borax buffer at pH 9.0).

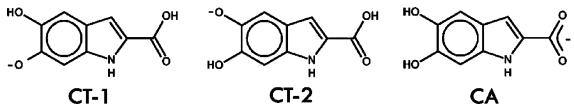


***Figure 2.*** Three DHICA monoanions with nomenclature.

Of the catecholate anions, **CT-1** is uniformly predicted to be energetically preferred in the ground state. We have calculated the $S_0-S_1$ and $S_0-S_2$ transitions of **CT-1** and **CA** with equation-of-motion coupled cluster singles and doubles[10] (EOM-CCSD) theory with a Dunning double-$\zeta$ basis set[11,12] (polarization functions on heavy atoms). These calculations predict $S_0-S_1$ and $S_0-S_2$ transitions at 3.60 and 4.39 eV for **CT-1** and 4.47 and 4.79 eV for **CA**. The excited states have $\pi\pi^*$ character and are dominated by single excitations from the two highest occupied orbitals of the restricted Hartree−Fock (RHF) reference into the lowest-lying virtual orbital. We used these orbitals to generate a four-electron, three-orbital complete active space self-consistent field (CASSCF)[13] wavefunction averaged[14] over three states (SA3-CAS(4,3)). We used this to optimize $S_0$ and $S_1$ equilibrium geometries for all three forms, where we evaluated the $S_0$, $S_1$, and $S_2$ energies via multistate multireference Rayleigh−Schrödinger second-order perturbation theory (MS-MRPT2)[15−17] with a Dunning double-$\zeta$ basis set with polarization functions on all atoms. At the $S_0$ configurations, these calculations predict $S_0-S_1$ and $S_0-S_2$ transitions at 4.04 and 4.91 eV (**CT-1**), 3.51 and 4.97 eV (**CT-2**), and 4.51 and 5.07 eV (**CA**). Corresponding excitations at the $S_1$ configurations were 3.99 and 4.90 eV (**CT-1**), 3.21 and 5.09 eV (**CT-2**), and 3.99 and 4.83 eV (**CA**). The $S_0$ energies for **CT-2** and **CA** were higher in energy than **CT-1** by 0.14 eV (3.2 kcal/mol) and 0.60 eV (13.8 kcal/mol), respectively, at the $S_0$ configurations. In the excited state, this situation is significantly altered, and the $S_1$ energy of **CT-2** is lower than that of both **CT-1** and **CA** by 0.54 eV (12.3 kcal/mol) and 1.46 eV

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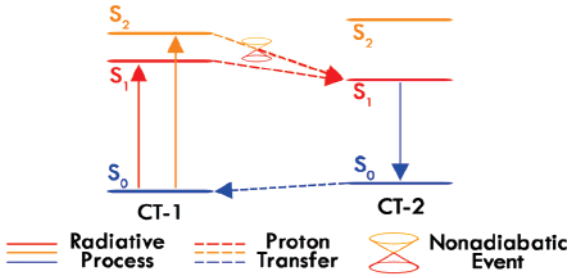


***Figure 3.*** Dual ESIPT photocycle schematic. Energy levels are scaled to MS-MRPT energies. No barrier height information should be inferred.

(33.7 kcal/mol), respectively, at the $S_1$ geometries. The norm of the $S_0-S_1$ difference dipole for **CT-2** is larger than that of **CT-1**, indicating a broadened transition within a simple Onsager model. RHF calculations in a conductor-like screening model (COSMO)[18] of a dielectric environment indicated differential stabilization of **CA** relative to the catecholate forms that was of the same order as the MS-MRPT2 ground-state energy difference, consistent with coexistence of carboxylate and catecholate forms in solution.

These data led us to a photochemical reaction mechanism such as that in Figure 3. Within this mechanism, the dominant absorbing form is the catecholate anion **CT-1**. The dual absorption features can be attributed to the $S_0-S_1$ and $S_0-S_2$ transitions of **CT-1**. The resulting excited species react via adiabatic ($S_1$) or nonadiabatic ($S_2$) ESIPT to form the $S_1$ state of **CT-2**, which decays by photon emission. **CT-1** is regenerated on the ground state via the reverse reaction.

There may be significant population of the **CA** at pH 9.0. Our results suggest that the $S_0-S_1$ transition of **CA** may contribute to the absorption at 4.2 eV, but that **CA** does not contribute to the emission. Photoexcited **CA** may form **CT-2** via multistep proton transfer with the solvent (in contrast to one-step internal transfer for **CT-1**). The close correspondence between the absorption and fluorescence excitation spectrum of DHICA at pH 9.0 suggests that the contribution of **CA** near 4.2 eV should be small (see Supporting Information). Accumulating populations of dianions may also contribute. The ratio of dianions to monoanions at pH 9.0 should be $10^{(pH-pKa)} = 0.17$. DHICA may exist as three possible dianions. Exploratory MS-MRPT2 calculations suggest that similar ESPT processes exist within the dianionic population. The calculated oscillator strengths are generally lower than those for the monoanions.

We postulate the existence of a nonadiabatic pathway which forms the $S_1$ state of **CT-2** from the $S_2$ state of **CT-1**. We have verified this via the optimization of a minimal energy[19] conical intersection characterized by intermediate O−H distances. Correlation of $S_2$ of **CT-1** and $S_1$ of **CT-2** is suggested by analysis of the orbital structure and the $S_N-S_0$ charge difference densities (see Supporting Information).

Reactions which transfer a light atom between two heavy atoms are known to be inherently multidimensional.[20] Barriers identified along one-dimensional reaction coordinates are of limited use in the analysis. Calculation of the full multidimensional reaction surface for proton transfer in DHICA will be the subject of future work.

Though we specifically address the photochemistry of DHICA, our results have profound implications within the broader context of melanin research. The broad absorbance of eumelanin is indicative of a dense manifold of states with varying degrees of localization.[21] For eumelanin to fulfill its role as a photoprotectant, photoexcitations into many high-lying states must be funneled into fewer low-lying states in a manner that avoids chemical degradation of the pigment and/or surrounding tissue. The convergent ESIPT photocycles in DHICA fulfill this requirement, albeit on a much smaller scale. This raises the possibility that intramonomer ESIPT is a mechanism for energy dissipation from high-lying macromolecular states. To the best of our knowledge, this is a new suggestion within the melanin literature. We are pursuing further investigations.

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**Supporting Information Available:** Complete citation for ref 22, sample preparation and characterization data, unscaled absorption and emission spectra, fluorescence excitation spectra, excitation dependence of the fluorescence, quantum yields, optimized Cartesian coordinates in angstroms, absolute energies, dipole moments, and state-averaged active orbitals and occupation numbers. This material is available free of charge via the Internet at http://pubs.acs.org.

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