THE JOURNAL OF PHYSICAL CHEMISTRY Letters

Letter

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Excited-State Proton-Transfer Processes of DHICA Resolved: From Sub-Picoseconds to Nanoseconds

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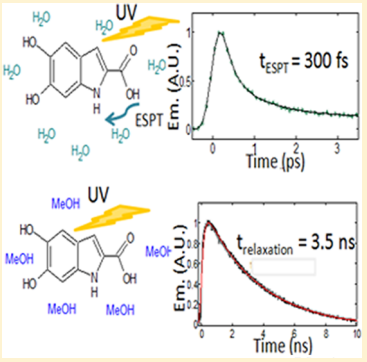
Supporting Information

ABSTRACT: Excited-state proton transfer has been hypothesized as a mechanism for UV energy dissipation in eumelanin skin pigments. By using time-resolved fluorescence spectroscopy, we show that the previously proposed, but unresolved, excited-state intramolecular proton transfer (ESIPT) of the eumelanin building block 5,6-dihydroxyindole-2-carboxylic acid (DHICA) occurs with a time constant of 300 fs in aqueous solution but completely stops in methanol. The previously disputed excited-state proton transfer involving the 5- or 6-OH groups of the DHICA anion is now found to occur from the 6-OH group to aqueous solvent with a rate constant of 4.0×10^8 s^{-1}.



SECTION: Spectroscopy, Photochemistry, and Excited States

The 5,6-dihydroxyindole-2-carboxylic acid (DHICA), a key product of tyrosine metabolism in cutaneous melanocytes, plays important roles in skin homeostasis as a major precursor with 5,6-dihydroxyindole (DHI) of eumelanin biopolymers, the dark pigments of human skin, hair, and eyes,[1] as an antioxidant,[2] and as a central mediator in cell−cell communication.[3] Eumelanins contain various proportions of DHICA-derived units ranging from only a few % up to >50% depending on the phenotype and organism. While eumelanins appear to be complex biopolymers in which the various units concur to determine the macroscopic properties at several levels of structural organization, redox states, and disorder, a detailed understanding of the photophysical behavior of key building blocks is crucial if structure−property−function relationships are to be drawn. The peculiar absorption features of eumelanin[4,5] and its former monomer DHI and DHICA might play a role in protecting the skin against UV radiation, though the UV-induced reaction mechanisms are largely unknown. Recent time-resolved spectroscopy work on DHICA[6−8] revealed a rich, pH-dependent photochemistry in aqueous buffer solution. At a pH where the carboxyl group of the molecule is fully protonated (e.g., pH 2.5), a red-shifted fluorescence band ($\lambda_{max} \approx 430$ nm) with a relatively short lifetime of 240 ps was observed and attributed to a zwitterionic species formed as a result of rapid excited-state intramolecular proton transfer (ESIPT) from the COOH group toward the NH group.[6] However, the actual transfer time and decay of the original excited state of the fully protonated molecule could not be resolved with the temporal resolution of the employed streak camera technique. By using femtosecond fluorescence upconversion (FU), we can now directly resolve this reaction step and show that it indeed proceeds on the sup-picosecond time scale.

The deprotonated carboxylate anion $DHICA^-$ was observed to have a long (~1.6 ns) lifetime in neutral (pH 7) buffer solution representing decay to a new species with a red-shifted fluorescence spectrum ($\lambda_{max} \approx 450$ nm) and a 2.4 ns lifetime.[6] This species was assigned to a complex between the excited $DHICA^-$ and a buffer species formed through a diffusion process.[6] On the other hand, calculations by Olsen et al.[9] suggested that the 5- and 6-OH groups of DHICA should be involved in an ESIPT process or multistep excited-state proton transfer (ESPT) to the solvent. This discrepancy between experimental and theoretical results motivated us to further examine the role of the OH groups of the carboxylate anion of DHICA in proton transfer. As we will show below, the previously reported slow excited-state decay (1.6 ns) of $DHICA^-$ in aqueous buffer solution indeed represents slow ESPT to the solvent, thus reconciling experimental and theoretical results. In methanol, the proton transfer stops, and the excited-state lifetime becomes 3.5 ns, representing the fluorescence decay of DHICA.

Received: February 27, 2013
Accepted: April 8, 2013

   dx.doi.org/10.1021/jz400437q | J. Phys. Chem. Lett. 2013, 4, 1383−1388

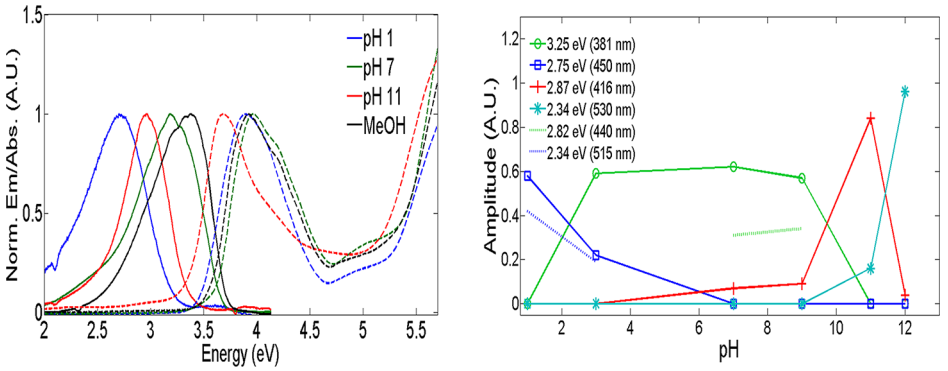


Figure 1. (Left) Normalized absorbance (dashed lines) and fluorescence (solid lines) spectra of DHICA measured at various pHs. (Right) Relative concentrations of the different DHICA species as a function of pH. The 450 nm band represents the protonated zwitterionic species, the 381 nm band the mono anion ($DHICA^-$), the 416 nm band the double anion ($DHICA^{2-}$), and finally the 530 nm band the trianion ($DHICA^{3-}$). In order to account for the nonsymmetric shape of the component spectra (due to their vibrational progressions), two broad Gaussian bands at 515 and 440 nm were added. Details of the band fitting are presented in the SI (Figure SI2A–C).

DHICA was prepared by oxidative cyclization of dopa,[10] and its purity was checked by ^{1}H NMR. Time-resolved fluorescence measurements were performed with a combination of FU, a streak camera, and time-correlated single-photon counting (see the Supporting Information (SI)).

Measurements of the steady-state fluorescence spectra of DHICA as a function of pH, from 1.0 to 12, allow us to identify the various protonation states of DHICA via their fluorescence spectra (Figure 1). We fitted the measured fluorescence spectra at various pH values with a sum of Gaussians in order to obtain the component spectra corresponding to the different protonation states of DHICA, and the pH dependences of their amplitudes are plotted (Figure 1). The result of the Gaussian band spectral deconvolution is shown in the SI (Figure SI1A–F). At low pH (1.0), we observe a strongly red-shifted fluorescence, with $\lambda_{max} \approx 450$ nm (and not 430 nm, as previously reported[6]) and a lifetime of 240 ps. On the basis of extensive time-resolved fluorescence work on DHICA[6,11] and experiments on indole-2-carboxylic acid (ICA),[12] as well as recent combined time-resolved fluorescence and quantum chemistry calculations[13] on ICA, this red-shifted fluorescence was assigned to a zwitterionic species formed through an unresolved sup-picosecond ESIPT process involving the COOH and NH groups. At the same time, solvent (water) molecules were shown to play an important role in the proton transfer,[13] implying that the exact nature of interaction between the proton dissociated from the COOH group and the NH group has still to be established. At high pH (11), when the molecule is doubly deprotonated, a red-shifted fluorescence peaking at ~416 nm is observed, which we consequently attribute (mainly) to the $DHICA^{2-}$ species. The measured fluorescence spectra at intermediate pH values are a result of coexisting species. For instance, at pH 7, the fluorescence spectrum has a broad red tail and can be described as a superposition of the $DHICA^{2-}$ spectrum and a (dominating) band at ~380 nm, which we assign to the $DHICA^-$ carboxylate anion, in agreement with previous reports.[6] The fluorescence spectrum of DHICA in methanol with a maximum at ~360 nm is also shown in Figure 1.

The weak fluorescence of the zwitterionic species at 450 nm only has significant amplitude at pH values when DHICA is fully protonated, that is, pH ≈ 1.5–5 (pK_a = 4.25). The carboxylate anion $DHICA^-$ species on the other hand shows fluorescence over a wide pH range, ~2.5–10; its pK_a implies that it will have a significant ground-state concentration in the pH interval of ~3–10, and its long fluorescence lifetime (as compared to the zwitterionic species) means that even at a pH substantially lower than the pK_a of the COOH group (4.25), it has significant contribution to the overall measured fluorescence. The double anion $DHICA^{2-}$ exhibits fluorescence also at pH values much lower than its ground state pK_a (9.7), the reason being its formation from $DHICA^-$ through ESPT and its 2.4 ns lifetime emphasizing its contribution to the steady-state spectrum (see also below). At pH 10–11, $DHICA^{2-}$ becomes the dominating ground-state species, which is reflected by the sharp increase in the amplitude of its spectral component, and at the highest pH values (>11), the fully deprotonated $DHICA^{3-}$ starts to appear (λ_{max} = 530 nm). As can be seen in the single-species fluorescence spectra in Figure 1, that is, for the zwitterionic DHICA (pH 1) and DHICA in methanol, the fluorescence spectrum of a species is generally not a symmetric Gaussian band (that these spectra belong to a single species is concluded from the fact that the whole band is characterized by the same fluorescence lifetime). In order to approximately account for this asymmetry of the spectra due to vibrational progressions, two additional Gaussian bands with lower amplitude and maxima at 440 and 515 nm were added in the band fitting (Figure SI1 (SI) and the dotted lines in Figure 1). It should be recognized that due to this asymmetry of the spectra, it is difficult to obtain good fits with accurate spectral amplitudes in pH regions of multiple species, that is pH ≈ 3–5 and ~8–10.

As described above, at low pH (e.g., pH 2.5), the fluorescence band observed at 450 nm is due to a zwitterionic species suggested to be formed through an unresolved sub-picosecond ESIPT process[6,11] involving the COOH and NH groups of DHICA. Figure 2 shows the FU decay of the originally excited molecule measured at 380 nm. It is dominated by a major ~300 fs component (~60% amplitude) followed by a slower one of ~1 ps, of low amplitude (~30%), and a much slower component (~10%), which appears to be constant on the studied time range. A blow-up of the initial ultrafast decay is shown as the inset in Figure 2. The slow decay represents the previously reported 240 ps decay[6] of the zwitterionic species formed through the ultrafast ESIPT process.

When the fluorescence of DHICA is measured in methanol, the picture is completely changed; the lifetime is very much

dx.doi.org/10.1021/jz400437q | J. Phys. Chem. Lett. 2013, 4, 1383–1388

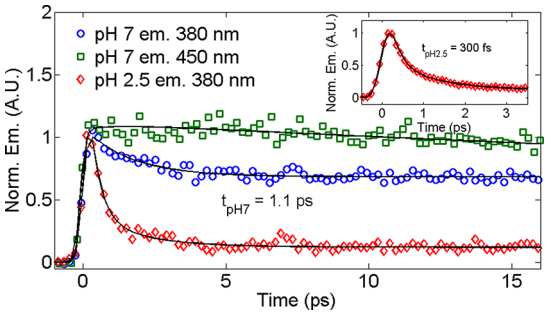


Figure 2. FU kinetics of DHICA in phosphate buffer, excited at 267 nm. The decay at pH 2.5 measured at 380 nm can be fitted by ∼300 fs (60%) and ∼1 ps (30%) time constants and a low-amplitude (∼10%) constant (on this time scale) background. At pH 7, the decay is characterized by a low-amplitude ∼1 ps decay on the blue side of the fluorescence spectrum and corresponding weak rise on the red side, characteristic of a solvation-dynamics-induced spectral red shift. The very slow dynamics at pH 7 represent the $DHICA^-$ to $DHICA^{2-}$ conversion. (Inset) Higher resolution of the pH 2.5 kinetics.

prolonged to ∼3.5 ns (Figure 3). A FU measurement of DHICA/MeOH reveals a few-picoseconds decay on the short-

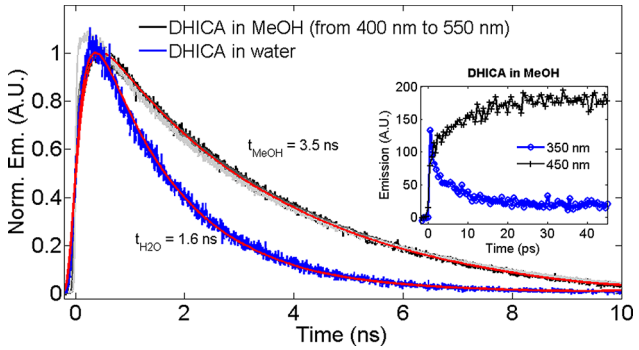


Figure 3. Fluorescence decay of (black) DHICA in methanol measured with TCSPC under 267 nm excitation, emission from 400 to 550 nm (50 nm step) showing a decay time of 3.5 ns independent of emission wavelength, and (blue) of DHICA in distilled water (pH ≈ 6) with emission at 400 nm, having a decay of 1.6 ns, for fluorescence kinetics measured at 450 nm, monitoring the $DHICA^-$ to $DHICA^{2-}$ proton transfer; see Figure SI2 (SI). (Inset) FU kinetics of DHICA in methanol, exhibiting picosecond decay on the blue side and a corresponding rise on the red side of the 380 nm fluorescence band due to solvation dynamics. The very slow decay following the initial picosecond dynamics is the 3.5 ns decay.

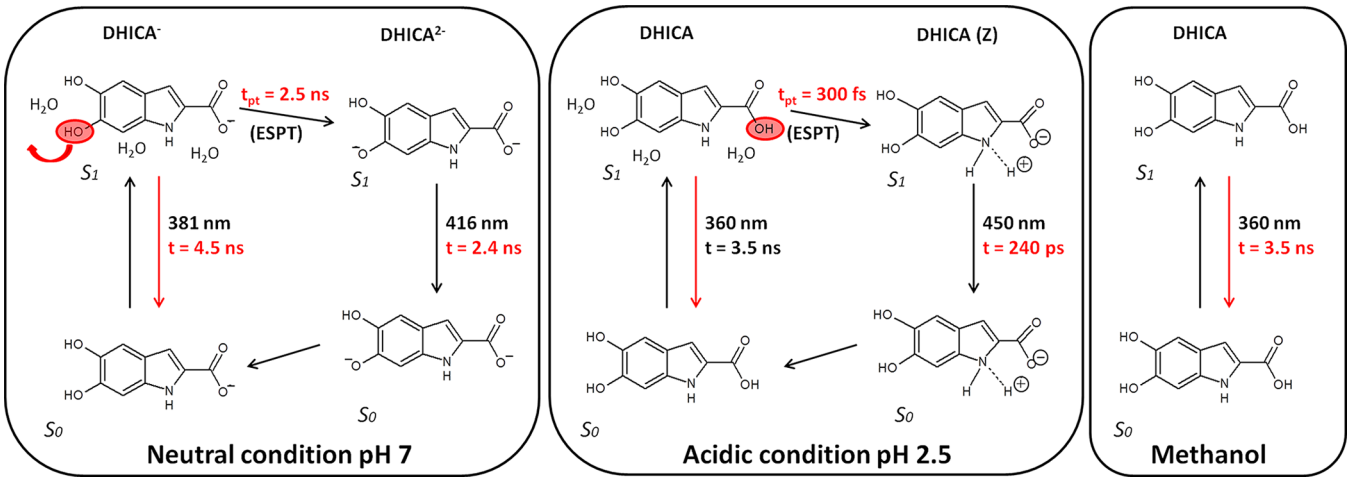
wavelength side of the fluorescence band accompanied by a corresponding fast rise on the red side of the fluorescence band (Figure 3 inset). Such a behavior is typical of a spectral red shift and has previously been shown to be the characteristics of excited-state solvation dynamics;[14,15] we consequently attribute this few-picoseconds dynamics of DHICA in MeOH to a solvation process of the excited-state molecule. Thus, in aqueous solution, the ESPT from COOH occurs with a time constant of 300 fs but is totally blocked in methanol, as monitored by the 3.5 ns fluorescence lifetime. The solvent sensitivity of the ESIPT process suggests direct involvement of solvent molecules and could be the reason for the nonexponential fluorescence decay of the initially prepared excited state (300 fs and ∼1 ps) of protonated DHICA in aqueous solution; solvent molecule reorientations could be envisaged to facilitate the proton transfer and their dynamics therefore imprinted on the proton-transfer process. Details of this behavior will be the target of future investigations.

The observed rate of proton transfer $(\sim 300\ \text{fs})^{-1}$ is faster than what has been observed for the fastest ESPT processes of photoacids[16] but a few times slower than the fastest reported ESIPT processes.[17,18] The reaction is strongly solvent-dependent, as shown by its total inhibition in MeOH. This is clearly contrary to the behavior of a typical ESIPT process, which occurs along a reaction coordinate predefined by an intramolecular hydrogen bond. The participation of water molecules in the ESPT process is reminiscent of what has been observed for organic chromophores like 7-hydroxyquinoline (7HQ) in solution[19−21] or solvent clusters (in this case, interpreted as hydrogen atom transfer).[22] Similar effects have been studied in several proteins.[23−26] The solvent involvement in the proton transfer in these systems is often described as a solvent wire mediating the proton transfer from proton (hydrogen atom) donor to acceptor. Alternatively, the picture of Tolbert et al.,[27,28] where intramolecular H-bond accepting groups together with solvent molecules define a preexisting network for solvating the expelled proton, could perhaps be envisaged to explain the results. The overall conclusion of this part is that femtosecond time scale ESPT from the COOH toward the NH group requires active participation of the solvent molecules and that water is essential for the rapid transfer to occur. Involvement of water molecules in the ESIPT of the closely related indole-2-carboxylic acid (ICA) was also concluded from combined fluorescence spectroscopy and quantum chemistry work.[13] The middle and right-hand panels of Scheme 1 illustrate the relaxation pathways of DHICA in acidic (pH 2.5) aqueous solution and in methanol, respectively.

The fluorescence kinetics of the DHICA carboxylate anion has already been investigated in a previous study in ref 6, and it was suggested that in the excited state, this species associates with a buffer component on the nanosecond time scale through a diffusion process and forms a complex that decays to the ground state with a 2.4 ns lifetime. Although it is known that the presence of buffer species and their concentration may influence the rate of proton transfer,[29] studies of DHICA fluorescence kinetics in buffer solutions with different cations and anions did not provide conclusive evidence supporting this interpretation (A. Corani et al., unpublished). We were therefore prompted to take into account other possibilities for the observed spectral and dynamic properties of $DHICA^-$. It was mentioned above that on the basis of calculations, Meredith and co-workers[8,9] suggested ESPT to occur from the OH groups of the photoexcited molecule. ESPT from prototype photoacid molecules like 2-naphthol[27,28,30] is known to be solvent-dependent; for medium strength photoacids, proton transfer does not occur in alcohols. We measured the fluorescence decay of $DHICA^-$ as a function of water/MeOH ratio (Figure 4), and the results show that the rate constant of the excited-state decay increases from 0.28×10^9 s^{-1} (3.5 ns) in neat MeOH to 0.63×10^9 s^{-1} (1.6 ns) in neat water, comparable to what has been observed previously for other photoacids.[27] On the sub-picosecond time scale, the $DHICA^-$ in aqueous solution exhibits a ∼1 ps solvation dynamics process preceding the proton transfer, similar to that observed for DHICA in methanol (Figure 3). These results consequently suggest that the nanosecond lifetime component of $DHICA^-$ fluorescence in aqueous solution is a result of a relatively slow ESPT to the solvent and not to the formation of a complex with the buffer, as previously reported.[6]

dx.doi.org/10.1021/jz400437q | J. Phys. Chem. Lett. 2013, 4, 1383−1388

Scheme 1. Scheme Summarizing the ESPT Processes of DHICA under Neutral and Anionic Conditions[a]



[a]For the time constant of S_1 to S_0 decay of DHICA$^-$ in the absence of ESPT, we use the measured excited-state lifetime of ICA at pH 7 (ICA$^-$) 4.5 ns, which does not contain the OH groups in positions 5 and 6, (SI, Figure SI3). With the measured fluorescence lifetime of DHICA$^-$ (1.6 ns), this leads to a time constant of 2.5 ns ($k = 4.0 \times 10^8$ s^{-1}) for the ESPT. For the S_1 to S_0 processes of neutral DHICA (in the absence of ESPT), we used the measured decay time of DHICA in MeOH (3.5 ns).

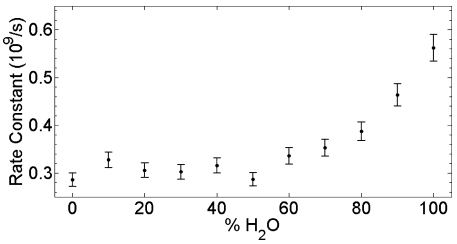


Figure 4. DHICA fluorescence decay rate constant in H_2O/MeOH solvent mixtures, extracted from the fluorescence decays measured at 350 nm with TCSPC using 267 nm excitation.

As shown above (Figure 1), the proton-transfer product of DHICA$^-$ and DHICA^{2-} is characterized by a red-shifted fluorescence spectrum with a maximum at ~415 nm. Measurements of fluorescence lifetimes in this band should therefore reflect the dynamics of its formation and decay. Indeed, time-resolved fluorescence measurement probing the red-shifted DHICA^{2-} band (at 450 nm) exhibits the expected ~1.6 ns time constant as a rise and decays with a 2.4 ns lifetime (Figure SI2, SI; this kinetic trace was previously published,[6] but it is shown again for clarity). Thermodynamic considerations of ground and excited states of a proton-containing molecule and its conjugate base predict that its excited state is a stronger acid than the ground state if the absorption or fluorescence spectra of the conjugate base are red-shifted as compared to the acid form.[31−33] The pK_a in the excited state can be estimated with a Förster cycle[34,35] calculation. From the fluorescence spectra of DHICA$^-$ (λ_{max} = 381 nm) and DHICA^{2-} (λ_{max} = 414 nm) shown in Figures SI1 and SI3 (SI), we can estimate the change of pK_a for the DHICA$^-$ excited state to five pH units ($\Delta pK_a^* = 5$). The 5-OH and 6-OH groups of DHICA are known to have different pK_a's, 13.2 and 9.7,[9,36] respectively, implying that in the excited state, the 6-OH group has a pK_a of ~4.7 and will therefore dissociate under neutral conditions. The rate of proton transfer (4.0×10^8 s^{-1}) is similar to that of other medium strength photoacids.[28,35,37]

With the help of quantum chemistry calculations, Olsen et al.[9] discussed ESPT between all three monoanions of DHICA (CT-1 (5-O$^-$), CT-2 (6-O$^-$), and CA (COO$^-$)). CT-1 to CT-2 ESIPT was concluded to occur and possibly a multistep proton transfer from CA with the solvent to form the rearranged anion CT-2. The ESPT involving the OH groups of DHICA$^-$ observed here, thus, is reminiscent of the process suggested in ref 9 involving the CA species, but our measurements give no evidence of the carboxylate group acting as a proton acceptor. In an aqueous solution with water molecules acting as proton acceptors, this is perhaps not surprising.

To summarize this part, the steady-state fluorescence spectra together with the fluorescence kinetics show that upon photoexcitation of DHICA$^-$ in aqueous buffer solution, the excited state decays through an ESPT process to form the excited state of the double anion, DHICA^{2-}, with a time constant of 1.6 ns. DHICA^{2-} then decays back to the ground state with a time constant of 2.4 ns (Scheme 1, left panel). In neat MeOH, the proton transfer is inhibited (Figures 3 and 4), and the excited state decays with a 3.5 ns time constant. In order to estimate the intrinsic rate of ESPT of DHICA$^-$, we take the measured fluorescence lifetime of the anionic form of the closely related indole-2-carboxylic acid (ICA$^-$), 4.5 ns[13] (Figure SI3, SI), as the sum of all excited-state deactivation processes in the absence of proton transfer. This leads to an ESPT rate of 4.0×10^8 s^{-1} (2.5 ns time constant).

As an additional proof of the ESPT picture of DHICA$^-$ in aqueous solution presented above, we also measured the fluorescence spectra and kinetics (Figure SI3, SI) of ICA, which is lacking the 5- and 6-OH groups purportedly responsible for the solvent-dependent fluorescence. These measurements (as well as those previously reported[22]) show that both fluorescence spectra and kinetics are practically independent of the solvent and that the fluorescence decay is wavelength-independent with a lifetime of 4.5 ns, very similar to that of DHICA in MeOH. Thus, when the 5- and 6-OH functionalities are removed, the solvent dependence of the fluorescence spectrum and its decay vanishes. This shows that the observed solvent dependence of the DHICA$^-$ fluorescence is related to the 5- and 6-OH groups, as discussed above, and not due to a general solvent dependence of the indole core excited-state decay.

dx.doi.org/10.1021/jz400437q | J. Phys. Chem. Lett. 2013, 4, 1383−1388

The excited state of DHICA, a key eumelanin building block and a diffusible photoprotective agent from metabolically active melanocytes, has been shown by time-resolved fluorescence spectroscopy to decay through two main ESPT pathways on the 100 fs-to-ns time scale. The presence of the photoionizable 6-OH group is critical for the excited-state decay of the anionic form (e.g., at pH 7), whereas fast deprotonation of the COOH group drives the sub-picosecond excited-state decay of the neutral form (e.g., at pH 2.5). Both pathways are critically dependent on water, being virtually stopped in methanol. These results fill an important gap in the current knowledge of the excited-state dyamics of DHICA and provide a new groundwork for further studies on UV photoprotection mechanisms in the eumelanin phenotype

■ ASSOCIATED CONTENT

Supporting Information

Fits of the fluorescence steady-state spectra are presented in Figure SI1A−F. The red-shifted in-growth corresponding to the formation of the dianion by the 1.6 ns proton transferred is shown on Figure SI2. Time-resolved measurment of the ICA molecule in different solvents is shown in Figure SI3, and the experimental methods are available. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

Financial support is acknowledged from the Swedish Research Council, the Knut & Alice Wallenberg Foundation, and the European Research Council (Advanced Investigator Grant 226136-VISCHEM). M.d'I. acknowledges financial support from the Italian MIUR, PRIN 2010-2011 project.

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