

Excited-State Intramolecular H Atom Transfer of Hypericin and Hypocrellin A Investigated by Fluorescence Upconversion

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Received: July 16, 1999

The excited-state intramolecular H atom transfer reaction occurring in hypericin and hypocrellin A is investigated by means of the fluorescence upconversion technique in a variety of solvents. Rising components of ~ 10 ps, attributable to intramolecular H atom transfer, are clearly observed in the fluorescence upconversion traces of *both* hypericin and hypocrellin A. The amplitude of the rising component is emission wavelength dependent and occurs on the blue edge of the emission spectra. Neither in hypericin nor in hypocrellin A does this fluorescent component exhibit a deuterium isotope effect. This result identifies it with the ~ 10 ps component, also lacking a deuterium isotope effect, observed in hypericin in transient absorption measurements monitoring stimulated emission. The clear and complementary observation in fluorescence of the ~ 10 ps component in both hypericin and hypocrellin A is a crucial link in refining a unified model of the hypericin and hypocrellin photophysics that we first proposed in *J. Phys. Chem. B* 1998, 102, 6098.

Introduction

The naturally occurring polycyclic quinones, hypericin and hypocrellin (Figure 1), have attained notoriety for their wide range of biological activities, many of which require light. These have been the subject of several reviews^{1–5} and have been referred to in detail in our previous work. One of the most interesting photophysical processes that these molecules execute is excited-state proton or H atom transfer, both *intramolecular*^{6–19} and *intermolecular*.^{20–22} We have suggested that the intermolecular proton transfer plays a role in certain aspects of the biological activity of hypericin and hypocrellin.^{20,23–25}

Among the most convincing evidence that intramolecular H atom transfer occurs in hypericin is the observation of a ~ 10 ps rise time in the fluorescence upconversion signal of hypericin in DMSO.¹¹ One of the reasons for using the fluorescence upconversion technique is that often in transient absorption experiments it is difficult to distinguish stimulated emission from transient absorption. For example, if there are overlapping excited-state spectra, an apparent rise in induced absorption might in fact correspond to a decay of stimulated emission superimposed on an absorption transient that appears instantaneously and that does not decay on the time scale of the measurement. Thus, transient absorbance data can be subject to complications because they measure ground-state bleaching, absorption of all excited states present (both singlet and triplet), and stimulated emission. Because fluorescence upconversion monitors emission *only from the fluorescent singlet state*, it is not subject to these complications and hence provides complementary information not subject to the same ambiguities.

Our argument for intramolecular excited-state H atom transfer in hypericin is as follows. The deshydroxy analogue of hypericin, mesonaphthobianthrone (phenanthro-[1,10,9,8,-*o,p,q,r,a*]perylene-7,14-dione (Figure 1), is nonfluorescent except in strong acids^{7,8,11} (e.g., sulfuric acid or triflic acid (CF₃SO₃H)) where it produces a fluorescence spectrum that has

nearly the same shape as that of hypericin in DMSO (Figure 2a). *These results demonstrate the importance of a protonated carbonyl group for producing hypericin-like fluorescence.* The hypericin emission spectrum grows in on a 6–12 ps time scale in all solvents except in sulfuric acid where it is instantaneous. On the basis of the results for mesonaphthobianthrone (see above), *the rise time for the appearance of the hypericin emission is taken as evidence for an excited-state H atom transfer.*⁷ Confirming this interpretation are the fluorescence upconversion measurements¹¹ of hypericin and O-methyl hypericin analogues, which are incapable of executing intramolecular excited-state H atom transfer reactions.

In this article, rising components of ~ 10 ps, attributable to intramolecular H atom transfer, are clearly observed in the fluorescence upconversion traces of *both* hypericin and hypocrellin A. The clear and complementary observation in fluorescence of the ~ 10 ps component in both hypericin and hypocrellin A is a crucial link in refining a unified model of their photophysics, which we proposed elsewhere.¹⁵

Experimental Section

Materials. Hypericin and hypocrellin A were obtained from Molecular Probes and were used as received. Anhydrous DMSO as well as HPLC grade methanol, ethanol, and octanol (Aldrich) were used, freshly opened, without further purification. Steady-state absorbance spectra were obtained on a Perkin-Elmer Lambda 18 double-beam UV–vis spectrophotometer with 1 nm resolution. Steady-state fluorescence spectra were obtained on a Spex Fluoromax with a 4 nm band-pass and corrected for lamp spectral intensity and detector response.

Time-Resolved Measurements. The fluorescence upconversion apparatus is described in detail elsewhere.¹⁸ The upconverted signal is sent directly into a H10 (8 nm/mm) monochromator (Jobin Yvon/Spex Instruments S.A. Group) coupled to a Hamamatsu R760 photomultiplier equipped with a UG11 UV-pass filter and operated at maximum sensitivity. The photomultiplier output is amplified in two stages (by a factor

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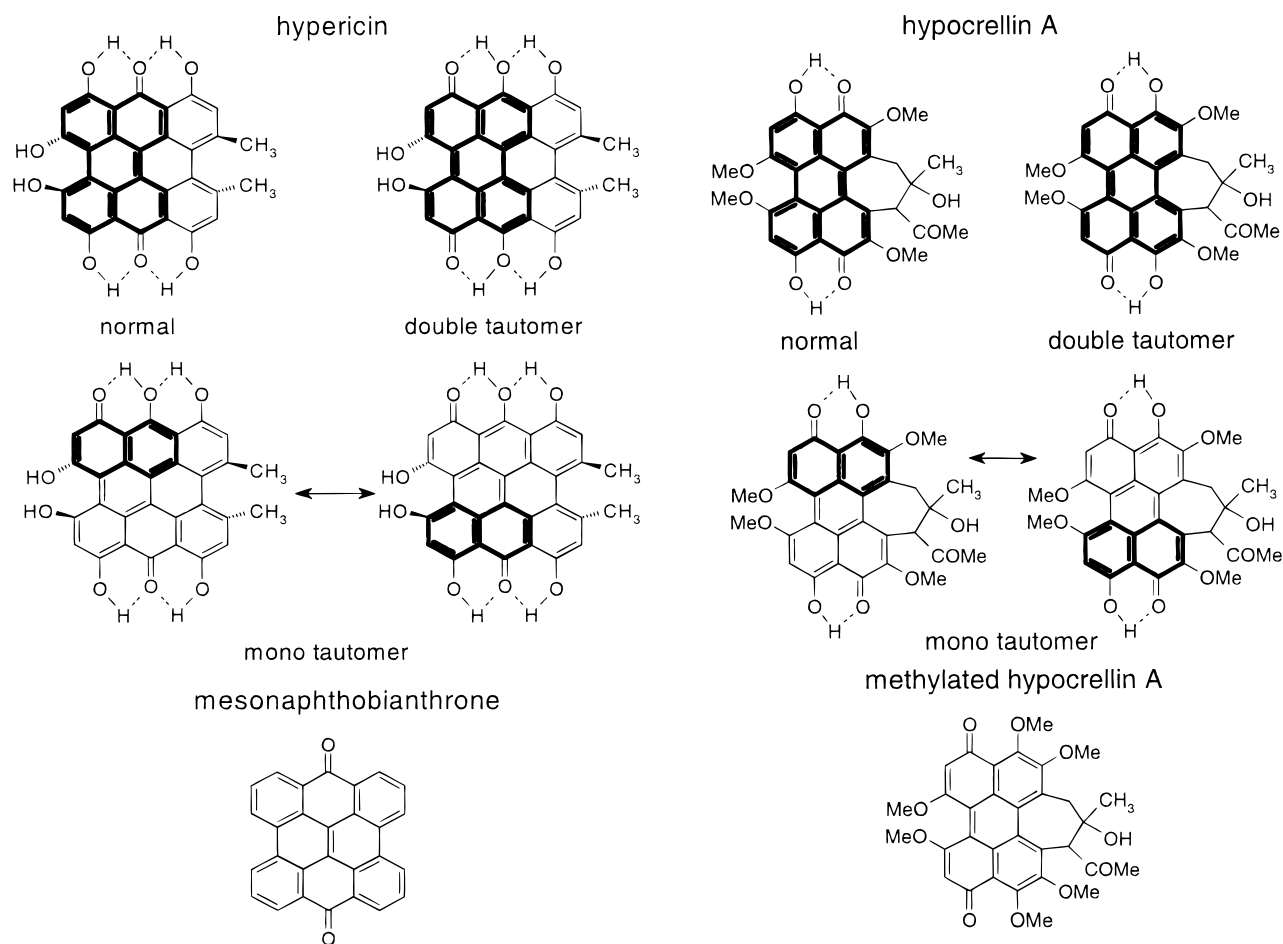


Figure 1. Structures of hypericin tautomers and mesonaphthobianthrone, and structures of hypocrellin A tautomers and its O-methylated analogue.

of 25) by a Stanford Research Systems SR 445 DC-300 MHz amplifier, terminated at 500 Ω and carefully calibrated after a long warm-up. Photon arrival events are registered with a SR400 gated photon counter operated in CW mode with a threshold level of 80–100 mV. At each delay step, the signal is obtained by averaging 3–5 samples collected for 1 s each. To reduce fluctuations due to laser flicker or sample instability (e.g., air bubbles in the flow cell), data are resampled if significant relative standard deviation or relative drift is detected. This approach also helps eliminate errors due to data transmission. A translation stage (Microcontrole, France) with a resolution of 1 step/ μm or 10 microsteps/ μm is used to delay gate pulses and is controlled by computer via an IEEE interface and a Klinger Scientific CD-4 motor driver. The instrument response function is obtained by collecting a cross correlation function of the second harmonic and the fundamental (with the long-pass filter removed); the resulting third harmonic is plotted against delay time. Cross correlation functions typically have a fwhm of 280–300 fs and, for time scales greater than 20 ps, are assumed to be δ -function-like. Average pump power was about 40–60 mW as measured at the second prism for precompensation. The spectral resolution was limited by the bandwidth of the upconversion crystal (BBO) and was estimated to be 5–6 nm.

A sample of a total volume of ~ 10 mL was constantly pumped through a 1 mm quartz flow cell by a dye circulator. All experiments, excluding sample preparation, were performed in an unlit room. Hypocrellin A/DMSO solutions required thorough deoxygenation in order to avoid artifacts owing to

sample degradation. Similar precautions were taken for the O-methyl analogue in alcohols.

Data Analysis. Fluorescence upconversion traces were fit with Spectra-Solve for Windows 95/98 data collection and processing software (Ames Photonics, Inc.) employing nonlinear least-squares iterative deconvolution subroutine and assuming Poisson noise model. Two contributions are generally considered when fitting the data: a short rising component and a long-lived component (assigned to a time constant of infinity for experiments on time scales up to 50 ps). The fit equation then assumes the following form:

$$F(t) = a_1[1 - \exp(-t/\tau_1)] + a_2 \exp(-t/\tau_2) \approx -a_1 \exp(-t/\tau_1) + (a_1 + a_2) \exp(-t/\infty)$$

As a matter of convenience, the fitting results are reported in a normalized form such that $(a_1 + a_2)$ is equal to unity. A rapid rising component of fluorescence is observed for both hypericin and hypocrellin A. The time constant for this component ranges from 4.5 to 10 ps. For simplicity, we refer to it throughout this article as the ~ 10 ps component.

O-Methylation of Hypocrellin A. To 3 mg of hypocrellin A in 1 mL of acetone under nitrogen were added 1 g of potassium carbonate and 0.5 mL of MeI. This solution was boiled for 24 h with stirring. The solvent was removed, and residue was extracted with ethyl acetate. After ethyl acetate was removed under vacuum, the red crude product was purified by flash column chromatography (ethyl acetate/hexane 4:1, then methanol/ethyl acetate 1:1), giving 2.9 mg of red solid. NMR

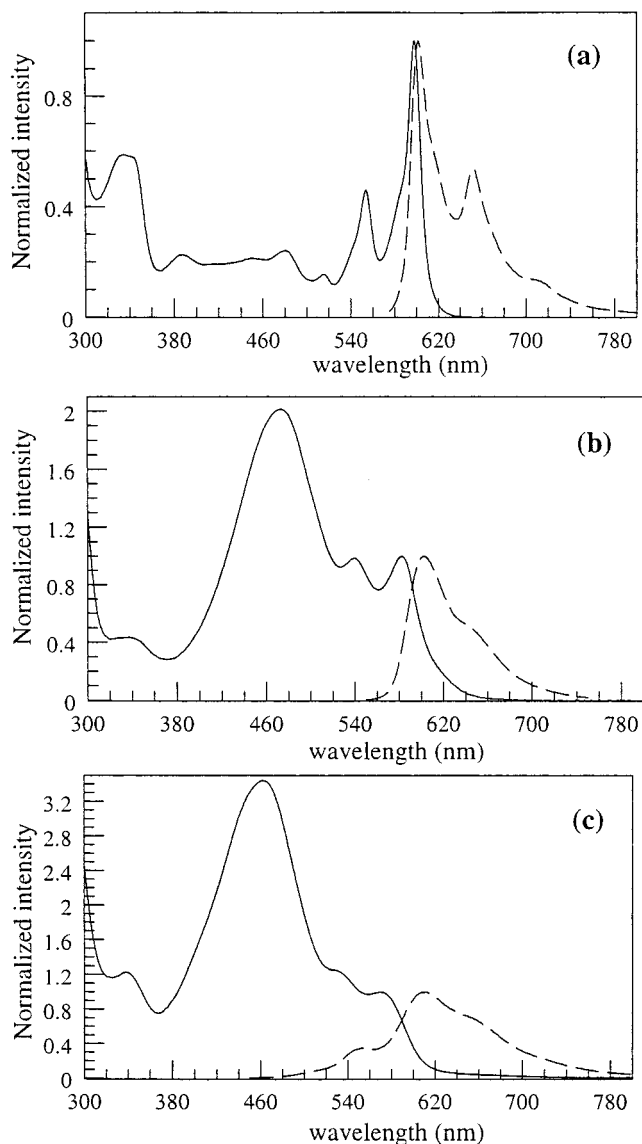


Figure 2. Absorbance and fluorescence emission spectra of hypericin (a), hypocrellin A (b) in DMSO and hypocrellin A O-methylated analogue (c) in ethanol at room temperature. The emission spectra were obtained using an excitation wavelength of 414 nm (the same as that used in the fluorescence upconversion experiments).

showed that the phenol protons had disappeared. δ ppm (CDCl_3): 6.83–6.82 (2H); 4.05–4.21 (18H); 3.27–3.40 (2H); 2.51–2.56 (1H); 1.91–1.94 (3H); 1.62–1.69 (3H).

Results

The steady-state absorption and emission spectra of hypericin, hypocrellin A, and its O-methyl analogue are presented in Figure 2.

Fluorescence upconversion traces of hypericin and hypocrellin A are presented in Figures 3–7. The significant results are that a ~ 10 ps rising component is observed on the blue edge of the emission spectra in all cases, except for hypocrellin A in DMSO; no measurable long-lived (i.e., 50–230 ps) rising or decaying component is observed in the hypocrellin (or hypericin) fluorescence upconversion traces; and no deuterium isotope effect is observed on the ~ 10 ps component for either hypericin or hypocrellin A.

Figure 7 presents the polarized fluorescence data of hypocrellin A in DMSO, ethanol, and octanol. The crucial result

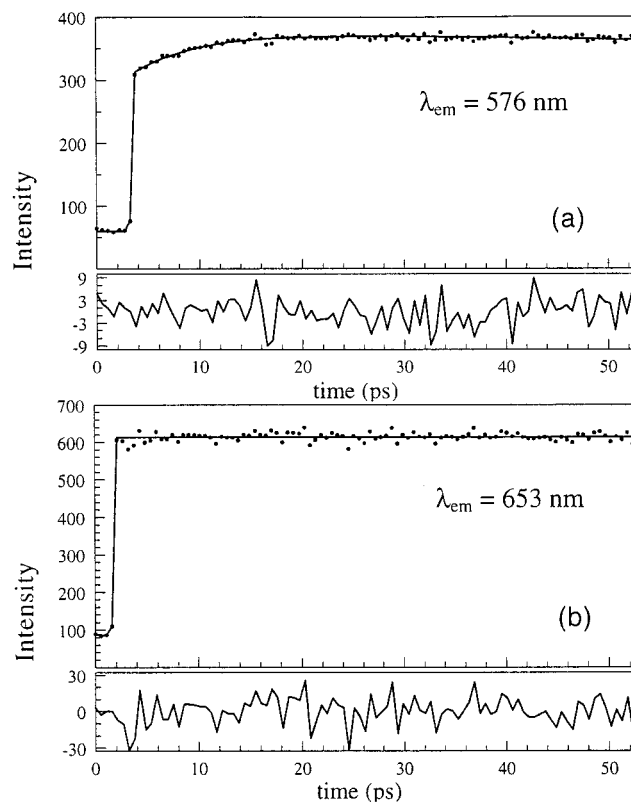


Figure 3. (a) Fluorescence upconversion transient for hypericin in DMSO at $\lambda_{em} = 576$ nm. The fit curve is described by the following equation (with background subtracted): $F(t) = -0.21 \exp(-t/6.5 \text{ ps}) + 1.00 \exp(-t/\infty)$. (b) Fluorescence upconversion transient for hypericin in DMSO at $\lambda_{em} = 653$ nm. The fit curve is described by the following equation (with background subtracted): $F(t) = 1.00 \exp(-t/\infty)$. The excitation wavelength was the second harmonic of our unamplified Ti:sapphire oscillator, 414 nm.

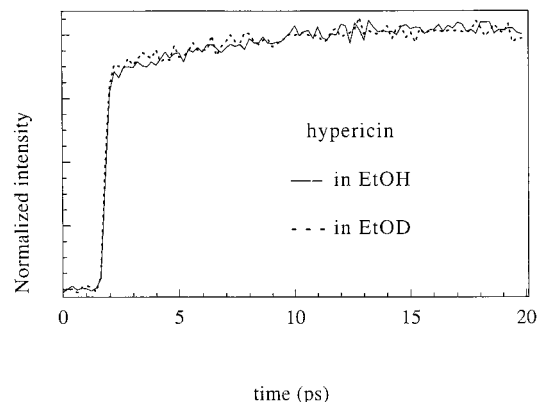


Figure 4. Lack of deuterium isotope effect in the hypericin excited-state H atom transfer in ethanol demonstrated by fluorescence upconversion with $\lambda_{ex} = 415$ nm and $\lambda_{em} = 576$ nm.

here is that no ~ 10 ps component is observed in the anisotropy decay, indicating that the origin of this component cannot be attributed to the relaxation of a higher lying excited state.

Figure 8 indicates the absence of the ~ 10 ps rising component in the O-methyl analogue of hypocrellin A (Figure 1), further suggesting that its origin is intramolecular H atom transfer.

Discussion

A. Assignment of the ~ 10 ps Component to Intramolecular H Atom Transfer. The original assignment of the ~ 10 ps component in hypericin to intramolecular H atom transfer was

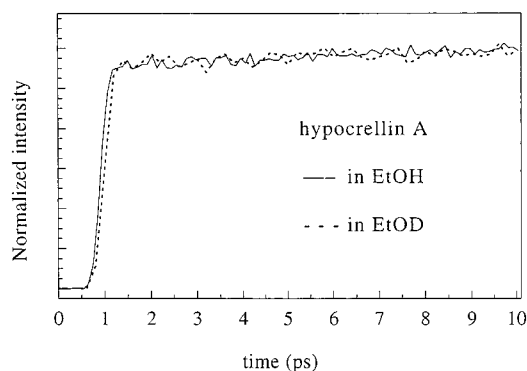


Figure 5. Lack of deuterium isotope effect in the hypocrellin A excited state H atom transfer in ethanol demonstrated by fluorescence upconversion with $\lambda_{\text{ex}} = 415$ nm and $\lambda_{\text{em}} = 576$ nm.

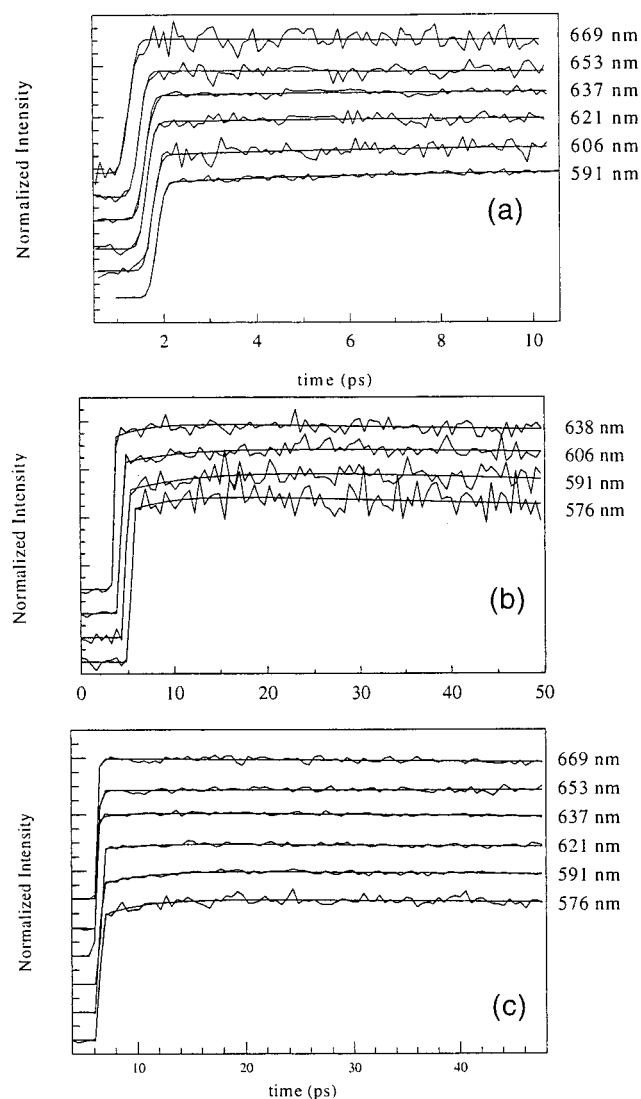


Figure 6. (a) A series of upconversion traces for hypocrellin A in octanol collected at different emission wavelengths. $F(t) = -0.10 \exp(-t/4.1 \text{ ps}) + 1.00 \exp(-t/\infty)$, $\lambda_{\text{em}} = 591$ nm. (b) A series of upconversion traces for hypocrellin A in acetonitrile collected at different emission wavelengths. $F(t) = -0.13 \exp(-t/5.4 \text{ ps}) + 1.00 \exp(-t/\infty)$, $\lambda_{\text{em}} = 591$ nm. (c) A series of upconversion traces for hypocrellin A in ethanol collected at different emission wavelengths. $F(t) = -0.10 \exp(-t/4.8 \text{ ps}) + 1.00 \exp(-t/\infty)$, $\lambda_{\text{em}} = 591$ nm.

based on the spectroscopic properties of the analogue meso-naphthobianthrone in various solvents and the observation of a rise time in the stimulated emission of hypericin (see Introduc-

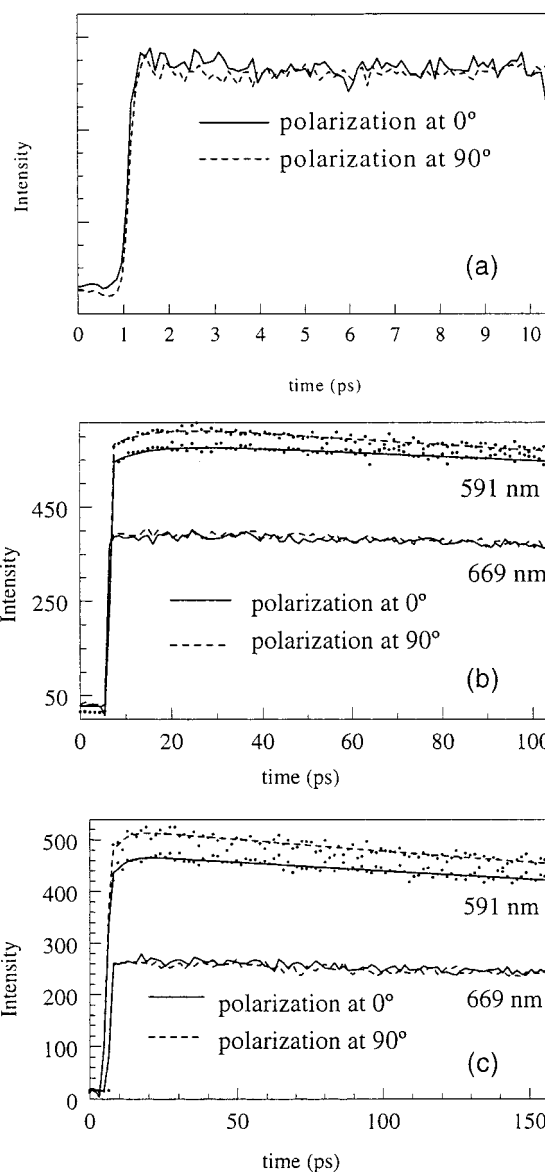


Figure 7. (a) Absence of fluorescence anisotropy decay of hypocrellin A in DMSO collected at 591 nm. Same was observed for other emission wavelengths up to 637 nm. (b) Fluorescence anisotropy decay of hypocrellin A in ethanol collected at 591 and 669 nm. Fit results for 591 nm: $F(t) = 1.00 \exp(-t/1012 \text{ ps}) - 0.0785 \exp(-t/7.9 \text{ ps})$; $r(t) = -0.032 \exp(-t/282 \text{ ps})$. (c) Fluorescence anisotropy decay of hypocrellin A in octanol collected at 591 and 669 nm. Fit results for 591 nm: $F(t) = 1.00 \exp(-t/1113 \text{ ps}) - 0.141 \exp(-t/3.65 \text{ ps})$; $r(t) = -0.034 \exp(-t/502 \text{ ps})$.

tion). Additional evidence was provided by the investigation of *O*-methyl analogues^{9–11} and the observation of the ~ 10 ps component in fluorescence upconversion.¹¹ The latter eliminates the possibility that the transient arises from spurious excited-state absorbance decays that might overlap the stimulated emission spectrum. Also, when hypericin is complexed with human serum albumin,¹⁸ this fast component is not observed. This further strengthens the assignment of the process to H atom transfer, since Raman measurements²⁶ have indicated that hypericin binds to HSA by means of a specific hydrogen bonded interaction between its carbonyl oxygen and the $\text{N}_1\text{-H}$ of the tryptophan residue in the IIA subdomain of HSA. We have suggested that this result indicates that when the carbonyl is no longer free to accept the H atom owing to coordination with HSA the H atom transfer process is completely impeded. This

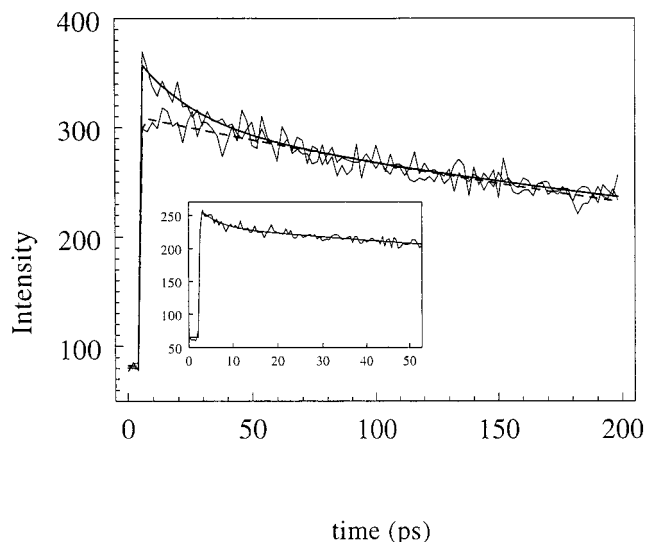


Figure 8. Fluorescence upconversion traces obtained at $\lambda_{em} = 576$ nm (solid) and $\lambda_{em} = 653$ nm (dashed) for the O-methyl hypocrellin A analogue in ethanol. The inset shows a fast decay component on a smaller time scale at $\lambda_{em} = 560$ nm, where it was the most prominent. $\lambda_{ex} = 414$ nm. Fit results: $F(t) = 1.00 \exp(-t/466 \text{ ps})$ for 653 nm; $F(t) = 0.20 \exp(-t/24 \text{ ps}) + 0.80 \exp(-t/560 \text{ ps})$ for 576 nm; and $F(t) = 0.14 \exp(-t/4.4 \text{ ps}) + 0.86 \exp(-t/300 \text{ ps})$ for 560 nm. Fast excited-state decay components are observed in several perylene quinone analogues and in hypericin itself.^{7–10} We have suggested that these arise from possible dynamic solvation of higher lying singlet states or from prompt intersystem crossing. These fast decaying components stand in contrast to the slower ≥ 10 ps rising components attributed to intramolecular H atom transfer.

seems to imply that the H atom transfer is a concerted process yielding the double tautomer (Figure 1).

The ~ 10 ps rise observed in the fluorescence upconversion signal for hypocrellin A in this work is also absent in the O-methyl analogue (Figure 8), which lacks labile H atoms. In addition, no component of this duration is observed in the fluorescence anisotropy decay of hypocrellin A. Hypocrellin A, like hypericin, has its $S_0 \rightarrow S_1$ transition polarized at large angles to its $S_0 \rightarrow S_2$ transition.¹⁶ If a ~ 10 ps fluorescence anisotropy decay were observed, it could be attributed to excited-state relaxation from S_2 to S_1 . On the other hand, a rapid relaxation was observed in the fluorescence anisotropy decay of 7-azaindole dimer,²⁷ which also possesses transition dipole moments to closely lying excited states at large angles to each other: in this case, the observation was used to argue quite convincingly that the proton-transfer reaction in the 7-azaindole dimer in the condensed phase does not proceed by means of an intermediate, as has been argued to be the case in the gas phase.^{28,29}

Vibrational cooling effects may be eliminated since, again, the ~ 10 ps component is not observed in the O-methyl analogues and also because in hypericin and hypocrellin, this component is observed using excitation wavelengths of 294, 415, and 588 nm.^{9–11} Last, solvent relaxation effects for S_1 can be discarded since no dynamic Stokes shifts have been observed¹¹ on this or any other time scale.

B. A Unified Picture of the Hypericin and Hypocrellin Photophysics. We had previously begun to construct a picture that attempts to unify the intramolecular photophysics of hypericin and hypocrellin A. The key points, now refined and augmented, upon which this picture is based are as follows.

(1) The H atom transfer time for hypericin is ~ 10 ps. For hypocrellin A, as observed in the fluorescence upconversion data and in the transient absorption data,^{12,15} a ~ 10 ps component is observed in addition to a longer-lived component

with a time constant from 50 to 250 ps in the solvents we have studied. The time constant for the shorter-lived (i.e., 10 ps) component in hypericin and hypocrellin A has a negligible dependence on solvent.⁸ For hypocrellin A, the time constant of the longer-lived H atom transfer is very strongly dependent on viscosity.^{14,17}

(2) No deuterium isotope effect is observed for hypericin⁷ or for the ~ 10 ps component of hypocrellin A (Figure 5). But a deuterium isotope effect of 1.4 is observed for the longer component of hypocrellin A.¹³ This isotope effect unambiguously identifies this excited-state process as an H atom transfer event.

(3) The rates of excited-state intramolecular H atom transfer reactions of hypericin and hypocrellin A have been measured as a function of temperature in an ethanol/methanol mixture. The data yield activation energies of 0.044 ± 0.008 and 2.12 ± 0.07 kcal/mol for hypericin and for the longer-lived component of hypocrellin A, respectively.¹⁷ Much of the barrier cited above for hypocrellin A results from the temperature dependence of the viscosity of the solvent mixture. The viscosity-independent part of the activation barrier, 0.41 ± 0.088 kcal/mol, is larger than that for hypericin but still quite small. The viscosity-dependent barrier in hypocrellin A and the negligible barrier in hypericin are most easily explained by NMR measurements, which show a flexible seven-membered ring in the bay region of hypocrellin A.¹⁹ Consistent with this is the observation that only one ground-state conformer/tautomer is present for hypocrellin B, whose seven-membered ring is more rigid owing to the presence of a double bond.¹⁹ The exact nature of the conformational changes that are coupled to the H atom transfer reaction in hypericin and hypocrellin A have yet to be identified and are currently under investigation.

(4) X-ray and fluorescence data suggest that hypocrellin A exists at least partially in a tautomeric form in the ground state (Figure 1). This conclusion has now been verified by NMR measurements.¹⁹ Recent 2D ROESY NMR results from our laboratory indicate that only one species (the "normal" species) is populated in the ground state for hypericin.¹⁹ Similar conclusions have been obtained by Falk and co-workers.³⁰

Structurally hypericin and hypocrellin are very similar. They both possess extended aromatic skeletons whose most important functional groups are the hydroxyls peri to the carbonyls. In this regard, the most significant structural difference between them is that hypocrellin possesses two fewer peri hydroxyls. The current picture that we have formed of the excited-state dynamics of hypericin and hypocrellin is that the different photophysical behavior that we have enumerated above of these two structurally very similar molecules arises because we are probing different regions of very similar potential energy surfaces. This picture is crudely illustrated in Figure 9 of ref 15. In this work, we demonstrated that we could simulate our experimental transient absorbance and linear dichroism data using reasonable parameters.

A crucial result in forming this hypothesis is the observation that under certain conditions we resolve a time constant in the hypocrellin transient absorption measurements that is comparable to that observed in hypericin. This ~ 10 ps component in hypocrellin A unifies our picture of the photophysics of hypericin and hypocrellin A if we can interpret it as an excited-state H atom transfer arising from another tautomeric species and if we can relate it to the corresponding process in hypericin. This is, in fact, very likely. Three significantly populated species are observed for hypocrellin A:¹⁹ two "normal" species differing in the orientation of the seven-membered ring (i.e., a gauche or

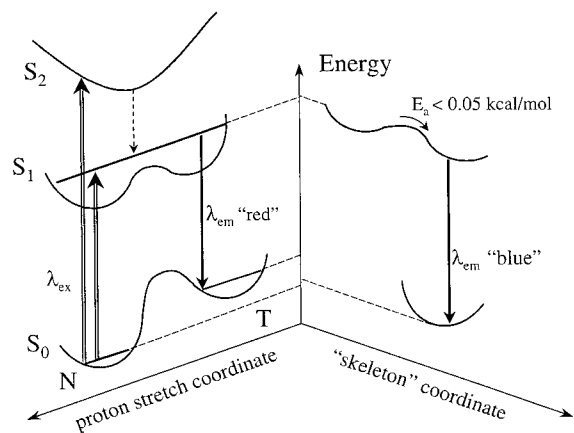


Figure 9. Potential energy surface for hypericin (and hypocrellin A). The oxygen–oxygen distance between which the H atom is transferred strongly modulates the magnitude of the matrix element that couples the reactant and product states and thus determines the size of the barrier separating them. When the O–O distance is $< 2.6 \text{ \AA}$, the *adiabatic limit* is obtained.^{47–51} Here, because the vibrational energy levels of the proton stretch mode lie *above* a small barrier in the proton coordinate separating the reactant and product species, an isotope effect will not be observed as a result of H atom transfer. We have argued that the $\sim 10 \text{ ps}$ reaction in hypericin and hypocrellin A falls into this adiabatic limit.¹⁵ The O–O distances in both hypericin and hypocrellin A are $\sim 2.5 \text{ \AA}$. Staib et al.⁴⁷ however, suggest an intriguing means of obtaining a deuterium isotope effect in the adiabatic limit. Deuterium substitution may lower the ground vibrational energy below the top of the barrier in the proton coordinate. Such a lowering of the ground-state energy level would now induce an isotope effect because now the proton could tunnel through the barrier or effect an activated crossing of it. We have proposed that the isotope effect observed in hypocrellin A for its longer-lived component has its origins in such an explanation. The absence of an isotope effect for the 10 ps component indicates that the zero-point vibrational level lies above the barrier in the proton coordinate and that the reaction coordinate for the H atom transfer cannot be identified with the proton coordinate. Trapping the system in the tautomer well is effected by a low-amplitude conformational change in the “skeleton” coordinate. An interesting feature revealed in Figures 3 and 6 is that the rise time for the fluorescence occurs on the *high-energy* or blue edge of the fluorescence spectrum. This diagram attempts to provide a plausible explanation of how a rise in “blue” emission can occur by making the energy difference between the ground- and excited-state double tautomer larger in the skeleton coordinate than in the proton coordinate.

anti conformation about the C14–C13 bond or the C14–C16 bond) and a double tautomer in the gauche conformation.

C. Construction of Potential Energy Surfaces. Our emerging model for the excited-state photophysics of hypericin and hypocrellin A is a refinement of that presented elsewhere.¹⁵ The most significant addition to the model is the detection and identification of three significantly populated ground-state species for hypocrellin A¹⁹ and the determination of the temperature dependence of the excited-state processes (see above).

The absence of a deuterium isotope effect on the excited-state H atom transfer for the $\sim 10 \text{ ps}$ component in hypericin and hypocrellin A is interpreted to mean that the reaction coordinate for this process cannot be identified with the proton coordinate and that other intramolecular motions are in fact responsible for the process. Recent temperature-dependent measurements indicate that these motions are extremely low-amplitude, $E_a = 0.044 \pm 0.008 \text{ kcal/mol}$ for hypericin.¹⁷ Because the nature of this motion is not yet identified, we refer to it as the *skeleton coordinate* (Figure 9).

On the basis of the importance of the conformation of the seven-membered ring of hypocrellin A in determining the

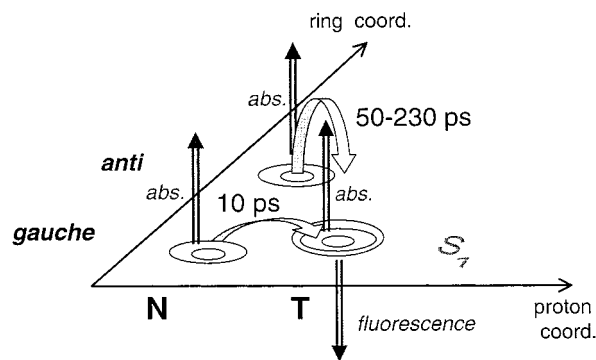


Figure 10. S_1 potential surface of hypocrellin A. Along with the hypericin-like proton and skeleton coordinates, this molecule exhibits an additional degree of freedom associated with the twisting motion of the seven-membered ring (the “ring” coordinate). NMR 2D ROESY data¹⁹ indicate that motion of the seven-membered ring is coupled to H atom transfer in the ground state. As discussed in the text, in the excited state the kinetic relationship between the conformational and tautomeric isomers is different than in the ground state. In the text, a rationalization for the absence of rising 50–250 ps components in the fluorescence signal is also presented. (Recently, a study of the excited-state kinetics of hypocrellin A based on its nonlinear absorptive and refractive properties has appeared. This study is highly model dependent and relies upon fitting the data to several variable parameters. Contrary to our conclusions, it was determined that only two singlet species are involved in the excited-state kinetics.^{52,53})

population of its ground-state species and on the strong viscosity dependence of its longer-lived H atom transfer component, we suggest the role of a *ring coordinate* that is coupled to the H atom transfer (Figure 10). Consequently, the two optically excited normal species of hypocrellin A decay into the fluorescent tautomer species with an $\sim 10 \text{ ps}$ time constant and a longer-lived, viscosity-dependent, time constant.

A potential problem with this latter picture is the inability to observe a 50–230 ps component in the hypocrellin A emission. This may, however, be rationalized by noting that such a component will not be observed on a short time scale if its amplitude is small or comparable to that of the $\sim 10 \text{ ps}$ component. We have simulated kinetic traces and have concluded that up to 15% of a 50 ps rising component and 30% of a 230 ps rising component would be undetectable given the signal-to-noise levels afforded by our apparatus (Figure 11). The observation of the 50–230 ps component on a longer time scale is also prohibited for similar reasons because of the presence of the dominating $\sim 1 \text{ ns}$ fluorescence decay process.¹⁰

D. Outstanding Problems. *i. Ground-State Heterogeneity.* As we note in the Conclusions section, a possible objection to our assignment of the excited-state reaction to H atom transfer in these systems is the observation of mirror image symmetry between the absorption and emission spectra, which indicates minimal structural changes between the absorbing and emitting species, not usually the case for excited-state tautomerization. Our first attempt to respond to this criticism was to suggest that the ground state was populated with at least one other species, for example, a monotaomer.^{7,8} This seemed to be reasonable, especially given the breadth of the visible absorption spectrum; there are no “gaps” of zero absorbance anywhere between the ultraviolet and $\sim 600 \text{ nm}$. This suggestion also seemed to be supported by the observation that the transient absorbance^{9,10} and upconversion¹¹ kinetics of hypericin differ with excitation wavelength and probe wavelength, respectively. For hypericin, however, evidence is emerging that the ground-state is much less heterogeneous than we had believed. On the basis of ab initio calculations (RMP2/6-31G(d) level of theory,

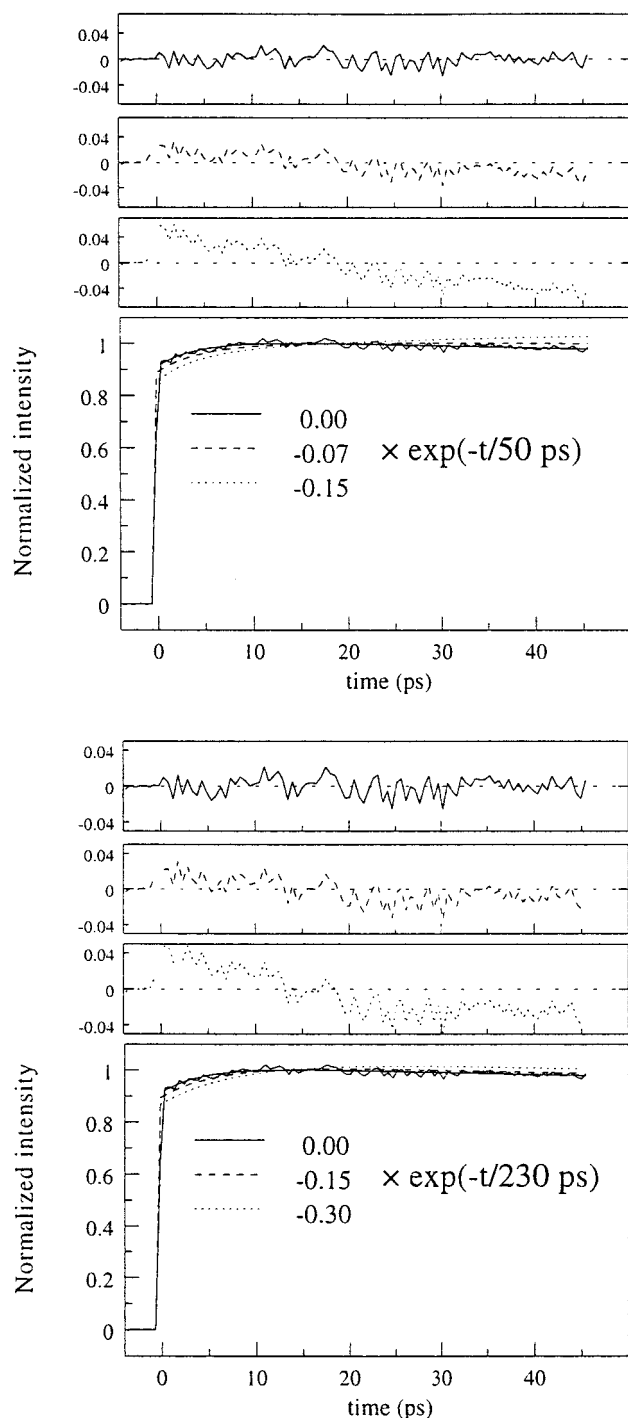


Figure 11. Simulated fluorescence upconversion traces with the increasing amplitudes of additional 50 ps (top) and 230 ps (bottom) rising components. The residuals show deviations from the data collected for hypocrellin A in octanol with $\lambda_{em} = 591$ nm. The experimental data are well described by the following function: $F(t) = -0.10 \exp(-t/4.5 \text{ ps}) + 1.0 \exp(-t/1200 \text{ ps})$.

using geometries obtained with the 3-21G basis and Hartree–Fock wave functions), only one hypericin species, the “normal” form, is populated in the ground state for an unionized gas-phase species.³¹ Furthermore, on the NMR time scale, only one species of hypericin appears to be present in the ground state.¹⁹ On the other hand, as noted above, three significantly populated species are observed for hypocrellin A.¹⁹

ii. Reconciling the Kinetics with the Ground-State Populations. First, we consider hypocrellin A. The excited-state dynamics modeled in Figure 9 and especially Figure 10 are an

extension of the model presented elsewhere,¹⁵ enhanced by the knowledge that on the NMR time scale three ground-state species are present. As noted above, *it is an assumption of the model that only the “tautomer,” not the “normal” species is significantly fluorescent.* Consequently, to justify the absence of 50–230 ps rise times in the fluorescence upconversion kinetics, it was necessary to conclude that the concentration of the species giving rise to them is smaller than or comparable to that producing the ~ 10 ps component.

The NMR measurements indicate that in the ground state there is 60% gauche double tautomer (gD), 30% anti normal tautomer (aN), and 10% gauche normal tautomer (gN). In the ground state, these species are related sequentially, by the following equilibria: $gD \leftrightarrow aN \leftrightarrow gN$. On the basis of our model¹⁵ and the requirement that only the tautomer is significantly fluorescent, we conclude that, in the excited state, the relation among the species is different: $gN^* \rightarrow gD^* \leftarrow aN^*$ (Figure 10).

Figure 7 indicates that, at the bluest emission wavelengths, the amplitude of the ~ 10 ps component is roughly 10% that of the instantaneously emitting component. In other words, this would seem to imply that, based upon the fluorescence upconversion measurements, the ground-state population is 90% gD and 10% gN (the decay of gN^* being assigned to the ~ 10 ps component because it does not involve a conformational change of the seven-membered ring).

The discrepancy in the reckoning of the ground-state population of the tautomers by NMR or fluorescence can be minimized by acknowledging that the fluorescence measurements may not detect between 15% and 30% of a longer-lived rising component. Thus, the populations determined from fluorescence may be correspondingly adjusted to 75% gD, 15% aN, and 10% gN. (Currently we have no clear explanation for why the 10 ps component is absent in the upconversion trace for hypocrellin A in DMSO.)

The problem, however, is more acute for hypericin; only one species is identified in the NMR and yet we observe an “instantaneous” rise in the upconversion signal as well as a rising component whose amplitude is $\sim 10\%$ that of the instantaneous component. On the basis of our previous reasoning, this would seem to suggest that there are at least two species present in the ground state. It is possible that there are ground-state species interconverting faster than the NMR time scale of 300 ms. If this were the case, the “instantaneous” rise observed in the fluorescence of hypericin and perhaps also of hypocrellin A might be explained by an ultrafast conversion (< 300 fs) of such a species to form the double tautomer. The resolution to this question clearly requires work using higher time resolution and a tunable excitation source.

Another possibility, related to the above explanation, can be formulated in terms of Figure 9. Immediately upon optical excitation, the H atom remains *delocalized* between the enol and keto oxygens. Thus, once in each period of the O–H vibration ($\sim 3000 \text{ cm}^{-1}$ in ground-state hypericin³¹ or 11 fs), the excited state is expected to be “tautomer-like,” and thus fluoresce. The H atom is subsequently localized on an ~ 10 ps time scale owing to a small structural change of the aromatic skeleton.

Conclusions

The fluorescence upconversion measurements of hypericin and hypocrellin A presented here provide detailed and complementary information to the transient absorption data of ref 15

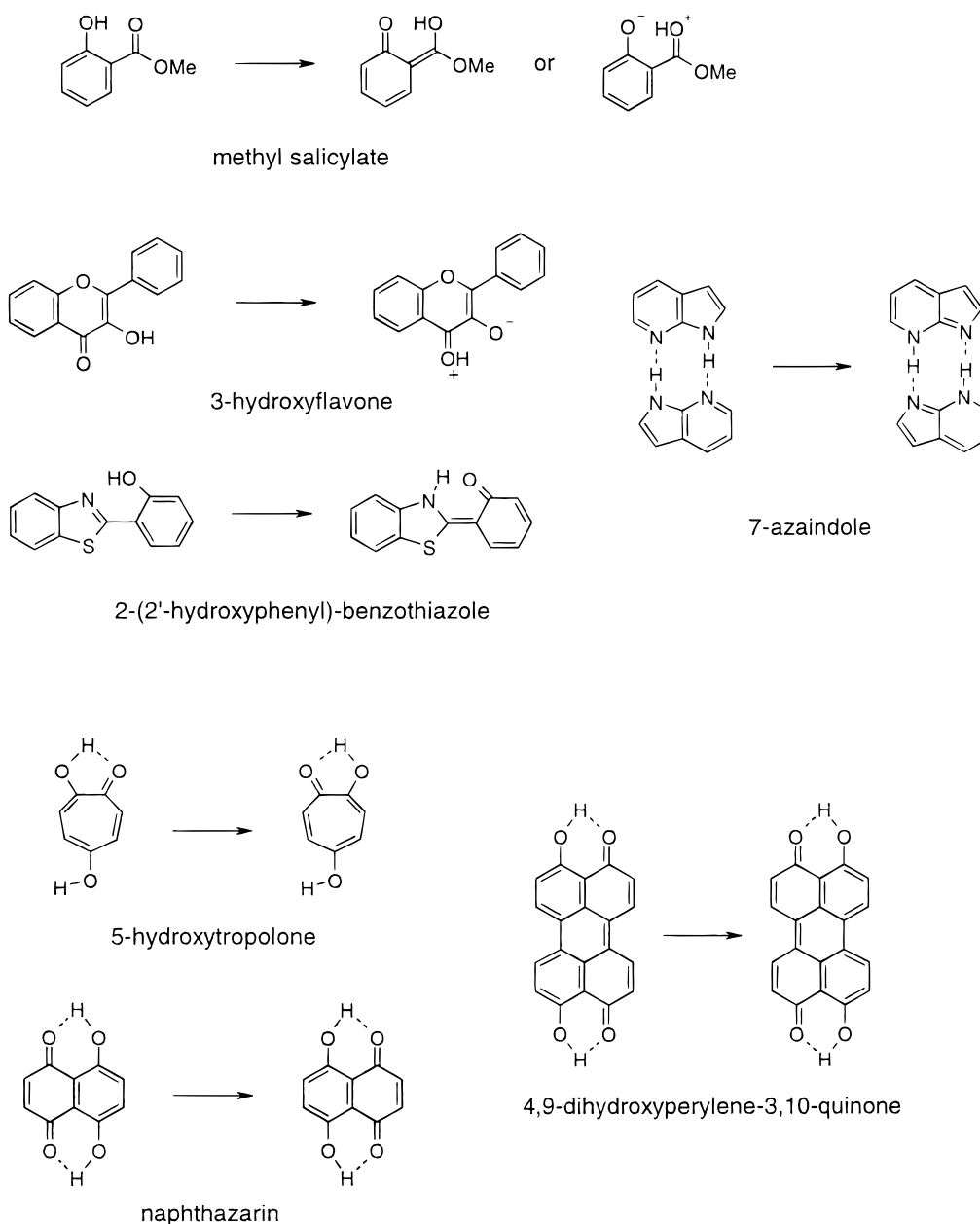


Figure 12. Proton transfer species. In the upper half of this figure the product of tautomerization is structurally and electronically different from the educt. In contrast, H atom transfer in 5-hydroxytropone results in nearly identical species and double tautomers of naphthazarin and 4,9-dihydroxyperylene-3,10-quinone are the mirror images of “normal” structures.

and permit a refinement of the model of the ground- and excited-state potential energy surfaces initially developed therein.

In conclusion, we address two objections that are still occasionally raised to the assignment of the excited-state processes in hypericin and hypocrellin to excited-state H atom transfer. These are the absence of a deuterium isotope effect in hypericin and in the shorter-lived component of hypocrellin A and the mirror image symmetry between the absorption and emission spectra in hypericin and hypocrellin.

We have treated the lack of a deuterium isotope effect in hypericin at length elsewhere. *Its absence is easily attributed to the reaction coordinate not being identified with the proton coordinate*, as we have depicted in Figure 9. There is precedent for this in other systems.^{32–34}

Requiring the *absence* of mirror image symmetry between the absorption and emission spectra assumes that the potential energy surface of the emitting species is significantly different from that of the absorbing species. Such a displacement in the

coordinate of the emitting species is clearly evident in the most commonly studied proton-transfer systems (Figure 12): methyl salicylate,^{33,35} 7-azaindole dimer,^{27,36} 2-phenyl-benzotriazole,³⁷ 3-hydroxyflavone.^{38–42}

If, however, we consider systems in which the normal and tautomer species are symmetric, or nearly so, this disparity no longer exists or is significantly minimized. 5-Hydroxytropone^{43–45} presents an excellent example of such a case. Other examples are the double H atom transfer in naphthazarin⁴⁶ and in the 4,9-dihydroxyperylene-3,10-quinone subunit of hypocrellin, producing entirely symmetric structures (Figure 12).

We argue that hypericin and hypocrellin A have very similarly symmetric normal and tautomeric forms as indicated by the highlighted bond systems in Figure 1. That is, regardless of the tautomeric form in which the molecule finds itself, there is always conserved an aromatic core to which is attached a hydroxyl group *peri* to a carbonyl group. Even in the case of the monotaomerized species, it is possible to draw resonance

forms that upon superposition restore the aromatic character of the substructure involved in the H atom transfer reaction. Consequently, we conclude that the mirror image symmetry observed in hypericin and hypocrellin is not at all surprising. If, on the other hand, the excited-state reaction were a genuine proton transfer, then the resulting charge-separated species would be expected to exhibit an emission spectrum significantly different from that of the absorption spectrum, as in 3-hydroxyflavone, and the rate of reaction should be very sensitive to solvent polarity, which is not the case for hypericin or hypocrellin.⁵⁴ To avoid any possible confusion in this matter, we now refer to the excited-state reactions in hypericin and hypocrellin as excited-state H atom transfer.

Acknowledgment. This work was supported by NSF Grant CHE-9613962 to J.W.P.

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- The hypocrellin A reaction does have a solvent dependence. But this dependence is upon viscosity and arises from conformational changes of the seven-membered ring that are coupled to the H atom transfer reaction.^{14,19} The reported dependence of the hypocrellin A reaction on solvent polarity is “indirect” and occurs only for solvents within a certain class, such as nitriles or alcohols. The apparent polarity dependence arises because the viscosity increases along with the polarity. A genuine polarity dependence would be expected if the reaction were a proton transfer, rather than an H atom transfer.