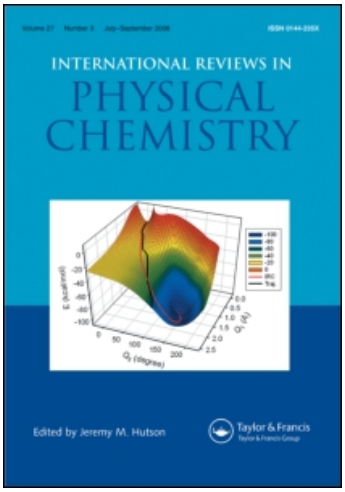

International Reviews in Physical Chemistry
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713724383

Excited-state intramolecular H-atom transfer in nearly symmetrical perylene quinones: Hypericin, hypocrellin, and their analogues

J. W. Petrich[a]
[a] Department of Chemistry, Iowa State University, Ames, Iowa, USA

Online publication date: 26 November 2010

To cite this Article Petrich, J. W.(2000) 'Excited-state intramolecular H-atom transfer in nearly symmetrical perylene quinones: Hypericin, hypocrellin, and their analogues', International Reviews in Physical Chemistry, 19: 3, 479 — 500
To link to this Article: DOI: 10.1080/01442350050034207
URL: http://dx.doi.org/10.1080/01442350050034207

INT. REVIEWS IN PHYSICAL CHEMISTRY, 2000, VOL. 19, NO. 3, 479–500

Excited-state intramolecular H-atom transfer in nearly symmetrical perylene quinones: hypericin, hypocrellin, and their analogues

J. W. PETRICH

Department of Chemistry, Iowa State University, Ames, Iowa 50011, USA

H-atom transfer and proton transfer reactions, like electron transfer reactions, are of fundamental importance in both the physical and biological sciences. H-atom transfer and proton transfer reactions lie at the heart of acid–base chemistry and of a wide range of catalytic reactions in biological systems. Although much progress has been made in understanding electron transfer reactions through the combination of experimental and theoretical work, many aspects of excited-state H-atom and proton transfer reactions are poorly understood, in particular, the way in which the solvent or the intramolecular modes of the solute couple to the reaction. We argue that hypericin and the hypocrellins undergo excited-state intramolecular H-atom transfer reactions. The hypericin and hypocrellin reactions are, relatively speaking, very slow, occurring in about 10 ps for hypericin and from 10–250 ps for hypocrellin A and may be explained in terms of a reaction coordinate that is dominated by intramolecular motions of the aromatic skeleton and the side chains. The observation of a 10 ps transient in hypocrellin A (which, like its analogue in hypericin, lacks a deuterium isotope effect) is essential in attaining a unified understanding of the hypericin and hypocrellin photophysics. Without this common 10 ps component, the photophysics of these two systems bear no similarities and are seemingly unrelated. Our assignment of intramolecular H-atom transfer to the excited-state kinetics has at times been controversial, owing largely to the mirror image symmetry between the absorption and emission spectra and to the absence of deuterium isotope effects. These topics are discussed in detail and we conclude that neither the absence of mirror image symmetry nor the presence of an isotope effect is a *conditio sine qua non* for a H-atom transfer reaction.

1. Introduction

Hypericin and hypocrellin (figure 1) are naturally occurring perylene quinones that have gained great interest recently owing to their light-induced biological activity [1–5]. They display virucidal activity against several types of viruses, including the human immunodeficiency virus (HIV) [6–8], as well as antiproliferative and cytotoxic effects on tumour cells [9–11]. Hypericin is also a potent antidepressant [12, 13], exhibits light-dependent inhibition of protein kinase C (PKC) [14], and is reported to possess numerous other types of biological behaviour [15–20]. Hypericin, like other anticancer drugs, also induces apoptosis [10, 21, 22].

Owing to this important biological activity, over the past few years we have been studying the photophysics of hypericin and hypocrellin [23–34]. By means of H/D substitution, investigation of *O*-methylated analogues, and complementary studies using both transient absorption and fluorescence upconversion spectroscopies, we have argued that the major primary photophysical process in hypericin and hypocrellin A in organic solvents is excited-state hydrogen atom transfer. Considerable effort was required to demonstrate this fact owing to the unusual mirror image symmetry between absorption and emission spectra, the lack of a H/D isotope effect on the proton transfer reaction in hypericin, and the occasional consideration of this ultrafast reaction in terms of equilibrium Förster-cycle type calculations [35].

International Reviews in Physical Chemistry ISSN 0144-235X print/ISSN 1366-591X online
http://www.tand.co.uk/journals

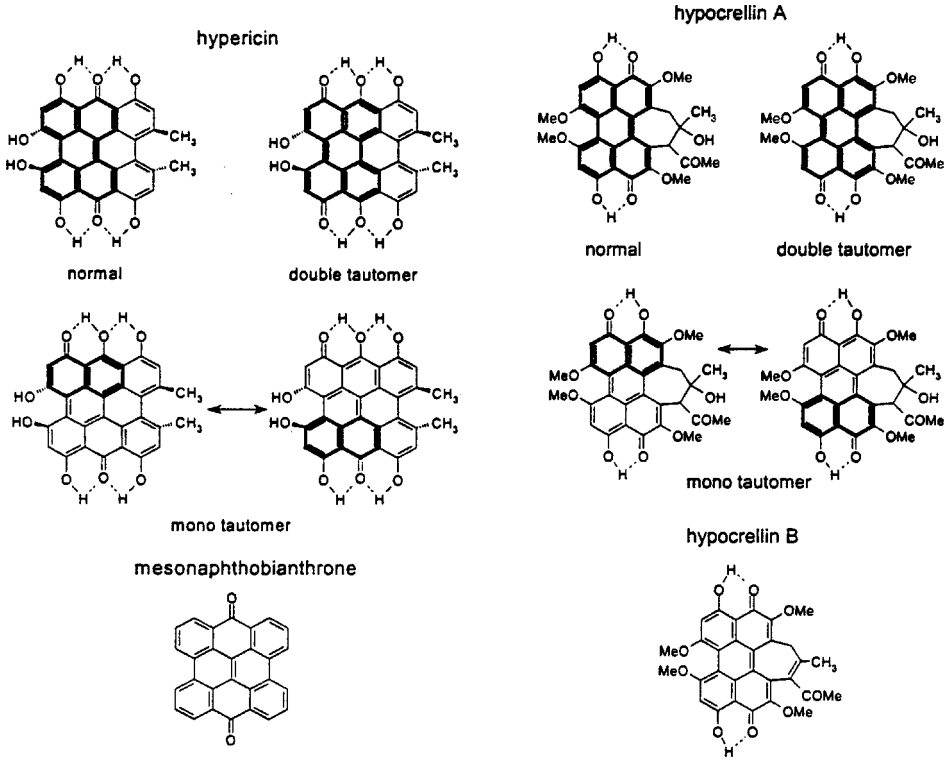

Figure 1. Hypericin (normal form, double tautomer, and monotautomer), mesonaphthobianthrone, hypocrellin A (normal form, double tautomer, and monotautomer), hypocrellin B.

We have suggested that the labile protons resulting from the intramolecular proton transfer reaction may be important for understanding the light-induced biological activity of hypericin and hypocrellin A. Notably, hypericin and hypocrellin A acidify their surroundings upon light absorption [36–38]. The role of photogenerated protons take on significance in the context of the growing body of literature implicating changes in pH with inhibition of virus replication [39], antitumour activity [40, 41], and apoptosis (programmed cell death) [42–44]. For example, comparative studies for nine perylenequinones, including hypericin, provide evidence that the quantum yield of singlet oxygen formation is not sufficient to explain the reported antiviral activities of these molecules and that other structural features of perylene quinones are involved [45]. In fact, the quantum yield of singlet oxygen from hypericin is much less than had initially been presumed. Recently, Jardon and co-workers have revised their earlier estimation of a singlet oxygen quantum yield of 0·73 [46, 47], essentially equal to the triplet yield, to 0·35 in ethanol and less than 0·02 in water [48]. Based on this result, mechanisms involving only oxygen clearly cannot explain all the activity of hypericin.

We had previously reported that hypericin does not require oxygen for its antiviral activity [4, 33, 49, 50]. This conclusion was based on an inability to estimate accurately low oxygen levels in our virus samples. Our most recent results indicate that while antiviral pathways independent of oxygen may exist, the role of oxygen in this activity is significant, although it seems to differ for hypericin and hypocrellin A [51]. For

example, the ability of photogenerated protons to enhance the activity of activated oxygen species may be of importance.

The plan of this review is as follows. First, we briefly describe the experimental techniques employed in our investigations of these systems and our reasons for using them (section 2). Second, we outline the salient experimental observations concerning hypericin, hypocrellin and their analogues and furnish evidence for excited-state H-atom transfer in these systems (section 3). Third, we discuss the reaction coordinate for the H-atom transfer and attempt to justify the apparently disparate photophysics of hypericin and hypocrellin (section 4). Fourth, we revisit objections that have been raised to our assignment of H-atom transfer as the fundamental primary photoprocess in hypericin and hypocrellin (section 5). Fifth, we discuss the major unresolved questions regarding the photophysics of these systems (section 6).

2. Experimental methods

Most of the data discussed in this review are based on time-resolved ultrafast spectroscopic methods. Two types of complementary experiments are involved: pump–probe absorption and fluorescence upconversion. It is not our intent to describe the mechanics of these experiments, for which the detailed descriptions of the apparatus may be found elsewhere [31, 33, 52, 53]. We wish, however, to point out some important aspects of these two techniques. Both derive their time resolution from the duration of the laser pulses involved. In each experiment, two pulses are used. The first and more intense of the two, the pump pulse, perturbs the system from equilibrium, usually putting it into an excited state. The second pulse probes this perturbation at various delay times subsequent to the arrival of the pump pulse. In the pump–probe 'absorption' technique, the probe pulse interrogates the sample by monitoring bleaching of absorbance, induced absorbance from excited states and photochemically generated species, and stimulated emission from emissive states. It is possible that all three of these phenomena may be observed simultaneously depending on the complexity of the system. In the fluorescence upconversion technique, a pump pulse populates the excited state of the sample. The molecular mission is collected efficiently and imaged into a nonlinear optical gating crystal. This spontaneous emission is then gated or 'upconverted' with a replica of the pump pulse: the sum of the emission light frequency and the gating pulse frequency is detected as a function of the delay of the gate pulse with respect to the pump pulse.

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, the interpretation of 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.

The power of the fluorescence upconversion technique is demonstrated in this review because it permits the observation of 10 ps transients in *both* hypericin and hypocrellin A [53], which is essential in attaining the unified view of the photophysics

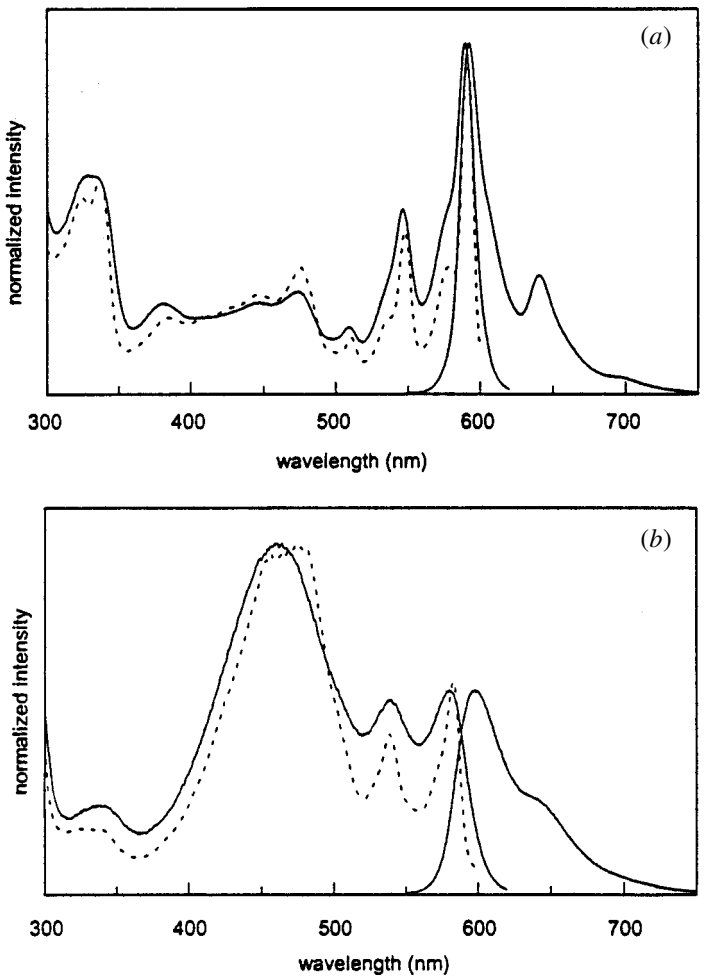

Figure 2. Comparison of the fluorescence excitation and emission spectra of (*a*) hypericin and (*b*) hypocrellin in a 1:1 ethanol/methanol mixture at room temperature. The excitation spectra were measured at 295 K (solid line) and at 77 K (dashed line). The excitation spectra were monitored at 650 nm at 295 K and at 620 nm at 77 K.

of these systems which is presented here. Using transient absorption techniques, the presence of these transients in hypocrellin A can be detected only in very viscous solvents or by studying the anisotropy decay of the system [29].

3. Excited-state intramolecular H-atom transfer in hypericin and hypocrellin

3.1. *Argument for H-atom transfer*

Our argument for intramolecular excited-state H-atom transfer in hypericin is as follows. The deshydroxy analogue of hypericin, mesonaphthobianthrone (figure 1), is non-fluorescent except in strong acids [24, 33] (e.g. sulphuric acid) where it produces a fluorescence spectrum that has nearly the same shape as that of hypericin in alcohols (figure 2). *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 sulphuric acid where it is instantaneous.

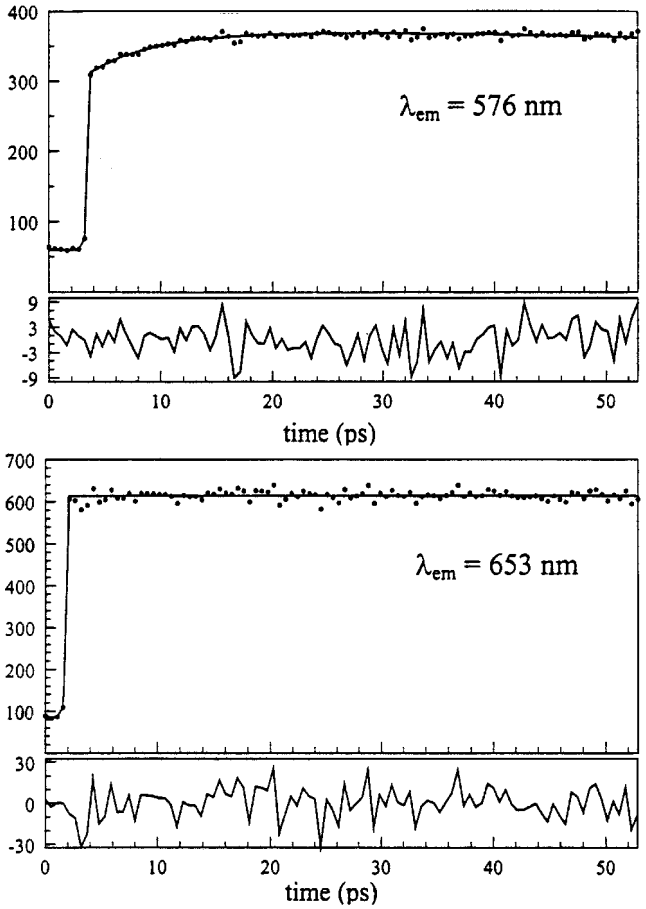

Figure 3. Fluorescence upconversion transient for hypericin in ethanol at λ_{em} = 576 nm. The fitted curve is described by the following (with background subtracted): $F(t) = -0{\cdot}21 \exp(-t/6{\cdot}5\text{ ps}) + 1{\cdot}00 \exp(-t/\infty)$. At λ_{em} = 653 nm, however, there is no rising component in the fluorescence trace. Similar behaviour is observed for hypocrellin A (see figure 4). The excitation wavelength was the second harmonic of our unamplified Ti:sapphire oscillator, 414 nm. The panel below the kinetic trace displays the residuals between the fit and the data.

Thus, *the rise time for the appearance of the hypericin emission is taken as evidence for an excited-state H-atom transfer* [24, 54]. Confirming this interpretation are the fluorescence upconversion measurements of hypericin and *O*-methyl hypericin analogues [33, 34, 52, 53], which are incapable of executing intramolecular excited-state H-atom transfer reactions. Hypericin exhibits a rise in its fluorescence signal whereas the methylated derivatives do not. Also consistent with this interpretation is the recent observation that the oxidative electron-transfer quenching of triplet hypericin cannot be adequately described in terms of the reduction potential of hypericin in its 'normal' form, thus suggesting that the triplet species is tautomerized [55].

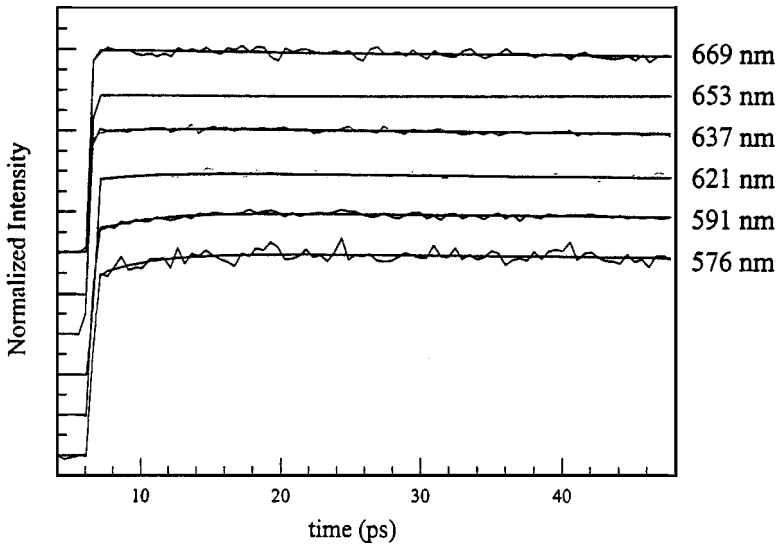

Figure 4. A series of upconversion traces for hypocrellin A in octanol collected at different emission wavelengths. $F(t) = -0{\cdot}10\exp(-t/4{\cdot}1\text{ ps}) + 1{\cdot}00\exp(-t/\infty)$, $\lambda_{em} = 591$ nm. Note that at redder emission wavelengths, the amplitude of the rising component is negligible.

3.2. *Comparison of hypericin and hypocrellin A*

3.2.1. *Range of time constants for the H-atom transfer*

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 (figures 3 and 4). For simplicity, in the rest of our discussion, we refer to this shorter-lived component at the '10 ps component'. The amplitude of the rising component is emission wavelength dependent and occurs on the blue edge of the emission spectra. The clear and complementary observation in fluorescence of the ~ 10 ps component in both hypericin and hypocrellin A is a crucial link in providing a unified model of the hypericin and hypocrellin photophysics that we first proposed elsewhere [29]. (For hypocrellin A in viscous solvents such as octanol and ethylene glycol, *an absorption transient of ~ 10 ps duration*, similar to the hypericin transient, can also be detected [29].) In addition to the 10 ps component in hypocrellin A, there is a longer-lived transient, also assigned to H-atom transfer, whose time constant ranges from 50–250 ps in the solvents we have studied [28].

3.2.2. *Deuterium isotope effects on the H-atom transfer in hypericin and hypocrellin A*

The 10 ps component neither in hypericin nor in hypocrellin A exhibits a deuterium isotope effect. The longer-lived component in hypocrellin A exhibits an isotope effect of 1·4 (in MeOD versus MeOH) [27]. This unambiguously identifies the process as a H-atom transfer event.

3.2.3. *Solvent dependence*

The H-atom transfer rate in hypericin has no significant solvent dependence [24, 54]. The H-atom transfer rate for the longer component in hypocrellin A has a strong dependence on the bulk viscosity [28]. The time constant for H-atom transfer in

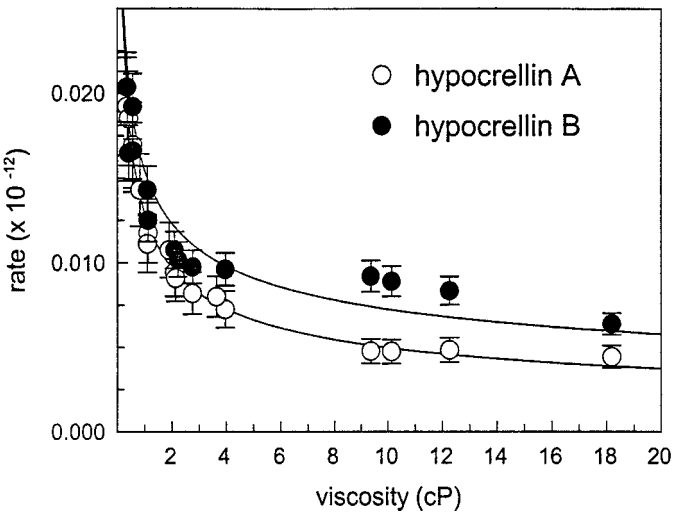

Figure 5. Intramolecular H-atom transfer rate of millimolar hypocrellin B and hypocrellin A in various solvents of different viscosity at 22 °C. The data are fit to a phenomenological expression $k = (C/\eta^a)\exp(-E_0/RT)$, where $C = 2{\cdot}17 \times 10^{12}$ s^{-1}, $a = 0{\cdot}33$ and $E_0 = 2{\cdot}92$ kcal mol^{-1} for hypocrellin B and $1{\cdot}9 \times 10^{12}$ s^{-1}, 0·42 and 3·0 kcal mol^{-1} for hypocrellin A, respectively. An iterative nonlinear least-squares fit is used to obtain the parameters in both the cases. The solvents used in this study are methanol, ethanol, propanol, pentanol, octanol, nonanol, decanol, ethylene gycol, dimethyl sulphoxide, formamide, dimethylformamide, acetonitrile, butyronitrile, 2,2,2-trifluoroethanol and cyclohexanone. Viscosity data are obtained from the text by Viswanath and Natarajan [56]. The data points represent an average of from 2 to 4 measurements. The error bars represent an estimated error of 10%. (Figure used with the permission of the American Society of Photobiology.)

hypocrellin ranges from 50 to 250 ps in the solvents we have studied (figure 5). The viscosity dependence is remarkable not only because it is absent in hypericin, but also because *it is exceedingly well described by a bulk effect* and does not require specific consideration of the structural aspects of the solvents, which vary considerably. It is often the case that trends are only followed for solvents of a given kind: alkane or alcohols; primary alcohol or higher degree alcohol; hydrogen bonding or non-hydrogen bonding, etc. Nuclear magnetic resonance (NMR) results cited below lead us to suggest that the viscosity dependence on the excited-state transfer process is a consequence of the coupling of the H-atom transfer to conformational changes of the 7-membered ring in hypocrellin. *Hypericin does not have such a ring and does not exhibit such viscosity dependent effects.*

3.2.4. *Temperature dependence of the H-atom transfer*

The H-atom transfer in hypericin and hypocrellin A was studied as a function of temperature in a 1:1 ethanol/methanol mixture. The Arrhenius plot constructed from the 10 ps component of hypericin yields an activation energy of $0{\cdot}044 \pm 0{\cdot}088$ kcal mol^{-1}. The Arrhenius plot constructed from the longer-lived component of the hypocrellin A reaction yields an activation energy of $2{\cdot}12 \pm 0{\cdot}070$ kcal mol^{-1} [32] (figure 6). Because the longer-lived component of the hypocrellin A reaction exhibits a viscosity dependence and because viscosity has a temperature dependence, the activation energy reported above cannot be totally indicative of the intrinsic barrier in the conformational coordinate. *In order to separate the contribution of solvent*

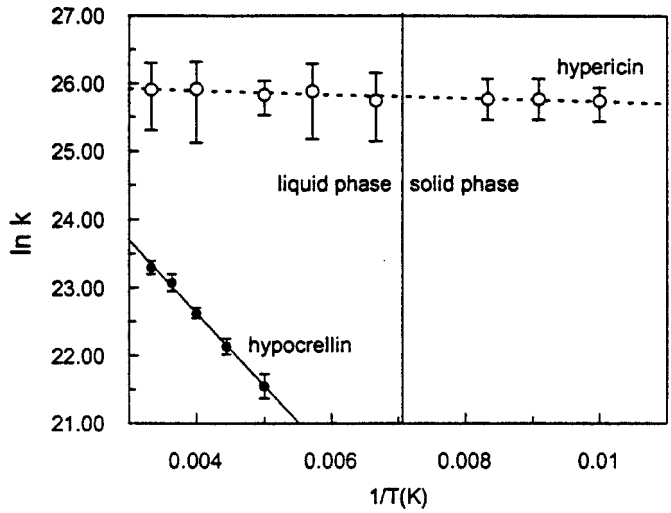

Figure 6. Arrhenius plots for hypericin and hypocrellin A [32] in a 1:1 methanol:ethanol mixture. Each point represents the average of at least five experiments. The error bars are ± one standard deviation from the mean. The Arrhenius prefactors and activation energies obtained from these plots are: hypericin, $A = 1{\cdot}96 \pm 0{\cdot}08 \times 10^{11}\ s^{-1}$ and $E_a = 0{\cdot}044 \pm 0{\cdot}008$ kcal mol^{-1}; hypocrellin, $A = 4{\cdot}97 \pm 0{\cdot}67 \times 10^{11}\ s^{-1}$ and $E_a = 2{\cdot}12 \pm 0{\cdot}070$ kcal mol^{-1}.

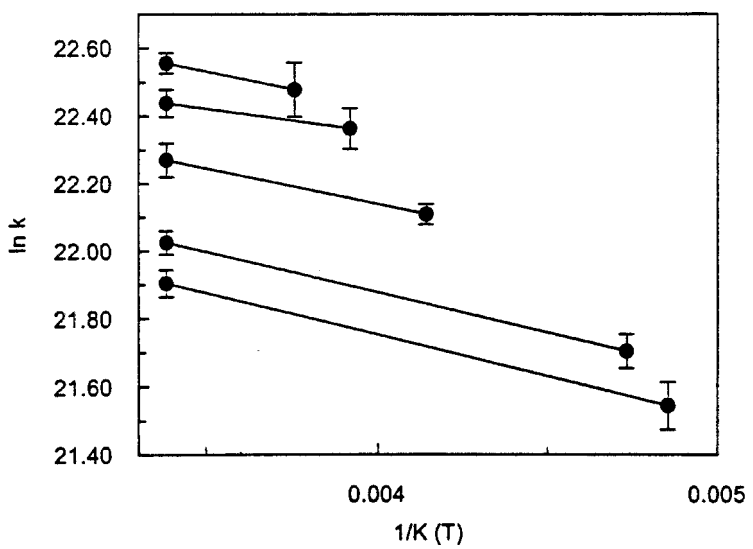

Figure 7. Isoviscosity plots for hypocrellin A. The solvents are (from top to bottom, left hand side of the graph): cyclohexanone, butanol, pentanol, nonanol and decylalcohol at room temperature. The corresponding viscosities of ethanol, at different temperatures are plotted at the right hand side. The energy of activation is $0{\cdot}41 \pm 0{\cdot}088$ kcal mol^{-1}. The probe wavelength is 595 nm.

viscosity from the internal barrier, we constructed isoviscosity plots in which the temperature dependence of the rate could be examined at constant viscosity. These plots, presented in figure 7, indicate that the intrinsic barrier is higher than that in hypericin, but still quite small: $0{\cdot}41 \pm 0{\cdot}088$ kcal mol^{-1}.

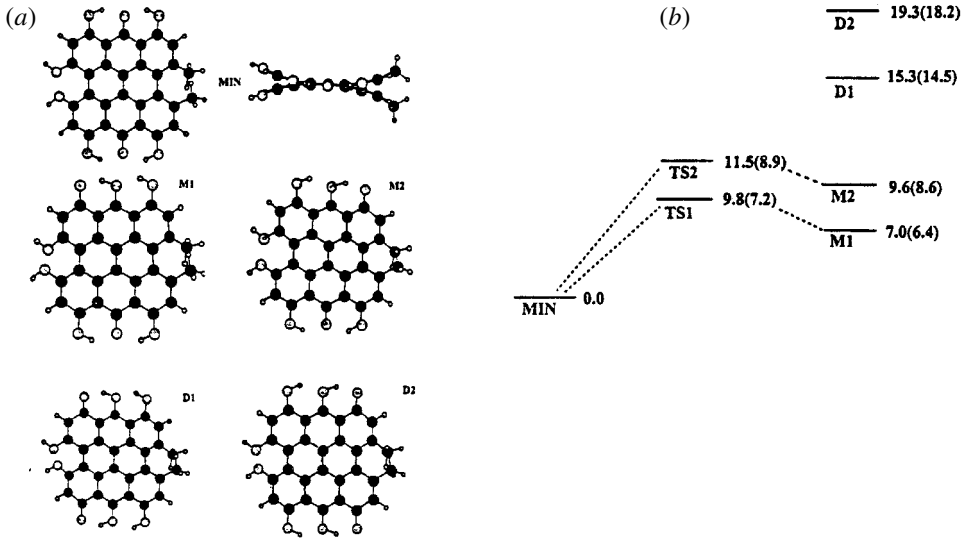

Figure 8. (*a*) Optimized structures for potential energy minima of hypericin based on the RMP2/6-31G(d) level of theory, using geometries obtained with the 3-21G basis and Hartree–Fock wavefunctions. At the top, two views are shown for the 'normal' form (figure 1), which is here referred to as MIN, i.e. the minimum energy structure. M1 and M2 are monotautomers; D1 and D2 are double tautomers. (*b*) Estimated relative energies (kcal mol^{-1}) of minima and transition states. Energies including zero-point corrections are given in parentheses.

The significant contribution of the solvent viscosity to the activation energy of the hypocrellin A reaction can also be deduced from the data presented in figure 5. The apparent activation energy is:

$$E_a = -R\frac{\mathrm{d}\ln k}{\mathrm{d}(1/T)}.$$

This may be written as a sum of viscosity independent and dependent terms:

$$-R\frac{\mathrm{d}\ln k}{\mathrm{d}(1/T)} = -R\frac{\partial \ln k}{\partial(1/T)}\bigg|_{\eta} - \frac{\partial \ln k}{\partial \eta}\bigg|_{T} R\frac{\partial \eta}{\partial(1/T)}.$$

The first term is the viscosity independent activation energy, $\partial \ln k/\partial(\eta)|_T$, which may be evaluated from the data in figure 5, and gives:

$$k = \left(\frac{C}{\eta}\right)^{0.42} \exp\left(-\frac{E_0}{RT}\right).$$

Therefore,

$$\frac{\partial \ln k}{\partial \eta}\bigg|_{T} = -\frac{0.42}{\eta}.$$

For a 1:1 methanol/ethanol mixture, η is about 0·8 cP at room temperature and thus $(\partial \ln k/\partial \eta)|_T \sim 0{\cdot}5$. Since the solvent viscosity activation energy, $R[\partial \eta/\partial(1/T)]$, is about 3 kcal mol^{-1}, it can be estimated that about 1·5 kcal mol^{-1} of the observed activation arises from the temperature dependence of the solvent viscosity, which is consistent with the results of the isoviscosity plots (figure 7).

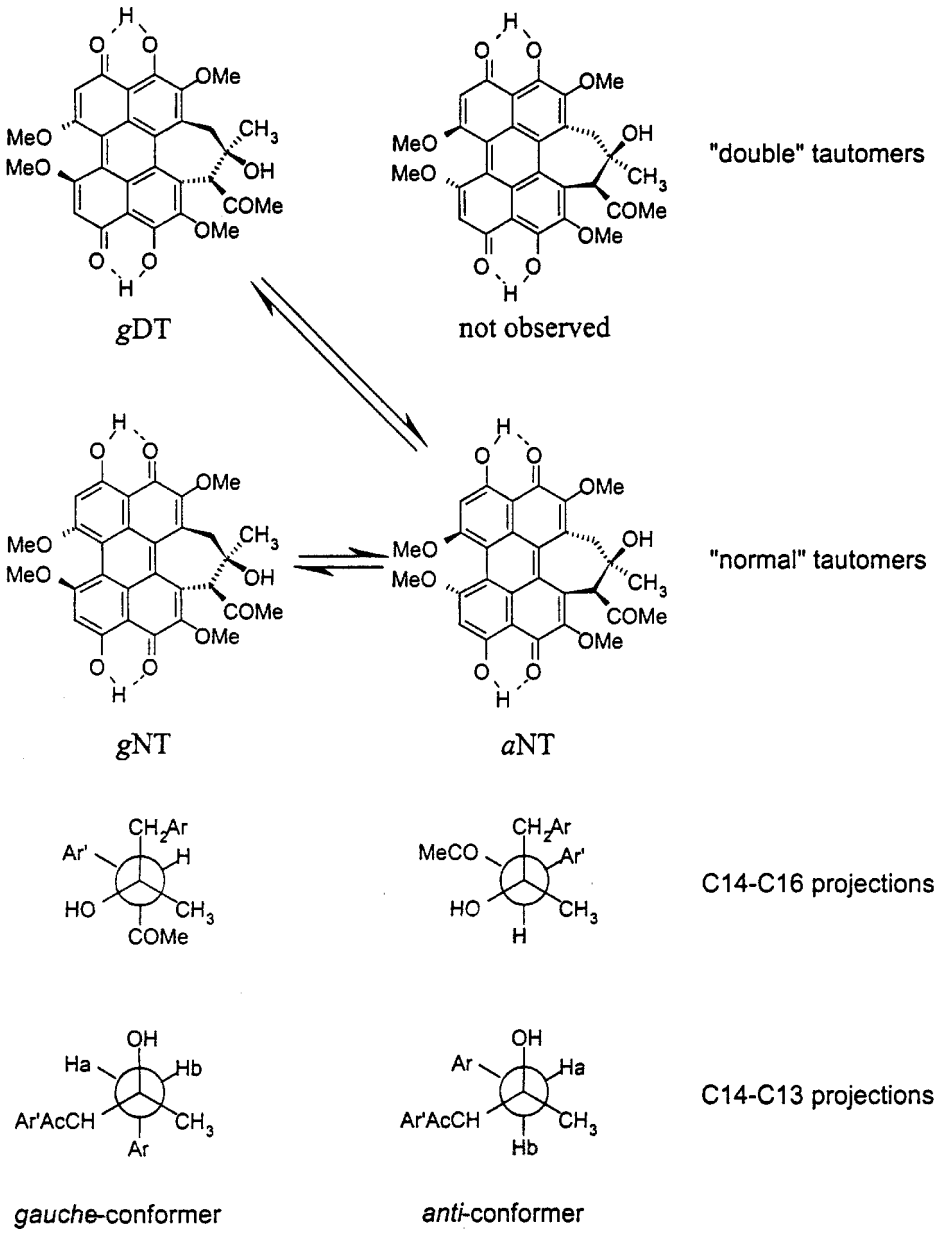

Figure 9. Conformational forms of the hypocrellin A normal and double tautomers. The *anti*/*gauche* nomenclature is based on the relative position of the methyl and acetyl moieties as depicted by the Newman projection: *gauche* double tautomer, *g*D, 60% of the ground-state population; *anti* normal tautomer, *a*N, 30%; *gauche* normal tautomer, *g*N, 10%. (Figure used with the permission of the American Society of Photobiology.)

3.2.5. *NMR and* ab initio *calculations*

Temperature-dependent ^{1}H NMR and 2D-ROESY studies of hypericin indicate that there is only one conformer/tautomer for hypericin in the ground state [57]. This is consistent with the theoretical predictions [58] (figure 8). On the other hand, the NMR measurements indicate that three ground-state species are significantly

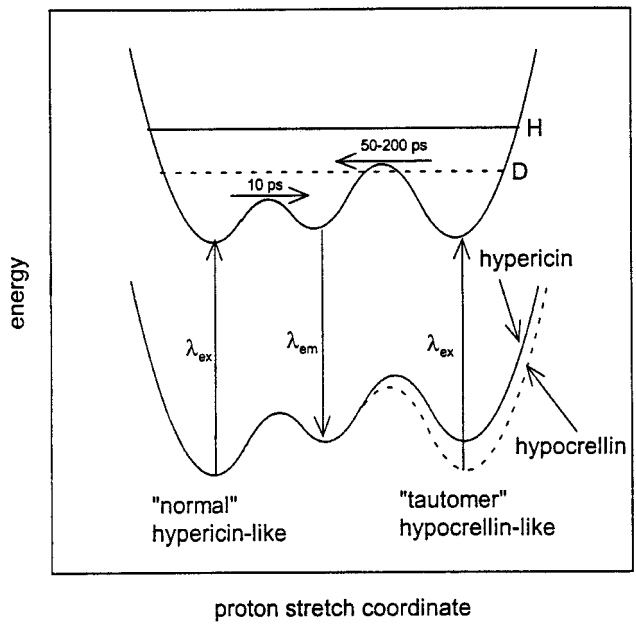

Figure 10. Unified picture depicting the ground- and excited-state potential energy surfaces for hypericin-like and hypocrellin-like molecules as a function of the proton stretch coordinate. The right-hand side of the ground-state potential energy surface indicates that the hypericin double tautomer is not populated in the ground state, whereas the hypocrellin A double tautomer (figure 1) is populated. On the excited-state surface, the zero-point vibrational levels for an OH···O or an OD···O system are depicted. The height of the zero-point level with respect to the barrier in the proton stretch coordinate determines whether an isotope effect will be observed. The third potential well, the middle of the figure represents either another possible tautomeric form or some other intermediate between, for example, the normal and the double tautomer species of hypericin. The arrows in the diagram are meant to remind the reader of the time constants for the H-atom transfer processes in hypericin and hypocrellin. One should not identify the proton coordinate for the reaction coordinate in this system.

populated for hypocrellin A (figure 9) [57], owing largely to the flexibility of the 7-membered ring. That the 7-membered ring plays an important role in determining the populations of conformers and tautomers in the ground state is demonstrated by the NMR study of hypocrellin B (figure 1). The 7-membered ring of hypocrellin B contains a double bond and is consequently more rigid than that of hypocrellin A. Only one ground-state conformer/tautomer is observed by NMR for hypocrellin B.

4. Discussion and synthesis of the hypericin and hypocrellin photophysics

4.1. *Origins (and lack of) an isotope effect*

Theoretical treatments for both non-adiabatic and adiabatic proton transfer [57–73] bear some similarities to those for electron transfer [74, 75]. But many aspects of excited-state H-atom and proton transfer reactions are poorly understood, in particular, the way in which the solvent or the intramolecular modes of the solute couple to the reaction. The absence of an isotope effect may be considered in the context of work by Hynes and co-workers [58–61, 64]. These workers discuss proton transfer of a linear system OH···O in the *non-adiabatic* and *adiabatic* limits. The limiting cases are determined by the extent to which the reactant and product species

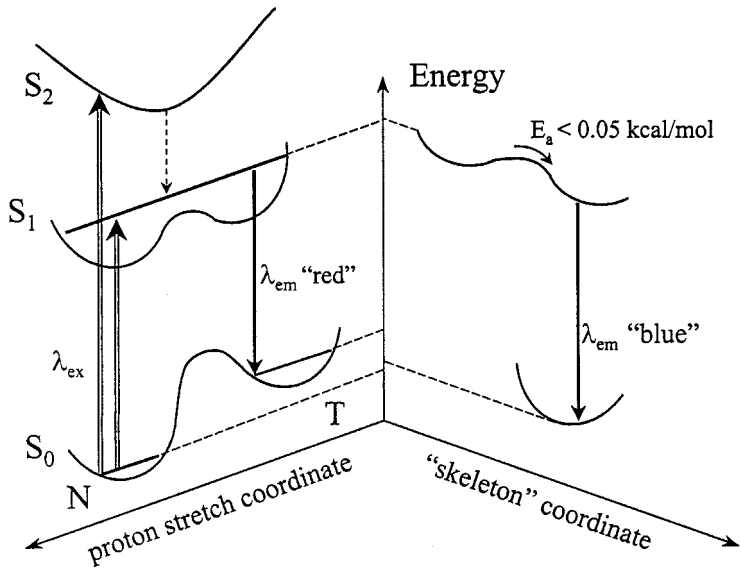

Figure 11. Generalized potential energy surface rationalizing the 10 ps H-atom transfer in hypericin (and hypocrellin A, in which case N and T are interchanged). The absence of an isotope effect for this component indicates that the zero point vibrational level lies above the barrier in the proton coordinate 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 4 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.

are separated by a barrier in the proton coordinate that is large with respect to the thermal energy, kT. When the barrier is large, the bound-state vibrational levels of the reactants and products lie well below the barrier and the reactants and products are consequently localized, the transfer event can be described by a tunnelling process, in which case very large isotope effects can be expected. This *non-adiabatic limit* is expected to hold for weak or intermediate strength hydrogen bonded systems that are characterized by heavy atom (O–O) distances between which the proton transfers of 2·6–2·7 Å. This heavy atom distance strongly modulates the magnitude of the matrix element that couples the reactant and product states and thus determines the size and the thickness of the barrier separating them [58, 64].

In the adiabatic treatment of proton transfer, a Born–Oppenheimer separation is used to distinguish the fast proton motion (~ 2500 cm^{-1}) from the slow heavy-atom motion (e.g. the O–O vibration) and from the even slower solvent motions (~ 100 cm^{-1}). The proton can thus adjust immediately to any instantaneous nuclear configuration of these slow degrees of freedom. The proton wavefunction depends on the proton coordinate, q; and it depends parametrically on the heavy-atom coordinates, Q, and the solvent coordinates, $\mathbf{S}$.

Whether the system is in the reactant or product configuration or in an intermediate configuration depends on the stabilization imparted by the Q and $\mathbf{S}$. In the theory, this stabilization is discussed mostly in terms of the solvent. Most reference to the heavy-

atom coordinate is made in terms of the O–O vibration (i.e. the heavy atoms between which the proton is transferred) because it usually has the most pronounced effect on the coupling between the reactant and product states. Because this distance is so short in hypocrellin and hypericin, we suggest that the proton transfer should always be considered to be in the adiabatic limit. More important heavy atom motions for hypocrellin and hypericin are likely to involve the twisting motion of the aromatic skeleton subsequent to or during transfer.

When the heavy atom distance is $< 2{\cdot}6$ Å, the *adiabatic limit* is obtained. 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 proton transfer (figure 10). We have argued that hypericin falls into the adiabatic limit because its relevant oxygen–oxygen distance is $\sim 2{\cdot}5$ Å [76, 77]. In hypocrellin, this distance is comparable [78].

Staib *et al.* [64], however, suggest the intriguing possibility that 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 induce an isotope effect because now the proton could tunnel through the barrier or effect an activated crossing of it. We propose that this is the origin of the isotope effect observed for the long-lived transient in hypocrellin A (figure 10). That the isotope effect is relatively small suggests that the vibrational ground state is not significantly lowered below the barrier and that the H-atom transfer is an activated process.

4.2. *Reaction coordinates for the H-atom transfer rates in hypericin and hypocrellin*

If the zero-point energy lies above the barrier in the proton coordinate, the hydrogen atom is effectively delocalized between the two oxygen atoms until a change in another coordinate can trap the system in the tautomerized form (figure 11). We propose that it is the time scale for this latter conformational change that determines the observed H-atom transfer time.

In the model system for excited-state proton transfer, 3-hydroxyflavone [46, 79–81], the reaction occurs in ~ 100 fs in dry non-hydrogen bonding solvents [80]. In hydrogen bonding solvents such as alcohols, the observed proton transfer time in 3-hydroxyflavone reflects the time required for the displacement of the solvent hydrogen-bonded to the enol proton of the solute, thus permitting the proton to effect its transfer. Specific hydrogen bonding interactions between hypericin and hypocrellin with solvent cannot explain the relatively slow H-atom transfer times ($\geqslant 10$ ps) because the hypericin reaction is insensitive to solvent [24, 54] and because the hypocrellin reaction, while sensitive to solvent, is only well correlated to the bulk viscosity of different solvents and not to solvents of a given 'type' [28]. The insensitivity of the hypericin reaction to solvent is most likely a result of the strong association of the proton with the oxygens of both the *peri* hydroxyl and the carbonyl groups. Since the oxygen–oxygen distance (in the ground state) is essentially the same in both hypericin and hypocrellin (2·5 Å), a different origin for the solvent dependence of the longer-lived H-atom transfer of the hypocrellin A reaction must be found.

Our emerging model for the excited-state photophysics of hypericin and hypocrellin A is a refinement of that presented elsewhere [29]. The most significant addition to the model is the detection and identification of three significantly populated ground-state species for hypocrellin A and the determination of the temperature dependence of the excited-state processes [32, 57].

The absence of a deuterium isotope effect on the excited-state H-atom transfer *for*

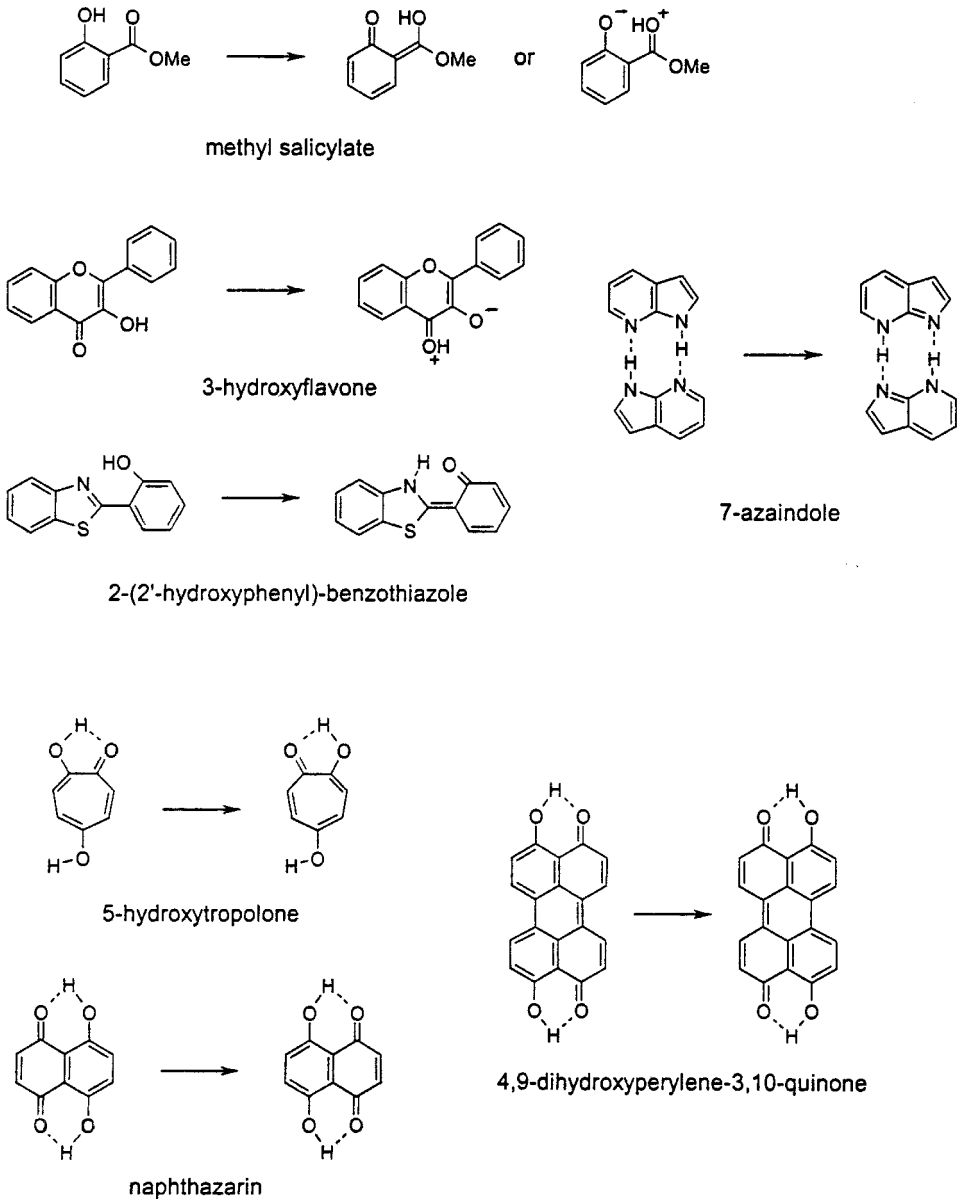

Figure 12. Proton-transfer and H-atom transfer species. In the upper half of this figure the product of tautomerization is *structurally and electronically* different from educt. In contrast, H-atom transfer in 5-hydroxytropolone results in nearly identical species and double tautomers of naphthazarin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

the ~ 10 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, $E_a = 0{\cdot}044 \pm 0{\cdot}008$ kcal mol^{-1} for hypericin. Because the nature of this motion is not yet identified, we refer to it as the '*skeleton coordinate*' (figure 11).

The $2{\cdot}12 \pm 0{\cdot}070$ kcal mol^{-1} activation energy *for the longer-lived H-atom transfer* in hypocrellin A, in addition to the strong dependence of its H-atom transfer reaction on viscosity [28], indicate that, unlike the 10 ps component, rather large amplitude conformational changes are involved. Consistent with the idea of a large-amplitude transition occurring in hypocrellin A is that the form of the kinetic traces changes drastically as the liquid–glass transition is approached.

Based on the importance of the conformation of the 7-membered ring of hypocrellin A in determining the 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 longer-lived H-atom transfer (figure 11). Consequently, the two optically excited normal species of hypocrellin A decay into the fluorescent tautomer species with an ~ 10 ps time constant and a longer-lived, viscosity dependent, time constant. The exact nature of the conformational changes that are coupled to the H-atom transfer reaction in hypericin and hypocrellin has yet to be identified and is currently under investigation.

4.3. *Towards a unified picture of the hypericin and hypocrellin photophysics*

The current picture that we have formed of the excited-state dynamics of hypericin and hypocrellin is that the different photophysical behaviour 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 [29]. This picture is crudely illustrated in figures 10 and 11.

A crucial result in forming this hypothesis is the observation that under certain conditions we resolve a time constant in the hypocrellin photophysics which is comparable to that observed in hypericin. This ~ 10 ps component in hypocrellin unifies our picture of the photophysics of hypericin and hypocrellin 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. A thoroughly studied system that bears many similarities to this one is that of stilbene. The *trans*-to-*cis* photoisomerization of stilbene bears distinct differences from that of the *cis*-to-*trans* photoisomerization. The former process has a much stronger viscosity dependence and occurs on a longer time scale than the latter. The differences in behaviour have been attributed to different reaction coordinates for the two isomerization processes [82–86].

5. Revisiting the assignment of intramolecular H-atom transfer in hypericin and hypocrellin

5.1. *Absence of isotope effect and presence of mirror-image symmetry*

Here 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 for the 10 ps component of hypericin and hypocrellin 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 above [24, 27, 29, 53, 54]. *Its absence is easily attributed to the reaction coordinate not being identified with the proton coordinate*, as we have depicted in figures 10 and 11. There is precedent for this in other systems [87–89].

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 system (figure 12): methyl salicylate [88, 90]; 7-azaindole dimer [91, 92]; 2-phenyl-benzotriazole [93]; and 3-hydroxyflavone [79–81, 94, 95].

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-Hydroxytropolone [96–98] presents an excellent example of such a case. Other examples are the double H-atom transfer in naphthazarin [99] and in the 4,9-dihydroxyperylene-3,10-quinon e 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 monotautomerized 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. In order 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. (The hypocrellin A reaction *does* have a solvent dependence. But this dependence is upon *viscosity* and arises from conformational changes of the 7-membered ring which are coupled to the H-atom transfer reaction. The reported dependence of the hypocrellin A reaction on solvent polarity [28] 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 a H-atom transfer.)

5.2. *Additional concerns*

Here we wish to comment on additional objections that we have encountered with regard to the assignment of the excited-state processes in hypericin and hypocrellin to intramolecular H-atom transfer. These are: (a) the viscosity dependence of the hypocrellin reaction; (b) the possibility that the process results from triplet dynamics; (c) the possibility that the process results from aggregation effects.

(a) It has been remarked that the viscosity dependence of the hypocrellin A H-atom transfer reaction is anomalous because 'hypocrellin is a very rigid molecule'. This claim is unfounded as demonstrated by the NMR results indicating three ground-state species for hypocrellin A whose populations are determined by the flexibility of the 7-membered ring. This flexibility is furthermore lost by the presence of the double bond in the 7-membered ring in hypocrellin B, which is shown by NMR to have only one ground-state conformer/tautomer.

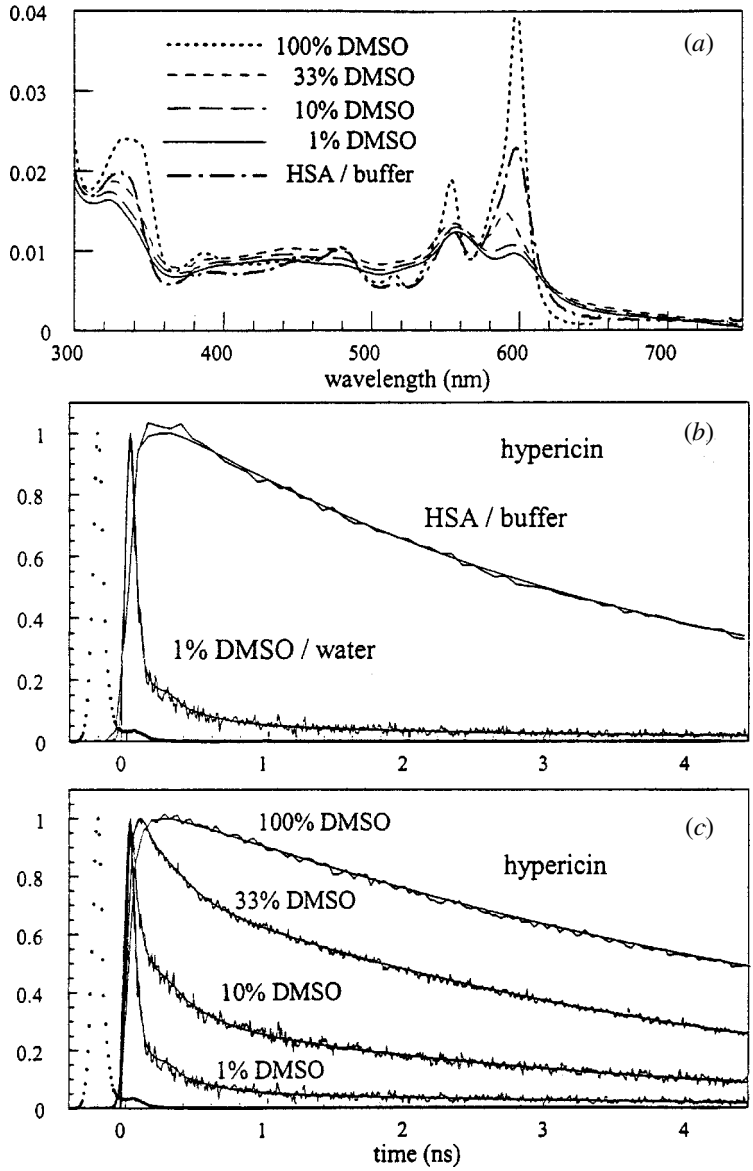

Figure 13. (*a*) Absorbance spectra of hypericin in 1:1 mixture with human serum albumin (HSA) in buffer (dash–dotted) and in dimethylsulphoxide (DMSO) water mixtures of various proportions: 1% (solid), 10% (long dashed), 33% (short dashed) and 100% DMSO (dotted). These spectra were scaled to compensate for slightly different chromophore concentrations. (*b*) Comparison of normalized lifetime decay traces of hypericin with HSA (1:1 ratio) and in 1% DMSO/water solution. $F(t) = 0{\cdot}96\exp(-t/12\text{ ps})+0{\cdot}03\exp(-t/304\text{ ps})+0{\cdot}01\exp(-t/3680\text{ ps})$ for 1% DMSO. $F(t) = 0{\cdot}75\exp(-t/3050\text{ ps})+0{\cdot}25\exp(-t/5770\text{ ps})$ for HSA/buffer solution. (*c*) Normalized lifetime decay traces of hypericin in DMSO/water mixtures of different proportions is shown in the insert. $F(t) = 0{\cdot}96\exp(-t/12\text{ ps})+0{\cdot}03\exp(-t/304\text{ ps})+0{\cdot}01\exp(-t/3680\text{ ps})$ for 1% DMSO. $F(t) = 0{\cdot}91\exp(-t/1{\cdot}4\text{ ps})+0{\cdot}05\exp(-t/264\text{ ps})+0{\cdot}04\exp(-t/3380\text{ ps})$ for 10% DMSO. $F(t) = 0{\cdot}41\exp(-t/190\text{ ps})+0{\cdot}59\exp(-t/3870\text{ ps})$ for 33% DMSO. $F(t) = 1{\cdot}00\exp(-t/5580\text{ ps})$ for 100% DMSO. The instrument response function is shown by the dotted line. It is also artificially shifted with respect to the fluorescence decays.

(b) The observed processes cannot be triplet dynamics because they are observed in the fluorescence upconversion experiment, which detects only *excited singlet photophysics* [31, 33, 53].
(c) Aggregation effects can be excluded [31] as demonstrated by figure 13. The absorption spectrum of hypericin is very sensitive to the presence of water, which induces aggregation (figure 13(*a*)). Aggregation also quenches the fluorescence very effectively (figures 13(*b*) and (*c*)). None of the hypericin or hypocrellin samples that we have studied has exhibited aggregation effects. In the solvents we have investigated, the absorption spectrum remains unchanged from the micromolar to millimolar concentration range. Furthermore, fluorescence upconversion data cannot be obtained in the presence of aggregates because they are insufficiently fluorescent.

To conclude this section, we note once again that neither the absence of mirror image symmetry nor the presence of an isotope effect is a *conditio sine qua non* for a H-atom transfer reaction. We suggest that we have reasonably and thoroughly responded to the presence of the former and the absence of the latter. We recognize, however, that a more direct demonstration of an excited-state H-atom transfer reaction is the observation of the bleaching of the carbonyl or hydroxyl stretching frequency as a function of time subsequent to laser excitation. Such measurements require a tunable infrared probe pulse coupled to a visible or ultraviolet pump pulse.

6. Outstanding questions

6.1. *Ground-state heterogeneity*

As we note above, a possible objection to our assignment of the excited-state reaction to H-atom transfer in these perylene quinone 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. Our first attempt to explain this symmetry was to suggest that the ground state of hypericin was populated with at least one other species, for example, a monotautomer [24, 54]. 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 ~ 600 nm. This suggestion also seemed to be supported by the observation that the transient absorbance and upconversion [33] 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. Based on *ab initio* calculations (RMP2/6-31G(d) level of theory, using geometries obtained with the 3-21G basis and Hartree–Fock wavefunctions), only one hypericin species, the 'normal' form, is populated in the ground state for an unionized gas phase species [58]. Furthermore, on the NMR time scale, only one species of hypericin appears to be present in the ground state [57]. On the other hand, as noted above, three significantly populated species are observed for hypocrellin A [57].

6.2. *Reconciling the kinetics with the ground-state populations*

First we consider hypocrellin A. The excited-state dynamics modelled in figure 11 are an extension of the model presented elsewhere [29] (figure 10), enhanced by the knowledge that on the NMR time scale three ground-state species are present. *It is an assumption of this model that only the 'tautomer', not the 'normal' species is significantly fluorescent*. Consequently, in order to justify the absence of 50–250 ps rise times in the fluorescence upconversion kinetics (figure 4), it is 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 (*g*D), 30% *anti* normal tautomer (*a*N) and 10% *gauche* normal tautomer (*g*N) (see figure 9). In the ground state, these species are related sequentially, by the following equilibria: $gD \leftrightarrow aN \leftrightarrow gN$. Based on our model [29] 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 11).

Figures 3 and 4 indicate 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% *g*D and 10% *g*N (the decay of *g*N* being assigned to the ~ 10 ps component because it does not involve a conformational change of the 7-membered ring, which occurs on a longer time scale).

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 15–30% of a longer-lived rising component (this has been demonstrated by simulations not shown here [53]). Thus, the populations determined from fluorescence may be correspondingly adjusted to 75% *g*D, 15% *a*N and 10% *g*N.

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 ~ 10% that of the instantaneous component. Based on 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 can be formulated in terms of figure 11. 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 (~ 3000 cm^{-1} in ground-state hypericin [58] 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.

7. Conclusions

The absence of an isotope effect for the 10 ps component in hypericin and hypocrellin indicates that the reaction coordinate for this H-atom transfer process is not the proton coordinate. The negligible activation energy for hypericin indicates that a very low-amplitude displacement in at least one 'skeleton' coordinate be taken into account in order to describe the reaction dynamics. The longer-lived H-atom transfer for hypocrellin A has a viscosity dependent activation energy, suggesting that a larger amplitude motion than for the case of hypericin comprises part of the reaction coordinate. The strong viscosity dependence of this longer-lived H-atom transfer suggests that the process is coupled to conformational changes of the 7-membered ring. The presence of the 10 ps component and the longer-lived component indicate that in hypericin and hypocrellin we are probing different portions of the excited-state potential energy surface and consequently different aspects of the reaction coordinate.

Acknowledgments

Over the years, this work has been supported by an Office of Naval Research (ONR) Young Investigator Award, an AASERT training grant from ONR, fellowships from Phillips Petroleum, Amoco, and GAANN, the Carver Trust, NIH grant GM57351, and NSF grants BIR9413969 and CHE-9613962. It is a pleasure to thank the undergraduate students, graduate students and postdoctoral associates who have made invaluable contributions to the understanding of this problem: J. Paterson, E. Dertz, and M. Snyder; F. Gai, D. S. English, A. V. Smirnov, M. Fehr, J. Park, K. Ashby and J. Wen; K. Das and A. Datta. In the course of this highly interdisciplinary enterprise, we have benefited enormously and obtained great enjoyment in our interactions, collaborations, and discussions with M. S. Gordon, G. A. Kraus, R. Larock, S. Carpenter, A. Andreotti, B. Fulton, M. A. McCloskey, P. G. Haydon, P.-S. Song, E. W. Castner Jr, J. T. Hynes, F. Sureau, P. de Witte, P. Miskovsky and P. Jardon. We thank Mr P. Chowdhury for his helpful comments and criticisms of this manuscript.

References

[1] Duran, N., and Song, P. S., 1986, *Photochem. Photobiol.*, **43**, 677.
[2] Lown, J. W., 1997, *Can. J. Chem.*, **75**, 99.
[3] Diwu, Z., 1995, *Photochem. Photobiol.*, **61**, 529.
[4] Kraus, G. A., Zhang, W., Fehr, M. J., Petrich, J. W., Wannemuehler, Y., and Carpenter, S., 1996, *Chem. Rev.*, **96**, 523.
[5] Falk, H., 1999, *Angew. Chem. Int. Ed.*, **38**, 3116.
[6] Meruelo, D., Lavie, G., and Lavie, D., 1988, *Proc. Natl Acad. Sci.* USA, **85**, 5230.
[7] Lenard, J., Rabson, A., and Vanderoef, R., 1993, *Proc. Natl Acad. Sci.* USA, **90**, 158.
[8] Hudson, J. B., Zhou, J., Chen, J., Harris, L., Yip, L., and Towers, G. H. N., 1994, *Photochem. Photobiol.*, **60**, 253.
[9] Couldwell, W. T., Gopalakrishna, R., Hinton, D. R., He, S., Weiss, M. H., Law, R. E., and Apuzzo, M. L., 1994, *Neurosurgery*, **35**, 705.
[10] Anker, L., Gopalakrishna, R., Jones, K. D., Law, R. E., and Couldwell, W. T., 1995, *Drugs of the Future*, **20**, 511.
[11] Zhang, W., Law, R. E., Hinton, D. R., and Couldwell, W. T, 1997, *Cancer Lett.* (Shannon, Irel.), **120**, 31.
[12] Linde, K., Ramirez, G., Mulrow, C. D., Pauls, A., Weidenhammer, W., and Melchart, D., 1996, *Br. Med. J.*, **313**, 253.
[13] Suzuki, O. K., Oya, M., Bladt, S., Wagner, H., 1984, *Planta Medica*, **50**, 272.
[14] Takahashi, I. N. S., Kobayashi, E., Nakano, H., Suzuki, K., and Tamaoki, T., 1989, *Biochem. Biophys. Res. Commun.*, **165**, 1207.
[15] Andreoni, A., Colasanti, A., Colasanti, P., Mastrocinque, M., Riccio, P., and Roberti, G., 1994, *Photochem. Photobiol.*, **59**, 529.
[16] Thomas, C., MacGill, R. S., Miller, G. C., and Pardini, R. S., 1992, *Photochem. Photobiol.*, **55**, 47.
[17] Vandenbogaerde, A. L., Delaey, E. M., Vantieghem, A. M., Himpens, B. E., Merlevede, W. J., and De Witte, P. A., 1998, *Photochem. Photobiol.*, **67**, 119.
[18] Agostinis, P., Donella-Deana, A., Cuveele, J., Vandenbogaerde, A., Sarno, S., Merlevede, W., and De Witte, P., 1996, *Biochem. Biophys. Res. Commun.*, **220**, 613.
[19] Vandenbogaerde, A. L., and De Witte, P. A., 1996, *Phytother. Res.*, **10**, S150.
[20] Vandenbogaerde, A. L., Cuveele, J. F., Proot, P., Himpens, B. E., Merlevede, W. J., and De Witte, P. A., 1997, *J. Photochem. Photobiol.* B, **38**, 136.
[21] Weller, M. T., Grimmel, C., Shabet, M., Bremen, D., Krajewski, S., and Reed, J. C., 1997, *Neurol. Res.*, **19**, 459.
[22] Mirossay, L. M., Kocisova, E., Radvakova, I., Miskovsky, P., and Mojzis, J., 1999, *Physiol. Res.* **48**, 135.
[23] Gai, F., Fehr, M. J., and Petrich, J. W., 1993, *J. Am. chem. Soc.*, **115**, 3384.
[24] Gai, F., Fehr, M. J., and Petrich, J. W., 1994, *J. phys. Chem.*, **98**, 5784.
[25] Gai, F., Fehr, M. J., and Petrich, J. W., 1994, *J. phys. Chem.*, **98**, 8352.

[26] DAS, K., ENGLISH, D. S., FEHR, M. J., SMIRNOV, A. V., and PETRICH, J. W., 1996, *J. phys. Chem.*, **100**, 18275.
[27] DAS, K., ENGLISH, D. S., and PETRICH, J. W., 1997, *J. phys. Chem.* A, **101**, 3241.
[28] DAS, K., ENGLISH, D. S., and PETRICH, J. W., 1997, *J. Am. chem. Soc.*, **119**, 2763.
[29] DAS, K., SMIRNOV, A. V., SNYDER, M. D., and PETRICH, J. W., 1998, *J. phys. Chem.* B, **102**, 6098.
[30] DAS, K., DERTZ, E., PATERSON, J., ZHANG, W., KRAUS, G. A., and PETRICH, J. W., 1998, *J. phys. Chem.* B, **102**, 1479.
[31] DAS, K., SMIRNOV, A. V., WEN, J., MISKOVSKY, P., and PETRICH, J. W., 1999, *Photochem. Photobiol.* **69**, 633.
[32] DAS, K., ASHBY, K., WEN, J., and PETRICH, J. W., 1999, *J. phys. Chem.* B, **103**, 1581.
[33] ENGLISH, D. S., DAS, K., ASHBY, K. D., PARK, J., PETRICH, J. W., and CASTNER, E. W. J., 1997, *J. Am. chem. Soc.*, **119**, 11585.
[34] ENGLISH, D. S., DAS, K., ZENNER, J. M., ZHANG, W., KRAUS, G. A., LAROCK, R. C., and PETRICH, J. W., 1997, *J. phys. Chem.* A, **101**, 3235.
[35] FALK, H., MEYER, J., and OBERREITER, M., 1992, *Monatsh. Chem.*, **123**, 277.
[36] FEHR, M. J., MCCLOSKEY, M. A., and PETRICH, J. W., 1995, *J. Am. chem. Soc.*, **117**, 1833.
[37] SUREAU, F., MISKOVSKY, P., CHINSKY, L., and TURPIN, P. Y., 1996, *J. Am. chem. Soc.*, **118**, 9484.
[38] CHALOUPKA, R., SUREAU, F., KOCISOVA, E., and PETRICH, J. W., 1998, *Photochem. Photobiol.*, **68**, 44.
[39] PINTO, L. H., HOLSINGER, L. J., and LAMB, R. A., 1992, *Cell*, **69**, 517.
[40] NEWELL, K. J., and TANNOCK, I. F., 1989, *Cancer Res.*, **49**, 4447.
[41] NEWELL, K. J., WOOD, P., STRATFORD, I., and TANNOCK, I., 1992, *Br. J. Cancer*, **66**, 311.
[42] GOTTLIEB, R. A. N., SKOWRONSKI, E., and BABIOR, B. M., 1996, *Proc. Natl Acad. Sci.* USA, **93**, 654.
[43] LI, J. E., 1995, *J. biol. Chem.*, **270**, 3203.
[44] BARRY, M. A., REYNOLD, J. E., and EASTMAN, A., 1993, *Cancer Res.*, **53**, 2349.
[45] HUDSON, J. B., IMPERIAL, V., HAUGLAND, R. P., and DIWU, Z., 1997, *Photochem. Photobiol.*, **65**, 352.
[46] JARDON, P., LAZORTCHAK, N., and GAUTRON, R, 1987, *J. Chim. Phys. Phys.-Chim. Biol.*, **84**, 1141.
[47] RACINET, H., JARDON, P., and GAUTRON, R., 1988, *J. chim. Phys.*, **85**, 971.
[48] DARMANYAN, A. P., BUREL, L., ELOY, D., and JARDON, P., 1994, *J. chim. Phys.*, **91**, 1774.
[49] FEHR, M. J., CARPENTER, S. L., and PETRICH, J. W., 1994, *Bioorg. Med. Chem. Lett.*, **4**, 1339.
[50] FEHR, M. J., CARPENTER, S. L., WANNEMUEHLER, Y., and PETRICH, J. W., 1995, *Biochemistry*, **34**, 15845.
[51] PARK, J., ENGLISH, D. S., WANNEMUEHLER, Y., CARPENTER, S., and PETRICH, J. W., 1998, *Photochem. Photobiol.* **68**, 593.
[52] ENGLISH, D. S., ZHANG, W., KRAUS, G. A., and PETRICH, J. W., 1997, *J. Am. chem. Soc.*, **119**, 2980.
[53] SMIRNOV, A. V., DAS, K., ENGLISH, D. S., WAN, Z., KRAUS, G. A., and PETRICH, J. W., 1999, *J. phys. Chem.* A, **103**, 7949.
[54] CHEN, Y., GAI, F., and PETRICH, J. W., 1994, *Chem. Phys. Lett.*, **222**, 329.
[55] DARMANYAN, A. P., JENKS, W. S., ELOY, D., and JARDON, P., 1999, *J. phys. Chem.* B, **103**, 3323.
[56] VISWANATH, D. S., and NATARAJAN, G., 1989, *Data Book on the Viscosity of Liquids* (New York: Hemisphere).
[57] SMIRNOV, A. V., FULTON, D. B., ANDREOTTI, A., and PETRICH, J. W., 1999, *J. Am. chem. Soc.*, **121**, 7979.
[58] PETRICH, J. W., GORDON, M. S., and CAGLE, M., 1998, *J. phys. Chem.* A, **102**, 1647.
[59] BORGIS, D., and HYNES, J. T. (editors), 1989, *The Enzyme Catalysis Process*, Vol. 178 (New York: Plenum Press).
[60] BORGIS, D., and HYNES, J. T., 1991, *J. chem. Phys.*, **94**, 3619.
[61] BORGIS, D., and HYNES, J. T., 1993, *Chem. Phys.*, **170**, 315.
[62] BORGIS, D., and HYNES, J. T., 1996, *J. chem. Phys.*, **100**, 1118.
[63] ANDO, K., and HYNES, J. T., 1999, *Adv. chem. Phys.*, **110**, 381.

[64] STAIB, A., BORGIS, D, and HYNES, J. T., 1995, *J. chem. Phys.*, **102**, 2487.
[65] LAASONEN, K., and KLEIN, M. L., 1994, *J. Am. chem. Soc.*, **116**, 11620.
[66] TUCKERMAN, M., LAASONEN, K., SPRIK, M., and PARINELLO, M., 1995, *J. chem. Phys.*, **103**, 150.
[67] HAMMES-SCHIFFER, S., and TULLY, J. C., 1994, *J. chem. Phys.*, **101**, 4657.
[68] HAMMES-SCHIFFER, S., and TULLY, J. C., 1995, *J. phys. Chem.*, **99**, 5793.
[69] HAMMES-SCHIFFER, S., 1998, *J. phys. Chem.* A, **102**, 10443.
[70] CUKIER, R. I., and MORILLO, M., 1989, *J. chem. Phys.*, **91**, 857.
[71] LI, D., and VOTH, G. A., 1991, *J. phys. Chem.*, **95**, 10425.
[72] SCHEINER, S., 1985, *Acc. Chem. Res.*, **18**, 174.
[73] WARSHEL, A., 1991, *Computer Modeling of Chemical Reactions in Enzymes and Solutions* (New York: Wiley).
[74] DE VAULT, D., 1984, *Quantum Mechanical Tunnelling in Biological Systems* (Cambridge: Cambridge University Press).
[75] ULSTRUP, J., 1979, *Charge Transfer Processes in Condensed Media* (Berlin: Springer).
[76] ETZLSTORFER, C., FALK, H., MUELLER, N., SCHMITZBERGER, W., and WAGNER, U. G., 1993, *Monatsh. Chem.*, **124**, 751.
[77] FREEMAN, D., FROLOW, F., KAPINUS, E., LAVIE, D., LAVIE, G., MERUELO, D., and MAZUR, Y., 1994, *J. chem. chem. Soc., Chem. Commun.*, 891.
[78] CHEN, W. S., CHEN, Y. T., WAN, X. Y., FRIEDRICHS, E., PUFF, H., and BREITMAIER, E., 1981, *Liebigs Ann. Chem.*, 1880.
[79] MCMORROW, D., and KASHA, M., 1984, *J. phys. Chem.*, **88**, 2235.
[80] SCHWARTZ, B. J., PETEANU, L. A., and HARRIS, C. B., 1992, *J. phys. Chem.*, **96**, 3591.
[81] STRANDJORD, A. J. G., and BARBARA, P. F., 1985, *J. phys. Chem.*, **89**, 2355.
[82] FLEMING, G. R., 1986, *Chemical Applications of Ultrafast Spectroscopy* (London: Oxford University Press).
[83] WALDECK, D. H., 1991, *Chem. Rev.*, **91**, 415.
[84] SALTIEL, J., and SUN, Y. P., 1990, *Photochromism: Molecules and Systems* (Amsterdam: Elsevier).
[85] TODD, D. C., JEAN, J. M., ROSENTHAL, S. J., RUGGIERO, A. J., YANG, D., and FLEMING, G. R., 1990, *J. chem. Phys.*, **93**, 8658.
[86] SENSION, R. J., REPINEC, S. T., SZARKA, A. Z., and HOCHSTRASSER, R. M. J., 1993, *J. chem. Phys.*, **98**, 6291.
[87] FREY, W., LAERMER, F., and ELSAESSER, T., 1991, *J. phys. Chem.*, **95**, 10391.
[88] HEREK, J. L., PEDERSEN, S., BANARES, L., and ZEWAIL, A. H., 1992, *J. chem. Phys.*, **97**, 9046.
[89] PETEANU, L., and MATHIES, R. A., 1992, *J. phys. Chem.* **96**, 6910.
[90] GOODMAN, J., and BRUS, L. E., 1978, *J. Am. chem. Soc.*, **100**, 7472.
[91] TAYLOR, C. A., EL-BAYOUMI, M. A., and KASHA, M., 1969, *Proc. Natl Acad. Sci.* USA **63**, 253.
[92] TAKEUCHI, S., and TAHARA, T., 1998, *J. phys. Chem.* A, **102**, 7740.
[93] KIM, Y. R., YARDLEY, J. T., and HOCHSTRASSER, R. M., 1989, *Chem. Phys.*, **136**, 311.
[94] SWINNEY, T. C., and KELLEY, D. F., 1991, *J. phys. Chem.*, **95**, 10369.
[95] BRUCKER, G. A., SWINNEY, T. C., and KELLEY, D. F., 1991, *J. phys. Chem.*, **95**, 3190.
[96] CROTEAU, R., and LEBLANC, R. M., 1978, *Photochem. Photobiol.* **28**, 33.
[97] ENSMINGER, F. A., PLASSARD, J., ZWIER, T. S., and HARDINGER, S., 1993, *J. chem. Phys.*, **99**, 8341.
[98] ENSMINGER, F. A., PLASSARD, J., ZWIER, T. S., and HARDINGER, S., 1995, *J. chem. Phys.*, **102**, 5246.
[99] MAZZINI, S., MERLINI, L., MONDELLI, R., NASINI, G., RAGG, E., and SCALIONI, L., 1997, *J. Chem. Soc., Perkin Trans. 2*, 2013.