Deuterium Isotope Effect on the Excited-State Photophysics of Hypocrellin: Evidence for Proton or Hydrogen Atom Transfer

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Received: October 3, 1996; In Final Form: February 3, 1997[⊗]

A deuterium isotope effect of 1.4 is observed in the excited-state proton or atom transfer of hypocrellin *A* in methanol and methanol-*O-d*. This is the most direct and convincing evidence to date that excited-state intramolecular proton or atom transfer occurs in hypocrellin. It is consistent with previous proposals (e.g., *Chem. Rev.* **1996**, *96*, 523) that such excited-state intramolecular processes occur generally in the class of polycyclic quinones possessing light-induced antiviral activity, of which hypocrellin and the related compound hypericin are members. The transfer reaction in these compounds is discussed in terms of being in the adiabatic limit in the proton stretch coordinate. We discuss the possibility of presenting a unified picture of the hypericin and hypocrellin photophysics.

Introduction

The naturally occurring polycyclic quinones hypocrellin and hypericin are of interest because of their light-induced antiviral and antitumor activity. We have discussed elsewhere several aspects of their excited-state photophysics[1−3] and have proposed a means of exploiting these properties.[1,4] Based on the precedent of molecules such as 3-hydroxyflavone,[5] it is well-known that the proximity of enol and keto groups in organic molecules provides an environment in which excited-state intramolecular proton or hydrogen atom transfer readily occurs. In our first investigations of hypericin, we proposed that based on its structure, it too would execute such an excited-state intramolecular transfer, and we interpreted absorption transients in terms of such a process. Objections to such an assignment were raised based upon the mirror-image symmetry noted above and the absence of any discernible isotope effect.[2] The former point may be responded to by invoking a ground-state heterogeneity of the hypericin population, which may include partially tautomerized species. Recent measurements are consistent with such heterogeneity.[3b] Also, as indicated in the previous article, hexa- and tetramethoxy analogues of hypericin display none of the transients that are interpreted in terms of proton or hydrogen atom transfer in hypericin or hypocrellin.[3d]

The absence of an isotope effect and whether proton or atom transfer is a significant nonradiative decay process in hypericin may be responded to in the context of work by Hynes, Borgis, and co-workers.[6] These workers discuss proton transfer of a linear system OH···O in the nonadiabatic and adiabatic limits.[7] The limiting cases are determined by the extent to which the reactant and product species 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 ground-state vibrational levels of the reactants and products lie well below the barrier, the reactants and products are consequently localized, and the transfer event can be described by a tunneling process, in which case very large isotope effects can be expected.[6a] This *nonadiabatic limit* is expected to hold for weak or intermediate strength hydrogen-bonded systems that are characterized by heavy-atom (O−O) distances of 2.6−2.7 Å between which the proton transfers.[6b,8] This heavy-atom distance 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 heavy-atom distance is <2.6 Å, the adiabatic limit is obtained.[6c−f] 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. No deuterium isotope effect is observed for hypericin; we have argued that hypericin falls into the adiabatic limit[2] because its relevant oxygen−oxygen distance is ≲2.5 Å.[9] In hypocrellin, this distance is comparable[10] (Figure 1). This article considers the excited-state photophysics of hypocrellin in light of what is known concerning hypericin and in terms of the theory of proton transfer referred to above.

Experimental Section

Hypocrellin *A* was obtained from Molecular Probes Inc. at >98% purity as determined from the supplied TLC and NMR data and was used without further purification. Methanol (Fischer, HPLC grade) was used as received. Methanol-*O-d* (Aldrich) was distilled prior to use, since it was observed that the time constants in the undistilled solvents are shorter. Pump−probe experiments were performed with either amplified dye laser pulses of ∼1−3 ps duration at 30 Hz[3a] or with 150−200 fs pulses at 2 kHz from a regeneratively amplified Ti:sapphire laser.[3b] The method of global data analysis,[3a] which permits the simultaneous fitting of kinetic traces obtained with different probe wavelengths, was employed to extract the time constants (Table 1). Kinetic traces were collected using time scales of 200 and 500 ps. The former are presented in Figure 2. The latter were collected in order to obtain a more accurate measure of the time constants.

Results

Because hypocrellin has a complex excited-state spectrum,[3a] conclusions based on the measurement of a transient at only one probe wavelength can be misleading if a spectral shift occurs between two different solvents or if several time constants are needed to describe the data. The most secure means of analysis then is to measure kinetic traces at several different probe wavelengths and to perform a global analysis on the data. Such data sets are presented for hypocrellin in MeOH and MeOD in Figure 2. The results of the global fits are summarized in Table

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[⊗] Abstract published in *Advance ACS Abstracts,* April 1, 1997.

S1089-5639(96)03047-2 CCC: $14.00

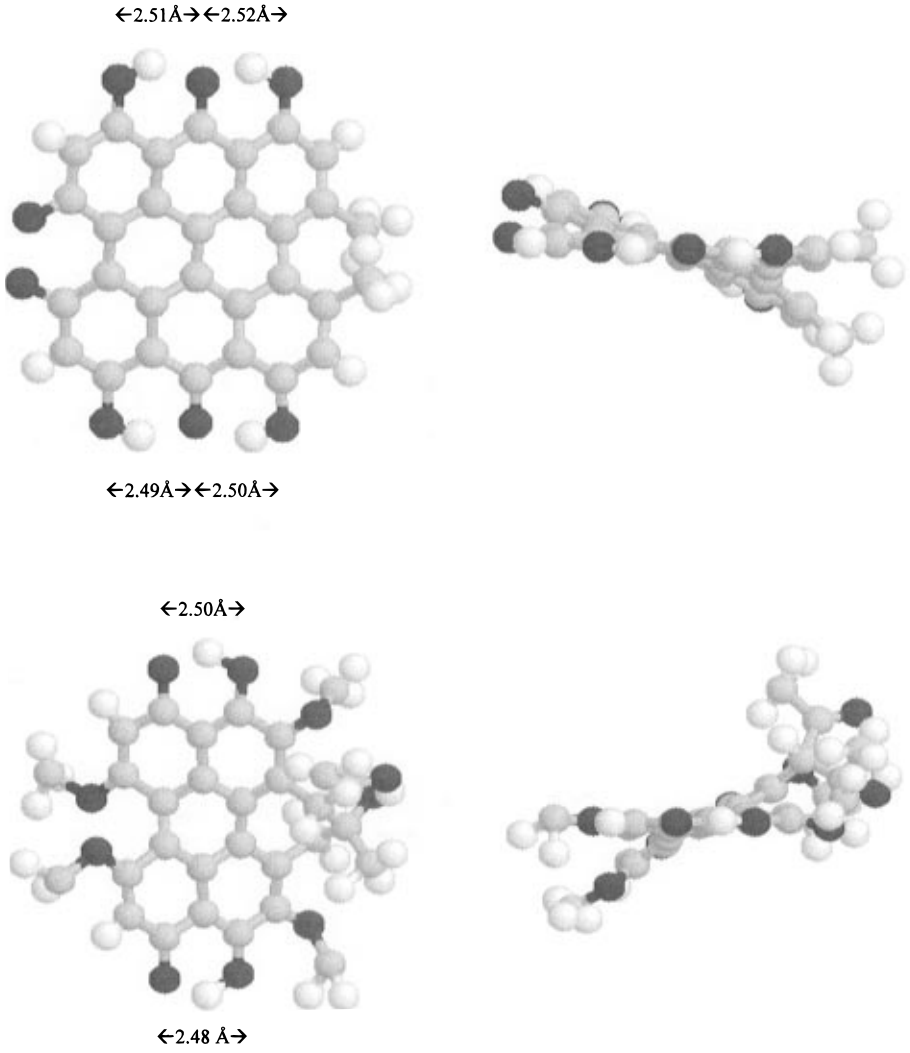

Figure 1. Structures of hypericin and hypocrellin obtained from X-ray data. Critical parameters for the consideration of proton transfer are indicated: O−O distance; the distance of the proton from the enol oxygen and the keto oxygen. Based on experimental data compiled for hydrogen bond strengths and a potential energy function obtained by Lippincott and Schroeder,[8] Hynes, Borgis, and co-workers[6] have proposed that proton-transfer occurs in the adiabatic limit for O−O distances less than 2.6 Å. The O−O distances for hypericin and hypocrellin are $\lesssim$2.5 Å and certainly fall in this regime. The barrier height in the proton coordinate is very sensitive to the O−O separation and, based on theoretical and experimental work,[6,8] is estimated to change ∼50 kcal mol^{-1} Å^{-1}; in general, the coupling between tautomeric forms is modulated by the O−O vibration. However, because the O−O distance is so small for both hypocrellin and hypericin, we assume that the barrier in the proton coordinate is almost negligible and that this coupling is "saturated." Thus, proton or atom transfer is believed to occur for all admissible values of the O−O coordinate and is dependent only upon the fluctuations of the solvent and of the solute skeleton. It is important, however, to recall that the theory[6] and the parameters[8] upon which certain aspects of this discussion of proton transfer are formulated are based upon the assumption of a linear OH···O system. The OH···O system in hypocrellin and hypericin deviates slightly from linearity, and so the qualitative conclusions presented in this article should be considered in this light.

TABLE 1: Global Fitting Parameters for Hypocrellin[a]

λ_{probe} (nm), solvent	a_1 (τ_1, ps)	a_2 (τ_2, ps)	a_3 (τ_3, ps)
595, MeOH	0.09 (67)	−0.06 (1308)	0.06 (∞)
570, MeOH	0.02 (67)	0.04 (1308)	0.01 (∞)
560, MeOH	0.01 (67)	0.08 (1308)	0.02 (∞)
550, MeOH	0.02 (67)	0.06 (1308)	0.02 (∞)
595, MeOD	0.09 (96)	−0.03 (1350)	0.04 (∞)
570, MeOD	0.04 (96)	0.05 (1350)	0.03 (∞)
560, MeOD	0.05 (96)	0.06 (1350)	0.07 (∞)
550, MeOD	0.04 (96)	0.05 (1350)	0.05 (∞)

[a] The data were taken on a 500 ps time scale and were fit to the following form: $\Delta A(t) = a_1[1 - \exp(-t/\tau_1)] + a_2 \exp(-t/\tau_2) + a_3 \exp(-t/\infty)$.

1. These data yield an isotope effect of 1.4 for the process, which we have assigned to a "back transfer" to an "untautomerized species".[11] For comparison, the excited-state transients for hypericin in MeOH and MeOD are presented in Figure 3. Consistent with our earlier report,[2a] there is no evidence for an isotope effect in hypericin. The fluorescence lifetime of hypocrellin in MeOH and MeOD is 1305 ± 15 and 1365 ± 25 ps, respectively. The fluorescence quantum yield of hypocrellin (taken relative to that of hypericin in DMSO, 0.35[3d]) in MeOH and MeOD is 0.15 ± 01 and 0.16 ± 01 ps, respectively. These results indicate that there is no isotope effect on the nonradiative processes affecting the longer-lived excited states.

Discussion

A. Origin of the Isotope Effect. In the adiabatic treatment of proton transfer by Hynes, Borgis, and co-workers,[6] 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 wave function depends on the proton

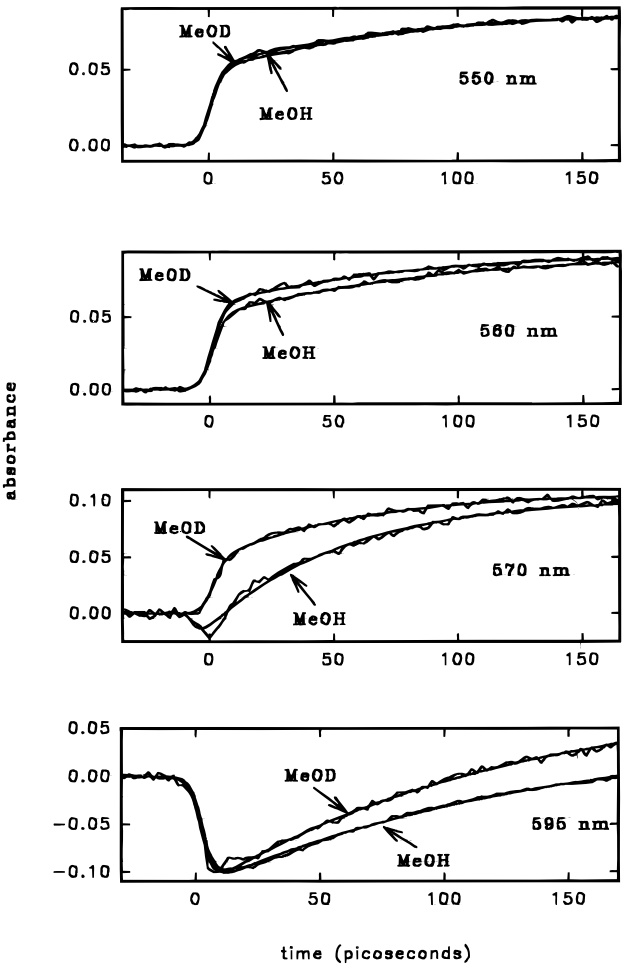

Figure 2. Kinetic traces of hypocrellin in MeOH and MeOD at four different probe wavelengths. Global fits to the data yield time constants of 67 and 97 ps for MeOH and MeOD, respectively. λ_{ex} = 588 nm.

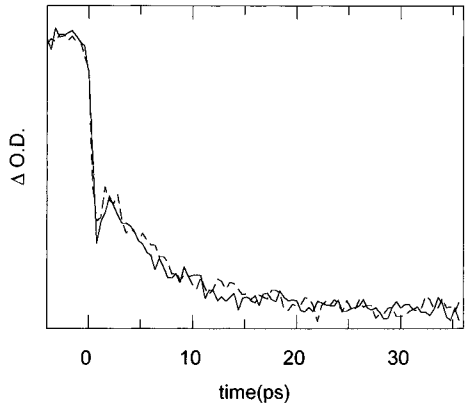

Figure 3. Kinetic traces of hypericin in MeOH (−) and MeOD (- - -). The lack of an isotope effect in the hypericin kinetics is apparent. λ_{ex} = 415 nm; λ_{probe} = 640 nm. The data are fit to a time constant of 6.0 ps. The spike at zero time is an artifact.[3b]

coordinate *q*, and it depends parametrically on the heavy-atom coordinates *Q* and the solvent coordinates *S*.

Figure 4 presents schematic potential energy surfaces as a function of the proton stretch coordinate for a system in the adiabatic limit. Whether the system is in the reactant or product configuration (part a or c of Figure 4) or in an intermediate configuration (Figure 4b) depends on the stabilization imparted by *Q* and *S*. In the theory, this stabilization is discussed largely 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

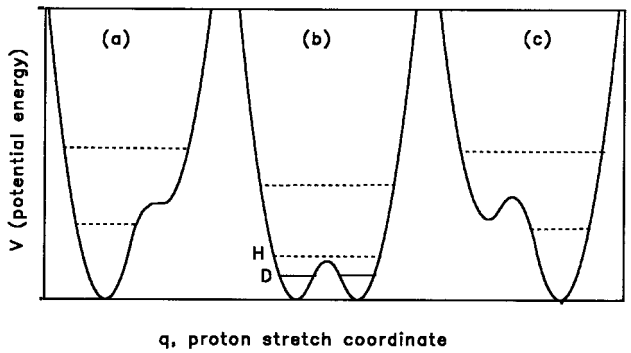

Figure 4. Potential energy curves in the proton stretch coordinate in the adiabatic limit adapted from ref 6f. The three curves indicate different configurations of the solvent and of the solute skeleton that contribute to the stabilization of (a) the "reactant", for example, the normal form or (c) the "product," the tautomer form. The curve depicted in (b) is obtained at the intersection of the energy surfaces in the coordinate representing the solvent (and solute) polarization whose fluctuation is responsible for trapping the molecule in one form or another. The dashed horizontal lines represent the vibrational energy levels for a proton, H. The solid horizontal line in (b) represents the ground-state vibrational energy level for a deuteron, D. If this level lies beneath the barrier in the proton/deuteron coordinate, conversion to products must occur either by tunneling or by an activated process. We suggest that isotopic substitution in hypocrellin produces such a lowering of the ground-state vibrational energy level, thus producing the observed isotope effect.

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 transfer reaction 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 and the rearrangement of double bonds in the aromatic skeleton after or during transfer.[3d]

As noted in the Introduction and illustrated in Figure 4b, in the adiabatic limit, because the vibrational energy levels of the proton stretch mode lie above a small barrier, an isotope effect will not be observed as a result of proton transfer. Staib et al.,[6f] 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 the isotope effect observed in hypocrellin has its origins in such an explanation. That the isotope effect is small suggests that the vibrational ground state is not significantly lowered below the barrier (as is indicated in Figure 4b) and that the proton or hydrogen atom transfer is an activated process. Temperature studies in MeOH and MeOD will permit us to confirm this hypothesis.

We might consider the existence of nonradiative processes other than proton or hydrogen atom transfer—or their possible relation to proton transfer. Zhang et al.[12] have proposed that the long-lived (nanosecond duration) fluorescent states and the triplet states of hypocrellin *A* and *B* can be quenched by accepting electrons from ground-state donors such as *N*,*N*-diethylaniline. Hypericin has also been shown to be a good electron donor and acceptor.[13] Song and co-workers[14] have recently demonstrated the ability of the long-lived fluorescent state of hypericin to transfer an electron to a series of acceptors. Although there is considerable precedent for the observation of deuterium isotope effects in electron-transfer reactions (ref 15 and citations therein), it is unlikely that the primary processes that are being probed in this work report on electron transfer.

In particular, there is no intermolecular quencher available and it is difficult to imagine that an intramolecular electron transfer would occur in hypocrellin (or hypericin), although this would be extremely interesting if it were the case.

Isotope effects can also be attributed to conformational changes. For example, the cis-to-trans photoisomerization rate of d_2-*tert*-stilbene is about 20% larger than that of the perprotio *tert*-stilbene.[16a] This isotope effect is assigned to the partcipation of a substantial amount of ethylenic carbon−hydrogen motion in the reaction coordinate. We have observed a very strong viscosity dependence on the hypocrellin process.[3c] But we have attributed it to a conformational change between twisted forms that is coupled to the proton or hydrogen atom transfer event because in hypocrellin only the enol hydrogens are exchangeable in methanol.

B. Toward a Unified Picture of the Hypericin and Hypocrellin Photophysics. Here is a summary of the salient results that we have obtained concerning the primary photoprocesses of these systems. (1) The transfer rate in hypericin does not depend on viscosity and depends only very weakly on solvent.[2] (2) The time constant in hypericin ranges from ∼6−12 ps.[2] (3) The transfer rate in hypocrellin has a strong dependence on the bulk viscosity and is very well correlated with polarity for solvents of a certain kind, for example, alcohols.[3c] (4) The time constant in hypocrellin ranges from 50 to 230 ps in the solvents we have studied.[3c] (5) For hypocrellin in viscous solvents such as octanol and ethylene glycol, a transient of ∼10 ps duration is also detected.[3a] (6) The transient absorbance kinetics of hypericin differ with excitation wavelength.[3b] The fluorescence properties of hypocrellin in H_2SO_4 and the X-ray structure of hypocrellin indicate that it exists in the tautomerized form in the ground state.[3a,11]

Structurally, hypericin and hypocrellin are very similar. They both possess extended aromatic skeletons whose most important functionalities are the enol and keto groups at positions β to each other. In this regard, the most significant structural difference between them is that hypocrellin possesses two fewer hydroxyl groups at positions β to the keto groups. 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. A crucial result in forming this hypothesis is the observation that under certain conditions we resolve a time constant in the hypocrellin photophysics that is comparable to that observed in hypericin (point 5 above). 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 proton or hydrogen 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 and to which we have made reference above is that of stilbene. The trans-to-cis photoisomerization of stilbene bears distinct differences from the cis-to-trans photoisomerization. For example, the former process has a much stronger viscosity dependence and occurs on a longer time scale than the latter. The differences in behavior have been attributed to different reaction coordinates for the two isomerization processes.[16,17]

Conclusions

The absence of a deuterium isotope effect on the excited state proton or hydrogen atom transfer in hypericin and the presence of such an effect in hypocrellin can be qualitatively understood in terms of a process that is adiabatic in the proton coordinate, in the sense of the theory developed by Borgis, Hynes, and coworkers.[6] For the tautomeric species excited in hypericin, the ground-state vibrational energy level in the proton coordinate lies above the energy barrier even upon deuterium substitution. For the species excited in hypocrellin, deuterium substitution lowers the vibrational energy level slightly below the energy barrier in the proton coordinate. Consideration of the excited-state potential surface in terms of this theory can also help to rationalize the difference in the time constant for the rates of these processes in the two molecules. It is ironic that hypocrellin, which, unlike hypericin, demonstrates no pH drop upon optical excitation under our experimental conditions,[18] provides the most unambiguous evidence for excited-state intramolecular proton or hydrogen atom transfer in this class or aromatic polycyclic quinones as manifested by the deuterium isotope effect. The results presented here suggest that polycyclic quinones such as hypocrellin and hypericin will be of great use in understanding proton-transfer reactions.

Acknowledgment. D.S.E. was supported by fellowships from Amoco and GAANN. Part of this work was supported by NSF Grants BIR9413969 and CHE-9613962. We thank Professor P.-S. Song for a preprint of his article.

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(11) The designation of "back transfer" is based upon the assumption in the literature that the most stable form of hypocrellin is that illustrated in Figure 1c of the previous article.[4d] The published X-ray structure indicates C(12)−O(12) and C(7)−O(7) bond lengths that are greater than those of their C(1)−O(1) and C(6)−O(6) neighbors within experimental error.[9] The

coordinates available from the Cambridge Crystallographic Data Bank suggest that all four bond lengths are comparable. The former data set is consistent with Figure 1d of the previous article, enol bonds being longer than keto bonds. The latter data set suggests a mixture of both the normal and the tautomer forms in the ground state.

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