Temperature Dependence of the Excited-State Intramolecular Proton Transfer Reaction in Hypericin and Hypocrellin A

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The excited-state intramolecular proton-transfer reactions of hypericin and hypocrellin A are measured as a function of temperature in an ethanol/methanol mixture. The data yield activation energies of 0.044 ± 0.008 and 2.12 ± 0.070 kcal/mol for hypericin and hypocrellin A, respectively. The negligible activation energy of hypericin is consistent with previous suggestions that the proton-transfer reaction is adiabatic (K. Das et al., *J. Phys. Chem.* **1997**, *101A*, 3241.) and that a very low-amplitude displacement in at least one other coordinate be taken into account in order to describe the reaction dynamics. The proton transfer for hypocrellin is also considered to occur in the adiabatic regime, but the significant activation energy suggests that a larger amplitude motion than that for the case of hypericin comprises part of the reaction coordinate. 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 is 0.41 ± 0.088 kcal/mol.

Introduction

The naturally occurring polycyclic quinones hypericin and hypocrellin (Figure 1) are of interest because of their light-induced antiviral (especially anti HIV) and antitumor activity (see refs 1−6 and citations therein). By applying the techniques of time-resolved absorption spectroscopy[7−13] and fluorescence upconversion[14] to hypericin, hypocrellin, and their methoxy analogues, we have demonstrated unequivocally that intra-molecular excited-state proton (or hydrogen atom) transfer is a dominant nonradiative process in hypericin and hypocrellin. This transfer occurs between one (or possibly more) of the hydroxyls peri to the carbonyls (Figure 1). Of special relevance to the role of labile protons for this light-induced biological activity is the observation that hypericin and hypocrellin acidify their surroundings upon light absorption.[5,15−17] The role of photo-generated protons takes on significance in the context of the growing body of literature implicating pH decreases with pharmacologically important functions (see refs 5,6,13,17−20 and citations therein).

At first glance, the similarity of the structures (Figure 1) and the spectra (Figure 2) of hypericin and hypocrellin would lead one to believe that they exhibit, at least superficially, similar excited-state photophysics. This is not the case. The time constant for excited-state proton transfer in hypericin is ∼10 ps and essentially independent of solvent,[7,8] whereas for hypocrellin it ranges from 50 to 250 ps in the solvents we have investigated.[12] Also, the proton-transfer reaction in hypericin exhibits no isotope effect,[7] whereas in hypocrellin a small isotope effect of 1.4 is observed.[11]

To gain additional information on these fascinating excited-state proton-transfer reactions, we have studied them as a function of temperature in 1:1 ethanol/methanol mixtures.

Experimental Section

Hypericin and hypocrellin A were obtained from Molecular Probes and were used as received. All the solvents (Aldrich,
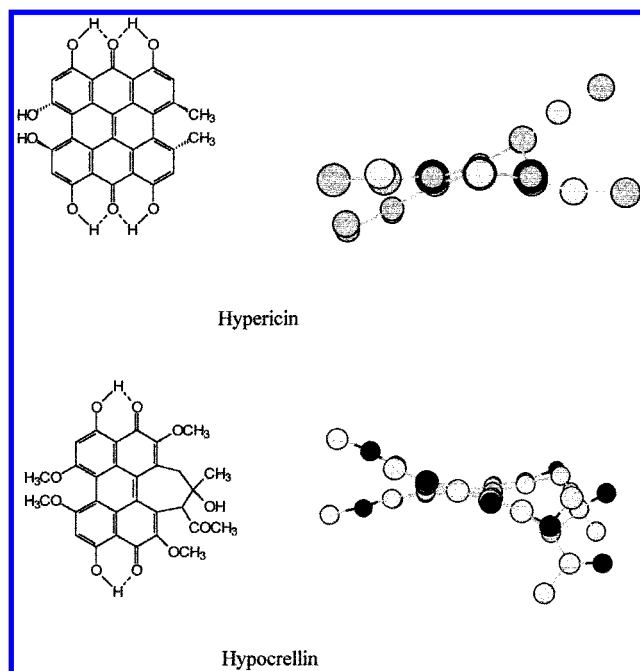

Figure 1. Structures of hypericin and hypocrellin A. Also indicated are side-on views indicating the magnitude of the out-of-plane deformations induced by the substituents in the bay regions.

spectroscopic grade) were also used as received. Pump−probe experiments were performed with amplified dye laser pulses of 1∼3 ps duration.[7] The temperature was controlled to within ± 0.2 K using a Joule−Thomson refrigerator along with a programmable temperature controller (K20) from MMR Technologies. For the low-temperature experiments, samples were prepared in neat ethanol, neat acetonitrile, or in a 1:1 ethanol/methanol mixture; then several freeze−pump−thaw cycles were performed to eliminate the presence of any dissolved gases. The samples were sealed in a quartz cuvette whose optical path length is 1 mm. The concentration of the sample was approximately millimolar. A freshly prepared sample was used

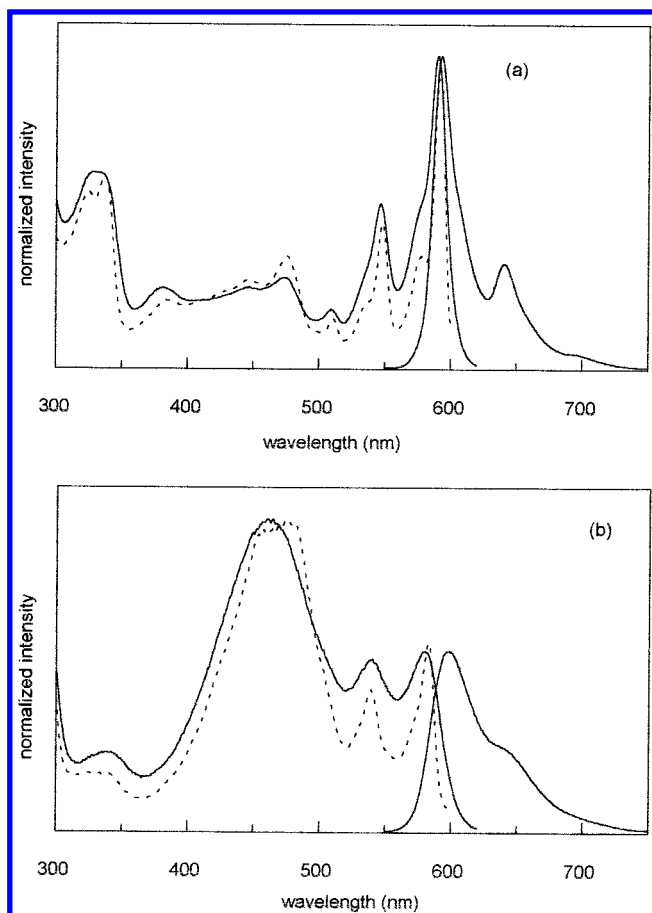* Author to whom correspondence should be addressed.

Figure 2. Comparison of the fluorescence excitation and emission spectra of hypericin and hypocrellin in a 1:1 ethanol/methanol mixture 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.

Figure 3. Transient absorbance traces of hypericin at various temperatures: $\lambda_{pump} = 588$ nm; $\lambda_{probe} = 600$ nm. Because the traces are virtually identical, they are displaced vertically with respect to each other.

Figure 4. Transient absorbance traces of hypocrellin at various temperatures: $\lambda_{pump} = 588$ nm; $\lambda_{probe} = 595$ nm.

after every three or four experiments. The pump wavelength for all the experiments was 588 nm. The probe wavelength for hypericin was 600 nm; that for hypocrellin was 595 nm. The probe wavelengths are those that have been previously determined[7,8,11,21] to be the clearest signatures of excited-state intramolecular proton transfer in these molecules. All the kinetic traces were obtained with the probe beam polarized at the magic angle (54.7°) to the pump beam. For hypericin the transients were collected on a 40 ps time scale; for hypocrellin, a 200 ps time scale. For hypocrellin at the lowest temperatures, to obtain a more accurate estimate of the time constant, a full scale of 500 ps was used. The time constants are extracted from the kinetic traces using Spectra Solve software. The rate constants for Arrhenius plots were constructed from the inverse of the proton-transfer times obtained from the fits to the data.

Finally, to determine the intrinsic activation energy of the proton-transfer process in hypocrellin A, it was necessary to measure the kinetics as a function of temperature in such a fashion that the temperature dependence of the viscosity did not intervene. Five two-point isoviscosity plots were thus constructed (Figure 8). The rate constants for hypocrellin were measured in five different solvents (cyclohexanone, butanol, pentanol, nonanol, and decyl alcohol) at room temperature. The rates were then measured in neat ethanol at the five different temperatures where the viscosity of ethanol is equal to that of the five solvents noted above. These measurements permitted the construction of five sets of isoviscous Arrhenius plots for hypocrellin. At least five measurements were made at each
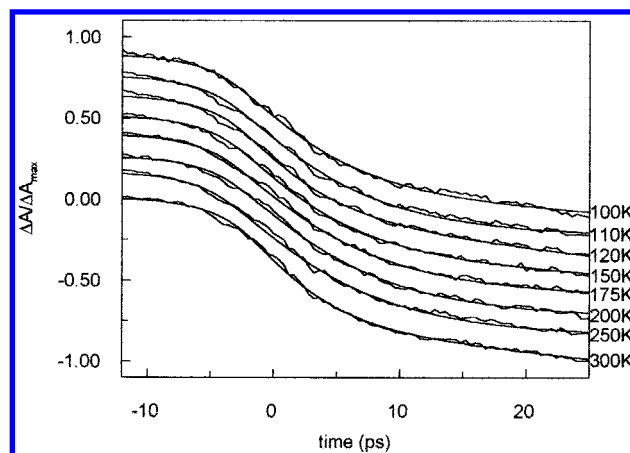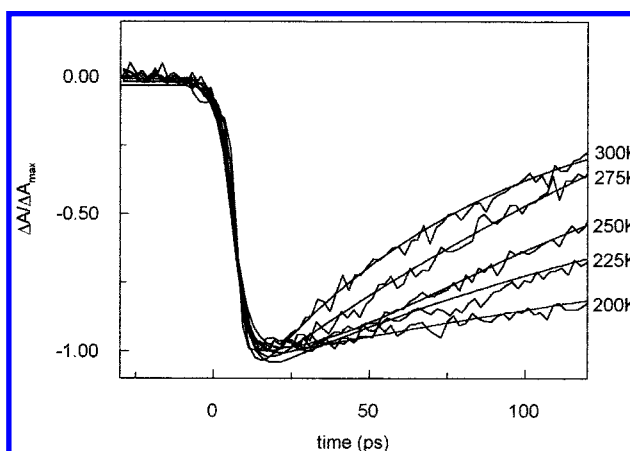
temperature. The error bars represent one standard deviation from the mean.

Results and Discussion

The transient absorption traces at various temperatures for hypericin and hypocrellin are displayed in Figures 3 and 4, respectively; and the proton-transfer times are compiled in Table 1. The Arrhenius plots constructed from these data are displayed in Figure 5. The activation energy for the hypericin reaction is 0.044 ± 0.008 kcal/mol; for the hypocrellin reaction, 2.12 ± 0.070 kcal/mol. (In a previous study we estimated an activation energy for the hypericin reaction, but this was over a very limited temperature range.[8]) The most striking features of the Arrhenius plots are (i) the difference in activation energies, (ii) the essentially zero activation energy of hypericin, and (iii) that the liquid−glass transition of the solvent does not have an effect on the hypericin plot; but, as this transition is approached, there is a dramatic effect on the hypocrellin kinetics (Figure 6).

The first two of these features are consistent with the lack of an isotope effect in hypericin and the small isotope effect in hypocrellin. In particular, the zero activation energy in hypericin is consistent with our previous proposal that the proton-transfer reaction is adiabatic in the proton coordinate and that the zero-point vibrational energy lies above the barrier in this coordinate (Figure 7). As discussed in the work of Hynes, Borgis, and co-workers,[22−25] a major factor in reducing the barrier is the

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TABLE 1: Proton Transfer Times for Hypericin and Hypocrellin A

sample	temp (K)	a_1	τ_1 (ps)	a_2	$\tau_2{}^c$
hypericin[a]	300	0.15 ± 0.03	5.6 ± 2.5	0.9 ± 0.1	∞
in 1:1 EtOH/MeOH	250	0.13 ± 0.04	5.5 ± 3.0	0.9 ± 0.3	∞
	200	0.11 ± 0.01	6.0 ± 1.5	0.9 ± 0.1	∞
	175	0.11 ± 0.03	5.7 ± 3.0	0.8 ± 0.3	∞
	150	0.15 ± 0.03	6.5 ± 3.0	0.9 ± 0.3	∞
	120	0.13 ± 0.02	6.4 ± 2.0	0.9 ± 0.1	∞
	110	0.14 ± 0.02	6.4 ± 2.0	0.9 ± 0.1	∞
	100	0.11 ± 0.01	6.6 ± 1.5	0.9 ± 0.1	∞
hypocrellin A[b]	300	0.8 ± 0.3	76 ± 7	0.01 ± 0.005	∞
in 1:1 EtOH/MeOH	275	0.9 ± 0.1	95 ± 13	0.01 ± 0.003	∞
	250	0.9 ± 0.2	149 ± 12	0.05 ± 0.02	∞
	225	0.7 ± 0.2	244 ± 28	0.02 ± 0.006	∞
	200	0.9 ± 0.1	438 ± 78	0.01 ± 0.003	∞
hypocrellin A[b]	300	0.7 ± 0.1	43 ± 6	0.02 ± 0.002	∞
in acetonitrile	275	0.7 ± 0.2	53 ± 7	0.03 ± 0.003	∞
	265	0.8 ± 0.3	58 ± 3	0.01 ± 0.002	∞
	255	0.9 ± 0.1	68 ± 6	0.02 ± 0.001	∞
	245	0.9 ± 0.3	79 ± 5	0.02 ± 0.001	∞

[a] The data for hypericin were fit to the following form: $\Delta A(t) = -a_1 [\exp(-t/\tau_1) - 1] - a_2 \exp(-t/\tau_2)$. [b] The data for hypocrellin were fit to the following form: $\Delta A(t) = -a_1 \exp(-t/\tau_1) - a_2 \exp(-t/\tau_2)$. [c] On the time scale on which the data were collected, the longer-lived component cannot be accurately determined and is given by infinity.
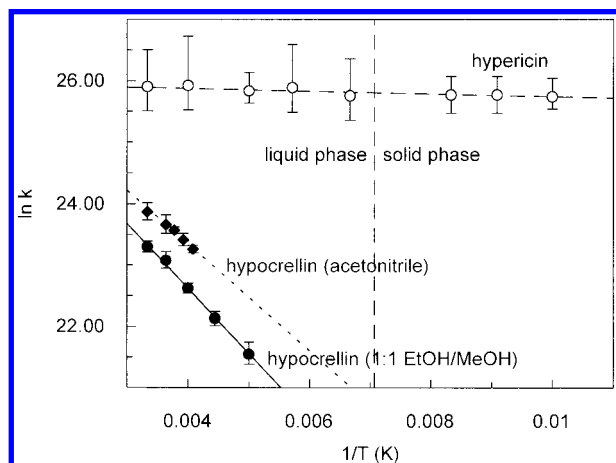

Figure 5. Arrhenius plots for hypericin and hypocrellin based on the data obtained from Figures 3 and 4. Each point represents the average of at least five experiments. The error bars are ± one standard deviation from the mean. The Arrhenius prefactors and activation energies obtained from these plots are the following: hypericin, $A = (1.89 \pm 0.06) \times 10^{11}$ s^{-1} and $E_a = 0.044 \pm 0.008$ kcal/mol; hypocrellin, $A = (4.65 \pm 0.62) \times 10^{11}$ s^{-1} and $E_a = 2.12 \pm 0.070$ kcal/mol. (This activation energy is close to the viscosity activation energies of methanol and ethanol, 2.61 and 3.54 kcal/mol, respectively.) Also included is a plot for hypocrellin in acetonitrile, for which we obtain $A = (4.54 \pm 1.11) \times 10^{11}$ s^{-1} and $E_a = 1.75 \pm 0.14$ kcal/mol. The dependence of the activation energy of hypocrellin in these two solvent systems clearly reflects the temperature dependence of the viscosity. (From 300 to 245 K, the viscosity of acetonitrile goes from 0.35 to 1.08 cP. Viscosity data are obtained from ref 33.)

Figure 6. Comparison of kinetic traces for hypocrellin at 200 and at 150 K: λ_{pump} = 588 nm; λ_{probe} = 595 nm. As discussed in detail elsewhere,[13] at probe wavelengths of 595 nm, two species whose transition dipole moments are at large angles to each other are simultaneously probed. From 300 to ∼200 K, the form of the parallel absorption traces, $\Delta A_{\parallel}(t)$, are nearly identical to those of the magic angle traces, $\Delta A(t) = \Delta A_{\parallel}(t) + 2\Delta A_{\perp}(t)$. (At λ_{probe} = 595 nm, $\Delta A_{\parallel}(t)$ and $\Delta A_{\perp}(t)$ have *opposite* signs.) This indicates that $\Delta A_{\perp}(t)$ fortuitously complements $\Delta A_{\parallel}(t)$ up to ∼200 K. Below ∼200 K, however, the form of the kinetic traces abruptly changes. This suggests a change in the relative population of ground-state species whose transition dipole moments are at large angles to each other. These phenomena are consistent with the glass transition inducing a perturbation in the population of ground-state isomers or tautomers. The differences in the hypericin and, especially, the hypocrellin excitation spectra between 295 and 77 K may reflect such changes in ground-state population (Figure 2).

proximity between the heavy atoms between which the proton is transferred. It is considered that if the heavy-atom distance is 2.5 Å or less, the proton-transfer reaction will lie in the adiabatic limit. X-ray data[26] and ab initio calculations[27] indicate that this is the case for hypericin. There are also X-ray data[28] indicating that hypocrellin lies in this limit as well.

If the zero-point energy lies above the barrier in the proton coordinate (Figure 7a), the proton (or 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 7b). We propose that it is the time-scale for this latter conformational change that determines the observed proton-transfer time.
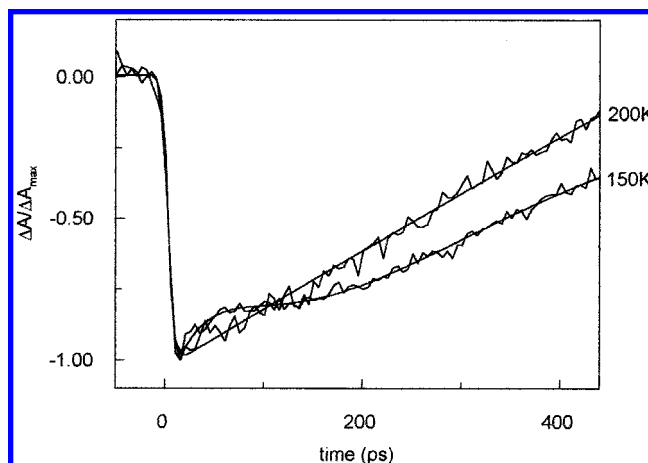
In the model system for excited-state proton transfer, 3-hydroxyflavone, the reaction occurs in ∼100 fs in dry nonhydrogen bonding solvents.[29] In hydrogen bonding solvents such as alcohols, the observed proton-transfer time in 3-hydroxyflavone reflects the time required for the displacement of the solvent hydrogen-bonded to the enol proton of the solute, thus permitting the proton to effect its transfer.[30] Specific hydrogen bonding interactions between hypericin and hypocrellin with solvent cannot explain the relatively slow proton-transfer times (≥10 ps) because the hypericin reaction is insensitive to solvent[7,8] and because the hypocrellin reaction, while sensitive to solvent, is only well correlated to the bulk viscosity of
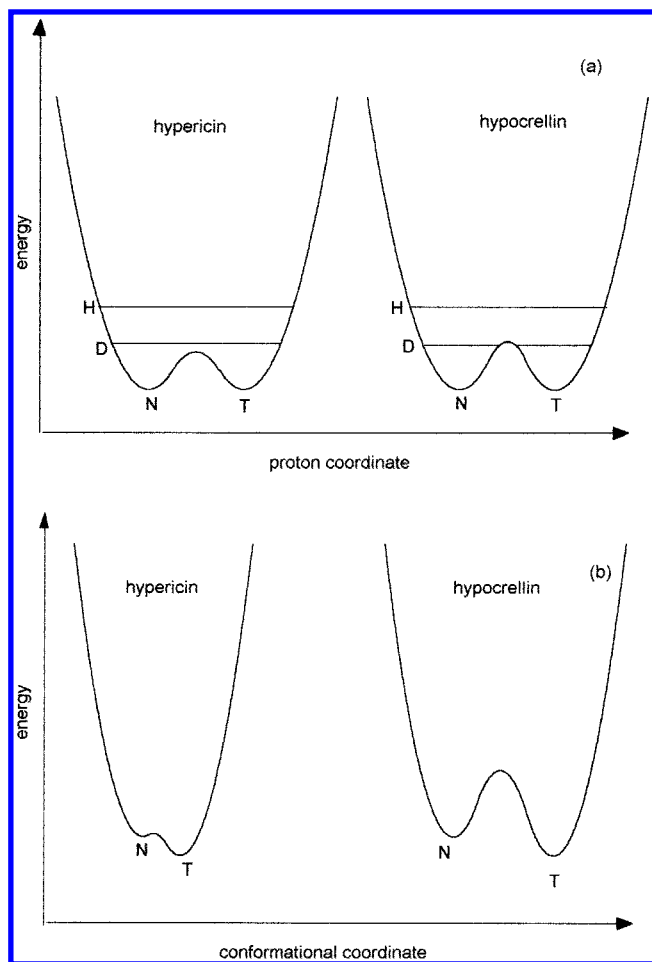
Figure 7. Schematic illustrations of the potential energy surfaces of hypericin and hypocrellin in the proton and "conformational" coordinates. Our justification for the placement of the zero-point level slightly below the barrier for deuterated hypocrellin is explained in detail elsewhere. Briefly, it provides an elegant means, suggested by Staib et al.,[25] to introduce an isotope effect in a situation where there is an adiabatic proton transfer and where the zero-point vibrational level for the protiated species lies above the barrier.

different solvents and not to solvents of a given "type".[12] 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 this oxygen–oxygen distance is essentially the same in both hypericin and hypocrellin (2.5 Å[11,26,28]), a different origin for the solvent dependence of the hypocrellin reaction must be found. The 2.12 ± 0.070 kcal/mol activation energy for hypericin, in addition to the strong dependence of its proton-transfer reaction on viscosity,[12] indicates that, unlike hypericin, its conformational change must be rather large in amplitude and may involve torsions of the aromatic skeleton or displacements of its seven-membered ring.[31] Consistent with the idea of a large-amplitude transition occurring in hypocrellin is that the form of the kinetic traces changes drastically as the liquid–glass transition is approached (Figure 6).

That the proton transfer reaction in hypocrellin A strongly depends on viscosity[12] strongly suggests that part of the activation barrier measured in the methanol/ethanol mixture arises from the temperature dependence of the viscosity of this solvent system. That the activation barrier in hypocrellin A depends on viscosity is indicated by constructing another Arrhenius plot in acetonitrile (Figure 5). To determine that part of the barrier that does not depend on viscosity (the "intrinsic
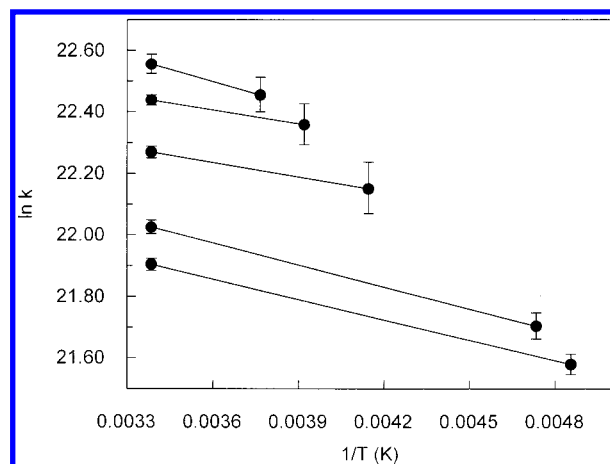

Figure 8. Five sets of isoviscosity plots for hypocrellin A. The points on the left-hand side of the figure correspond to five different solvents: from top to bottom, cyclohexanone (2.12 cP), butanol (2.73 cP), pentanol (3.93 cP), nonanol (10.03 cP), and decyl alcohol (12.13 cP) at room temperature. The points on the right-hand side of the figure correspond to pure ethanol at a temperature adjusted so that its viscosity is the same as that of its solvent partner. The viscosity-independent Arrhenius activation energy obtained from these plots is $E_a = 0.41 \pm 0.088$ kcal/mol.

barrier" to the reaction), we constructed isoviscosity plots[32] as described in the Experimental section and in the caption for Figure 8. The plots indicate that the viscosity-independent barrier to the reaction is 0.41 ± 0.088 kcal/mol. Thus, the viscosity-independent barrier in hypocrellin A is small on an absolute scale, but still 10 times larger than that for hypericin.

Conclusions

The negligible activation energy for hypericin is consistent with previous suggestions that the proton-transfer reaction is adiabatic[7,8,11,13] and requires that displacement in at least one other coordinate be taken into account in order to describe the reaction dynamics. The negligible activation energy for hypericin also indicates that the displacement in the "conformational" coordinate is very low-amplitude. The proton transfer for hypocrellin is also considered to occur in the adiabatic regime, but the activation energy for the process is significant (2.12 ± 0.070 kcal/mol), consistent with the strong correlation for the proton-transfer reaction on bulk viscosity,[12] and suggesting that a larger amplitude motion than for the case of hypericin comprises part of the reaction coordinate. The intrinsic barrier for the hypocrellin A reaction, 0.41 ± 0.088 kcal/mol, is larger than that for hypericin but still quite small. The conformational changes that are coupled to the proton transfer reaction in hypericin and hypocrellin have yet to be identified and are currently under investigation.

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