Solvent Dependence on the Intramolecular Excited-State Proton or Hydrogen Atom Transfer in Hypocrellin

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Hypocrellin and its analog, hypericin (Figure 1), are naturally occurring quinones that have been used for centuries as folk medicines in the orient and the occident and that have attracted much interest because of their light-induced toxicity toward the human immunodeficiency virus (HIV).[1] The structural similarities of hypocrellin and hypericin would seem to suggest that hypocrellin exhibits excited-state and antiviral behavior similar to that of hypericin. Although they both execute excited-state proton or atom transfer[2,3] between the keto and enol oxygens (Figure 1), there are many important differences between them. Hypocrellin absolutely requires oxygen for antiviral activity whereas hypericin does not.[3] Hypocrellin does not provide a light-induced pH drop of its surroundings under conditions in which hypericin does.[4] Here we discuss two other important differences. First, whereas in hypericin the excited-state photophysics depend only negligibly on solvent,[2b] in hypocrellin there is a very pronounced dependence on the solvent, which is related to bulk viscosity and to polarity in primary alcohols and, perhaps, nitriles. Second, the excited-state transfer process in hypocrellin occurs on a time scale at least 10 times longer than the analogous event in hypericin.[2] The elucidation of these *primary* photophysical processes provides a basis for understanding the different modes of activity of hypocrellin and hypericin and will be significant in the exploitation of their properties against viruses and tumors and in the design of other analogous systems.

Hypocrellin A (Molecular Probes) was used as received at >98% purity as determined from the supplied TLC and NMR measurements. Time constants were obtained from transient pump−probe measurements. Within experimental error, the time constants were identical regardless of whether the probe pulse was polarized parallel or at 54.7° (the magic angle) to the pump pulse. For each solvent, using an excitation wavelength of 588 nm, transients were collected at four probe wavelengths: 550, 560, 570, and 600 nm. A time constant was extracted using a global fitting analysis.[5]

The viscosity dependence is remarkable not only because it is absent in hypericin, but also because it appears to be exceedingly well described by a bulk effect; that is, it 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 (see Figure 3 for the polarity dependence): alkane or alcohols;[6] primary alcohol or higher degree alcohol;[7] hydrogen bonding or non-hydrogen bonding;[8] etc.

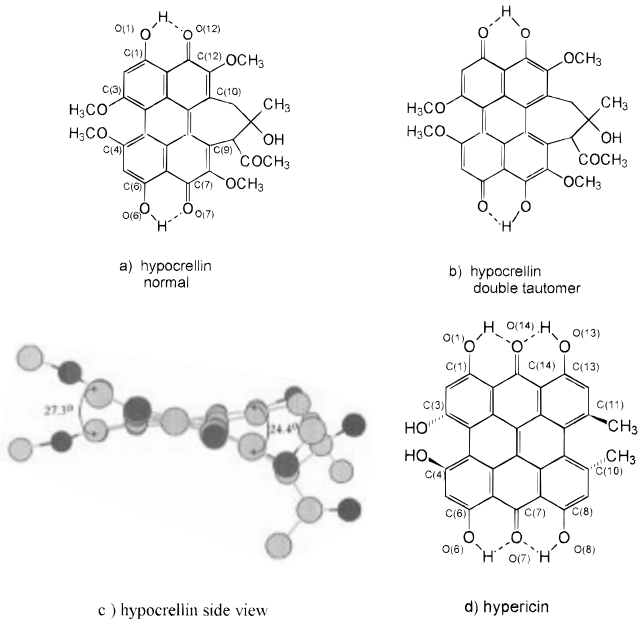

Figure 1. Two-dimensional structures of the "normal" form of hypocrellin A (a) and the bitautomer of hypocrellin (b). The distortion of the hypocrellin skeleton due to the interactions of the side chain groups is indicated in (c). The normal form of hypericin is indicated in (d). For hypocrellin, twist angles of 27.3° and 24.4° are measured with respect to C(3) and C(4) and C(9) and C(10), respectively. Comparable twist angles are observed in hypericin.[10] The numbering of the carbon atoms on the periphery of the aromatic skeleton is that used by Freeman et al.[10c] for hypericin.

We suggest that the viscosity dependence on the excited-state transfer process is a consequence of its coupling to a conformational change between twisted configurations[9] about the long molecular axis (Figure 1c). It is important to note, however, that similar twisted configurations exist in hypericin,[10] which exhibits no viscosity dependence. An important distinguishing factor may be that hypocrellin possesses a seven-membered ring in the "bay region" containing the C(9) and C(10) carbons. It may be that dragging this ring through the viscous medium during the twisting motion is more significant in distinguishing the kinetics of hypocrellin than the twisting motion of the aromatic skeleton itself, which at most goes through an angle of ∼30°.

The magnitude of the time constant in hypocrellin is of interest because, even at the lowest viscosity investigated here, it is ∼50 ps and is considerably larger than that in hypericin (∼6−12 ps).[2] Part of this difference is likely due to the reorganization energy associated with the rearrangement of the bonding in the aromatic skeleton upon interconversion between tautomers (Figure 1a,b), but this alone cannot explain the

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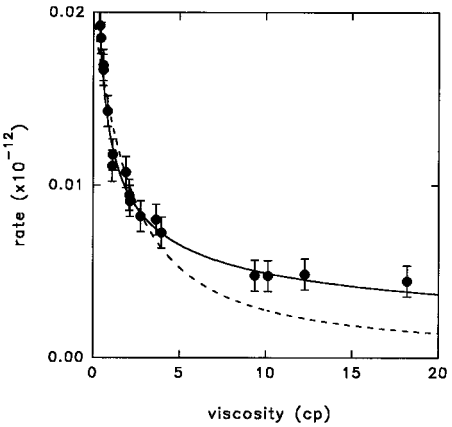

Figure 2. Intramolecular transfer rate of hypocrellin in 17 solvents of different viscosity at 22 °C. For viscosities up to 1.1 cP, the data were collected on a full scale of 200 ps. In order to determine more accurately the time constants at higher viscosities, a full scale of 1000 ps was employed. The data are fit to Kramers' expression and to a phenomenological expression.[6] The fit to the Kramers' equation (- - -), $k = A/(B/\eta)\{[1 + (B/\eta)^2]^{1/2} - 1\} \exp(-E_0/RT)$, yields $A = 2.94 \times 10^{12}$ s^{-1}, $B = 2.84$ cP, and $E_0 = 3.0$ kcal/mol. The solid line is the expression $k = (C/\eta^a) \exp(-E_0/RT)$, where $C = 1.90 \times 10^{12}$ s^{-1}, $a = 0.42$, and $E_0 = 3.0$ kcal/mol. A nonlinear least-squares fit is used to obtain the parameters in the Kramers and phenomenological expressions. The procedure is iterative in both cases: one parameter is kept constant, and two are varied; then another fit is performed holding one of the previously varied parameters constant, and varying the other two. This process is repeated until the parameters converge. Kramers' theory is the first to take into account diffusive crossings and recrossings of an activation barrier. The breakdown of this theory, as illustrated in the Figure, and efforts to address it have been discussed extensively. References can be found in several excellent reviews.[13]

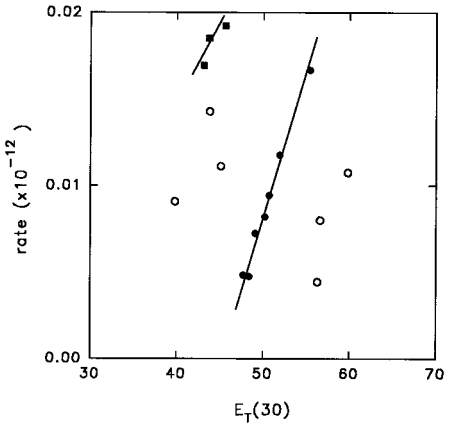

Figure 3. Intramolecular transfer rate of hypocrellin plotted against solvent polarity as measured by $E_T(30)$ for the same 17 solvents in Figure 2. The rate is well correlated with polarity for the alcohols (●, methanol to pentanol, and octanol to decanol). A correlation is also suggested for the three nitriles studied (■, aceto-, propio-, and butyronitrile). The other solvents (○), in order of increasing $E_T(30)$ are cyclohexanone, *N*,*N*-dimethylformamide, DMSO, ethylene glycol, formamide, and 2,2,2-trifluoroethanol. The solid lines are meant only to guide the eye.

difference in rate with respect to hypericin, which must also undergo similar rearrangement. A higher activation barrier for the conformational change referred to above (as is suggested by the fits in Figure 2) would provide an obvious contribution as well. On the basis of the hypericin data in ethylene glycol,[2b] this barrier is expected to be >1.5 kcal/mol for the hypocrellin reaction we measure here. An additional explanation may be that the process in hypocrellin is a "back transfer".[11] This assignment[5a] is based on the weak fluorescence of hypocrellin in sulfuric acid, the similarity of its lifetime in sulfuric acid with the time constants observed in transient absorption, and the X-ray structure, which indicates that in the ground state the tautomer may be the most stable species.[12] It is likely that in hypericin and hypocrellin we are probing different portions of *very similar* excited-state potential energy surfaces and consequently different aspects of the reaction coordinate. It is obvious that hypocrellin and hypericin are not the same molecule and consequently cannot have *identical* potential surfaces. Examination of their structures and their steady-state optical properties indicate, however, many qualitative similarities. We suggest, in terms of the data presented here and our emerging picture of the hypericin and hypocrellin photophysics, that the ground-state hypocrellin species excited in our experiments is very similar to the ground-state hypericin (double) tautomer *that would be excited if it were not thermodynamically inaccessible.*

Finally, whereas the molecular aspects of the solvent are not apparent in the viscosity dependence of our data, a plot of the transfer times against solvent polarity does reveal *specific* solute−solvent interactions (Figure 3). A good correlation of the proton transfer time is obtained in alcohols; another correlation is suggested in three nitriles. (In hypercin, only a very weak dependence on polarity is obtained.[2b]) The transfer times obtained in other solvents are scattered about the plot. It is therefore difficult to determine to what extent the excited-state transfer reaction involves charge-separated states: that there is in some cases a dependence upon polarity should not be taken to imply that the reaction is a proton rather than an atom transfer process. Solvent polarization coupling to a chemical reaction enhances the reaction rate if the polarization fluctuations can provide a lower free energy path and if the time scale for those fluctuations is short compared to those of competitive paths. On the other hand, specific solute−solvent interactions can certainly affect which intramolecular modes are coupled to the reaction coordinate.[14]

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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 1a.

(12) The published X-ray structure indicates C(12)−O(12) and C(7)−O(7) bond lengths that are within experimental error greater than those of their C(1)−O(1) and C(6)−O(6) neighbors.[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 1b, 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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