Chapter 13

Probing Solvation by Alcohols and Water with 7-Azaindole

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The nonradiative pathways of 7-azaindole are extremely sensitive to solvent. In alcohols, 7-azaindole executes an excited-state double-proton transfer. In water, this tautomerization is frustrated. Proton inventory experiments suggest a concerted double-proton transfer in the alcohols and point to another nonradiative process in water. We propose the following idealized picture. Whereas at room temperature 7-azaindole can form a cyclic hydrogen-bonded intermediate with a single alcohol molecule facilitating tautomerization, in water more than one solvent molecule coordinates to the solute and thus prohibits the concerted process. More detailed measurements, however, indicate that water and alcohols do not solvate 7-azaindole in fundamentally different ways, but rather that they represent two extremes of the same phenomenon.

7-Azaindole (Figure 1) is the chromophoric moiety of the nonnatural amino acid, 7-azatryptophan. Recently, we have proposed 7-azatryptophan as an alternative to tryptophan as an optical probe of protein structure and dynamics (*1-8*, Gai, F.; Rich, R. L.; Petrich, J. W. *J. Am. Soc. Chem.* in press). 7-Azatryptophan can be incorporated into synthetic peptides and bacterial protein (*1,2*, Smirnov, A. V.; Rich, R. L.; Petrich, J. W. *Biochem. Biophys. Res. Commun.*, in press; Rich, R. L.; Gai, F.; Lane, J. W.; Petrich, J. W.; Schwabacher, A. W. *J. Am. Soc. Chem.*, in press). Its steady-state absorption and fluorescence spectra are sufficiently different from those of tryptophan that selective excitation and detection may be effected. Most important for its use as an optical probe, however, is that the fluorescence decay for 7-azatryptophan over most of the pH range, when emission is collected over the entire band, is single exponential. For tryptophan, on the other hand, a nonexponential fluorescence decay is observed (Chen, Y.; Gai, F.; Petrich, J. W. *J. Phys. Chem.*, in press). The potential utility of 7-azatryptophan as an optical probe suggests a thorough investigation of the photophysics of its chromophore, 7-azaindole, in

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order to characterize its fluorescence properties and to elucidate its pathways of nonradiative decay.

7-Azaindole has undergone considerable study in nonpolar solvents (*6,9-13*). Kasha and coworkers (*9*) discovered that 7-azaindole can form dimers that undergo excited-state tautomerization (Figure 1a). It has also been demonstrated that excited-state tautomerization occurs for 7-azaindole in alcohols (*6,11-13*). In alcohols the fluorescence spectrum of 7-azaindole is bimodal. In methanol, for example, the maximum of the higher energy band is at 374 nm and that of the lower-energy band is at 505 nm. The former band arises from the so-called "normal" species that decays into the latter band by double-proton transfer. In alcohols, the tautomerization or double-proton transfer reaction has been traditionally depicted (Figure 1b) as being mediated by one solvent molecule, which forms a cyclic complex with the solute. In water, on the other hand, significantly different behavior is observed as illustrated by the fluorescence emission with a single maximum at 386 nm and the single-exponential fluorescence decay when emission is collected with a wide bandpass, 910 ps (*4-6*).

The aims of this article are to investigate the apparent difference between alcohols and water on the excited-state reactivity of 7-azaindole and to obtain more detailed information on the nature of the tautomerization process.

Solvation of 7-Azaindole in Water

7-Azaindole exhibits a single-exponential fluorescence decay of 910 ± 10 ps in water at neutral pH and 20°C if emission from the entire band ($\lambda_{em} \geq 320$ nm) is collected (*4-6*). The fluorescence decay, however, deviates from single exponential if emission is collected with a limited bandpass. For $\lambda_{em} \leq 450$ nm, a single exponential does not provide a satisfactory fit. An acceptable fit is obtained using two exponentially decaying components and indicates that about 20% of the fluorescent emission decays with a time constant between 40 to 100 ps (depending on the full-scale time base chosen for the experiment). A component with a 70-ps decay time is also detected in the transient absorbance of 7-azaindole in water (*3*). There is no such rapid component in the fluorescence decay or the transient absorption of the 7-methyl- and 1-methyl-derivatives of 7-azaindole (*3,4*). We have thus attributed this rapid component to a *small* population of 7-azaindole molecules that undergo excited-state tautomerization. For the duration of the discussion, we shall refer to this transient as the 70-ps component because it is more clearly resolved in the transient absorption measurements (*3*).

The 910 ps component that is resolved for $\lambda_{em} \leq 450$ nm or when emission is collected over the entire band is attributed to the majority of the 7-azaindole molecules that are not capable of excited-state tautomerization because they exist in a "blocked" state of solvation (Figure 1c). This assignment will be described in more detail below.

When $\lambda_{em} \geq 505$ nm, the fluorescence decay can be fit to the form $F(t) = -0.69 \exp(-t/70\text{ ps}) + 1.69 \exp(-t/980\text{ ps})$. The long-lived component is observed to lengthen from 910 to 980 ps. This lengthening of the lifetime at long emission wavelengths was reported earlier (*6*), but no significance was drawn to it. If the rise time of the fluorescence emission can be attributed to the

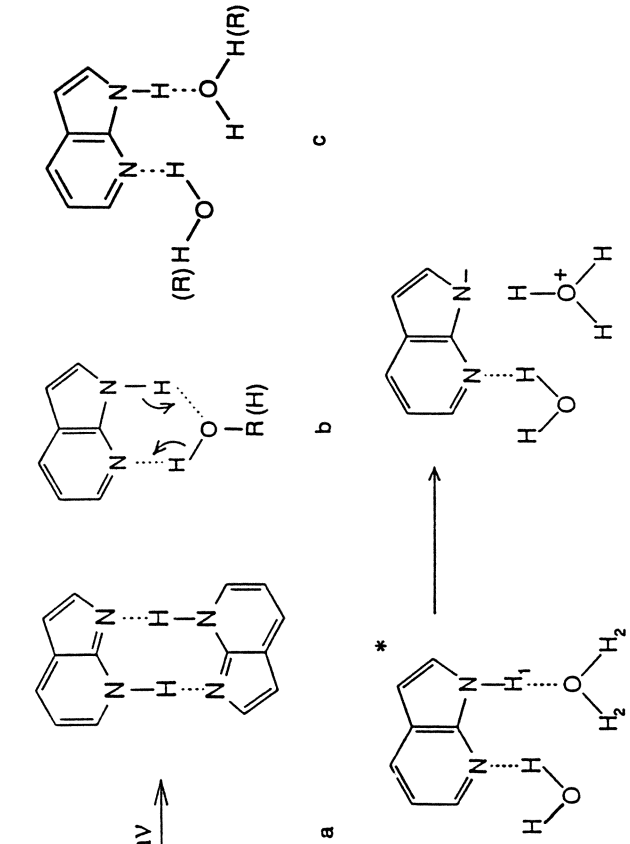

Figure 1. Idealized structures for excited-state tautomerization in (a) dimers of 7-azaindole and in (b) complexes of 7-azaindole with linear alcohols. We have argued that water (6), and to a certain extent, alcohols (13) can solvate 7-azaindole in such a fashion (c) that excited-state tautomerization is frustrated. We suggest, however, that abstraction (d) of the N_1 proton by the coordinated water molecule is an important nonradiative pathway.

appearance of tautomer, then for $\lambda_{em} \geq 505$ nm $|0.69/1.69| \sim 0.40$ is the fraction of tautomer present. The rest of the emission arises from 7-azaindole molecules incapable of tautomerization and characterized by a 910 ps lifetime. Thus, 980 ps represents the weighted average of 910 ps and a longer lifetime, namely ~ 1100 ps. This decay time is *identical* to that of protonated (pH < 3) 7-azaindole (*4*).

Comparison of 7-Azaindole in Water and Alcohols

At ambient temperature, the fluorescence decay of the normal band of 7-azaindole (commercially-available or purified) in alcohols can always be fit well by a single exponential plus a small amount of longer-lived component. Figure 2 illustrates the increase in the magnitude of this long component with decreasing temperature for 1-butanol. The amplitude of the longer-lived component increases from about 5% at 20°C to about 44% at -6°C. This result renders the assignment of this component in alcohols to an impurity untenable. Assuming that the extinction coefficient and the radiative rate of a putative impurity are relatively insensitive to temperature, such a large change in the amplitude is unlikely. The longer-lived component is *predominant* in polyalcohols even at 20°C: ethylene glycol, F(t) = 0.31exp(-t/141ps) + 0.69exp(-t/461ps); and propylene glycol, F(t) = 0.31exp(-t/197ps) + 0.69exp(-t/816ps). This longer-lived component is taken as evidence for the presence of a blocked state of solvation in alcohols such as has been already discussed for water (Chen, Y.; Gai, F.; Petrich, J. W. *Chem. Phys. Lett.*, submitted).

Application of the Proton Inventory to the Nonradiative Process in 7-Azaindole

7-Azaindole in Methanol. The isotope effect on proton transfer reactions is rarely a linear function of solvent deuterium content. Gross and Butler explained this phenomenon by noting that either the H/D composition in the proton site can be different with respect to the solvent or more than one proton is in flight during the rate-limiting step (*14*). The Gross-Butler equation (below) relates the rate of the process in the protiated solvent, k_o, to the rate in a solution of mole fraction n of the deuterated solvent and to all the protons in the reactant and transition states involved:

$$k_n = k_o \frac{\prod_i^{\nu} (1-n+n\phi_i^T)}{\prod_i^{\nu} (1-n+n\phi_i^R)} = k_o \prod_i^{\nu} (1-n+n\phi_i^T)$$

where ν is the total number of protons involved. The $\phi^{T,R}$ are the fractionation factors in the transition and the reactant states, respectively. ϕ is the ratio of the preference in a site in a molecule for deuterium over protium relative to the preference for deuterium over protium in a solvent molecule (*14*). In other words, ϕ is the equilibrium constant for the generalized reaction: XH + ROD $\rightleftharpoons$ XD + ROH. It is customary in most analyses to take $\phi^R = 1$ for an NH or an OH site, as indicated above. (These ϕ^R are for *the ground state*. In order to

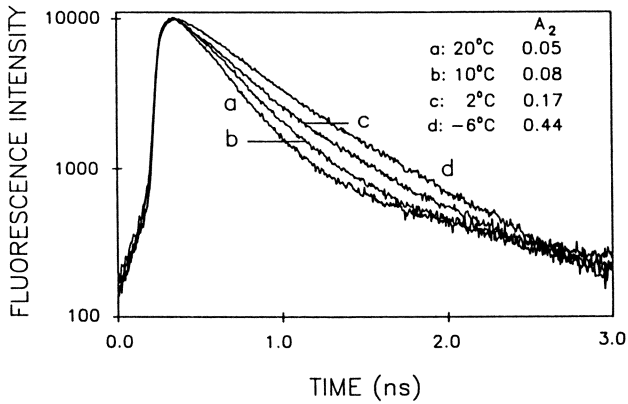

Figure 2. Fluorescence decay of the normal band of 7-azaindole in 1-butanol as a function of temperature (320 nm < λ_{em} < 460 nm): (a) F(t) = 0.95exp(-t/234 ps) + 0.05exp(-t/1818 ps); (b) F(t) = 0.92exp(-t/280 ps) + 0.08exp(-t/1406 ps); (c) F(t) = 0.83exp(-t/329 ps) + 0.17exp(-t/916 ps); (d) F(t) = 0.56exp(-t/360 ps) + 0.44exp(-t/760 ps). Because the full-scale time base for the photon-counting measurement is only three nanoseconds, an accurate determination of the duration of the longer-lived component is difficult.

apply them directly to our problem, we must assume that the ϕ^R are identical in the excited state. We have considered this possibility elsewhere (*8*).)

The downward bulging curve for 7-azaindole in methanol (and in ethanol (*8*)) (Figure 3) is such that a plot of $(k_n/k_o)^{½}$ vs n yields a straight line. This result suggests that only two protons are involved in the excited-state tautomerization of 7-azaindole in alcohols. This result is also consistent with the "cyclic complex" of 7-azaindole and alcohol (Figure 1b) that has been traditionally assumed to be required for the tautomerization to proceed.

7-Azaindole in Water. The downward bulging of the curve obtained for 7-azaindole in H_2O/D_2O mixtures suggests that more than one proton is involved in the transition state of the nonradiative deactivation process. Fitting k_n/k_o vs n to a quadratic model (i.e., a two-proton process) gives imaginary ϕ^T for the data in water (ϕ^T = 0.43 ± i0.30). Imaginary ϕ^T can be obtained when there are two or more competing parallel pathways and if at least one of the transition states involves *at least* two protons (*14*). We have, however, argued elsewhere (*3-5,8*) that not more than 20% of the 7-azaindole population in water is capable of executing double proton transfer and that this process can be observed only under conditions of sufficient wavelength and time resolution (*3,4*). In fact, double proton transfer of 7-azaindole in water is a minor nonradiative pathway compared to monophotonic ionization (*3,7*, Chen, Y.; Gai, F.; Petrich, J. W. *J. Phys. Chem.*, in press). The failure of the quadratic model to fit the proton inventory data coupled with the previous evidence against the importance of excited-state tautomerization in water argue against a concerted two-proton process in this solvent. (If two protons are being transferred by 7-azaindole in water, they are not transferred concertedly between N_1 and N_7).

For the sake of simplicity and because of experimental precedent with another system, we discuss the proton inventory data of 7-azaindole in water as a three-proton process. This three-proton process involves the abstraction of hydrogen from N_1 by a coordinated water molecule.

For the three-proton process shown in Figure 1d, the data in Figure 4b yield an excellent fit to the equation $k_n/k_o = (1-n+0.48n)(1-n+0.69n)^2$. Furthermore, a plot of $(k_n/k_o)^{½}$ vs n does not yield a straight line, which is inconsistent with a concerted two-proton process as observed in the alcohols.

Wang et al. observed essentially identical behavior in ribonuclease (*14*). In this enzyme there is an isomerization between two of its conformations that are characterized by $pK_a > 8$ and $pK_a = 6.1$. These workers measured a solvent isotope effect of 4.7±0.4. Their proton inventory measurements were best described by the relation $k_n/k_o = (1-n+0.46n)(1-n+0.69n)^2$. They assigned the rate-limiting step in this isomerization to proton transfer to a water molecule from the protonated imidazole group of a histidine (*14*). Within experimental error, the proton inventory rate parameters for 7-azaindole in water are identical to those for the isomerization of ribonuclease. In both cases, the shuttling of a proton from nitrogen to a water molecule is proposed to be the rate-determining step.

A fundamental assumption made in deriving the Gross-Butler equation is that the rate of H/D exchange between the solute and the solvent is significantly greater than the rate of proton transfer being investigated. In other words, *the decay of the entire reactant population must be characterized by a rate*

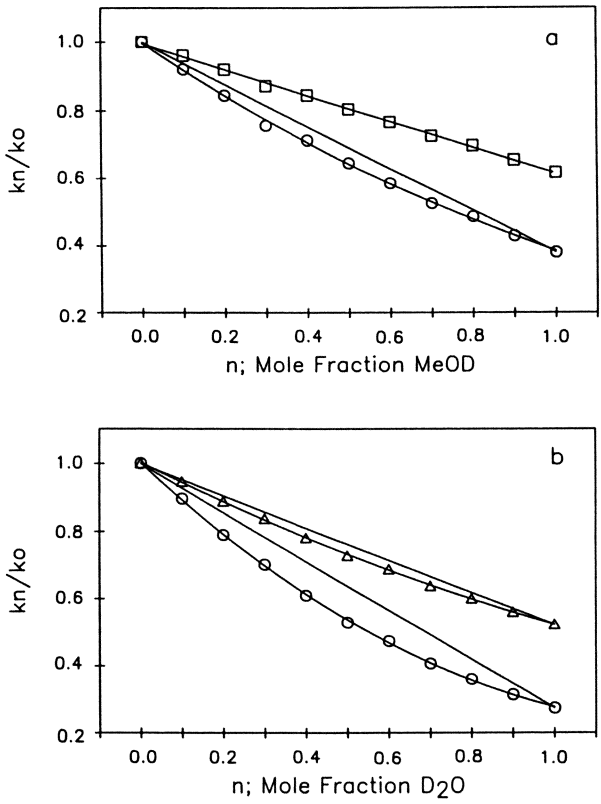

Figure 3. (a) Ratio of tautomerization rate of 7-azaindole in MeOH, k_o, to that in a mixture of protiated and deuterated methanol that is mole fraction, n, in MeOD, k_n. The open circles represent k_n/k_o vs n. The solid line through the data represents the fit assuming a two-proton process with $\phi^T = 0.62$. Directly above is plotted the straight line that would result from a one proton process, i.e., the average of k_o and k_1 weighted by the respective mole fractions of protiated and deuterated solvents (19). The open squares represent $(k_n/k_o)^{½}$ vs n. The linearity of this plot verifies the two-proton process in methanol, assuming the validity of the Gross-Butler equation. (b) Proton inventory data for 7-azaindole in H_2O and D_2O at 20°C. The open circles represent k_n/k_o vs n. The pH at n=0 is 6.8. The solid line through these data represents the fit assuming a three proton process: $k_n/k_o = (1 - n + 0.48\ n)(1 - n + 0.69\ n)^2$. The straight line plotted directly above is the result expected for a one-proton process. The open triangles represent $(k_n/k_o)^{½}$ vs n. The solid line through the open triangles is only meant to guide the eye. This plot deviates significantly from the straight line just above it. Hence, the proton inventory data in water are different from those in the alcohols. Assuming the validity of the Gross-Butler equation, the water data are inconsistent with a two-proton process. In all cases, the error bars lie within the symbols.

constant that does not change with time; that is, first-order decay kinetics must be obtained. If solvent exchange is not rapid, then the observed decay is a superposition of the decays of the individual isotopically substituted species. For the case of 7-azaindole, at least four individual rate constants may be involved (see below). In practice, it is often very difficult to distinguish experimentally between genuine first-order kinetics, which are characterized by a single exponential decay time, and the superposition of several single-exponential decays characterized by different time constants. For this reason, we have presented (*8*) several different methods of analyzing the 7-azaindole data (Table I).

In order for application of the Gross-Butler equation to the excited-state process of 7-azaindole to be valid, we require that 7-azaindole exchange its N_1 ligand with solvent protium or deuterium much faster than the actual tautomerization reaction depicted in Figure 1b. Since the fluorescence lifetime of 7-azaindole in the solvents used here ranges from 140 to 900 ps, an appropriate time constant for ligand exchange with the solvent would be a few picoseconds. Such a rapid exchange seems unlikely. NMR measurements of ground-state indoles indicate that N_1 exchanges its proton on a time scale of seconds with the solvent (*20*). The strong likelihood of slow exchange in the excited state requires us to consider the kinetics in more detail.

The Criteria for a Concerted Reaction. Figure 4 presents the four cases that may arise if two protons are involved in the deactivation of excited-state 7-azaindole. In Figure 4, the reactants and products are denoted A and D, respectively. B and C denote *intermediates* that would exist if the excited-state tautomerization of 7-azaindole proceeded by either the stepwise pathway ABD or ACD involving first the breaking of the N_1-H bond and then the formation of the N_7-H bond, and vice versa. Given such a reaction scheme, in order to demonstrate that the tautomerization is a concerted process, it is necessary, but not sufficient, to show that $k^{HD} = k^{DH}$ and that $k^{HD} = (k^{HH}k^{DD})^{1/2}$. This latter criterion is referred to as "the rule of the geometric mean." Use of the Gross-Butler equation assumes the applicability of the rule of the geometric mean. This relationship is very restrictive and demands that many requirements be satisfied (*8,16*). For the examples illustrated in Figure 4, one of the most important of these requirements is that for the concerted double-proton transfer, the secondary isotope effect at the N_7 (or N_1) site is *equal* to the primary isotope effect at the N_1 (or N_7) site. We shall also see that in order for this relationship to be satisfied, the reaction must be "symmetric"; that is, the rate constants for the decay of the intermediate B (or C) to A and D must be equal.

The significance of the rule of the geometric mean is that if there is a concerted reaction, both protons must be "in flight" in the transition state. Under these circumstances and in the absence of other effects such as tunneling (*16*), one thus expects the multiple sites in a single transition state to behave independently with respect to isotopic substitution.

The Nonradiative Process of 7-Azaindole in Water. Glasser and Lami (*17*) and Wallace and coworkers (*18*) have discussed the importance of fission of the NH bond as a nonradiative process in gas phase indole. Barkley and

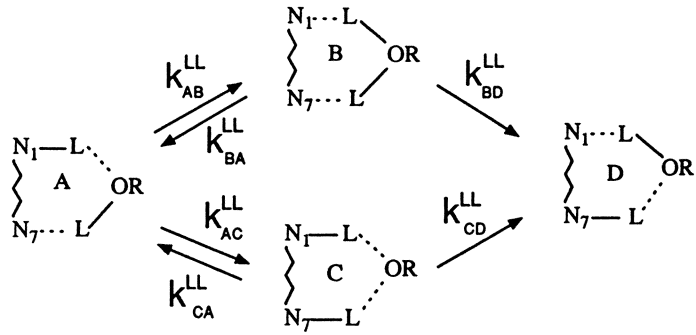

Figure 4. Excited-state tautomerization reactions for each of the four cases of isotopic substitution considered in the text. L = H or D. The paths ABD and ACD represent *stepwise* processes where B and C are distinct intermediates.

Table I
Rate Constants for Proton Transfer Steps[a]

rate constant ($s^{-1} \times 10^{-9}$)	MeOH/MeOD	EtOH/EtOD	H_2O/D_2O
k^{HH}	7.19 ± 0.10	5.43 ± 0.08	1.13 ± 0.02
k^{DD}	2.74 ± 0.04	1.93 ± 0.02	0.31 ± 0.01
$(k^{HH}k^{DD})^{1/2}$	4.43 ± 0.05	3.24 ± 0.03	0.59 ± 0.01
$k^{HD,b}$	4.42 ± 0.06	3.25 ± 0.02	
$k^{DH,b}$	4.59 ± 0.04	3.27 ± 0.02	
$k^{HD,c}$	4.29 ± 0.11	3.24 ± 0.09	0.48 ± 0.02

[a] Fluorescence lifetime measurements from which the rate constants were obtained were performed at 20 °C.
[b] Obtained from equations 18 and 19 of reference 12. Because this method requires fitting the data to a double-exponential fluorescence decay, the corresponding rate constants could not be determined for water, where a single exponential is sufficient to describe the decay curves.
[c] Obtained from equation 22 of reference 12. This method of analysis assumes that $k^{HD} = k^{DH}$. The values cited are for n = 0.5. If n = 0.2, then for MeOH/MeOD and EtOH/EtOD, k^{HD} is 4.14 × 10^9 s^{-1} and 3.18 × 10^9 s^{-1}, respectively. If n = 0.7, then for MeOH/MeOD and EtOH/EtOD, k^{HD} is 4.18 × 10^9 s^{-1} and 3.37 × 10^9 s^{-1}, respectively.

coworkers (*19*) have performed detailed investigations of the deuterium isotope effect on the photophysics of tryptophan, indole, and some of their derivatives. They have proposed at least six different mechanisms to explain the isotope effect ranging from photoionization, hydride transfer from the NH, proton transfer from the solvent to the ring, solvent mediated NH exchange, tautomerization resulting in NH abstraction, and exciplex formation.

We propose that the isotope effect observed in indole derivatives can be rationalized by the same mechanism that we illustrate for 7-azaindole in Figure 1d. We suggest that in indole this process is much less efficient because there is no N_7 nitrogen coordinated with a solvent proton. Such an interaction could establish a partial positive charge on N_7 that would help to stabilize the negative charge generated on N_1.

The Origin of the Isotope Effect. Finally we must comment on the origin of the isotope effect. In large part because of the rapid (1.4 ps) tautomerization observed in dimers of 7-azaindole (*10*), the tautomerization of dilute solutions of 7-azaindole in alcohols has been discussed in terms of a two-step process (*11-13*). The first step involves obtaining the correct solvation of the solute by the alcohol; the second step, double-proton transfer. The interpretation of our isotopic substitution experiments depends on whether the two-step model is appropriate and, if it is, whether the solvation step is slow, fast, or comparable to tautomerization. If the rate-limiting step in the double-proton transfer reaction is the formation of the cyclic complex, then the isotope effects we discuss above require reinterpretation. Additional experimental and theoretical work is necessary in order to answer this question definitively. For the moment, we suggest that if solvation were the rate-limiting step in the excited-state tautomerization of 7-azaindole in alcohols, it would be extremely fortuitous that the rule of the geometric mean holds (Table I). In addition, dimers of 7-azaindole may not be an appropriate paradigm for the tautomerization of the 7-azaindole-alcohol complex. For example, Fuke and Kaya (*20*) observe that in supersonic jets the rate of excited-state double-proton transfer of 7-azaindole dimers is 10^{12} s^{-1}, while in dimers of 1-azacarbazole and in complexes of 7-azaindole with 1-azacarbazole the rate is 10^9 s^{-1}. The reduction in rate by a factor of 10^3 is initially surprising given the very similar hydrogen bonding in the three types of complexes. It is therefore most likely premature to assume that tautomerization in a 7-azaindole complex occurs as rapidly as in a 7-azaindole dimer. Fuke and Kaya suggest that detailed considerations of the coupling of proton motion with intermolecular vibrational motion are required in order to predict the rate of such tautomerization reactions (*20*).

Figure 5 presents a plot of the time constant for excited-state proton transfer in 7-azaindole at 20°C against pK_{auto} for a wide range of solvents. K_{auto} is the equilibrium constant for autoprotolysis and characterizes both the proton accepting and proton donating abilities of a solvent (S) for the reaction: $2SH \rightleftharpoons SH_2^+ + S^-$ (*21*). The correlation is exceptionally good, especially when one considers that previous correlations attempted between the proton transfer times and viscosity or polarity ($E_T(30)$) *are strongly dependent upon the molecular structure of the solvent* (e.g., primary as opposed to secondary alcohols or

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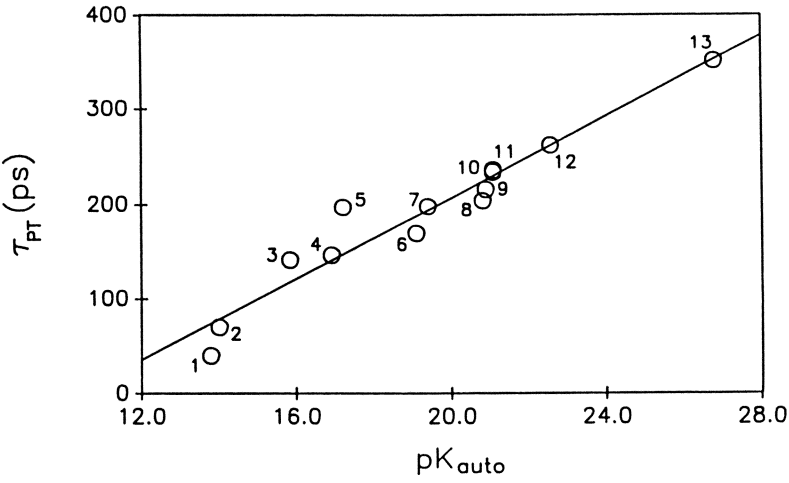

Figure 5. Correlation of the time constant for excited-state tautomerization of 7-azaindole in various solvents with pK_{auto}. (1) 2,2,2-trifluoroethanol; (2) water; (3) ethylene glycol; (4) methanol; (5) propylene glycol; (6) ethanol; (7) 1-propanol; (8) 1-pentanol; (9) 1-butanol; (10) 2-propanol; (11) 2-methyl-1-propanol; (12) 2-butanol; (13) 2-methyl-2-propanol. For 2,2,2-trifluoroethanol, the pK_{auto} is estimated from the pK_a.

polyalcohols or water) and in general are quite scattered (*13,22*). The linear free energy relation presented in Figure 5, however, comprises very disparate kinds of solvents. Even water fits well into this relationship. This correlation is consistent with the requirement of a cyclic solute-solvent complex for excited-state tautomerization and with the proton-transfer event being the rate-limiting step. The larger the autoprotolysis constant (the smaller the pK_{auto}), the easier it is for the solvent to accept a proton from N_1 and to donate a proton to N_7.

Summary and Conclusions

Recently two related studies of 7-azaindole in water have been performed. Chou et al. (*23*) investigated 7-azaindole in mixtures of water and aprotic solvents. Small additions of water to polar aprotic solvents produced tautomer-like emission. They proposed that excited-state tautomerization is possible only when there are significant concentrations of 1:1 complexes of 7-azaindole and water. They further proposed that in pure water the formation of higher-order aggregates inhibits tautomerization during the excited-state lifetime.

Chapman and Maroncelli have studied 7-azaindole fluorescence in water and in mixtures of water and diethyl ether (*22*). They too observe long-wavelength, tautomer-like emission at low water concentrations. In pure water they also observe a rapid rise time at long wavelengths. They, however, take a different point of view, namely that excited-state tautomerization occurs for the entire 7-azaindole population in pure water and that the 7-azaindole fluorescence lifetime is dominated by this reaction. Using a two-state kinetic model in conjunction with steady-state spectral data they conclude that the rapid rise time is associated with the nonradiative decay rate of the tautomer. They propose that the longer, ~ 900 ps, decay time of the entire emission band is a measure of the tautomerization rate. Their scheme requires that the nonradiative decay rate of the tautomer is greater than the rate of tautomerization. They estimate that the rate of tautomerization is $1.2 \times 10^9\ s^{-1}$.

Our observations and conclusions more nearly approach those of Chou et al., although there is a small population of 7-azaindole molecules that do tautomerize in addition to the majority of the population in which this reaction is frustrated. That the fluorescence lifetime of 7-azaindole is not dominated by excited-state tautomerization is demonstrated by the observation of three distinct fluorescence lifetimes: ~ 70 ps, the normal decay time; ~ 980 ps (i.e., 1100 ps (*4*)), the tautomer decay time; and 910 ps, the decay time of the blocked solute. Further evidence is provided by the spectral inhomogeneity of the emission band (*4*). Our major conclusions concerning water can be summarized as follows:

Only a small fraction ($\lesssim$ 20%) of 7-azaindole molecules in pure water are capable of excited-state tautomerization on a 1-ns time scale.

The majority of the 7-azaindole molecules are solvated in such a fashion that tautomerization is blocked. More than 10 ns (*4*) are required to achieve a state of solvation that facilitates tautomerization, that is, to convert the "blocked" species into a "normal" species.

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No significant emission intensity is observed for 7-azaindole in water at 510 nm because so little tautomer is produced and because the tautomer that is produced is rapidly protonated and has an emission maximum at ~ 440 nm.

Most importantly these results clarify the photophysics of 7-azaindole for use as the intrinsic chromophore of the probe molecule, 7-azatryptophan. In particular, the minor amount of tautomerization will contribute to the decay kinetics only if emission is collected at wavelengths red of 505 nm or with a relatively narrow spectral bandpass (with adequate temporal resolution). This is not a serious restriction since experiments are not likely to be performed with such spectral resolution owing to the low fluorescence intensity. When emission is collected over a large spectral region and on a full-scale time base coarser than 3 ns, the tautomerization reaction is imperceptible. On the other hand, the appearance of long-wavelength emission of a protein containing 7-azatryptophan in water would definitely signal a change of environment that facilitates tautomerization.

By analogy with the types of solvation possible in water, we propose that the long-lived fluorescence decay component observed for 7-azaindole in alcohols can be understood by attributing it to a "blocked" form of solvation. *In other words, alcohols and water represent different extremes of solvation; but in neither case is excited-state tautomerization completely permitted or completely prohibited.* Similar blocked states of solvation have been observed in argon matrices at 10 K for the much studied model of excited-state proton transfer, 3-hydroxyflavone (*24*). The groups of Barbara (*25*), Kasha (*26*), and Harris (*27*) have discussed the importance of intermolecular hydrogen bonding, cyclic hydrogen-bonded complexes with one solvent molecule, and doubly solvated hydrogen bonded complexes.

We have performed the first application of the proton inventory technique to an excited-state process. The data suggest that the excited-state tautomerization of 7-azaindole in alcohols proceeds by a concerted, two-proton process that is consistent with the structure of the cyclic solute solvent complex presented in Figures 1a,b. (The data, however, do not prove the existence of a cyclic complex of one solvent molecule with the solvent. There is the possibility that the double proton transfer involving N_1 and N_7 occurs via two different alcohol molecules that interact with each other sufficiently strongly to effect the concerted reaction.) These proton inventory experiments provide further evidence to support the model (Figure 1c) of 7-azaindole being solvated by water in such a way that double-proton transfer—as it occurs in alcohols at room temperature—is negligible.

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