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LETTERS

Influence of Medium Dynamics on Solvation and Charge Separation Reactions: Comparison of a Simple Alcohol and a Protein "Solvent"

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Dielectric relaxation in apomyoglobin and in 1-octanol are studied by using time-resolved Stokes shift measurements of the chromophore anilino-2-aminonaphthalene-6-dimethylsulfonamide. We find that the protein dielectric function can be represented by a Debye form with longitudinal time scale τ_L = 9.2 ns. The octanol measurements are consistent with the dielectric continuum model. The implications of these results on predicting the rates of adiabatic electron-transfer processes are discussed.

Dielectric Relaxation and Electron-Transfer Rates

The intense interest in biological electron-transfer processes has led to growing insight[1-7] into the factors that control donor–acceptor electronic coupling and nuclear reorganization in proteins. By contrast, the dynamics of this nuclear reorganization (solvation) remain poorly understood. It has been shown[8-18] that when

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solvation dynamics are slow relative to electron-transfer rates, such rates are limited not by electronic coupling but by solvent dynamics. It has been argued that in that limit the preexponential factor ν should become equal to some typical inverse solvent time scale τ^{-1}. Since a solvent may have many different time scales, the precise definition of τ is not obvious. As we will show, this problem may be exacerbated when the reacting chromophore is embedded in a protein "solvent". Kosower and Huppert[18a] have measured the intramolecular electron-transfer rate in anilinoaminonaphthalenedimethylsulfonamide (ANSDMA) in a series of alcohols. The dielectric function of an alcohol is given by

$$\epsilon(\omega) = \epsilon_\infty + (\epsilon_0 - \epsilon_\infty)\sum_{j=1}^{3}\frac{c_j}{1 + i\omega\tau_j} \tag{1}$$

ϵ_0 and ϵ_∞ are the static and optical values of the dielectric function, and τ_1, τ_2, and τ_3 are three time constants characterizing the dielectric relaxation. Kosower and Huppert[18a] found that the rate was approximately equal to $1/\tau'_1$ with

$$\tau'_1 = (\epsilon_\infty/\epsilon_0)\tau_1 \tag{2}$$

τ_1 being the longest of the time scales τ_j. For a Debye solvent with a single relaxation time, τ'_1 is equal to the characteristic relaxation time scale of longitudinal dielectric fluctuations and is called the *longitudinal relaxation time*. A considerable theoretical effort was made in order to calculate ν and to unravel the precise definition of the relevant solvent time scale τ. Zusman[19-22] had proposed a simple model for electron transfer which interpolated between the nonadiabatic and adiabatic limits. The formulation of Zusman is stochastic and assumes that the solvent has a single relevant time scale. Nadler and Marcus[23] and Rips and Jortner[24,25] have analyzed the transition to the adiabatic limit and discussed the role of the solvent time scale. Sparpaglione and Mukamel[26a] have calculated the electron-transfer rate in an arbitrary polar solvent characterized by the frequency- and wavevector-dependent dielectric function $\epsilon(\mathbf{k},\omega)$. The ω and $\mathbf{k}$ dependencies of ϵ reflect the temporal and spatial variations, respectively, of the dielectric fluctuations in the medium. In the dielectric continuum approximation, ϵ is taken to be independent of $\mathbf{k}$ and one uses $\epsilon(\omega) \equiv \epsilon(\mathbf{k}=0,\omega)$. The resulting expression of the rate is $K = \nu \exp(-q_a^2/2)$ with

$$\nu = \frac{V^2\left(\frac{\pi}{\hbar^2\lambda k_B T}\right)^{1/2}}{1 + V^2\left(\frac{\pi}{\hbar^2\lambda k_B T}\right)^{1/2}[\tau(q_a) + \tau(q_b)]} \tag{3}$$

Here $\tau(q_m)$ is the solvent time scale function

$$\tau(q_m) = \exp\left(\frac{-q_m^2}{2}\right)\int_0^\infty dt\left\{\frac{1}{[1 - M^2(t)]^{1/2}}\exp\left[\frac{q_m^2 M(t)}{1 + M(t)}\right] - 1\right\} \tag{4}$$

Also

$$q_a^2 \equiv \frac{(\Delta G - \lambda)^2}{2\lambda k_B T} \tag{5a}$$

$$q_b^2 \equiv \frac{(\Delta G + \lambda)^2}{2\lambda k_B T} \tag{5b}$$

where λ is the solvent reorganization energy and q_a^2 and q_b^2 are the activation free energies for the forward and reverse reactions divided by $^1/_2(k_B T)$. The electronic states representing the reactant and product are denoted by a and b, respectively. Similar to other theories[19-25] eq 3 interpolates between the adiabatic and the nonadiabatic limits. $M(t)$ is the solvent relaxation function which carries all the relevant information on the solvent dynamics. It can be evaluated as follows. We assume that the interaction between the charge-transfer system and the solvent is electrostatic. We denote the field created by the system's charge distribution in the j state by $D_j(\mathbf{r})$, j = a, b. The solvent is polar, and its polarization at point $\mathbf{r}$ is denoted by $P(\mathbf{r})$. The interaction between the solvent and solute is

$$V_j = -\int d\mathbf{r}\, D_j(\mathbf{r})\, P(\mathbf{r}) \qquad j = a, b \tag{6}$$

The solvation coordinate which controls the rate is given by

$$U \equiv V_b - V_a = \int d\mathbf{r}\, D_{ab}(\mathbf{r})\, P(\mathbf{r}) \tag{7}$$

with

$$D_{ab}(\mathbf{r}) \equiv D_a(\mathbf{r}) - D_b(\mathbf{r})$$

We further define the Fourier transform of $D_{ab}(\mathbf{r})$

$$D_{ab}(\mathbf{k}) = \int dr\, D_{ab}(\mathbf{r})\exp(i\mathbf{k}\cdot\mathbf{r}) \tag{8}$$

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$M(t)$ is related to the correlation function of the solvation coordinate

$$M(t) = \langle U(t)\, U(0)\rangle / \langle U^2\rangle \tag{9}$$

In eq 9, $U(t)$ is the solvation coordinate evolving in time following the unperturbed Hamiltonian of the medium (without the charge-transfer system). Equation 9 is derived by assuming a weak interaction of the system with the medium and using linear response theory to account for that interaction. Since U is related to the polarization, we can relate $M(t)$ to the correlation function $\langle P(r,t)\, P(r',0)\rangle$ which in turn may be related to $\epsilon(\mathbf{k},\omega)$. The general expression for $M(t)$ is

$$M(t) = \frac{\frac{1}{2\pi i}\int_{-\infty}^{\infty} d\mathbf{k}\, |D_{ab}(\mathbf{k})|^2 \int_{-\infty}^{\infty} \frac{d\omega}{\omega} \exp(i\omega t)\left[\frac{1}{\epsilon(\mathbf{k},\infty)} - \frac{1}{\epsilon(\mathbf{k},0)}\right]}{\int_{-\infty}^{\infty} d\mathbf{k}\, |D_{ab}(\mathbf{k})|^2 \left[\frac{1}{\epsilon(\mathbf{k},\infty)} - \frac{1}{\epsilon(\mathbf{k},0)}\right]} \tag{10}$$

In the dielectric continuum approximation we neglect the spatial variation of the dielectric fluctuations and replace $\epsilon(\mathbf{k},\omega)$ by $\epsilon(\omega) \equiv \epsilon(\mathbf{k}=0,\omega)$. Equation 10 then simplifies considerably:

$$M(t) = \frac{1}{2\pi i}\int_{-\infty}^{\infty} \frac{d\omega}{\omega} \exp(i\omega t) \frac{1/\epsilon(\omega) - 1/\epsilon_0}{1/\epsilon_\infty - 1/\epsilon_0} \tag{11}$$

In general, $M(t)$ depends on the solvent and on the charge-transfer system through the field $D_{ab}(\mathbf{k})$. In the continuum limit $M(t)$ depends solely on the solvent through $\epsilon(\omega)$. Equation 4 provides a precise definition for the solvent time scale controlling the adiabatic electron-transfer rate. It is important to note that the time scale $\tau(q)$ depends on the thermodynamic driving force and reorganization energy of the system. It therefore cannot be considered a purely solvent quantity. $\tau(q_a)$ is the time it takes for a solvent dielectric fluctuation at the transition state to relax to its equilibrium value in the reactant (a) potential well. $\tau(q_b)$ is the same quantity for the product (b) potential well. Adiabatic activationless electron-transfer processes have been studied extensively in numerous polar solvents such as alcohols and nitriles, revealing the relevant solvent time scale. However, there has been no direct measurement of the relevant time scale for a protein. Whether the dynamics of protein response to charge separation may modulate electron-transfer rates in biological systems is still an open question. The experiment described here is an attempt to determine the correlation function $M(t)$ and the time scale function $\tau(q)$ for a chromophore in a protein, employing data from time- and frequency-resolved fluorescence spectra.

Time-Dependent Stokes Shift in Alcohols and Myoglobin

Optical measurements may provide a direct means of probing the solvent polarization correlation function. Consider a polar chromophore in a polar solvent. Following a short pulse excitation the fluorescence spectrum will show a time-dependent Stokes shift. This reflects the solvent reorganization following the change in the chromophore's charge distribution upon optical excitation. Let us denote the position of the emission maximum at time t by $\nu(t)$. The time- and frequency-resolved fluorescence measurements may be used to obtain the dimensionless Stokes shift correlation function:[17,30]

$$C(t) \equiv \frac{\nu(t) - \nu(\infty)}{\nu(0) - \nu(\infty)} \tag{12}$$

Note that $C(0) = 1$ and $C(\infty) = 0$.

Consider a two-level chromophore with a ground state $|g\rangle$ and excited state $|e\rangle$. Using the same electrostatic coupling model used for the charge transfer (eq 6), it can be shown that $C(t)$ is given by eq 10 provided $D_{ab}(\mathbf{k})$ is replaced by $D_{eg}(\mathbf{k})$. In the dielectric continuum limit, $M(t)$ does not depend on the fields D_j at all. The correlation function $C(t)$ obtained in the fluorescence Stokes shift measurements is therefore equal to the correlation function $M(t)$ which controls charge-transfer processes. This is to be understood since in either case $M(t)$ reflects the relaxation of solvent polarization following a sudden change in the system's charge distribution. *This profound relationship allows the direct use of information obtained in optical measurements to predict charge-transfer rates in the same solvent.*[26e]

It should be emphasized that the use of eq 12 is appropriate only when there is one excited state and the system is sliding down this single potential energy surface. When there are two such states, the treatment is more complex. In addition, the inner shell reorganization energy λ_i should be incorporated. These effects are not included in the present expression.

This project follows much of the same methodology employed by Brand[27] several years ago, who used time-resolved emission spectroscopy (TRES) to study the interaction of fluorescent probes to apomyoglobin. These probes bind directly to the vacant heme pocket of apomyoglobin, as shown by competition experiments with heme ($k_B \approx 10^5$).[27] Brand[27] observed spectral shifts on a nanosecond time scale, using *N*-(*p*-tolyl)-2-aminonaphthalene-6-sulfonate (2,6-TNS). It is important to note that in their work Brand and co-workers showed that ANSDMA apparently binds to a single site, so that inhomogeneity of probe binding does not seem to significantly affect the observed photophysics. We have here tested the assumption that the fluorescence of such dyes is modulated by protein dynamics in a manner analogous to solvent relaxation. We selected anilino-2-aminonaphthalene-6-dimethylsulfonamide (2,6-ANSDMA) as the fluorescent probe of choice. This particular dye has convenient spectral properties, binds to apomyoglobin similarly to 2,6-TNS, and removes any potential contributions of hydrogen-bonding effects by the sulfonate group to the protein dynamics, and the photophysics of 2,6-ANSDMA in various organic solvents has been extensively studied by Kosower.[9,11,18] The dye exhibits fluorescence from two different excited states. After excitation, the first excited state populated is termed $S_{1,NP}$, for "nonpolar". This state is localized on the naphthalene ring of the dye and has a negligible dipole moment associated with it. Since the charge rearrangement in this initial excitation process is very small, it will have a negligible reorganization energy for the surrounding solvent dipoles, and the fluorescence lineshape from this state will not change with time. The $S_{1,NP}$ state may undergo an internal charge-transfer reaction to a "charge-transfer" state denoted $S_{1,CT}$, exhibiting charge separation between the naphthalene and phenyl rings. The $S_{1,CT}$ has a large dipole moment associated with it and so will be stabilized as the surrounding dipoles of the medium relax. The resulting fluorescence from the $S_{1,CT}$ level will therefore shift to lower energy with time, and the time evolution of this spectral shift reflects the dynamics of solvent relaxation.

We first reexamined 2,6-ANSDMA dissolved in 1-octanol. This well-studied[18a] system represents a test case for our methodology. Due to the presence of two excited states for ANSDMA, distinctly different fluorescence decay profiles are observed at short and long wavelengths when the dye is dissolved in alcohol. At short wavelengths, an instrument response limited fluorescence risetime dut to direct population of the $S_{1,NP}$ state is observed. At long wavelengths, delayed rise times are found due to subsequent formation of the $S_{1,CT}$ level. The values we obtain for the rise and decay times for the two excited states ($\tau_{d,NP} \approx \tau_{r,CT} \approx 200$ ps; $\tau_{d,CT} \approx 8$ ns) are consistent with those obtained by Kosower.[11] Time-resolved fluorescence spectra were reconstructed from the decay curves and the static emission spectrum. Ideally, the dielectric relaxation of the solvent should be probed by using a simpler chromophore which does not undergo intramolecular charge transfer. In the present system we have to disentangle the charge transfer from the solvent dynamics. We believe, however, that we can still extract the pure solvation dynamics. We have chosen the frequency of fluorescence at half-height on the blue

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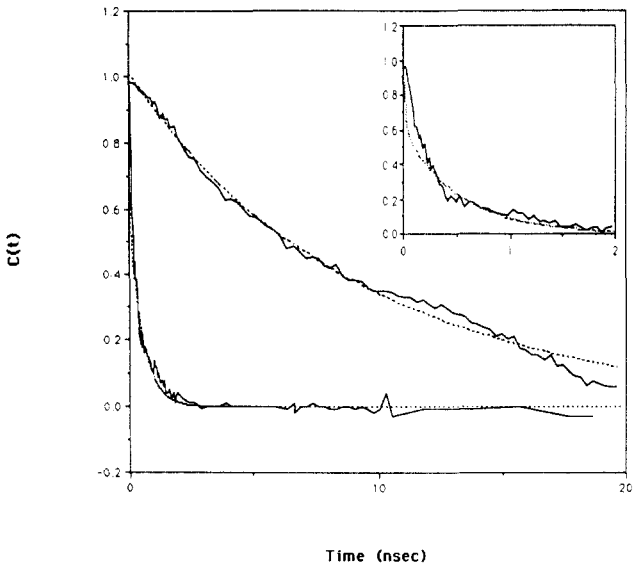

Figure 1. (a) Lower solid line, normalized Stokes shift curve ($C(t)$) for 2,6-ANSDMA in 1-octanol. The total Stokes shift is $\nu(0) - \nu(\infty) = 800$ cm^{-1}. (b) Upper solid line, normalized Stokes shift curve $C(t)$ for 2,6-ANSDMA complexed to apomyoglobin. $\nu(0) = \nu(\infty) = 1100$ cm^{-1}. (c) Lower dashed line, the calculated $C(t)$ curve for 1-octanol in the dielectric continuum approximation (eq 11 using the experimental dielectric function $\epsilon(\omega)$).[26] (d) Upper dashed line, the calculated $C(t)$ curve in the dielectric continuum approximation (eq 11) for a Debye solvent.[26] This curve is exponential $\exp(-t/\tau_L)$ with Debye longitudinal time scale of $\tau_L = 9.1$ ns. The inset shows the 1-octanol data and dielectric continuum calculation (curves a and c) at short times. It demonstrates that in our measurements we do not resolve the shortest of the three decay times of octanol.

side as a measure of the time-dependent Stokes shift and used it as $\nu(t)$ in eq 12. This choice was made following Fleming's analysis[17e] of various criteria for $\nu(t)$. With this definition we found a solvent-controlled time-dependent Stokes shift of $\nu(\infty) - \nu(0) = -800$ cm^{-1}, which is essentially completed in <2 ns compared with τ_e of 8 ns for fluorescence decay. The resulting solvent relaxation correlation function $C(t)$ is shown in Figure 1. We have also calculated $C(t)$ using eq 11 and the known dielectric function of octanol which has the form of eq 1. This calculation[26a] is also displayed in Figure 1. The experimental curve is in good agreement with the continuum theory except for the shortest component which is not resolved in our measurements. This is illustrated in the inset in Figure 1 which shows the short time behavior of both curves on an expanded scale.

We next repeated the same measurements for the dye/protein complex. The fluorescence decay curves for this sytem exhibit fast fluorescence risetimes at both long and short wavelengths. The fluorescence decay times are still wavelength dependent, however, implying that the $S_{1,CT}$ state is formed. An appropriate kinetic scheme which might account for these observations is not obvious. Clearly the protein dramatically alters the photophysics of the dye, presumably due to restrictions on conformational changes and/or free rotation of the phenyl group. Use of a polarizing filter may permit differentiation of $S_{1,NP}$ and $S_{1,CT}$ formation not observed in these spectra. Destabilization of the $S_{1,NP}$ state leading to direct excitation into the $S_{1,CT}$ level would also lead to identical fluorescence risetimes at all wavelengths. The TRES were again reconstructed from the fitted decay curves and the static fluorescence spectrum. Typical time-resolved spectra are shown in Figure 2. The results clearly show a shift of the 2,6-ANSDMA fluorescence to lower energy with time, along with a broadening in spectral shape. We have defined $\nu(t)$ in a similar way to the octanol system. The overall spectral shift of $\nu(\infty) - \nu(0) = -1100$ cm^{-1} is similar to the 1-octanol case. In contrast to the alcohol, however, the shift occurs over at least 20 ns. The $C(t)$ function can be represented by a single-exponential decay with a time scale of 9.1 ns. The experimental Stokes shift correlation function $C(t)$ and its single-exponential fit are displayed

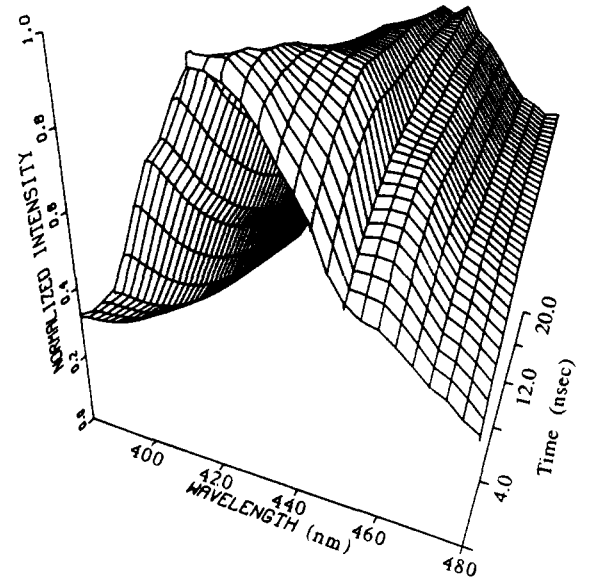

Figure 2. Time-resolved spectra of 2,6-ANSDMA/apomyoglobin complex. Temporal resolution is 9.8 ps.

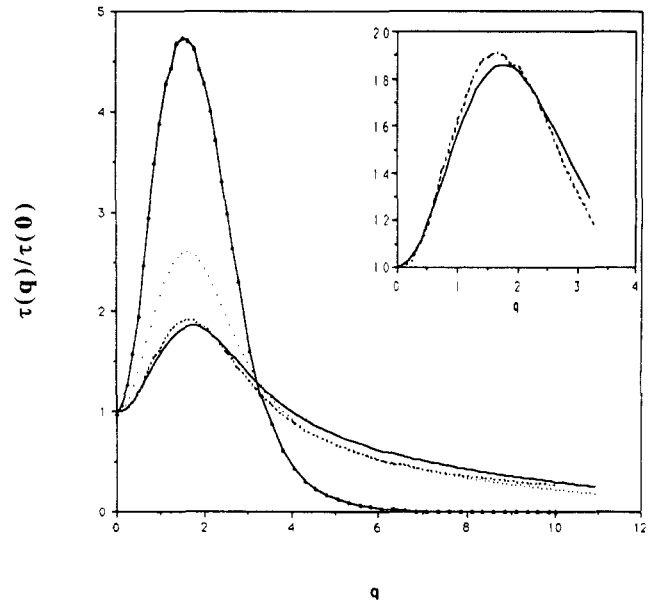

Figure 3. The solvent time scale function $\tau(q)$. The curves are normalized by $\tau(0)$ which corresponds to the solvent time scale for zero activation energy. Calculations were made by numerically integrating eq 4 taking $M(t) = C(t)$ using the four Stokes shift curves displayed in Figure 1. Curves a–d were calculated by using curves a–d, respectively, in Figure 1. The calculation yields also the values of $\tau(0)$ which are as follows: (a) dotted line, $\tau(0) = 150$ ps; (b) solid line, $\tau(0) = 6.2$ ns; (c) solid line plus triangles, $\tau(0) = 61.1$ ps; (d) dashed line, $\tau(0) = (\ln 2)(\tau_L)$. Inset shows an expanded view of the apomyoglobin data (curve b) and the Debye solvent calculation (curve d).

in Figure 1. These results are consistent with those obtained by Brand.[27] Hence, while the static fluorescence properties of the dye/protein are quite similar to those of the dye alcohol, the dynamics of the two systems occur on a very different time scale.

Implications on Adiabatic Electron-Transfer Rates

The main goal of the present article is to explore the use of solvent dielectric relaxation, obtained in time-resolved Stokes shift measurements, in the prediction of adiabatic electron-transfer rates. In the dielectric continuum approximation, the solvent correlation function $M(t)$ necessary for calculating the solvent time scale function $\tau(q)$ is identical with the Stokes shift correlation function $C(t)$. In Figure 1 we displayed the experimental $C(t)$ obtained by using the known dielectric function $\epsilon(\omega)$ of octanol. The parameters used for $\epsilon(\omega)$ (c_j, τ_j, ϵ_0, and ϵ_∞) were tabulated

in ref 26a. We have used both curves as well as the correlation function for a Debye solvent (eq 11) to calculate the time scale function $\tau(q)/\tau(0)$. The calculation was performed by numerically integrating eq 4. The results are displayed in Figure 3a,c. The value of $\tau(0)$ obtained by using the continuum approximation is $\tau(0) = 61$ ps, whereas the experimentally determined value is found here to be 150 ps. We consider this reasonable agreement, which illustrates that the experimental procedures and theoretical formalism used are valid for the simple alcohols. The adiabatic rate for intramolecular electron transfer for ANSDMA in octanol measured by Huppert and Kosower[18a] is 150–200 ps. It should be emphasized that the agreement with the continuum limit is not quantitative. The complete time scale function $\tau(q)$ is quite different for both cases. One possible reason for the difference could be that with our time resolution we do not resolve the initial fast decay part of $C(t)$. This results in a large calculated time scale $\tau(0)$. It should be emphasized that the experimental $\epsilon(\omega)$ is known only for a limited frequency range, and its high-frequency components are not available. This implies that the actual $\tau(q)$ may be much shorter. The short time behavior may correspond to the initial relaxation near the transition point which is most relevant to the electron transfer. Such a discrepancy is therefore a result of an incomplete knowledge of $\epsilon(\omega)$ rather than a deficiency of the current procedure.

We next turn to the protein data. The results of the $\tau(q)/\tau(0)$ calculations performed with the apomyoglobin data are shown in Figure 3. As expected from the single-exponential fit of Figure 1, the curve is very close to that of a Debye solvent. The $\tau(0)$ value is 6.2 ns. It is clearly unreasonable to assume that the activationless intramolecular electron transfer in the dye/protein system could be that slow. In fact, the fast risetime of the charge-transfer level in our measurements shows that the electron-transfer rate must be faster than 50 ps. There are several possible reasons for this discrepancy. First, as with the alcohol, the fastest relaxation times are probably not resolved in our measurements. Experiments with better temporal resolution (e.g., femtosecond pump–probe[31]) could reveal ultrashort dielectric relaxation time scales which could contribute to $C(t)$ and eventually to $\tau(q)$. Second, the dielectric continuum approximation is certainly an oversimplification. The relaxation dynamics in a protein is expected to be strongly dependent on the distance from the chromophore. This implies that we should use the full expression for $M(t)$ (eq 10) which depends on the wavevector- and frequency-dependent dielectric function. Since the relevant electric field in electron transfer $D_{ab}(\mathbf{k})$ is different than $D_{eg}(\mathbf{k})$, relevant for Stokes shift, we expect the resulting $M(t)$ to be different from $C(t)$. Including the spatial dispersion of the dielectric function is expected to be much more important in the protein, which has a well-defined and structured environment around the chromophore. In the alcohols, ensemble averaging over the various solvent configuration makes the continuum approximation more realistic. Third, proteins are characterized by multiple configurations which translate into distributions of barriers and reaction rates. Consequently, the electron-transfer rate may not be described by a simple rate equation, and a generalized rate equation with a frequency-dependent rate $K(s)$ should be used instead. The ordinary rate considered in this Letter is just the $s = 0$ component of $K(s)$. At short times, we expect the large s components of $K(s)$ to be important. $K(s=0)$ is then not a good measure of the electron-transfer rate. The complete generalized rate $K(s)$ for the present model was discussed earlier.[26f]

In conclusion, the data presented here provide a direct experimental test of the rate expression (11). The approach accurately predicts the solvation dynamics of a simple alcohol, when a continuum approximation is employed. It is apparent, however, that protein dynamics on picosecond–nanosecond time scales are not appropriately described by such a simplified picture. Such dynamics are on a time scale that would affect long-distance electron-transfer rates, though, and so must be taken into account when thermodynamic parameters are derived and compared between systems. The relaxation properties of a protein depend, not surprisingly, on the spatial relationship of particular amino acids to the charge or dipole being formed or eliminated.

Experimental Section

2,6-ANSDMA was prepared and purified by published procedures.[18k] 1-Octanol (Aldrich, anhydrous, 99+%) was used as received. Apomyoglobin was prepared by a variation of the method of Teale.[28] The apoprotein was then dialyzed twice against distilled water for 0.5 h each followed by two dialyses, for 1 h each, vs pH 6.4, 20 mM BIS-TRIS buffer. The protein was then further dialyzed against doubly distilled water to remove all ions. The solution was then passed down a 1-cm Sephadex G-25 column equilibrated at 0.1 mM KPhos, pH 6.8, immediately before use. Samples (20–40 μM protein, 1:4 dye/protein ratio, doubly distilled H_2O) were degassed by gently stirring over 2 h in a continuously purged (N_2) glovebox. 1-Octanol was obtained from Aldrich and distilled over magnesium. Dye solutions were degassed by stirring several hours in a continually flushed drybox. Data collection took 4–5 h and was performed at 24 °C. The fluorescence decay curves were obtained by using standard single photon counting (SPC) techniques and fit with a least-squares fitting routine. All fits had χ^2 values of 1.5 or less and residuals less than 5. Excitation pulses (295 nm) were produced from a mode-locked Nd:YAG pumped Coherent Model 700 dye laser. The cavity dumped dye laser, fitted with a single plate birefringent filter and a saturable absorber, produced 1-ps pulses as measured by a Femtochrome model FR103 autocorrelator. Subsequent second-harmonic generation was produced with β-barium borate. The detector was a broad-band Hamamatsu R2809U-11 6 μm proximity focus microchannel plate. A Spex 0.25 m emission monochromator was used with a 600 line/nm grating. Slits on either side of the monochromator were opened to 2 mm or less. The total instrument response was approximately 50 ps. Sample integrity was monitored by comparing decay curves measured periodically at one wavelength. The time-resolved emission spectra (TRES) of the samples were reconstructed (as described by O'Connor,[29] software adapted by Mark Prichard) from the static fluorescence spectra and the fluorescence decay curves measured every 2 or 4 nm throughout the static emission band. Temporal resolution of the TRES was 9.8 ps, and spectra were followed out to 20 ns past excitation. The static fluorescence spectra were recorded on either Spex DM1B or Perkin-Elmer MPF-44A fluorescence spectrometers.

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