Spectral Diffusion in Proteins: A Simple Phenomenological Model

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Received: September 16, 1998; In Final Form: December 17, 1998

Previous experimental studies on a modified cytochrome *c* have shown that optical hole widths have a power-law dependence on waiting time. We show that a phenomenological model, which assumes Gaussian random frequency fluctuations whose two-point time-correlation function is a stretched exponential, is consistent with the experimental data.

Optical spectroscopy has proven to be a useful technique for probing the dynamics of proteins at low temperatures.[1] In this paper, we are concerned with recent optical hole burning experiments on protoporphyrin IX-substituted cytochrome *c* in dimethylformamide/glycerol glass, performed by Fritsch et al.[2] at 4 K. In these experiments, the optical transition of the chromophore (protoporphyrin IX) is inhomogeneously broadened and a narrow-band laser is used to selectively excite only a small fraction of the chromophores (those on resonance with the laser). Some of the excited molecules undergo a photochemical reaction, and the photoproduct absorbs light in a spectral region different from the chromophore. Therefore, when the chromophore's absorption line shape is subsequently scanned, a dip or "hole" appears at the frequency of the burning laser.

In a waiting time experiment, the width of the hole is measured as a function of the waiting time, t_w, between burning and scanning. Typically, the hole width increases with t_w as a result of "spectral diffusion". That is, the transition frequency of an individual chromophore is not static in time, but rather fluctuates due to changes in the chromophore's local environment. As the ensemble of chromophores evolves in time, this leads to a broadening of the hole. In particular, this experiment measures the convolution of the initial hole shape with the waiting-time-dependent "spectral diffusion kernel". The latter is the conditional probability density that a chromophore has a transition frequency ν at time t_w given that it had transition frequency ν_0 at time 0.

In the simplest scenario, both the initial hole and the (spectral diffusion) kernel are Lorentzian functions of frequency, in which case one can simply subtract the initial hole width from the hole width at t_w to obtain the width of the kernel. And there is at least one physical model that leads to a Lorentzian kernel: when the chromophore is interacting in a dipolar manner with a collection of point defects[3] whose internal states change with time. A particular realization of this picture, believed to be appropriate for chromophores in glasses, takes the point defects to be two-level systems (TLS) whose energy asymmetries and tunneling matrix elements are widely distributed.[4−7] In the usual TLS model, it is known that the width of the kernel increases logarithmically with t_w, and indeed, this t_w dependence has been seen for many different chromophore/glass systems.[6]

The protein experiments, when analyzed by assuming a Lorentzian kernel, show a power-law dependence of the width of the kernel on t_w, with an exponent of about 1/2,[2] rather than the logarithmic dependence predicted by the standard TLS model. In addition, these experiments are independent of equilibration or aging time[2] (the time between cooling to cryogenic temperatures and hole burning), in contrast to predictions of the standard TLS model.[8] Other more involved thermal cycling hole burning experiments also provide evidence that the standard TLS model does not apply to proteins.[9] To understand the protein experiments then, one approach is to extend the standard TLS model to include TLS−TLS interactions.[2] Such a theoretical study was performed recently[10] in order to explain a similar power-law dependence of the kernel for chromophores in glasses at extremely long times.[11] Another approach is to extend the TLS model by invoking a new set of double well potentials, thought to be induced by the presence of the chromophore.[12]

In this paper, we consider yet another approach,[2] discarding the TLS model for proteins altogether. Our model is completely phenomenological; we simply assume that the chromophore's frequency fluctuations are described by a stationary Gaussian random process and that the two-point time-correlation function of the fluctuating frequency has a Kohlrausch (stretched exponential) form. These assumptions do not seem unreasonable for a complex interacting many-body system such as a protein.

To be specific, we take the chromophore's transition frequency to have zero mean, $\langle\nu\rangle = 0$, and a variance of $\langle\nu^2\rangle = \sigma^2$, so that (within the Gaussian assumption) the distribution of transition frequencies (inhomogeneous line shape) is the Gaussian function

$$P(\nu) = \frac{1}{\sqrt{2\pi\sigma^2}} \exp\left\{-\frac{\nu^2}{2\sigma^2}\right\} \tag{1}$$

The fwhm (inhomogeneous line width) of this distribution is $\Gamma = 2\sqrt{2\ln 2}\sigma$. We further assume that the two-point time-correlation function is given by $\langle\nu(t)\nu(0)\rangle \equiv \sigma^2 C(t)$, with $C(t) = e^{-(t/\tau)^x}$. τ is the relaxation time for frequency fluctuations, and x is the exponent characterizing the nonexponentiality of the relaxation. Within the Gaussian assumption the kernel is[13]

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10.1021/jp983751k CCC: $18.00 © 1999 American Chemical Society
Published on Web 02/09/1999

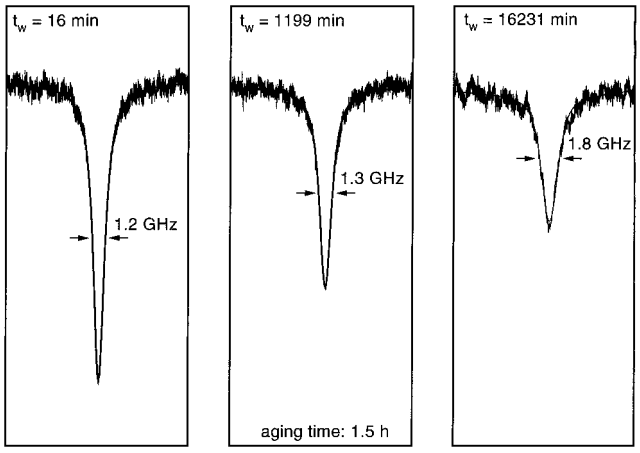

Figure 1. Experimental hole shapes for different waiting times together with Voigtian (convolution of Lorentzian and Gaussian) fits.

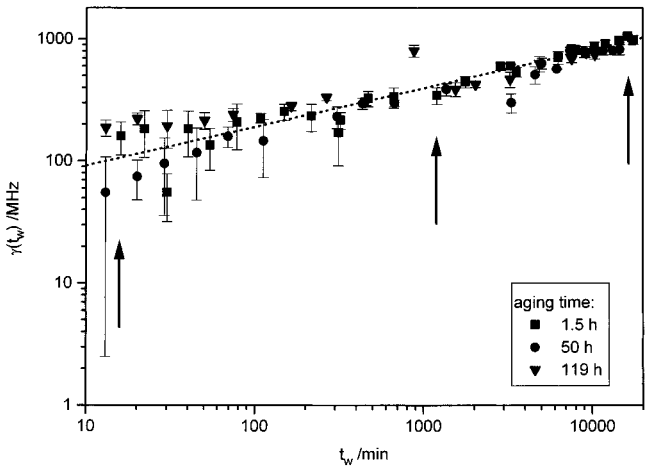

Figure 2. Width of the spectral diffusion kernel, $\gamma(t_w)$, as a function of waiting time, t_w. The arrows indicate the waiting times for the holes shown in Figure 1. The dotted line is the fit to eq 3.

$$P(\nu, t_w|\nu_0) = \frac{1}{\sqrt{2\pi\sigma(t_w)^2}} \exp\left\{-\frac{[\nu - \nu_0 C(t_w)]^2}{2\sigma(t_w)^2}\right\} \quad (2)$$

where $\sigma(t)^2 = \sigma^2[1 - C(t)^2]$. Thus, the kernel is a Gaussian function of frequency whose waiting-time-dependent fwhm is $\gamma(t_w) = 2\sqrt{2\ln 2}\sigma(t_w)$. It is precisely this width (within this model) that is measured in the waiting time experiment. If, in fact, t_w is short compared to τ, then $\gamma(t_w) \simeq \sqrt{\ln 2}(t_w/\tau)^{x/2}\sigma$. Writing σ in terms of Γ (which can be determined experimentally), we then have

$$\gamma(t_w) = \sqrt{2\Gamma}(t_w/\tau)^{x/2} \quad (3)$$

To analyze the experimental[2] hole widths within this model, for each t_w we fit the hole shape to a convolution of the initial (Lorentzian) hole shape with a Gaussian kernel. Examples of these fits are shown in Figure 1 for three different waiting times. As shown, the fits are excellent, as the convolutions are nearly indistinguishable from the experimental data. This yields $\gamma(t_w)$, which is shown in Figure 2 on a log−log plot for several different values of the aging time. The experimental data can be fit to eq 3 using two parameters, x and $\Gamma\tau^{-x/2}$. The best fit, shown in Figure 2, yields a value for x of 0.64. The inhomogeneous line width, Γ, for this system is 110 cm^{-1}. With this, we find that $\tau = 5 \times 10^{15}$ min. These numbers are not unreasonable; x values for many complex systems range from about 1/3 to 1, and the relaxation times for large-scale rearrangements in low-temperature glasses must be extremely long—certainly much longer than any reasonable experimental time scale. Note that this very large value for τ is consistent with our earlier assumption that $t_w \ll \tau$. Note also that although eq 2 predicts a waiting-time-dependent frequency shift, for $t_w \ll \tau$, this would not be observable, as is borne out experimentally.

In summary, we have shown that a very simple phenomenological model, with apparently reasonable assumptions, is consistent with the long-time spectral diffusion of a modified cytochrome *c* at low temperatures. It seems likely that the model is also consistent with the extremely long-time spectral diffusion observed for chromophores in glasses.[11] Our model provides a distinct alternative to models based on two-level systems. It will be important to analyze experimental data at different temperatures. If the model remains consistent with experiment, it will be interesting to try to understand the temperature dependence of x and τ and indeed the origin of the model assumptions themselves, from a more microscopic perspective.

Acknowledgment. J.L.S. is grateful for support from the National Science Foundation (Grants CHE-9526815 and CHE-9522057). J.F. gratefully acknowledges the collaboration with Prof. J. Vanderkooi, from the University of Pennsylvania, on heme proteins and financial support from the Fonds der Chemischen Industrie.

References and Notes

(1) [1] Leeson, D. T. ; Wiersma, D. A.; Fritsch, K.; Friedrich, J. *J. Phys. Chem. B* **1997**, *101*, 6331.
(2) Fritsch, K. et al., *Europhys. Lett.* **1998**, *41*, 339.
(3) Stoneham, A. M. *Rev. Mod. Phys.* **1969**, *41*, 82.
(4) Narasimhan, L. R. et al., *Chem. Rev.* **1990**, *90*, 439.
(5) Jankowiak, R.; Small, G. J. *Phys. Rev. B* **1993**, *47*, 14805.
(6) Silbey, R.; Koedijk, J. M. A.; Völker, S. *J. Chem. Phys.* **1996**, *105*, 901.
(7) Geva, E.; Skinner, J. L. *J. Chem. Phys.* **1997**, *107*, 7630.
(8) Fritsch, K.; Friedrich, J.; Kharlamov, B. M. *J. Chem. Phys.* **1996**, *105*, 1798.
(9) Fritsch, K.; Friedrich, J.; Parak, F.; Skinner, J. L. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 15141.
(10) Neu, P.; Reichman, D. R.; Silbey, R. J. *Phys. Rev. B* **1997**, *56*, 5250.
(11) Maier, H.; Kharlamov, B. M.; Haarer, D. *Phys. Rev. Lett.* **1996**, *76*, 2085.
(12) Heuer, A.; Neu, P. *J. Chem. Phys.* **1997**, *107*, 8686.
(13) Stephens, M. D.; Saven, J. G.; Skinner, J. L. *J. Chem. Phys.* **1997**, *106*, 2129.