Non-Markovian relaxation observed in photon echoes of iron-free myoglobin

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Non-Markovian relaxation has been observed from photon echo signals of iron-free myoglobin for temperatures below 8 K. The transition to the fast modulation limit above 10 K is considered to be initiated by the phonon-assisted conformational relaxation of the protein. From the analysis using stochastic theory, we point out that although the observed echo signals exhibit nonexponential behavior, the corresponding hole-burning spectra happen to be quasi-Lorentzian.

Since the work of Aihara[1] on the non-Markovian theory of the photon echo, a large number of works have been reported on this subject from both experimental[2,3] and theoretical[4-6] points of view. In particular, non-Markovian theories based on the stochastic model have progressed[4-7] a great deal. In order to confirm the theoretical predictions, in particular of the non-Markovian relaxation (the deviation from the exponential decay of the echo signal), two groups of Shank[2] and Wiersma[3] have recently performed photon echo experiments for dye solutions by using femtosecond optical pulses.

As far as the dye-doped polymer samples are concerned, there has already been a large number of reports on the experimental results on their photon echoes, mostly performed at low temperatures.[8] However, even with the use of optical pulses with tens of femtosecond time resolution, the echo signal of the zero-phonon line showed roughly an exponential decay. This might be due to the fact that the frequency modulation, which is induced by the electron-phonon coupling, is in the fast modulation limit $p=\Delta\tau_c \ll 1$, where Δ is the magnitude of the frequency modulation and τ_c is the correlation time. However, around the time origin of the echo signal, the narrow spike, due to the phonon sideband, and the quantum beat, due to the low frequency vibrational modes, might mask the temporal behavior around that region.[9] This has serious consequences for the investigation of the non-Markovian behavior of the zero-phonon line, because the transition from the fast modulation limit to the intermediate modulation case ($p \cong 1$) manifests itself as a change of the leading edge of the echo signals. Therefore, the preferred candidates for this study are samples in which the linear electron-phonon coupling and also the low frequency vibrational mode coupling are weak. Recently, we have found[10] that the linear electron-phonon coupling is extremely weak in iron-free hemeproteins such as myoglobin and cytochrome-*C*, and that the spike due to the phonon sideband is weak at the leading edge of the echo signal. Therefore, detailed investigation of the spectrum of the zero-phonon line is made possible for these materials.

By using the incoherent two-pulse photon echo technique,[11] we investigated the temperature dependence of the temporal behavior of the echo signal. The sample was iron-free myoglobin (H_2-Mb) as in our previous experiment.[10] The sulforhodamine 640 dye laser pumped by the second harmonic of the Nd:YAG laser was used as the excitation light for the echo experiment. The time resolution of the dye laser was 0.5 ps and the center wavelength of 620 nm was almost coincident with that of the absorption spectrum. The echo signal emitted at the $2K_2$-K_1 direction was detected, where K_1 and K_2 are the wave vectors of the first and the second excitation pulses, respectively.

The temporal behavior of the observed echo signal is shown in Fig. 1(A). The signal intensity, normalized by the signal magnitude at the time origin, was plotted in a logarithmic scale. The dimensionless abscissa is the delay time divided by the delay time t_c at the half maximum point of the signal. The echo decay curves changes significantly below 10 K. Above 10 K, the echo signal decays exponentially. As the temperature increases even more, the narrow spike due to the phonon sideband appears. However, the echo decay of the zero-phonon line completely obeys an exponential function. On the other hand, as the temperature decreases below 10 K, the transition away from the exponential decay was clearly observed. For example, the signal at 4.5 K, shown in Fig. 1[A(d)], cannot be fitted by exponential decay.

By using stochastic theory, we tried to simulate numerically the observed signals.[12] The third-order nonlinear polarization responsible for the two-pulse photon echo is written as

$$P^{(3)}(t)=\int_0^\infty\int_0^\infty\int_0^\infty d\tau_1 d\tau_2 d\tau \int_0^\infty g(\omega_{21})d\omega_{21}\exp[i(\omega_{21}-\omega)(\tau-\tau_2)-\gamma(\tau_2+2\tau_1+\tau)] \times E_2(t-\tau_2-t_{21})E_2(t-\tau_2-\tau_1-t_{21})E_1^*(t-\tau_2-\tau_1-\tau)\langle\exp\phi_2\rangle\ , \quad (1)$$

where

$$\phi_2 = -i \int_{\tau+\tau_1}^{\tau+\tau_1+\tau_2} \delta\omega(t)dt + i \int_0^{\tau} \delta\omega(t)dt \ . \quad (2)$$

2γ is the natural decay rate of the excited state, $g(\omega_{21})$ is the inhomogeneous broadening, ω_{21} is the transition frequency, ω is the center frequency of the excitation pulse, t_{21} is the delay time, and the modulation of the transition frequency $\delta\omega$ is a Gaussian stochastic variable which obeys

$$\langle \delta\omega(t_1)\delta\omega(t_2)\rangle = (2\pi\Delta')^2 \exp\left[-\frac{|t_2-t_1|}{\tau_c}\right] = \Delta^2 \exp\left[-\frac{|t_2-t_1|}{\tau_c}\right] . \quad (3)$$

Here, τ_c is the correlation time, Δ and Δ' are the magnitudes of the frequency modulation (we adopt Δ, following convention.) $\langle \cdots \rangle$ is the average over different realizations of a random perturbation. The function $\langle \exp\phi_2 \rangle$, named the relaxation function,[13] is given by[4,5]

$$\langle \exp\phi_2 \rangle = \exp\left\{-p^2\left[e^{-\tau_2/\tau_c} + \frac{\tau_2}{\tau_c} + e^{-\tau/\tau_c} + \frac{\tau}{\tau_c} - 2 - e^{-\tau_2/\tau_c}(e^{-\tau_2/\tau_c}-1)(e^{-\tau/\tau_c}-1)\right]\right\} \quad (4)$$

with $p = \Delta\tau_c$.

Since the bandwidth of the excitation pulse and the width of the inhomogeneous spectrum are much broader than that of the zero-phonon line (at liquid helium temperature, about several hundred megahertz for H_2-Mb), it is permissible to approximate a δ function for the excitation field and to assume an extremely broad inhomogeneous spectrum. Considering that $\tau = \tau_2 = t_{21}$, $t = 2t_{21}$, $\tau_1 = 0$, under the above approximations, the expression for the signal intensity for the two-pulse photon echo is

$$I_{2P}(t_{21}) = |\langle \exp\phi_2 \rangle|^2 = \exp\left\{-4p^2\left[\frac{t_{21}}{\tau_c} + e^{-t_{21}/\tau_c} - 1 - \tfrac{1}{2}(e^{-t_{21}/\tau_c}-1)^2\right]\right\} , \quad (5)$$

where the natural decay rate 2γ is omitted because the fluorescence lifetime of H_2-Mb is about 20 ns, which is much longer than the phase relaxation time. This expression indicates that at the limit of fast modulation ($p \ll 1$, $t_{21}/\tau_c \gg 1$) $I_{2P}(t_{21}) = \exp(-4p^2 t_{21}/\tau_c)$, while at the limit of slow modulation ($p \gg 1$, $t_{21}/\tau_c \ll 1$) $I_{2P}(t_{21}) = \exp[-(\frac{4}{3})p^2(t_{21}/\tau_c)^3]$. This $\exp(-ct_{21}^3)$ dependence has recently been pointed out by Yan and Mukamel.[7]

By fitting Eq. (5) to the observed signal we determined the values for the parameters p, Δ, and τ_c for each temperature. The calculated echo signals and the fitted parameters are shown in Fig. 1(B). It should be noted that these parameters can be determined with relatively high accuracy. The results show that Δ and τ_c greatly change around 10 K in this sample. With these parameters, we can also calculate the heterodyne-detected accumulated echo signal (hereafter referred to as the heterodyne echo), Fig. 2(A), and the persistent hole-burning spectrum, Fig. 2(B). As we have previously pointed out,[9] the hole-burning spectrum $H(\omega)$ can be obtained by the Fourier-cosine transform of the heterodyne echo signal $I_{\mathrm{HAP}}(t)$:

$$H(\omega) = \int I_{\mathrm{HAP}}(t)\cos\omega t \, dt \ . \quad (6)$$

With the notation used in Eq. (5), the expression for the heterodyne-detected echo signal is given as

$$I_{\mathrm{HAP}}(t_{21}) = |\langle \exp\phi_1 \rangle|^2 = \exp\left[-2p^2\left[\frac{t_{21}}{\tau_c} - 1 + e^{-t_{21}/\tau_c}\right]\right] , \quad (7)$$

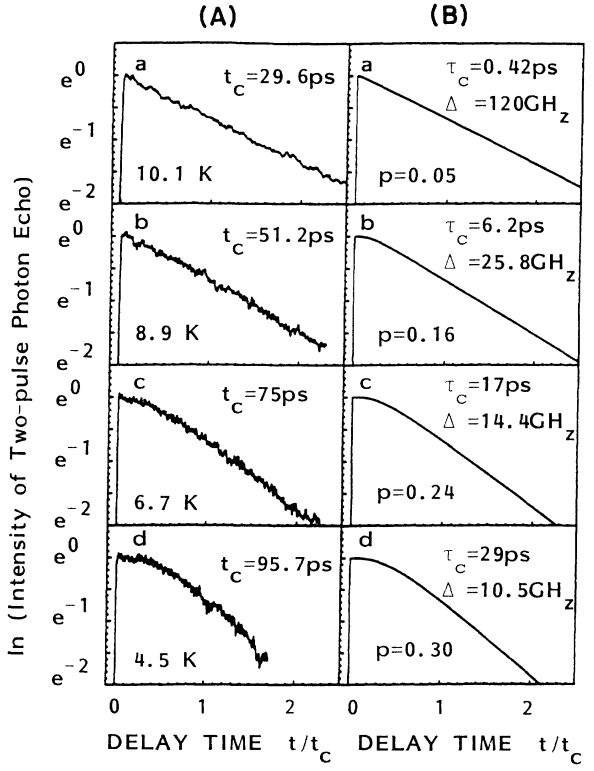

FIG. 1. (A) Two-pulse photon echoes observed in iron-free myoglobin. (B) The numerical simulation according to Eq. (5). The modulation parameters p, τ_c, and Δ were determined for each temperature.

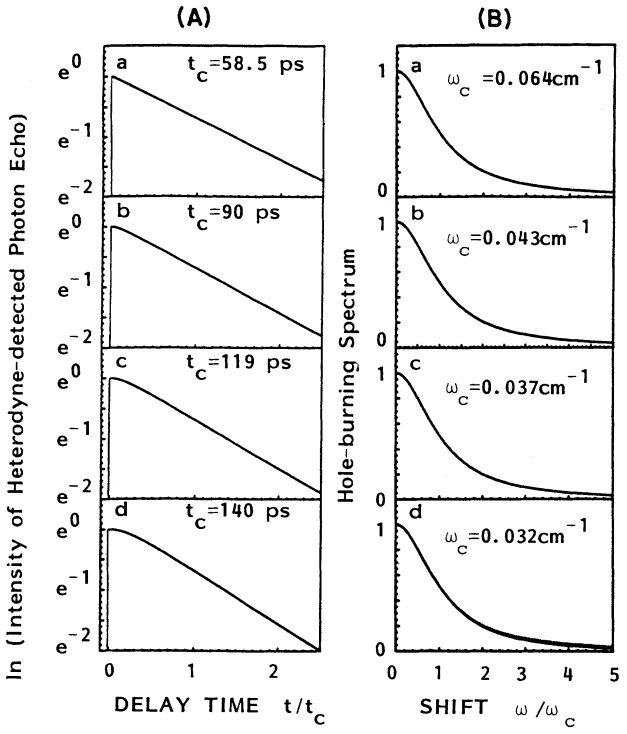

FIG. 2. (A) The numerical calculation for the heterodyne-detected accumulated photon echo according to Eq. (7), using the parameters determined in Fig. 1(B). (B) The numerical calculation for the hole-burning spectrum according to Eq. (6), using parameters determined in Fig. 1(B). In B(d), B(a) was superimposed (in dotted lines) for comparison. The two spectra almost coincide.

where

$$\phi_1 = i \int_0^\tau \delta\omega(t)dt \ .$$

This expression is the same as the square of the relaxation function $\langle \exp\phi_1 \rangle$ for the first-order optical process (absorption spectrum), because the factorization approximation exactly holds for the relaxation function of the heterodyne echo.[9] Equation (7) reduces to $\exp(-2p^2 t_{21}/\tau_c)$ in the fast modulation limit and to $\exp[-p^2(t_{21}/\tau_c)^2]$ in the slow modulation limit, which is in striking contrast with the $\exp[-(\frac{4}{3})p^2(t_{21}/\tau_c)^3]$ dependence in the two-pulse photon echo case. With the use of the continued fraction method for the calculation of the Fourier transform of the relaxation function,[13] the hole-burning spectrum was calculated, as shown in Fig. 2(B). It is interesting that the hole-burning spectra have a quasi-Lorentzian spectrum, while the corresponding two-pulse photon echo signals [Fig. 1(B)] exhibit a nonexponential temporal behavior. The Fourier-transform relation does not generally hold between the two-pulse echo and the persistent hole-burning spectrum.[7] On the contrary, this relation is always valid between the heterodyne echo and the hole-burning spectrum, irrespective of the values of Δ and τ_c.[9]

We now discuss the reason why the frequency modulation changes drastically in a small temperature interval in this sample. As was pointed out by Fayer's group,[14] the temperature dependence of the homogeneous width in the samples of dye-doped polymers is controlled by two terms, namely, one due to the electron-TLS (two-level system) interaction and the other due to the electron-phonon interaction. While the former interaction gives a T^α dependence, with $1 \le \alpha \le 2$ for the temperature dependence of the homogeneous width, the latter interaction shows activated exponential behavior. The temperature dependence of the homogeneous width for H_2-Mb is shown in Fig. 3. The open circles represent the results obtained by the two-pulse photon echo and the solid circles correspond to that of the heterodyne echo.[10] As mentioned above, since the exponential decay constant cannot be determined below 10 K due to the nonexponential temporal behavior, we used the calculated width for the hole-burning spectrum. The dotted line is the functional fit to the temperature dependence of the homogeneous width $\Delta\omega$,

$$\Delta\omega(\ \mathrm{cm}^{-1}\ ) = 0.006T^{1.0} + 0.95\sinh^{-2}(35/T)\ , \qquad (8)$$

where the first term is due to the electron-TLS interaction and the second term comes from the two-phonon Raman process of the quadratic electron-phonon interaction. Rather than using the Arrhenius plot,[15] we found that the fit of the $\sinh^{-2}$ function to the observed homogeneous width is fairly good at high temperatures.[16] The phonon frequency with the strongest contribution to the homogeneous width was determined to be 48 cm^{-1}. Compared with the other samples of dye-doped polymers, the contribution of the TLS term is relatively small in H_2-Mb, and the homogeneous width rises steeply around 10 K due to the onset of the two-phonon Raman process. Therefore, the homogeneous width below 10 K results from the interaction with the TLS.

In the present case, the TLS is closely related to the conformational substate of the protein. Recently, from the hole-filling experiment of porphyrin substituted hor-

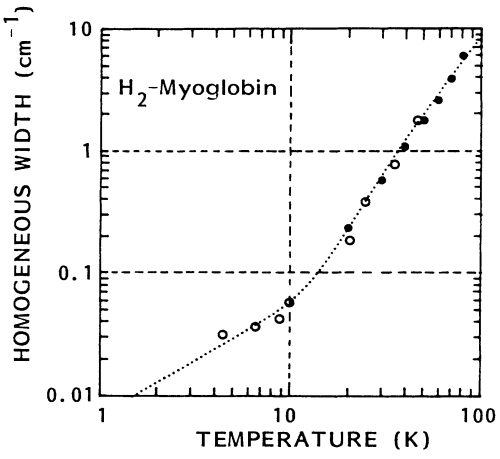

FIG. 3. The temperature dependence of the homogeneous width in iron-free myoglobin. The data consisting of solid circles and the open circles are, respectively, for those obtained from heterodyne echo (Ref. 10) and the two-pulse photon echo. The dotted line is the numerical simulation according to Eq. (8).

seradish peroxidase, Zollfrank *et al.*[17] reported that the conformational relaxation of the protein occurs in a step-like manner at around 14 K. From this observation, they concluded that the density of the TLS is relatively small in this sample due to the ordered structure of the protein. In our case, the transition to the fast modulation limit observed above 10 K is closely correlated with the onset of the two-phonon Raman process; in other words, the phonon-assisted conformational relaxation occurs around 10 K. Below this temperature, the frequency modulation caused by the electron-TLS interaction results in a non-Markovian relaxation in the two-pulse photon echo. This originates from the small number of TLS in the hydrophobic pocket of the apomyoglobin.

[1]M. Aihara, Phys. Rev. B **25**, 53 (1982).
[2]J. Y. Bigot, M. T. Portella, R. W. Schoenlein, C. J. Bardeen, A. Misgusand, and C. V. Shank, Phys. Rev. Lett. **66**, 1138 (1991).
[3]E. T. J. Nibbering, D. A. Wiersma, and K. Duppen, Phys. Rev. Lett. **66**, 2464 (1991).
[4]E. Hanamura, J. Phys. Soc. Jpn. **52**, 2258 (1983).
[5]B. D. Fainberg, Opt. Spectrosc. **55**, 669 (1983).
[6]R. F. Loring and S. Mukamel, Chem. Phys. Lett. **114**, 426 (1985).
[7]Y. J. Yan and S. Mukamel, J. Chem. Phys. **94**, 179 (1991).
[8]*Persistent Spectral Hole Burning: Science and Applications*, edited W. E. Moerner (Springer-Verlag, Berlin, 1987).
[9]S. Saikan, T. Nakabayashi, Y. Kanematsu, and N. Tato, Phys. Rev. B **38**, 7777 (1988).
[10]J. W-I. Lin, T. Tada, S. Saikan, T. Kushida, and T. Tani, Phys. Rev. B **44**, 7356 (1991).
[11]N. Morita and T. Yajima, Phys. Rev. A **30**, 2525 (1984).
[12]Although the present experiment was performed with incoherent light, we apply tentatively the theory of coherent-pulse photon echo. More rigorous analysis using the theory of incoherent-pulse photon echo is now in progress.
[13]R. Kubo, in *Fluctuation, Relaxation and Resonance in Magnetic Systems*, edited by D. ter Haar (Oliver and Boyd, Edinburgh, 1962), p. 23.
[14]M. Berg, C. A. Walsh, L. R. Narasimhan, K. A. Littau, and M. D. Fayer, J. Chem. Phys. **88**, 1564 (1988).
[15]A. Elschner, L. R. Narasimhan, and M. D. Fayer, Chem. Phys. Lett. **171**, 19 (1990).
[16]I. S. Osad'ko, Phys. Rep. **206**, 43 (1991).
[17]J. Zollfrank, J. Freidrich, J. M. Vanderkooi, and J. Fidy, Biophys. J. **59**, 305 (1991).