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COMMUNICATIONS

Nonphotochemical holeburning in a protein matrix: Chlorophyllide in apomyoglobin

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The techniques of photochemical and nonphotochemical holeburning have been widely applied to determine the homogeneous linewidths associated with electronic transitions of impurities in crystalline and amorphous hosts.¹ We wish to report the first example of nonphotochemical holeburning for a chromophore in a well-defined protein matrix and the temperature dependence of the holewidth. We find that the holeburning efficiency, the holewidth, and its temperature dependence are comparable for the same chromophore in the protein matrix and in an amorphous polymeric matrix. These results provide information on electron-phonon coupling in a protein matrix which may have important implications for understanding the coupling of protein dynamics with energy or electron transfer. The results are in striking contrast to the very broad photochemical holes our group^{2,3} and Meech *et al.*⁴ have observed in photosynthetic reaction centers.

We have shown that a one-to-one complex can be formed between a wide variety of chlorophyllide derivatives and the protein apomyoglobin⁵ and that the macrocycle substitutes specifically for heme in the heme pocket.⁶ For the present study the Zn-pyrochlorophyllide *a*-myoglobin (sperm whale apoMb) complex (Zn-PChlaMb) was prepared as previously described.⁵ The protein was embedded in a poly(vinyl alcohol) film (Aldrich, 125 000 average molecular weight, 0.1–0.2 mm thickness, 1×10^{-4} M) which forms an optical quality glass at cryogenic temperatures. At 1.4 K the Q_Y absorption band has a maximum at $14\,975\text{ cm}^{-1}$ and an inhomogeneous linewidth (FWHM) of approximately 500 cm^{-1} . For comparison, Zn-pyrochlorophyllide *a* esterified with 3-(3'-hydroxypropyl)pyridine as a ligand was embedded in a polystyrene film (Aldrich, 321 000 average molecular weight, 0.1–0.2 mm thickness, 1×10^{-4} M).

Nonphotochemical holeburning was performed using methods⁷ closely related to those described by Völker and co-workers.⁸ The apparent homogeneous linewidth was obtained from the best fit of the observed holes to a Lorentzian

line shape. The temperature dependence of the homogeneous linewidth was studied between 1.35 and 2.5 K. The data were fit to an expression of the form: $\Gamma_h = \Gamma_0 + bT^\alpha$, where Γ_0 , the lifetime-limited homogeneous width, was assumed to be 40 MHz corresponding to the room temperature singlet lifetime of the chromophore.^{5,9} A convenient representation of the data is the log-log plot shown in Fig. 1, from which one obtains the slope (α) equal to 1.26 ± 0.07 . A parallel study of Zn-PChla esterified with 3-(3'-hydroxypropyl)pyridine in polystyrene gave very similar results for both the absolute holewidth and the temperature dependence. A preliminary report for chlorophyll *a* in polystyrene gave a similar result.¹⁰

Several noteworthy observations emerge from the re-

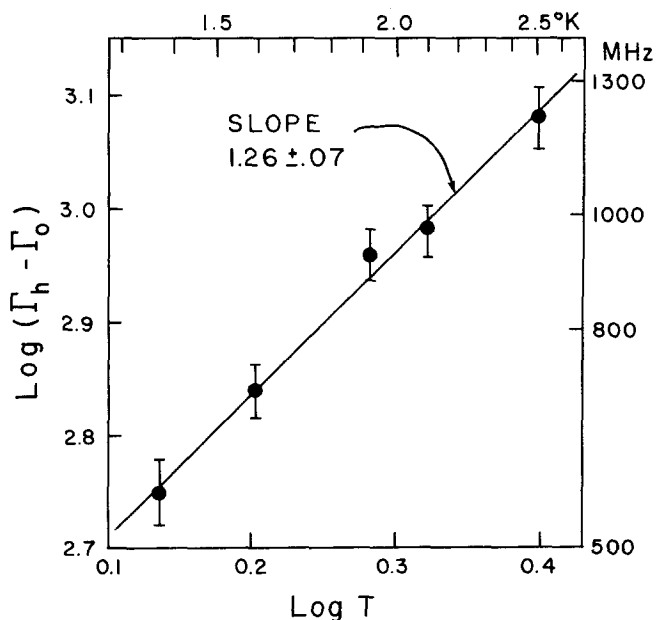


FIG. 1. Log-log plot of the temperature dependence of the pure dephasing contribution to the homogeneous linewidth for Zn-PChlaMb.

sults presented above. (i) Nonphotochemical holeburning occurs in protein matrices as it does in simpler polymeric or organic glasses. Although no quantitative comparison was undertaken, the efficiency of holeburning in the protein matrix is comparable to that in polystyrene. (ii) The homogeneous linewidths obtained at very low burn powers and pumped liquid He temperatures are comparable for a chromophore in a protein matrix and in polystyrene.¹¹ (iii) The $T^{1.3}$ temperature dependence of the dephasing contribution to the homogeneous linewidth for a chromophore in a protein matrix is very similar to that of the same chromophore in a polymeric glassy matrix or that of many organic chromophores in glassy matrices.¹²

The homogeneous linewidth for an electronic transition is determined by both the excited state lifetime and pure dephasing. Given the results in Fig. 1,⁹ it is evident that pure dephasing dominates at the lowest temperatures we have studied to date, as has been observed for many ordinary glasses. Comparisons of holeburning and photon echo data suggest that holeburning, if carefully performed, provides an accurate measure of the homogeneous linewidth,^{12,13} though an exception has been noted.¹⁴ The temperature dependence of the homogeneous linewidth measured by both methods agrees well for temperatures below 4 K which are considered in this communication.

A variety of models has been introduced to rationalize the temperature dependence of the homogeneous linewidth in glasses. Most are related to the physical model of a distribution of nearly isoenergetic two-level systems (TLS) of the glassy matrix coupled to the excited state of the chromophore. Such models were first introduced by Anderson and co-workers¹⁵ and Phillips¹⁶ to explain the anomalous low-temperature specific heat of glasses, and were introduced by Small and co-workers¹ to explain the phenomenon of non-photochemical holeburning. The $T^{1.3}$ temperature dependence at liquid He temperatures which has been widely observed in amorphous systems, and now in a protein matrix, can be derived from the TLS model, though many assumptions are required.¹ By contrast the temperature dependence in crystalline matrices generally follows a well-defined Arrhenius behavior.¹

At a qualitative level it appears that the guest-host interactions responsible for producing the $T^{1.3}$ temperature dependence in many ordinary glasses are also present in the chlorophyllide-apoMb complex. In contrast to ordinary glassy matrices, the protein has a well-defined average structure, known with great precision from extensive x-ray crystallographic¹⁷ and neutron scattering¹⁸ studies. The chromophore is not an impurity in the conventional sense, as its binding site is an intrinsic property of the protein.¹⁹ Although one might be tempted to suggest that a structured globular protein like Mb has characteristic phonon modes, it is much more likely that the huge number of available conformational substates involving very small variations in the positions of individual residues combined with weak inter-residue couplings will produce a broad distribution of low-frequency modes. There has been a great deal of discussion of this point, and information, specifically for Mb, is available from temperature-dependent specific heat and dielec-

tric constant measurements on metMb crystals (where glass-like behavior was observed)²⁰, Mössbauer²¹ and Raman²² spectroscopies, low-temperature ligand recombination kinetics,²³ and molecular dynamics simulations.²⁴ To the extent that the temperature dependence of the pure-dephasing contribution to the homogeneous linewidth is a measure of the coupling between electronic states of the chromophore and its environment, our results suggest that the protein is glass-like.

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¹J. M. Hayes, B. L. Fearey, T. P. Carter, and G. J. Small, *Int. Rev. Phys. Chem.* **5**, 175 (1986), and references therein.

²S. G. Boxer, D. J. Lockhart, and T. R. Middendorf, *Chem. Phys. Lett.* **123**, 476 (1986).

³S. G. Boxer, T. R. Middendorf, and D. J. Lockhart, *FEBS Lett.* **200**, 237 (1986).

⁴S. R. Meech, A. J. Hoff, and D. A. Wiersma, *Chem. Phys. Lett.* **121**, 287 (1985).

⁵K. A. Wright and S. G. Boxer, *Biochem.* **20**, 7545 (1981).

⁶The orientation of the chlorophyllide macrocycle in the heme pocket has been determined by NMR spectroscopy and is the same as that for heme [D. Lambright and S. G. Boxer (submitted)]. An x-ray crystal structure is in progress [see S. G. Boxer, A. Kuki, K. A. Wright, B. A. Katz, and N. H. Xuong, *Proc. Natl. Acad. Sci. U.S.A.* **79**, 1121 (1982)].

⁷Holes were burned in the (0,0) band for 30-120 s using a high resolution dye laser (Coherent 699-29 Autoscan, effective linewidth < 1 MHz) at very low power (10-40 $\mu\text{W}/\text{cm}^2$). The absorption spectrum was then probed as the fluorescence (front-face) excitation spectrum by scanning the same laser at about 1/10 or less of the burn power. The holewidth was found to be independent of the burn frequency over the range 15 000-15 050 cm^{-1} and the hole depths were approximately 5%-15%. The burn-power and burn-time dependence of the holewidth were measured at each temperature to avoid the "pitfalls" described in Ref. 8.

⁸H. P. H. Thijssen and S. Völker, *Chem. Phys. Lett.* **120**, 496 (1985).

⁹Since the observed holes are more than one order of magnitude broader than the room temperature lifetime-limited value, small variations in the excited state lifetime with temperature are of no consequence.

¹⁰T. P. Carter and G. J. Small, *Chem. Phys. Lett.* **120**, 178 (1985). The holewidths we observe are a factor of 2-3 smaller at 1.9 K. Carter and Small used a burn and probe power about one order of magnitude greater than in our experiments. It is not surprising that the absolute holewidth for Zn-PChla in polystyrene and in Mb are similar (within 20%) given the strong similarity between many other spectral properties of the chromophore in the protein and in organic solvents (see Ref. 5).

¹¹Photochemical holeburning has been reported for the multichromophoric proteins phycocyanin and phycoerythrin [J. Friedrich, H. Scheer, B. Zickendraht-Wendelstadt, and D. Haarer, *J. Am. Chem. Soc.* **103**, 1030 (1981); *J. Chem. Phys.* **74**, 2260 (1981)]. The observed holewidths were about 1-2 cm^{-1} , more than an order of magnitude greater than what we observe for Zn-PChlaMb. This difference is likely due to the effects of much higher burn power and energy transfer.

¹²H. P. H. Thijssen, R. van den Berg, and S. Völker, *Chem. Phys. Lett.* **120**, 503 (1985).

¹³R. van den Berg and S. Völker, *Chem. Phys. Lett.* **127**, 525 (1986).

¹⁴C. A. Walsh, M. Berg, L. R. Narasimhan, and M. D. Fayer, *Chem. Phys. Lett.* **130**, 6 (1986).

¹⁵P. W. Anderson, B. I. Walperin, and C. M. Varma, *Philos. Mag.* **25**, 1 (1972).

¹⁶W. A. Phillips, *J. Low Temp. Phys.* **7**, 351 (1972).

¹⁷T. Takano, *J. Mol. Biol.* **110**, 537, 569 (1977).

¹⁸J. C. Hanson and B. P. Schoenborn, *J. Mol. Biol.* **153**, 117 (1981).

¹⁹In this sense the protein host matrix is very different from a polymer glass or a crystal: there is no strain as occurs when a guest is substitutionally incorporated into a crystalline host, there is no problem of pockets of trapped solvent which often complicate a microscopic description of a polymeric glass, and the mean-squared displacement of individual atoms in the matrix is known in great detail [H. Frauenfelder, G. A. Petsko, and D. Tsernoglou, *Nature* **280**, 558 (1979)].

²⁰G. P. Singh, H. J. Schink, H. V. Lohneysen, F. Parak, and S. Hunklinger, *Z. Phys. B* **55**, 23 (1984).

²¹F. Parak, E. W. Knapp, and D. Kucheida, *J. Mol. Biol.* **161**, 177 (1982).

²²G. C. Wagner, J. T. Colvin, J. P. Allen, and H. J. Stapelton, *J. Am. Chem. Soc.* **107**, 5589 (1985).

²³A. Ansari, J. Berendzen, S. F. Bowne, H. Frauenfelder, I. E. T. Iben, T. B. Sauke, E. Shyamsunder, and R. D. Young, *Proc. Natl. Acad. Sci. U.S.A.* **82**, 5000 (1985).

²⁴R. M. Levy, R. P. Sheiden, J. W. Keepers, G. S. Dubey, S. Swaminathan, and M. Karplus, *Biophys. J.* **48**, 509 (1985).

Molecule-corrugated surface scattering calculations using the close coupling wave packet method^{a)}

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In this letter we report numerically exact quantum mechanical calculations of rotational-diffractive scattering of N_2 molecules off a model corrugated surface. The molecule-surface potential was taken to be the rigid dumbbell model of Gerber, Beard, and Kouri.¹ The close coupling wave packet (CCWP) method recently developed by Mowrey and Kouri^{2,3} was used to carry out the calculations. This method employs a close coupling expansion for the internal (rotor) states but treats the motion of the diatom center of mass via wave packet propagation. Associated with each rotor state is its own subpacket.²⁻⁴ Because the time dependent Schrödinger equation is a first order partial differential equation in time, the CCWP method is an initial value approach. At the initial time $t = 0$, only one of the subpackets contains any amplitude. As that subpacket evolves, the evolution operator causes the other subpackets to become populated. The long time subpackets are Fourier analyzed to determine the transition probability amplitudes (S -matrix elements) into specific internal states at the specific collision energy. The formal proportionality factors which must be multiplied times the Fourier transform of the subpacket to obtain the

S -matrix element for transition to a given rotor-diffraction state at a particular energy have been determined by a contour integration analysis recently developed by us.^{5,6} This procedure enables us to determine both the phase and modulus of the elements of one column of the S matrix at any energy contained in the original wave packet.^{3,5,6} The computational effort scales as the number of rotor basis states squared rather than total number of channels cubed, as a consequence of the initial value nature of the CCWP method. This feature, combined with the efficiency of the wave packet technique for treating the diffraction portion of the problem, has enabled us to obtain these numerically exact results for a collision system modeling the N_2 molecule scattering off a rigid corrugated lattice. The largest calculations we have done so far were done for a rotor basis set of even $j = 0-12$, all m_j and an x, y, z grid capable of describing diffraction quantum numbers $-8 \leq m \leq 8$, $-4 \leq n \leq 4$. This yields a total of 13 923 channels and took 231 minutes of computer time on the CRAY2 at the University of Minnesota Supercomputer Center. The interaction $V(z, \mathbf{p}, \hat{\mathbf{r}})$ is given by¹

$$V(z, \mathbf{p}, \hat{\mathbf{r}}) = A e^{-\alpha z} \left(2 \cosh\left(\frac{\alpha r}{2} \cos \theta\right) + 2\beta \left[\cosh\left(\frac{\alpha r}{2} \cos \theta\right) \times \left\{ \cos\left(\frac{2\pi x}{a}\right) \cos\left(\frac{\pi r}{a} \sin \theta \cos \phi\right) + \cos\left(\frac{2\pi y}{b}\right) \cos\left(\frac{\pi r}{b} \sin \theta \sin \phi\right) \right\} + \sinh\left(\frac{\alpha r}{2} \cos \theta\right) \left\{ \sin\left(\frac{2\pi x}{a}\right) \sin\left(\frac{\pi r}{a} \sin \theta \cos \phi\right) + \sin\left(\frac{2\pi y}{b}\right) \sin\left(\frac{\pi r}{b} \sin \theta \sin \phi\right) \right\} \right] \right), \quad (1)$$

where z is the vertical distance of the rotor center of mass above the surface, \mathbf{p} gives the x and y position of the rotor center of mass relative to a set of coordinates in the surface unit cell, and $\hat{\mathbf{r}}$ is the unit vector defined by θ and ϕ , orienting the rotor axis. The parameter α equals $1.94012 a_0^{-1}$, β is

taken to be 0.1, and the scattering is independent of A . A rectangular lattice with $a = 4 \text{ \AA}$, $b = 2 \text{ \AA}$ is employed. The time dependent Schrödinger equation in the CCWP formalism^{2,3} was solved for transitions out of the $j = m_j = 0$ initial rotor state, thereby yielding 13 923 S -matrix elements