Dynamics of Myoglobin−CO with the Proximal Histidine Removed: Vibrational Echo Experiments

K. D. Rector,[†] J. R. Engholm,[‡] J. R. Hill,[§] D. J. Myers,[†] R. Hu,[†] Steven G. Boxer,[†] Dana D. Dlott,[§] and M. D. Fayer*,[†]

Department of Chemistry, Stanford University, Stanford, California 94305, Stanford Free Electron Laser Center, Stanford University, Stanford, California 94305-4085, and School of Chemical Sciences, University of Illinois at Urbana-Champaign, Urbana, Illinois 61801

Received: September 15, 1997; In Final Form: November 11, 1997[⊗]

Picosecond infrared vibrational echo measurements from 60 to 300 K on CO bound to the active site of a mutant myoglobin, H93G(*N*-MeIm), are presented and compared to measurements on native myoglobin and on the mutant H64V. Although in H93G(*N*-MeIm) (the proximal histidine replaced by glycine, with exogenous *N*-methylimidazole in the proximal histidine pocket, covalently bound to Fe), the only covalent linkage between heme−CO and the protein is broken, there is no change in the temperature-dependent vibrational pure dephasing time, T_2*. The results demonstrate that severing the only covalent bond between the heme and the globin has little or no effect on the protein dynamics detected by vibration of the CO at the active site of myoglobin.

I. Introduction

Vibrational echo experiments have recently been applied to the study of the vibrational dynamics of CO bound to the active site of myoglobin.[1,2] Here, we extend that work by presenting the results of a vibrational echo study of the sperm whale mutant, myoglobin H93G(*N*-methylimidazole)(CO),[3,4] hereafter H93G(*N*-MeIm). The heme at the active site of Mb has only one covalent linkage to the protein, the iron−histidine 93 bond. Histidine 93 is contained in the F α-helix of the globin. In the mutant, proximal histidine 93 is replaced by glycine, leaving a cavity on the proximal side of the heme.[5] Many exogenous ligands (L) to the heme iron, such as imidazoles and pyridines, can be substituted into this cavity, producing a series of proteins, H93G(L).[3] Although these proteins retain a covalent linkage between the heme iron and the proximal ligand, the covalent connection to the protein backbone is severed. When *N*-methylimidazole is used as the exogenous proximal ligand, the hydrogen bond between the proton on the imidazole imino nitrogen and the hydroxyl group on serine 92 is also absent.[4] As with native Mb, the open heme coordination site on the distal side binds biologically important diatomic molecules such as O_2, CO, and NO.

The H93G(*N*-MeIm) protein has been shown to have a structure very similar to native.[4,6] The CO transition frequency and room-temperature vibrational CO lifetime of the mutant are almost identical with the native value.[4,7,8]

The vibrational echo is the vibrational analogue of the well-known spin echo technique in magnetic resonance[9] and the photon echo technique used in the study of electronic transitions.[10,11] The vibrational echo experiment measures the homogeneous dephasing time, T_2, of the vibrational transition of interest. When combined with pump−probe measurements of the vibrational lifetime, T_1, the pure dephasing time, T_2*, can be determined. Pure vibrational dephasing of the CO bound to heme in myoglobin is caused by dynamics of the protein medium, which lead to fluctuations of the CO oscillator frequency (vibrational transition energy) occurring on a fraction of a picosecond to ∼100 ps time scale.[12]

For protein conformational changes to cause pure dephasing, there must be a mechanism that couples the protein dynamics to the CO vibrational energy. Recently, temperature-dependent vibrational echo experiments have been performed on the stretching mode of CO bound to the active site of native horse heart Mb[1] and on the sperm whale myoglobin mutant denoted H64V, where H64 (the distal histidine) was replaced by valine.[2] Vibrational echo experiments on H64V resulted in a pure dephasing temperature dependence with a functional form identical with native Mb, but with the rate of pure dephasing ∼20% less than that of native Mb at all temperatures.[2] Because a relatively polar histidine is replaced by nonpolar valine in H64V, this suggests that global fluctuations in the electric field felt by the CO contribute to the pure dephasing.

Here we consider another plausible contribution to pure dephasing: local fluctuations of the position or orientation of the proximal histidine 93 caused by motions of the protein backbone. Such motions will act directly on the Fe in the heme because of the covalent linkage. The contribution of this "mechanical" mechanism can be tested by using H93G(*N*-MeIm), as this system retains the chemical and structural features of the proximal heme pocket, without the covalent connection of the Fe ligand bound to the protein.[4,6]

II. Experimental Method and Procedures

The vibrational echo experimental method and procedures have been discussed in detail elsewhere.[1,2,13] The vibrational echo is a two-pulse time-domain experiment that removes inhomogeneous broadening to give the homogeneous line shape for a vibrational transition. The integrated intensity of the echo pulse, which is emitted following the two excitation pulses, is measured as a function of the delay τ between the two input pulses. The Fourier transform of this echo decay curve is the

* To whom correspondence should be addressed.
[†] Department of Chemistry, Stanford University.
[‡] Stanford Free Electron Laser Center, Stanford University.
[§] School of Chemical Sciences, University of Illinois.

S1089-5647(97)03004-6 CCC: $15.00
Published on Web 01/08/1998

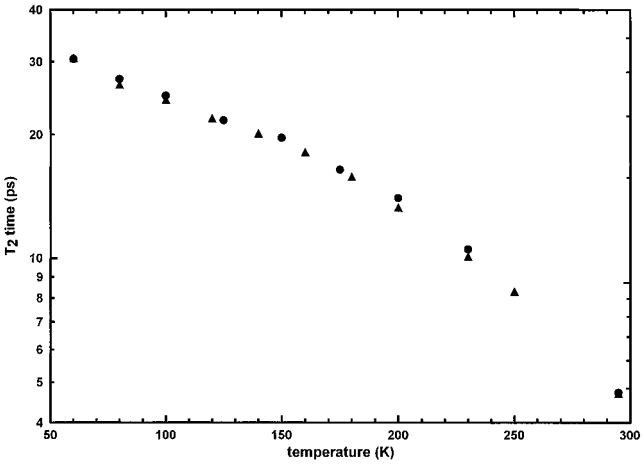

Figure 1. Homogeneous dephasing (T_2) time constants versus temperature for H93G(*N*-MeIm)−CO (circles) and native Mb−CO (triangles). The H93G(*N*-MeIm) mutation has no effect on T_2.

homogeneous line shape.[14] The vibrational echo experiment is performed on the $\upsilon = 0-1$ transition of the CO vibration, $\nu_{CO} = 1946 \pm 1$ cm^{-1},[4] which lies, within experimental error, at the same frequency as the A_1 state in native Mb.[8,15] For a Lorentzian homogeneous line having width $\Gamma = 1/\pi T_2$, the echo signal amplitude decays as

$$I(\tau) = I_0 \exp(-4\gamma_{10}\tau) \tag{1}$$

The homogeneous dephasing time constant, T_2, has contributions from the vibrational lifetime, T_1, and the pure dephasing time, T_2*. In myoglobin, there is no orientational relaxation contribution to the homogeneous dephasing.[12] T_1 can be measured by pump−probe experiments, and T_2* can be obtained using the relation

$$\frac{1}{T_2} = \frac{1}{T_2^*} + \frac{1}{2T_1} \tag{2}$$

The H93G(*N*-MeIm) mutant of sperm whale Mb was prepared as described previously.[3,5] The procedure for preparing the infrared samples from lyophilized H93G(*N*-MeIm) protein was identical with that used for native Mb.[16]

III. Results and Discussion

Vibrational echo data were obtained on H93G(*N*-MeIm) in the temperature range of 60−300 K. At lower temperatures, the data were fit to a single-exponential decay. At the highest temperatures, the decay times approach the laser pulse duration (∼1 ps). When this occurred, the data were fit with an algorithm that calculates a vibrational echo decay properly accounting for the excitation pulse duration and shape.[17] The cited values of T_1, T_2, and T_2* have error bars of <5%.

Temperature-dependent vibrational echo T_2 measurements of H93G(*N*-MeIm) are shown in Figure 1 (circles). Figure 1 also displays T_2 data taken previously on native Mb (triangles).[1] Within experimental uncertainty, the two data sets are identical. Thus, the temperature-dependent homogeneous line width of the CO bound at the active site of the protein is not influenced by removing the only covalent linkage between the CO−heme and the protein backbone.

A log plot of $1/T_2$* vs temperature is shown in Figure 2 for H93G(*N*-MeIm) (circles), for native Mb (triangles), and for H64V (squares). The upper solid line is a fit to a power law plus an exponentially activated process[1]

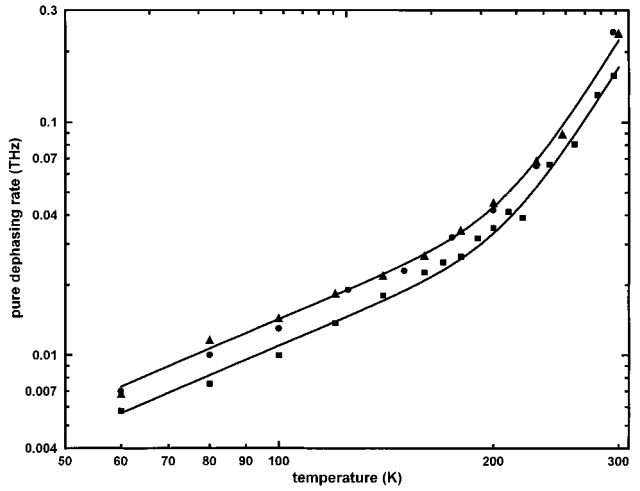

Figure 2. Pure dephasing rates versus temperature for H93G(*N*-MeIm)−CO (circles), compared to Mb−CO (triangles) taken from earlier work.[1] The temperature dependences are identical. The curve is a fit to eq 3. H64V data (squares) are also shown for comparison.[2]

$$\frac{1}{T_2^*} = a_1 T^{\alpha} + a_2 \exp\left(\frac{-\Delta E}{kT}\right) \tag{3}$$

where $\alpha = 1.3$ and $\Delta E = 1250$ cm^{-1} for both Mb and H93G(*N*-MeIm). Within the small experimental uncertainty, the temperature-dependent pure dephasing rates of CO bound to the active sites of the two proteins are identical. The temperature-dependent pure dephasing rates of H64V (lower solid line) have the same functional form as the other two proteins, but at every temperature T_2* is ∼20% slower.[2] The pure dephasing time constant, T_2*, was computed for H93G(*N*-MeIm) using eq 2 and the native Mb T_1 data. The temperature-dependent behavior of T_1 in Mb−CO systems has been studied in native Mb in a variety of solvents and in the H64V mutant.[1,2] There is only a slight (∼10%) increase in T_1 in all systems between 60 and 300 K. Furthermore, the lifetime of H93G(MeIm) at room temperature is identical with that of native Mb. Therefore, negligible error is introduced into the H93G(*N*-MeIm) T_2* values by use of the native Mb T_1 data.

In the mechanical pure dephasing mechanism being tested here, local protein conformational fluctuations may move the proximal histidine, pushing and pulling the Fe in and out of the plane of the heme ring system. If such motions change ν_{CO}, they will cause pure dephasing.

However, the temperature-dependent vibrational echo data displayed in Figure 2 demonstrate that the local mechanical mechanism described above does not contribute significantly to the pure dephasing of the CO stretching mode. Eliminating the covalent bond and replacing the proximal histidine with the *N*-MeIm ligand decouples the heme−CO from the local motions of the F helix at residue 93. The heme is still coupled to the protein dynamics through many van der Waals contacts.

The pure dephasing results give further support to the fluctuating global electric field mechanism proposed to explain pure dephasing in previous vibrational echo experiments on H64V.[2] When the polar histidine is replaced by nonpolar valine, H64V, the pure dephasing is reduced (see Figure 2) because one contributor to the fluctuating electric field is gone. The current experiments show that pure dephasing does not arise from local motions of the Fe−CO caused by movement of H93, but rather it is suggested that fluctuating electric fields are the dominate cause of pure dephasing in MbCO.[7,8]

V. Concluding Remarks

Vibrational echo data obtained on H93G(*N*-MeIm), in which the covalent linkage between heme−CO and the protein is broken, show that the temperature dependence of vibrational pure dephasing is identical with that in native Mb but faster than in H64V. The experiments support the proposition that fast global protein fluctuations rather than local mechanical motions of the proximal histidine are responsible for vibrational energy fluctuations (pure vibrational dephasing) of CO bound at the active site of myoglobin.

Acknowledgment. We thank Professors Alan Schwettman and Todd Smith and their research groups at the Stanford Free Electron Laser Center for assistance with these experiments. M.D.F., S.G.B. and D.D.D. acknowledge support from Office of Naval Research N00014-94-1-1024. M.D.F. also acknowledges support from the National Science Foundation, Division of Materials Research DMR-9610326. D.D.D. also acknowledges support from Office of Naval Research N00014-95-1-0259 and National Science Foundation, Division of Materials Research DMR-9404806. S.B.G. also acknowledges support from NIH GM27738.

References and Notes

(1) Rella, C. W.; Rector, K. D.; Kwok, A. S.; Hill, J. R.; Schwettman, H. A.; Dlott, D. D.; Fayer, M. D. *J. Phys. Chem.* **1996**, *100*, 15620.

(2) Rector, K. D.; Rella, C. W.; Kwok, A. S.; Hill, J. R.; Sligar, S. G.; Chien, E. Y. P.; Dlott, D. D.; Fayer, M. D. *J. Phys. Chem. B* **1997**, *101*, 1468.

(3) DePillis, G. D.; Decatur, S. M.; Barrick, D.; Boxer, S. G. *J. Am. Chem. Soc.* **1994**, *116*, 6981.

(4) Decatur, S. M.; DePillis, G. D.; Boxer, S. G. *Biochemistry* **1996**, *35*, 3925.

(5) Barrick, D. *Biochemistry* **1994**, *33*, 6546.

(6) Barrick, D. Personal communication, 1997.

(7) Hill, J. R.; Tokmakoff, A.; Peterson, K. A.; Sauter, B.; Zimdars, D. A.; Dlott, D. D.; Fayer, M. D. *J. Phys. Chem.* **1994**, *98*, 11213.

(8) Hill, J. R.; Dlott, D. D.; Rella, C. W.; Peterson, K. A.; Decatur, S. M.; Boxer, S. G.; Fayer, M. D. *J. Phys. Chem.* **1996**, *100*, 12100.

(9) Hahn, E. L. *Phys. Rev.* **1950**, *80*, 580.

(10) Kurnit, N. A.; Abella, I. D.; Hartmann, S. R. *Phys. Rev. Lett.* **1964**, *13*, 567.

(11) Abella, I. D.; Kurnit, N. A.; Hartmann, S. R. *Phys. Rev. Lett.* **1966**, *14*, 391.

(12) Tokmakoff, A.; Fayer, M. D. *J. Chem. Phys.* **1995**, *102*, 2810.

(13) Tokmakoff, A.; Zimdars, D.; Urdahl, R. S.; Francis, R. S.; Kwok, A. S.; Fayer, M. D. *J. Phys. Chem.* **1995**, *99*, 13310.

(14) Farrar, T. C.; Becker, D. E. *Pulse and Fourier Transform NMR*; Academic Press: New York, 1971.

(15) Ansari, A.; Beredzen, J.; Braunstein, D.; Cowen, B. R.; Frauenfelder, H.; Hong, M. K.; Iben, I. E. T.; Johnson, J. B.; Ormos, P.; Sauke, T.; Schroll, R.; Schulte, A.; Steinback, P. J.; Vittitow, J.; Young, R. D. *Biophys. Chem.* **1987**, *26*, 337.

(16) Hill, J. R.; Dlott, D. D.; Rella, C. W.; Smith, T. I.; Schwettman, H. A.; Peterson, K. A.; Kwok, A. S.; Rector, K. D.; Fayer, M. D. *Biospectroscopy* **1996**, *2*, 227.

(17) Zimdars, D. A.; Graduate Thesis, Stanford University, 1996.