Evidence for Conformational and Diffusional Mean Square Displacements in Frozen Aqueous Solution of Oxymyoglobin

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The temperature dependence of the Mössbauer spectra of ^{57}Fe in the active center of oxymyoglobin is analyzed. The effectvie mean square displacement $\langle x^2\rangle$ of the iron is a sum of three parts: a vibrational term $\langle x^2\rangle_v$, a conformational term $\langle x^2\rangle_c$, and a diffusional term $\langle x^2\rangle_d$. For $T > 240$ K diffusional line broadening $\Delta\Gamma \propto \langle x^2\rangle_d$ is observed, along with a decrease in quadrupole splitting due to random reorientation of the electric field gradient.

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Experimental[1-5] and theoretical[6] studies suggest that structural fluctuations are an essential feature of biological macromolecules. Recent x-ray diffraction measurements[1] on the oxygen storage protein myoglobin have shown that even in the crystalline state certain regions of the protein experience large atomic displacements. An understanding of the motions involved is of interest from the molecular dynamics point of view and is needed, eventually, to rationalize the biological activity of these macromolecules. The polypeptide chain of myoglobin forms a pocket for the heme group, a planar molecule with an iron atom at its center. The heme iron is the O_2-binding site and also forms a single covalent bond to the protein. Thus Mössbauer spectroscopy can be used to study the dynamics of the active center in this protein. Specifically, an effective mean square displacement (msd)[7] of the iron can be deduced from the cross section for recoilless absorption, and the line shape is affected by fluctuations of the hyperfine interaction[8] and by diffusion.[9-11]

We report measurements of the msd, linewidth, and quadrupole splitting on frozen aqueous solutions of oxymyoglobin between 4 and 260 K. The data show a clear division into three distinct dynamical regions, which are dominated by vibrational, conformational, and diffusional motion, respectively. Diffusional motion leads to line broadening and a decrease in quadrupole splitting.[8,11] We find empirically that the line broadening $\Delta\Gamma$ is proportional to the diffusional msd.

All Mössbauer measurements were done on ^{57}Fe-enriched sperm whale oxygmyoglobin in frozen aqueous solution.[12] The spectra are approximated by two Lorentzians. The quadrupole splitting $\Delta(T)$ is well reproduced by a polynomial of fourth degree in T up to 240 K. In order to analyze the decrease in Δ above 240 K we use extrapolated values Δ_0 (Table I). The relative areas $A(T)$ of the absorption lines, corrected for nonresonant background, are taken as a measure of the recoilless fraction $f(T)$. For $f(T)$ we write[7]

$$f(T) = \exp(-k^2\langle x_{\mathrm{eff}}^2\rangle) \propto A(T), \quad (1)$$

where $\langle x_{\mathrm{eff}}^2\rangle$ is an effective msd along the wave vector $\vec{k}$ of the γ rays. Figure 1 shows the temperature dependence of the relative msd and the linewidth $\Gamma(T)$. For $T < 230$ K, Γ depends linearly on T.[13] The rapid increase of Γ above 240 K is accompanied by a decrease in Δ as listed in Table I. Figure 2 demonstrates that there are three distinct temperature regions in which $\langle x_{\mathrm{eff}}^2\rangle$ and Γ are linearly related. This result is significant since at high temperatures both $\langle x_{\mathrm{eff}}^2\rangle$ and Γ are nonlinear functions of T.[10,14]

Following Frauenfelder, Petsko, and Tsernaglou,[1] we assume that the total msd of the iron may be approximated by a sum of three statistically independent terms,

$$\langle x_{\mathrm{eff}}^2\rangle = \langle x^2\rangle_v + \langle x^2\rangle_c + \langle x^2\rangle_d, \quad (2)$$

where the subscripts v, c, and d refer to vibration, conformation, and diffusion, respectively. At low temperatures only $\langle x^2\rangle_v$ is observed; we assume that it follows, for all T, the classical, linear temperature dependence[7] evident below 160 K. The difference $\langle x_{\mathrm{eff}}^2\rangle - \langle x^2\rangle_v = \langle x^2\rangle_{cd}$ [cf. Eq. (2)] is plotted in Fig. 3. The term $\langle x^2\rangle_c$ is

TABLE I. Comparison of measured quadrupole splittings Δ_M with values Δ_R calculated from Eq. (6). The values Δ_0 are extrapolated from a polynomial of fourth degree valid for $T < 240$ K.

T (K)	Δ_M (mm/s)	Δ_0 (mm/s)	Δ_R (mm/s)
253.3	1.77 ± 0.01	1.82	1.80
256.3	1.75 ± 0.02	1.81	1.76
259.3	1.63 ± 0.05	1.80	1.67

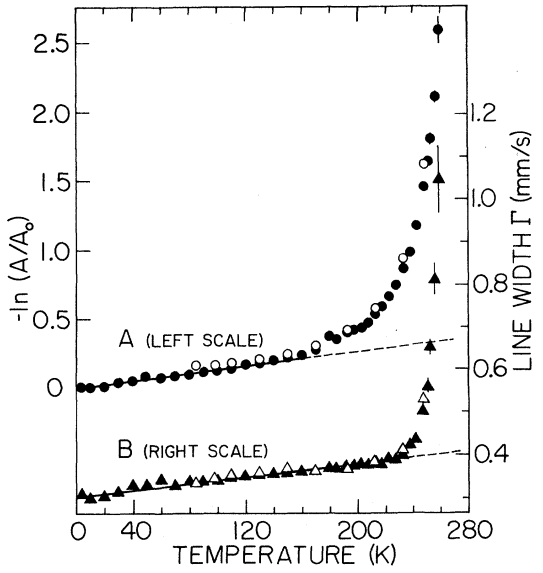

FIG. 1. Temperature dependence of the relative $\langle x_{\mathrm{eff}}{}^2\rangle$ (curve A) and the linewidth Γ (curve B) [full width at half maximum (FWHM)] (circles, triangles: remeasured points).

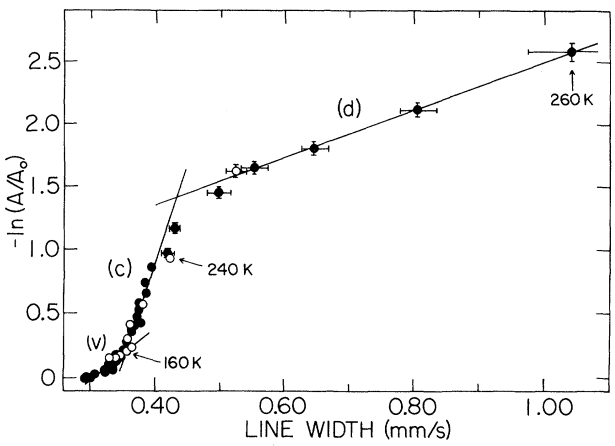

FIG. 2. Correlation of the relative $\langle x_{\mathrm{eff}}{}^2\rangle$ and the linewidth Γ (FWHM) (circles: remeasured points). (v), (c), and (d) refer to regions dominated by vibrations, conformations, and diffusion, respectively.

postulated to arise from thermally activated transitions between different conformational substates of the molecule.[1] In contrast to conformational fluctuations, diffusive motions lead to a msd $\langle x^2\rangle_d$ with no upper bound. As a result, the Mössbauer intensity vanishes at 260 K.

It is instructive to compare our msd with that found in crystals. Parak *et al.*[4] reported Mössbauer measurements on polycrystalline metmyoglobin. If we treat the msd data of Ref. 4 in the same way as ours by subtracting $\langle x^2\rangle_v$, the triangles in Fig. 3 are obtained. We note the coincidence of the two data sets up to 240 K and the characteristic increase of the msd in the frozen solution due to diffusion above 240 K.

The temperature dependence of $\langle x^2\rangle_c$ can be reproduced by the two-state model[15] indicated in Fig. 3. The iron is bound in one of the two potential wells, L and R, and jumps from one to the other at rates k_L and k_R, respectively. If d is the distance between the wells and τ_N the nuclear lifetime, the msd is given by[15]

$$\langle x^2\rangle_c = d^2 k_L k_R/[(k_L+k_R)(k_L+k_R+\tau_N{}^{-1})]. \quad (3)$$

If we assume that $k_L \ll k_R$, Eq. (3) reduces to

$$\langle x^2\rangle_c = d^2 \exp(\Delta S/k_B)\exp(-Q/k_B T)Z(T), \quad (4)$$

$$Z(T) = k_R/(k_R+\tau_N{}^{-1}).$$

Here $\exp(\Delta S/k_B)$ is an entropy term, the transition rate is $k_R = \nu_0 \exp(-E/k_B T)$, and $\nu_0 \sim 10^{13}$ s^{-1} is a typical vibrational frequency. To test this model we fit both sets of $\langle x^2\rangle_c$ values in Fig. 3 by Eq. (4). The logarithm of the preexponential factor in k_R was found to be $\log[\nu_0/(\mathrm{s}^{-1})] = 13.1$

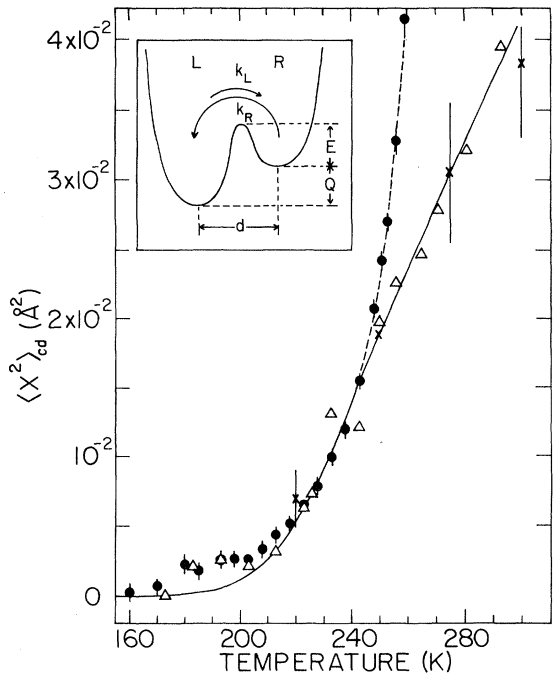

FIG. 3. $\langle x^2\rangle_{cd} = \langle x^2\rangle_c + \langle x^2\rangle_d$ as a function of temperature. Solid circles, MbO_2 aqueous solution (this work); triangles, polycrystalline MetMb (see Ref. 4); crosses, MetMb x-ray data (see Ref. 1). See text for inset.

TABLE II. Parameters deduced from Eq. (4) with $\nu_0 = 1.3 \times 10^{13}$ s^{-1}.

Sample	Q (kJ/mole)	E (kJ/mole)	c (Å^2)[b]
MetMb,[a] 200 K$<T<$300 K	6.2 ± 1.5	27.8 ± 1	0.5 ± 0.3
MbO_2, 200 K$<T<$245 K	6 ± 2	27.0 ± 1	0.2 ± 0.1

[a]Data of Ref. 4.

[b]$c = d^2 \exp(\Delta S/k_B)$.

± 0.6. As summarized in Table II, the fitted parameters of the two data sets agree within error and reproduce the measured points quite well (Fig. 3). We note that for x-ray diffraction, which is a fast process, the factor Z in Eq. (4) is unity. Indeed, if we multiply the $\langle x^2 \rangle_c$ values deduced from x-ray studies[1] by Z (crosses in Fig. 3), they agree well with the Mössbauer data. The near identity of $\langle x^2 \rangle_c$ in frozen solutions and crystals in surprising, especially since different chemical states of myoglobin are compared. It suggests that conformational fluctuations occur independently of the matrix and thus are an intrinsic property of the protein.

To estimate the diffusional msd, $\langle x^2 \rangle_d$, which is evident in Fig. 3 for $T > 240$ K, we subtract $\langle x^2 \rangle_c$ of the crystalline metmyoglobin sample[4] from our data using a linear interpolation between the measured points (Fig. 3). We find that both $\langle x^2 \rangle_d$ and the line broadening $\Delta\Gamma$ follow an Arrhenius law with activation energies $E_a = 82 \pm 2$ kJ/mole and $E_a = 79 \pm 4$ kJ/mole, respectively. These values are comparable with $E_a = 66 \pm 8$ kJ/mole found for self-diffusion of ^{3}H in ice.[16] Since the activation energies for $\langle x^2 \rangle_d$ and $\Delta\Gamma$ are the same within error, there is no doubt that the same process is responsible for both $\langle x^2 \rangle_d$ versus $\Delta\Gamma$ in Fig. 4 shows that the two quantities are proportional to each other. Similar empirical correlations have been noted before.[10,14] If $\Delta\Gamma$ of Fig. 4 is expressed as a frequency $\nu = \Delta\Gamma/2\pi\hbar$, the proportionality constant $\langle x^2 \rangle_d / \nu$ is $(2.5 \pm 0.1) \times 10^{-9}$ Å^2 s. This value is equal to $\tau_N/k^2 = 2.6 \times 10^{-9}$ Å^2 s, where τ_N is the nuclear lifetime, and k is the wave number of the γ ray. Thus we can write the empirical relation[17]

$$\langle x^2(T) \rangle_d \sim (\tau_N/k^2)\nu(T). \quad (5)$$

Evidence for random reorientation of the iron complex as a result of diffusion comes from the decrease in quadrupole splitting Δ observed for $T > 240$ K. The stochastic model of line shape of Dattagupta and Blume[8] predicts a reduced quadrupole splitting Δ_R according to

$$\Delta_R = \Delta_0[1 - (\Delta\Gamma/\Delta_0)^2]^{1/2}, \quad (6)$$

where $\Delta\Gamma$ is the line broadening and Δ_0 is the quadrupole splitting in the absence of relaxation. The analysis presented in Table I demonstrates that the data are compatible with the fluctuation model.

While all experimental results indicate the presence of diffusive motion, the microscopic mechanism of the process is not clear. The decrease in quadrupole splitting observed above 240 K demonstrates that the surrounding of the iron nucleus is fluctuating and that the motion of the iron complex is not of pure translational character.[8,11] A possible explanation could be ligand fluctuations that may modulate the electric field gradient thermally. These fluctuations are presumably correlated with the melting process of the solvent. Calorimetric measurements show premelting effects starting near 240 K.[18] Most likely the water around the protein starts melting at this temperature, and whole protein or parts of it may perform diffusive motions. The Arrheniuslike temperature dependence of $\langle x^2 \rangle_d$ and $\Delta\Gamma$ suggests a jump diffusion process. Reorientation of H_2O molecules or proton jumps may be re-

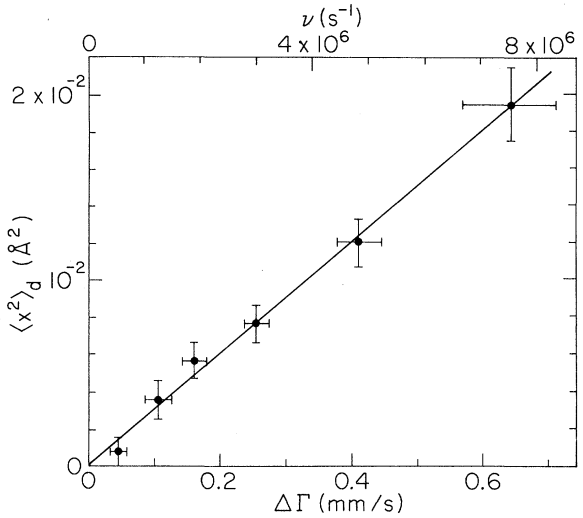

FIG. 4. $\langle x^2 \rangle_d$ vs $\Delta\Gamma$ in the temperature range 240 K $< T <$ 260 K.

sponsible for the diffusion process as suggested by the similarity of the activation energies with that found for ^{3}H in ice.[16]

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[18]H. Keller, unpublished.

Cosmological and Astrophysical Implications of Heavy Majorana Particles

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It is pointed out that heavy Majorana particles contemplated in certain grand unified theories may explain the cosmological baryon excess. Induced neutrino mixing can give oscillation lengths consistent with reactor and accelerator experiments, but capable of explaining the solar-neutrino "puzzle."

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It is somewhat puzzling that neutrinos appear massless in sharp contrast to all other quarks and charged leptons. This may simply be a reflection of extremely large masses of their chiral partners (Majorana type), in which case a sizable mixing comparable to usual mass terms is allowed between neutrinos and heavy Majorana particles. This situation can happen[1] in a number of grand unified models, including some SO(10) models,[2] that are more left-right symmetric and therefore aesthetically more appealing than the minimal SU(5) model.[3] In this note we shall demonstrate two possible implications of these heavy Majorana particles: generation of the cosmological baryon asymmetry and a large oscillation length of neutrinos that may explain the solar-neutrino "puzzle."[4] In the picture that we shall develop below, these two separate issues in cosmology and astrophysics are correlated and have a common origin in extremely large masses ($\gtrsim 10^{10}$ GeV) of Majorana particles.

In order to clarify our main points we shall take as an illustration a simple model that extends the minimal SU(5) model[3] by simply adding SU(5)-singlet Majorana particles N_i ($i = 1 \sim$ number of generations). This particular example

