Mössbauer Effect in Proteins

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In proteins, the Mössbauer effect and neutron scattering show a broad line and a rapid increase of the conformational mean-square displacement above about 180 K. The increase, dubbed the "dynamical transition," is controversial. We introduce a new interpretation of the Mössbauer effect in proteins and demonstrate that no dynamical transition is required. The increase in the mean-square displacement and the broad line are caused by fluctuations in the protein's hydration shell. Using the dielectric spectrum of these fluctuations, we predict the shape of the Mössbauer spectrum from 80 to 295 K with one dimensionless coefficient.

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Proteins are prototypes of complex systems. Fluctuations in the bulk environment and the hydration shell of proteins are essential for their functions [1]. The Mössbauer effect [2] is well suited for studying the connection between external fluctuations and internal motions. In the Mössbauer effect, a gamma ray is emitted and absorbed without recoil; it has the natural width Γ_n determined by its mean lifetime τ. For studies on heme proteins, the central iron atom is relevant when it is the nuclide ^{57}Fe. The ^{57}Fe gamma rays have an energy $E_0 = 14.4$ keV and a mean life $\tau = 140$ ns or characteristic rate $k_{\mathrm{Mo}} = 1/\tau$, corresponding to a natural linewidth $\Gamma_n = 4.7$ neV. This rate coincides with a range of protein motions so that the Mössbauer effect can yield data relevant for biology. The Mössbauer spectrum is normally determined by the transmission of gamma rays from a ^{57}Fe source moving with a velocity v through a stationary absorber containing the protein sample. The transmission $\mathrm{Tr}(\Delta E_{\mathrm{exp}})$, where $\Delta E_{\mathrm{exp}} = E_0 v/c$, is related to the scattering amplitude $S(\Delta E_{\mathrm{exp}})$ by $\mathrm{Tr}(\Delta E_{\mathrm{exp}}) = 1 - \mathrm{const} \times S(\Delta E_{\mathrm{exp}})$ in the thin absorber limit including convolution with the emission spectrum. Mössbauer spectra in proteins possess characteristic features. Figure 1 shows typical transmission spectra at four temperatures, measured by Parak and collaborators [3–5] in hydrated myoglobin (Mb) polycrystals. Below about 180 K, the Mössbauer line remains sharp, but its area decreases with increasing temperature. The decrease is due to thermal vibrations and characterized by the Lamb-Mössbauer relation $f(T) = \exp[-q^2\langle x^2\rangle_V]$, where $q = 7.29$ Å^{-1} is the wave vector of the gamma rays, $f(T)$ the recoilless fraction, and $\langle x^2\rangle_V$ the vibrational mean-square displacement (MSD) of the iron atom. In dehydrated proteins, the line remains sharp to well above 180 K. In a hydrated protein, however, a broad line appears above about 180 K. Conventionally, the spectrum is decomposed into a narrow line and a broad band [6–9]. The Lamb-Mössbauer relation is used to calculate the MSD of the sharp line. At about 180 K, this MSD increases sharply with temperature [10,11] as later also observed in neutron scattering [12] and dubbed the "protein dynamical transition" (PDT). The conventional treatment has problems: The Lamb-Mössbauer relation is valid only for a harmonic potential [6], but proteins are not harmonic. The separation into a narrow line and a broad band is misleading. The entire spectrum is inhomogeneous, composed of sharp lines. In the conventional treatment, the broad component is homogeneous. The Heisenberg uncertainty relation then implies that the nuclear lifetime is shortened by as much as a factor of 100. No nuclear model exists, to our knowledge, to understand such a shortening. The PDT is caused by the incorrect separation into a sharp and a broad component and so is not a valid implication of the Mössbauer data. Our unified model solves these problems [1].

We have shown earlier that dielectric fluctuations in the hydration shell of Mb predict the onset of the PDT and the temperature dependence of the Lamb-Mössbauer $f(T)$ above 180 K without a fitting parameter [1]. Here we address the full Mössbauer spectrum. Two types of fluctuations in the protein environment, called α and β_h, are involved. Their properties are known from the physics of supercooled liquids and glasses [13,14]; their spectra are measured by using dielectric relaxation spectroscopy [15]. The α fluctuations are structural. Their rate coefficient is inversely proportional to the viscosity of the medium. They can be neglected in protein crystals, but they are important in viscous liquids [9,16,17]. The β_h fluctuations originate in the hydration shell and influence internal protein motions [1]. They depend on hydration and vanish if the protein is dehydrated. The spectrum of β_h fluctuations can be described by the Havriliak-Negami function [18]

$$\varepsilon''_\beta(k_\beta, T) = -\Delta\varepsilon \mathrm{Im}\{1 + [ik_\beta/k_h(T)]^b\}^{-c}. \quad (1)$$

Here k_h, b, and c are fit parameters. The rate coefficient $k_h(T)$ follows an Arrhenius law [1]. The normalized distributions $\rho_\beta(k_\beta, T) = \varepsilon''_\beta(k_\beta, T)/a''$, where a'' is the area of the loss spectrum ε'', are shown as insets in Fig. 1. Their

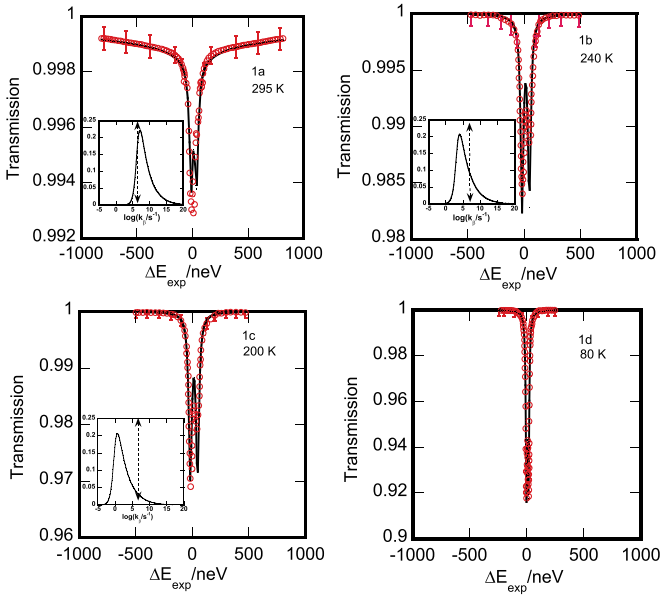

FIG. 1 (color online). Mössbauer transmission spectra of myoglobin. The fact that the spectra are doublets is caused by quadrupole interactions and is irrelevant for the present discussion. Red circles: Characteristic fits adapted from original experimental references indicated below. Error bars are from the scatter of actual experimental points. Solid lines: Current fits with hydration shell fluctuation rate distributions as in Eqs. (1)–(3). $h = 0.4$. Insets: Hydration shell fluctuation rate distributions $\rho(k_\beta, T)$ between 0 and 0.25 plotted for $\log(k_\beta/\mathrm{s}^{-1})$ between -5 and 20 [1,26]. Vertical lines determined by experimental rate k_{Mo}. (a) Metmyoglobin (metMb), 295 K [4]; (b) metMb, 240 K [5]; (c) metMb, 200 K [5]. (d) Carbonmonoxymyoglobin (MbCO), 80 K [3]. No inset because the rate distribution is shifted outside the rate window of the plot. The parameter $\chi = 1.8 \pm 0.2$. Quadrupole doublet peak positions and experimental FWHM (Γ): (a) $(-12, 36)$ neV, FWHM = 34 neV, (b) $(-17, 48)$ neV, FWHM = 28 neV, (c) $(-17, 47)$ neV, FWHM = 32 neV, and (d) $(-2, 16)$ neV, FWHM = 14 neV. Errors are $\pm 15\%$. Logarithm of the minimized sum of square residuals divided by the degrees of freedom: (a) -6.6, (b) -5.6, (c) -5.1, and (d) -4.8; fit obtained by using a Levenberg-Marquardt algorithm.

shape is temperature-independent, an effect known from polymer physics and called time-temperature superposition.

Mössbauer emission and absorption processes are recoilless as proven, for instance, by the celebrated gravity experiment of Pound and Rebka [19]. The emission and absorption lines have essentially the natural linewidth Γ_n. A source moving with velocity $|v| \geq 0$ relative to the absorber allows the source line to scan over the absorber line resulting in a line centered at $v = 0$, with a sharp width $2\Gamma_n$. However, if an extranuclear effect changes the gamma ray energy seen by the system by E_β, the line is centered at $v_\beta/c = E_\beta/E_0$ but is still sharp with a width $2\Gamma_n$. If E_β is distributed [see Eqs. (5) and (6)], a broad inhomogeneous band results. The sharp central line is due to the nuclei that have not moved appreciably during the lifetime τ. The question then is the origin of the energy E_β. In a dehydrated protein in a crystal, the PDT and the broad line are absent. The α fluctuations in the bulk solvent and the β_h fluctuations in the hydration shell are thus candidates for producing E_β [20]. The data in Fig. 1 were taken by using Mb crystals, where the hydration h, the weight ratio of water to protein, is 0.4 [21] but where the α fluctuations are absent. The β_h rate distributions in the insets in Fig. 1 were taken at the same hydration; they provide intuitive understanding of the dynamics: Well below 200 K, all β_h fluctuations are much slower than k_{Mo} and do not affect the recoilless absorption. At about 180 K, a small fraction of proteins have rate coefficients $k_\beta > k_{\mathrm{Mo}}$; they shift out of rest-frame resonance into a broad band. With increasing temperature, the dielectric spectrum moves to higher frequencies, the area above k_{Mo} increases, and more proteins move into the broad band. At 295 K, nearly all proteins are in the inhomogeneous wings seen in Fig. 1(a).

The quantitative analysis of the full Mössbauer spectrum is based on the seminal work of Singwi and Sjölander [22]. The standard interpretation in which the ^{57}Fe atom makes a Brownian walk within a rigid cage is inappropriate; the atom is surrounded by residues that fluctuate continuously [1]. Moreover, the ^{57}Fe atom plus the Mössbauer gamma ray comprise a quantum mechanical system coupled to the hydration shell and the crystal environment [23]. The motion of the iron atom in the absorber therefore cannot simply be considered to occur in ordinary 3D space; it is forced diffusion in a very large conformation space where each step is given by χk_β. χ is a dimensionless parameter to be determined experimentally. The time correlation function for the absorption of the gamma ray by an iron nucleus is $\langle \exp[-i\vec{q} \cdot \vec{R}(0)] \exp[i\vec{q} \cdot \vec{R}(t)] \rangle$ with brackets denoting a quantum and ensemble average. The position operators are sums of independent vibrational (V) and conformational (C) components so the time correlation function factorizes. The vibrational component involves phonon modes that are fast on the time scale of the 140 ns lifetime of the ^{57}Fe nuclear level and results in the vibrational recoilless fraction $f_V(T) = \exp[-q^2\langle x^2(T)\rangle_V]$. The conformational part of the time correlation function is the quantum transition amplitude between the quantum states described by operators $\vec{R}_C(0)$ and $\vec{R}_C(t)$. The transition amplitude is a measure of dephasing due to coupling of the iron absorber to hydration shell fluctuations, which are coupled to the macroscopic crystal environment. It has been shown (see [23] and references cited therein) that quantum dephasing occurs exponentially in time as a result of coupling of the quantum system (here the iron plus the gamma ray) with its macroscopic environment. The transition amplitude then is given for the

ensemble of proteins by an integral over the fluctuation rate distribution with the dephasing occurring exponentially in time with rate χk_β:

$$\langle \exp[-i\vec{q}\cdot\vec{R}_C(0)]\exp[i\vec{q}\cdot\vec{R}_C(t)]\rangle = \int d(\log k_\beta)\rho(k_\beta, T)\exp(-\chi k_\beta|t|). \quad (2)$$

The transition amplitude over the protein ensemble is unity at zero time and decays to zero at long times, reflecting continuing diffusion of an individual protein-hydration shell system in the large conformation space with a vanishing probability to return to a particular substate. The intermediate correlation function for the Mössbauer effect in heme proteins is given by

$$I(t) = f_V(T)\exp[-(i\Delta E_{\rm exp}t + \Gamma|t|/2)/\hbar] \times \int d(\log k_\beta)\rho(k_\beta, T)\exp[-\chi k_\beta|t|]. \quad (3)$$

The integration of Eq. (3) over time gives the scattering function $S(\Delta E_{\rm exp})$:

$$S(\Delta E_{\rm exp}) = f_V(T)\int d(\log k_\beta)\rho(k_\beta, T) \times \frac{1}{\pi}\frac{\Gamma/2 + \chi\hbar k_\beta}{\Delta E_{\rm exp}^2 + (\Gamma/2 + \chi\hbar k_\beta)^2}. \quad (4)$$

The distributions of β_h rates shown in the insets in Fig. 1 result in a broad spectrum in energy space. Equations (3) and (4) have only the dimensionless parameter χ to be determined by fitting. The 295 K data in Fig. 1(a) give $\chi \approx 1.8$, and this value was kept fixed for the three other temperatures. The resulting scattering function $S(\Delta E_{\rm exp})$ agrees with the experimental data.

The individual recoilless lines are recovered from Eq. (4) by applying the Fourier convolution theorem to the Lorentzian integrand to get

$$S(\Delta E_{\rm exp}) = f_V(T)\int dE_\beta w(E_\beta, T) \times \frac{1}{\pi}\frac{\Gamma/2}{(\Delta E_{\rm exp} - E_\beta)^2 + (\Gamma/2)^2} \quad (5)$$

with the probability density given by

$$w(E_\beta, T) = \int d(\log k_\beta)\rho(k_\beta, T)\frac{1}{\pi}\frac{\chi\hbar k_\beta}{E_\beta^2 + (\chi\hbar k_\beta)^2}. \quad (6)$$

The physical Mössbauer emission and absorption is sharp, and what appears as a broad band describing the protein ensemble is composed of a distribution of individual recoilless lines. Each individual protein molecule is in a different conformational substate with its own β_h fluctuation rate coefficient k_β. The broad band is inhomogeneous with an envelope described by $w(E_\beta, T)$.

The fact that the model introduced here describes the Mössbauer spectrum from 80 to 295 K by using the independently determined spectrum of the β_h fluctuation in the hydration shell leads to a number of conclusions. (i) The Mössbauer spectrum does not consist of a narrow line and a broad band but is an inhomogeneous ensemble of recoilless lines. (ii) The temperature and energy dependence of the internal protein fluctuations resulting in quantum dephasing are predicted by the external hydration shell fluctuations with only one dimensionless parameter determined by experiment. (iii) The separation into elastic and quasielastic components is arbitrary—there is no distinction between ''narrow and broad.'' (iv) The ''protein dynamic transition,'' described by [24,25] as ''an abrupt onset of atomic displacements on the microscopic length and time scale... now known to be a generic property of hydrated proteins...,'' is not required since the full Mössbauer spectrum is explained without invoking a dynamical transition. In reality, the apparent abrupt increase in the MSD occurs when k_β becomes larger than $k_{\rm Mo}$ and is described by the probability density $w(E_\beta, T)$ by including only proteins with energy between $\pm E_{\rm Mo} = \pm\hbar k_{\rm Mo}$:

$$f_C(T) = \int_{-E_{\rm Mo}}^{+E_{\rm Mo}} dE_\beta w(E_\beta, T). \quad (7)$$

$f_C(T)$ is the conformational recoilless fraction. This prediction is in agreement with the measured data [1]. (v) The treatment here is restricted to β_h fluctuations because the α fluctuations are very slow in protein crystals. In cases where the α fluctuations are also present, for instance, in viscous liquids [9,16,17], they also affect the Mössbauer spectrum. Neutron scattering studies of hydrated proteins [26] can be analyzed as done here for the Mössbauer effect. The neutron experiments also show an elastic line and a quasielastic band [27]. The analysis given here suggests that the broad band is inhomogeneous and consists of a large number of lines whose width is determined by the instrumental resolution. Hydrogen atoms are distributed throughout the protein with different environments [28]. However, if the relevant dielectric spectrum is known, it should predict the shape and the temperature dependence of the broad band in powder and polycrystal samples. (vi) The mechanism of the interaction between the hydration shell and the protein interior that leads to the broad lines is a major unsolved question. More experiments and theoretical calculations are needed to solve this problem.

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