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Protein dynamics: determination of anisotropic vibrations at the haem iron of myoglobin

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Abstract

The phonon assisted Mössbauer effect is used to determine the anisotropic harmonic vibrations labelled by the iron in the active centre of myoglobin at room temperature. A single crystal of metmyoglobin is investigated in five different orientations. Several modes are assigned by the projection of the vibrational amplitude onto the beam directions. The density of phonons below 1 meV shows a quadratic increase with the energy like that in a Debye solid. An anisotropic velocity of sound in the protein crystal is extracted with a mean sound velocity of 1657 m s^{-1}. Modes between 4 and 5 meV are identified as haem sliding motions. Vibrations between 30.2 and 36.5 meV are mainly within the haem plane; those between 19 and 25.6 meV are perpendicular to the plane. These results together with phonon assisted Mössbauer effect measurements on hydrated myoglobin powder and polycrystal samples in the temperature range between 50 K and room temperature are compared with the results of Mössbauer absorption experiments. While the mean square displacements at the iron obtained by the phonon assisted Mössbauer effect increase linearly with temperature up to room temperature, Mössbauer absorption reveals a dynamical transition temperature. Above this temperature a much stronger increase of the mean square displacements occurs, indicating protein specific and functionally important dynamics. Since these displacements are not registered by the phonon assisted Mössbauer effect the involved energy transfer is beyond the energy resolution of the method i.e. smaller than 1 meV. Actually the energy transfer is in the nanoelectronvolt energy regime as seen from the energy profile of the Mössbauer absorption spectrum. Protein specific dynamics can be explained as diffusive motion of molecular segments in limited space.

1. Introduction

For several years, the investigation of protein dynamics has been at the centre of interest of many groups working in the field of 'biological physics'. A strong impact came from the first study of the rebinding kinetics of CO in myoglobin [1]. In these experiments the CO bound

to the haem iron was photolysed by a laser flash. Its rebinding was followed optically in a large time interval from 10^{-6} to 100 s at temperatures from 40 to 160 K. The obtained non-exponential kinetics proved that the individual myoglobin molecules differ slightly in their structure. Although all molecules are in the 'unligated' conformation after the photolysis, the individual molecules are in so called 'conformational substates'. The structural distribution within an ensemble of protein molecules also reveals itself in the mean square displacements, $\langle x^2 \rangle$, of the individual atoms of a molecule as obtained from the Debye–Waller factor of x-ray structure analysis [2]. The temperature dependence of the $\langle x^2 \rangle$-values gave some insight into energy differences of the conformational substates [3, 4].

For a long time, Mössbauer experiments on the haem iron have been used in order to study protein dynamics. First experiments on myoglobin crystals are described in [5–9]. Mean square displacements of the iron were determined from the Lamb–Mössbauer factor in the temperature range of 4.2 K to room temperature. Two temperature regimes have been seen. Below about 200 K, the $\langle x^2 \rangle$-values at the position of the iron are typical for iron compounds or frozen solutions of iron ions. Above 200 K the $\langle x^2 \rangle$-values show a very pronounced temperature dependence and increase strongly up to room temperature. Today, one calls the temperature at the border between the two regimes the dynamical transition temperature, T_c. A similar behaviour for the mean square displacements of the hydrogen atoms was found by incoherent neutron scattering [10].

There are a number of papers giving an overview of the development of the investigation of protein dynamics. We refer to [11–15]. In this contribution we want to concentrate on the determination of phonon densities in myoglobin by 'phonon assisted Mössbauer spectroscopy'. This method uses synchrotron radiation; the Mössbauer effect is measured in the time domain. We describe new measurements on a myoglobin single crystal in several orientations at room temperature. The results are compared with previous temperature dependent experiments on polycrystalline and powder material, which averaged over orientations. Together with the conventional Mössbauer spectroscopy in the energy domain and x-ray structure investigation on a myoglobin mutant one obtains some insight into the physics of protein dynamics and its connection with protein function.

2. Material and methods

Sperm whale myoglobin was enriched in the Mössbauer isotope ^{57}Fe according to [16]. Crystals with the space group $P2_1$ were grown from a saturated ammonium sulfate solution. A metmyoglobin crystal with the size of $3 \cdot 2 \cdot 1$ mm^3 was used. The experiments on this crystal have been performed on beam line ID18 at the ESRF in Grenoble.

The phonon assisted Mössbauer effect allows the determination of the density of phonons coupling to the iron. The principle of the experiment is explained in figure 1. Two sets of monochromators select a narrow energy band of 0.5 meV from the synchrotron radiation. Its absolute energy can be varied in the range $-80\,\text{meV} < \varepsilon < +80\,\text{meV}$ where $\varepsilon = 0$ corresponds to the Mössbauer resonance energy of 14.413 keV of the isotope ^{57}Fe. The beam is scattered by the protein sample. The scattered radiation is registered by the avalanche photodiodes APD1a, APD1b and APD2.

Let us first consider the case where the monochromators are adjusted to $\varepsilon = 0$; one obtains elastic nuclear forward scattering: with a certain probability, the ^{57}Fe nuclei of the sample absorb x-rays. The deexcitation occurs by emitting radiation with a time delay, $\tau_N = 140$ ns, characteristic for the lifetime of the 14.413 keV level. However, the overwhelming quantity of scattered radiation stems from elastic and inelastic x-ray scattering by the electrons of the sample. The ratio of the intensity coming from the electronic scattering and the nuclear

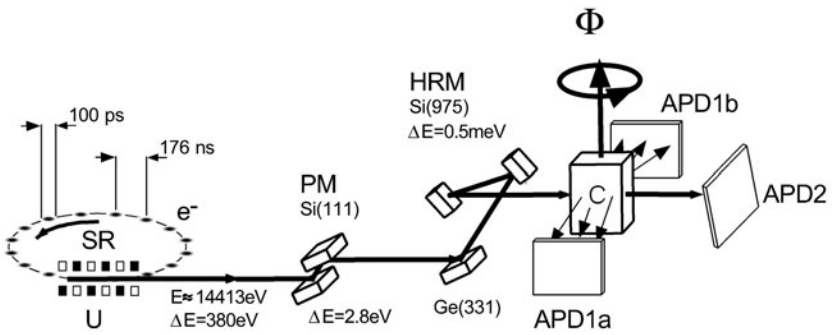

Figure 1. Set-up of the phonon assisted Mössbauer spectroscopy. The synchrotron storage ring SR is filled with electron bunches with time structure. The undulator U produces synchrotron radiation in the energy regime of the ^{57}Fe Mössbauer level. The monochromator system consists of a high heat load pre-monochromator PM of two Si(111)/Si(111) crystals and a Ge(331), Si(975)/Si(975) high resolution monochromator HRM. The myoglobin crystal C is mounted on a diffractometer (not shown). The rotation angle is Φ. Two avalanche photo-diodes, APD1a and APD1b, of $10 \cdot 10\ \mathrm{mm}^2$, are situated in the scattering direction 3 mm beneath the sample. APD2 detects only the forward scattered radiation.

resonance scattering can be estimated by comparing the energy dependences of the two processes. While the electrons scatter the whole radiation within the available energy interval of about 1 meV the iron nuclei select only the energy with the width, Γ, of 5×10^{-6} meV. The time delay of the nuclear deexcitation is used to separate it from the electronic scattering which is a prompt process without time delay. This is possible because the synchrotron radiation has a time structure. At the ESRF an x-ray flash of 100 ps length is emitted every 176 ns. APD1a/b and 2 are gated during the x-ray flash and 10 ns after; all registered quanta after 10 ns come from nuclear deexcitation.

We now change the adjustment of the monochromators so that ε becomes significantly larger than 0.5 meV. This means that the x-rays no longer fulfil the resonance condition of the Mössbauer nuclei. Nevertheless, the avalanche photo-diodes register time delayed radiation. This comes from the fact that phonons coupling to the iron can balance the missing or excessive energy. The delayed counting rate becomes a measure of phonons transferring the energy ε to the iron.

3. Data evaluation and results

Figure 2 shows the results for the single-crystal experiment. The crystal was rotated around its b-axis by an angle Φ. By an independent experiment the orientation of the crystal was determined. $\Phi = 0$ corresponds to an incoming beam perpendicular to the $\vec{a}^*\vec{b}^*$-plane in the reciprocal lattice which means parallel to the c-axis. Five orientations of the sample were measured. The sample was kept at room temperature. In all experiments the incoming beam was perpendicular to the b-axis. In the following the direction of the incoming beam, $\vec{k}/|k|$, is called the x-axis, where $k = 2\pi/\lambda$ is the wavevector of the radiation.

For the data analysis one has to remember that the measured spectrum is proportional to a convolution of the resolution function, $R(\varepsilon)$, of the monochromator system with the multiple phonon spectrum $S_{\mathrm{exp}}(\varepsilon)$ of the sample.

$$I_{\mathrm{exp}}(\varepsilon) = cR(\varepsilon) \otimes S_{\mathrm{exp}}(\varepsilon) \tag{1}$$

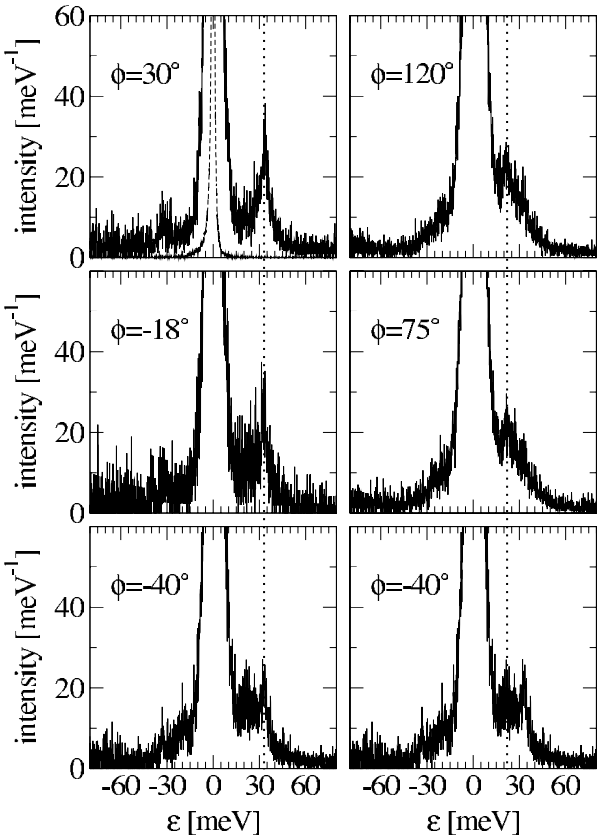

Figure 2. Phonon spectra of iron in a single crystal of metmyoglobin oriented with the crystalline *b*-axis perpendicular to the synchrotron beam. Dashed curve: energy resolution of the experiment. Vertical dotted lines mark two pronounced intensities at 33 and 22 meV. Φ is the rotation angle around the *b*-axis. $\Phi = 0°$ is perpendicular to the a^*b^*-direction in reciprocal space.

with

$$S_{\mathrm{exp}}(\varepsilon) = f\left\{(a-1)\cdot\delta(\varepsilon) + \left(\delta(\varepsilon) + S_1 + \frac{1}{2!}S_1 \otimes S_1 + \frac{1}{3!}S_1 \otimes S_1 \otimes S_1 + \cdots\right)\right\}. \tag{2}$$

c and a are constants; f is the Lamb–Mössbauer factor. Using the convolution theorem of the Fourier transformation (FT), $I_{\mathrm{exp}}(\varepsilon)$ is the Taylor series of the exponential function of the FT of the one-phonon scattering $S_1(\varepsilon)$:

$$\exp[\mathrm{FT}\{S_1(\varepsilon)\}] = \frac{\mathrm{FT}\{R(\varepsilon) \otimes S_{\mathrm{exp}}(\varepsilon)\}}{f \cdot \mathrm{FT}\{R(\varepsilon)\}} = \frac{1}{f}\mathrm{FT}\{S_{\mathrm{exp}}(\varepsilon) + f(1-a)\delta(\varepsilon)\}. \tag{3}$$

$R(\varepsilon)$ is known from the forward scattering spectrum measured by APD2 at $\varepsilon = 0$. The proportional constant c, the absorption correction for the elastic line a and the Lamb–Mössbauer factor f are obtained by a normalization procedure using Lipkins' sum rule [17]. From the one-phonon scattering function $S_1(\varepsilon)$ one can calculate the density of phonons coupling to the iron according to

$$D(\varepsilon) = \frac{\varepsilon}{E_R}(1 - \exp(-\varepsilon/k_B T))S_1(\varepsilon). \tag{4}$$

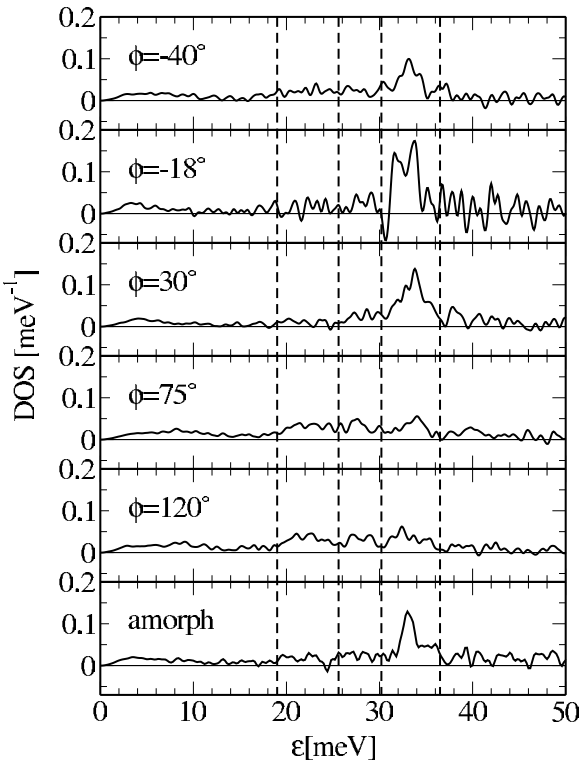

Figure 3. Density of phonon states coupling to the iron in the active centre of a single crystal of metmyoglobin. For the orientation and definition of Φ see figure 2. Vertical dashed lines mark energy regimes for the evaluation of the vibrational mean square displacement $\langle x^2(\varepsilon_1, \varepsilon_2)\rangle$ according to equations (5) and (6)).

Figure 3 shows the density of states for the five orientations of the sample and for an amorphous sample measured with an energy resolution of 0.85 meV at SRI-3-CAT, APS, Argonne. The angular dependence is obvious.

The density of states can be used to calculate the mean square displacement at the position of the iron, $\langle x^2(\varepsilon_1, \varepsilon_2)\rangle$, due to phonon modes in the energy interval from ε_1 to ε_2:

$$\langle x^2(\varepsilon_1, \varepsilon_2)\rangle = \frac{\hbar^2}{m_{\mathrm{Fe}}} \int_{\varepsilon_1}^{\varepsilon_2} \frac{D(\tilde{\varepsilon})}{\tilde{\varepsilon}} \left(\frac{1}{\exp(\tilde{\varepsilon}/k_B T) - 1} + \frac{1}{2} \right) \mathrm{d}\tilde{\varepsilon}. \tag{5}$$

In order to get the intended anisotropic mean square displacements, we have to consider the symmetry of the myoglobin crystal. There are two molecules related by the symmetry of $P2_1$. Figure 4 gives an impression of the relative orientations of the two myoglobin molecules. The rotation axis b is sketched as an arrow; the haem group and the HIS93 are highlighted to correlate Φ with the orientation of the haem planes. The haem normals form an angle of 43.7°, whereas in-plane vectors can be oriented parallel to the incoming x-ray beam. This corresponds with the $\Phi = 0°$ orientation. If a vibrational amplitude, $\vec{r}(t)$, of the iron has an angle θ to the incoming beam direction $\vec{k}/k$, only the component $|\vec{r}(t)| \cdot \cos(\Theta)$, the projection onto the incoming beam, is essential for the absorption process [18–20]. For amorphous samples or samples consisting of a huge number of small crystals, $D(\varepsilon)$ and $\langle x^2\rangle$ in equation (5) are averages over all possible angles θ yielding $\langle x^2\rangle = \frac{1}{3}\langle r^2\rangle$. The situation is different in an experiment with a single crystal. In myoglobin the axis b is perpendicular to the axes a and c.

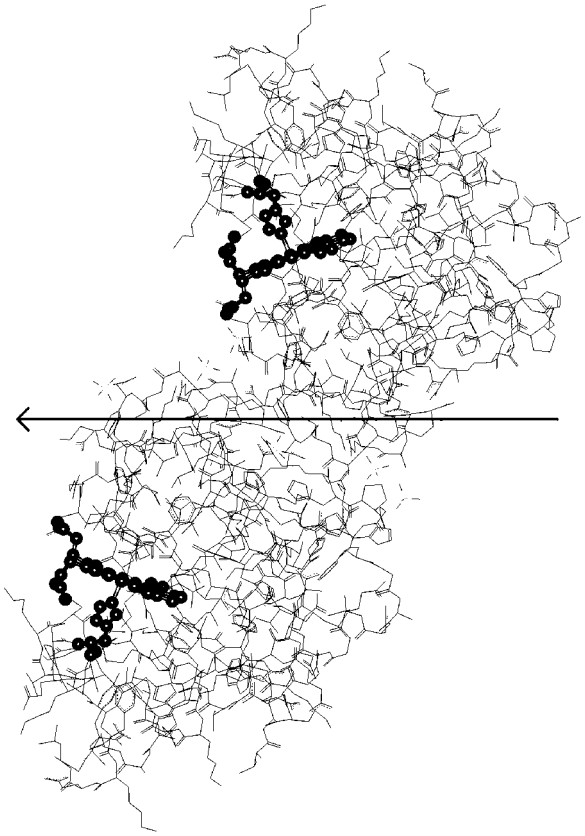

Figure 4. Two metmyoglobin molecules in the unit cell with $P2_1$ symmetry. The amino acid chain is given as sticks, the haem plane and the HIS93 are indicated by balls. The arrow indicates the crystallographic b-axis which is the rotation axis of the experiment. The view is shown for a rotation angle of $\Phi = 0°$.

In our experiment $\vec{k}/k$ is always in the $\vec{a}, \vec{c}$-plane. The contribution of a mode $\vec{r}(t)$ with the energy ε on the x-axis defined by $\vec{k}/k$ is

$$\langle x^2(\varepsilon)\rangle = \langle r^2(\varepsilon)\rangle \cos^2(\beta)\cos^2(\Phi). \tag{6}$$

β is the angle between $\vec{r}(t)$ and the $\vec{a}, \vec{c}$-plane of the crystal. Note that each vibration $\vec{r}(t)$ within one myoglobin molecule has a twin $\vec{r}'(t)$ in the other molecule related by the symmetry of $P2_1$. However, the involved 180° rotation axis does not change $\cos(\beta)$ or $\cos(\Phi)$.

In order to analyse the anisotropy of the mean square displacements we have selected five energy regions where ε_1–ε_2 was 0–1 meV, 4–5 meV, 19–25.6 meV, 25.6–30.2 meV and 30.2–36.5 meV respectively (compare figure 3). Equations (5) and (6) allow the calculation of $\langle x^2(\varepsilon_1, \varepsilon_2)\rangle$ which are shown in figure 5. For all energy intervals the angular dependence of the mean square displacements obeys the $\cos^2$ law according to equation (6). However, there remains an angular independent part. This indicates that a number of different modes contribute to the mean square displacement in the energy interval. These modes have other directions of the vibration amplitudes and, therefore, different angular dependences. From the orientation of the crystal we can correlate the maxima of the $\cos^2$ curves with the orientation of the haem planes. $\langle x^2(\varepsilon_1 = 30.2, \varepsilon_2 = 36.5)\rangle$ has its maximum at $\Phi = 5.3°$. This is

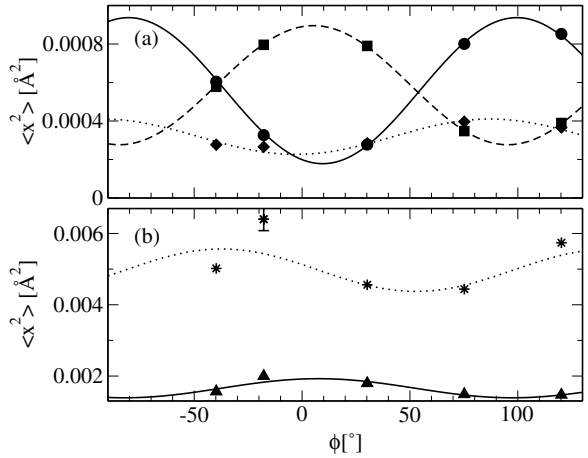

Figure 5. Mean square displacement $\langle x^2(\varepsilon_1, \varepsilon_2)\rangle$ of modes belonging to an energy regime from ε_1 to ε_2. Closed symbols are measurements at five different orientations Φ (see text and figure 3). Error bars are as small as or smaller than the symbols except for the single one given in figure 5(b). Lines are fits of equation (6) to the data of an energy regime. (a) Closed circles and solid curve: $\varepsilon_1 = 19$ meV, $\varepsilon_2 = 25.6$ meV. Closed diamonds and dotted curve: 25.6–30.2 meV. Closed squares and dashed curve: 30.2–36.5 meV. (b) Closed triangles and solid curve: haem sliding in the energy regime of 4–5 meV. Asterisks and dotted curve: $\langle x^2(\varepsilon_1, \varepsilon_2)\rangle$ due to acoustic phonons of the energy regime below 1 meV.

Table 1. Mean square displacements at the iron coming from modes in three different energy intervals. ϕ describes the rotation around b. The $\langle x^2\rangle$-value NM stems from a normal mode analysis [26].

Energy interval (meV)	0–1	4–5	19.0–25.6	25.6–30.2	30.2–36.5
Φ (deg)	−37.2	7.5	99.5	87.1	5.3
$\langle x^2\rangle$ (Å) anisotr.	0.001 19	0.000 54	0.000 76	0.000 18	0.000 62
$\langle x^2\rangle$ (Å) NM$\perp$ haem			0.0009		

the angle of the incoming beam with the haem plane. Hence, in this energy interval the main contributions come from modes within the haem plane. Rotating the crystal by about 90° we obtain the maximum for $\langle x^2(\varepsilon_1 = 19.0, \varepsilon_2 = 25.6\rangle$ at $\Phi = 99.5°$. Due to the $P2_1$ symmetry, the contributing modes are 22° tilted to the haem normal; the crystal symmetry makes it impossible to select modes parallel to the haem normal. Figure 5(b) also shows the angular dependence of the $\langle x^2(\varepsilon_1 = 0, \varepsilon_2 = 1\rangle$- and the $\langle x^2(\varepsilon_1 = 4, \varepsilon_2 = 5\rangle$-values. The modes below 1 meV are acoustic phonons. The modes between 4 and 5 meV show the same angular dependence of the mean square displacements as the modes between 30.2 and 36.5 meV. This indicates that the motion is mainly in the haem plane direction. Table 1 summarizes the result.

The phonon density within the energy regime below 1 meV allows the determination of the velocity of sound [17] using equation (7):

$$D_{Debye}(\varepsilon) = \frac{m_{\mathrm{Fe}}}{2\pi^2 \rho v_g^3 \hbar^3}\varepsilon^2 \tag{7}$$

v_g is the sound velocity averaged over the longitudinal, v_l, and the transversal, v_t, velocity in the direction of the incoming beam. The density of myoglobin was taken as $\rho = 1.23$ g cm^{-3} and the mass of the iron atom is $m_{\mathrm{Fe}} = 57 \cdot 1.66 \times 10^{-27}$ kg. Results are summarized in table 2.

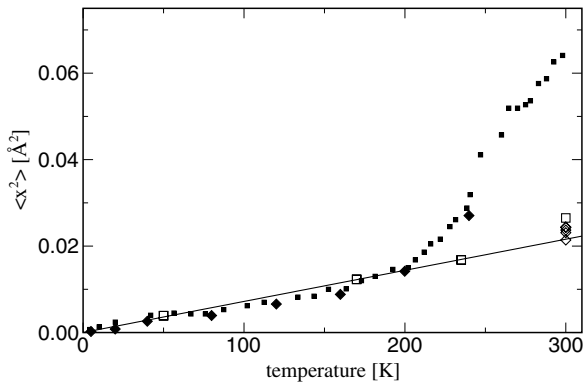

Figure 6. Temperature dependent mean square displacement $\langle x^2 \rangle = \frac{1}{3}\langle r^2 \rangle$ labelled at the iron in myoglobin. Filled squares: Mössbauer absorption on deoxymyoglobin crystals [26]. Filled diamonds: Mössbauer absorption on metmyoglobin crystals. Open squares: phonon assisted Mössbauer spectroscopy on an amorphous metmyoglobin sample with the same water content as in crystals [17]. The solid line is a linear fit of the three low temperature phonon assisted Mössbauer data. Diamonds at 300 K: present results for the mean square displacement projected onto the five orientations of a single crystal of metmyoglobin. The energy range from $\varepsilon_1 = 0$ to $\varepsilon_2 = 50$ meV was taken into account for each spectrum.

Table 2. Velocity of sound determined for different orientations of the crystal.

Φ (deg)	42	64	112	157	202	Amorphous
v_g (m s^{-1})	1659 ± 16	1531 ± 17	1717 ± 18	1766 ± 15	1611 ± 10	1605 ± 9

The average over the five angles gives a sound velocity for the crystal of 1657 ± 16 m s^{-1}. The amorphous sample yields an average over all directions.

4. Discussion

The anisotropic vibrations at the position of the iron reveal themselves in the whole energy regime. An estimation using an average sound velocity in proteins obtained by laser induced ultrasound [21] yields an energy of about 1 meV for the acoustic phonons at the first Brillouin zone. This is in agreement with normal mode calculations [22]. The lowest optical modes have an energy of about 0.8 meV. The $\langle x^2 \rangle$-values coming from modes between 0 and 1 meV show an angular dependence. This reflects an anisotropic velocity of sound in a myoglobin crystal. The average value of 1657 ± 16 m s^{-1} fits very well with the value of 1605 ± 9 m s^{-1} obtained from the amorphous sample [17]. We note in passing that the present results on a single crystal of Mbmet do not show any enhancement of the low energy density of states over the Debye behaviour and therefore no indication of the so-called boson peak. This peak was found, however, in an amorphous myoglobin sample (compare [17]).

The energy interval between 4 and 5 meV can be assigned to phonons with wavelengths between 12 and 16 Å. This is a little bit larger than the diameter of the haem plane. The contributing modes have a maximum at $\Phi = 7.5°$. They represent a sliding in the haem plane. The mean square displacements from modes in a direction tilted by 22° to the haem normal can be compared with those calculated by a normal mode analysis in the direction of the haem normal. The agreement is surprisingly good; the difference may reflect the difference of the mode directions.

The experiment described has improved our knowledge of the phonon spectrum of a protein. However, mean square displacements, calculated from the phonon density, do not show 'protein specific' motions. This problem becomes clear from figure 6. The plot depicts the temperature dependence of the mean square displacement, $\langle x^2\rangle^\gamma$, obtained in a wide temperature region by Mössbauer absorption spectroscopy on Mbmet and Mbdeoxy samples consisting of randomly oriented small crystals. These values are compared with mean square displacements obtained by the phonon assisted Mössbauer effect. It is obvious that the $\langle x^2\rangle^\gamma$ values are much larger above a characteristic temperature, T_c, of about 200 K than those obtained from the phonon density spectra. Since the mean square displacements calculated from the phonon density do not show this effect, the strong increase of $\langle x^2\rangle^\gamma$ above T_c cannot be explained by an increasing anharmonicity of the phonon modes.

First of all, the discrepancy between the mean square displacements obtained by different experiments is a result of the different energy resolutions and the correlated time sensitivity. The phonon assisted Mössbauer effect has an energy resolution of about 1 meV. That means that this method is sensitive to energy transfers larger than 1 meV. Consequently, the strong increase of $\langle x^2\rangle^\gamma$ above T_c reflects processes with a smaller energy transfer. The Mössbauer absorption spectroscopy on ^{57}Fe has a resolution of 5×10^{-6} meV. The Mössbauer spectra above T_c show in addition to the narrow Lorentzians broad lines indicating quasi-diffusive processes [13, 14, 23]. From the broad lines one can deduce that the protein specific dynamics is mainly determined by processes on a timescale between 1 and 100 ns. This corresponds to an energy transfer of only 10^{-4} meV. The quasi-diffusive processes are hidden under the elastic scattering line of 1 meV width in the phonon assisted Mössbauer effect. Phonons reflect essentially fast processes while the Mössbauer absorption experiments are also sensitive to rather slow motions. At present the best interpretation of the $\langle x^2\rangle^\gamma$ values above T_c uses a two-state model. Below the dynamical transition temperature T_c, the protein molecules are in a 'rigid' state and behave in a good approximation like solids. The dynamics is determined by the phonons yielding $\langle x_v^2\rangle$. These values are in good agreement with those determined by phonon assisted Mössbauer effect. Above T_c, there is a temperature dependent non-vanishing probability of reaching another 'flexible' state where the segments of the molecule perform quasi-diffusive motions in limited space yielding $\langle x_t^2\rangle$. The total mean square displacement is given by $\langle x^2\rangle^\gamma = \langle x_v^2\rangle + \langle x_t^2\rangle$. Why are two separated states introduced? The main argument is the fact that there seems to be a gap between the typical phonon energies and the quasi-diffusive motions.

It should be mentioned that the results from incoherent neutron scattering are also discussed in a similar two-state model [24].

The modes yielding $\langle x_t^2\rangle$ are called 'protein specific' motions. They are essential for the function of the protein. This was recently proved by an x-ray structure analysis of the myoglobin mutant L29W ligated with CO [25]. The structure was determined at 105 K with and without illumination by laser light. Beside of the photolysis of the CO molecule, no change of the structure has been obtained. When the photolysis was performed slightly above 200 K, which means above T_c, a dramatic change of the conformation was observed. Conformational changes which are essential for the function only occur above T_c. For such changes the quasi-diffusive motions are necessary as a lubricant.

Acknowledgments

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References

[1] Austin R H, Beeson K W, Eisenstein L, Frauenfelder H and Gunsalus I C 1975 *Biochemistry* **14** 5355–73
[2] Frauenfelder H, Petsko G A and Tsernoglou D 1979 *Nature* **280** 558–63
[3] Parak F, Hartmann H, Aumann K D, Reuscher H, Rennekamp G, Bartunik H and Steigemann W 1987 *Eur. Biophys. J.* **15** 237–49
[4] Chong S-H, Joti Y, Kidera A, Go N, Ostermann A, Gassmann A and Parak F G 2001 *Eur. Biophys. J.* **30** 319–29
[5] Parak F and Formanek H 1971 *Acta Crystallogr.* A **27** 573–8
[6] Berg A I, Knox P P, Kononenko A A, Frolov E N, Chrymova I N, Rubin A B, Lichtenstein G I, Goldanskii V I, Parak F, Buckel M and Mössbauer R L 1979 *Mol. Biol.* **13** 81–9
[7] Parak F 1980 *J. Physique Coll.* **41** C1 71–8
[8] Keller H and Debrunner P G 1980 *Phys. Rev. Lett.* **45** 68–71
[9] Parak F, Finck P, Kucheida D and Mössbauer R L 1981 *Hyperfine Interact.* **10** 1075–8
[10] Doster W, Cusack S and Petry W 1989 *Nature* **337** 754–6
[11] Frauenfelder H, Parak F and Young R D 1988 *Annu. Rev. Biophys. Biophys. Chem.* **17** 451–79
[12] Parak F and Frauenfelder H 1993 *Physica* A **201** 332–45
[13] Parak F G 2001 *Proc. 1st Workshop on Biological Physics (2000)* ed L M Virulh Sa-yakanit and Hans Frauenfelder (Bangkok: World Scientific) pp 7–18
[14] Parak F G and Nienhaus G U 2002 *Eur. J. Chem. Phys. Phys. Chem.* **3** 249–54
[15] Parak F 2002 *Rep. Prog. Phys.* **66** 103–29
[16] Teale F W J 1959 *Biochim. Biophys. Acta.* **35** 543–50
[17] Achterhold K, Keppler C, Ostermann A, van Bürck U, Sturhahn W, Alp E E and Parak F G 2002 *Phys. Rev.* E **65** 051916-1–13
[18] Chumakov A I, Rüffer R, Baron A Q R, Grünsteudel H, Grünsteudel H F and Kohn V G 1997 *Phys. Rev.* B **56** 10758–61
[19] Kohn V G, Chumakov A I and Rüffer R 1998 *Phys. Rev.* B **58** 8437–44
[20] Paulsen H, Benda R, Herta C, Schünemann V, Chumakov A I, Duelund L, Winkler H, Toftlund H and Trautwein A X 2001 *Phys. Rev. Lett.* **86** 1351–4
[21] Edwards C, Palmer S B, Emsley P, Helliwell J R, Glover I D, Harris G W and Moss D S 1990 *Acta Crystallogr.* A **46** 315–20
[22] Melchers B, Knapp E W, Parak F, Cordone L, Cupane A and Leone M 1996 *Biophys. J.* **70** 2092–9
[23] Knapp E W, Fischer S F and Parak F 1982 *J. Phys. Chem.* **86** 5042–7
[24] Zaccai G 2000 *Science* **288** 1604–7
[25] Ostermann A, Waschipky R, Parak F G and Nienhaus G U 2000 *Nature* **404** 205–8
[26] Parak F, Knapp E W and Kucheida D 1982 *J. Mol. Biol.* **161** 177–94