ment of Energy under Contract No. DE-AC02-77ER04387 and by the Petroleum Research Fund administered by the American Chemical Society. One of us (J.L.W.) wishes to acknowledge support by the Alexander von Humboldt Foundation. Work began at the Lehrstuhl für Theoretische Chemie der Universität Bonn.

[a]Permanent address: Centro del Consiglio Nazionale delle Ricerche per lo Studio delle Relazioni tra Struttura e Reattività Chimica, Milano, Italy.

[1]J. L. Whitten and T. A. Pakkanen, Phys. Rev. B 21, 4357 (1980).

[2]The diffuse H 1s′ orbital when orthogonalized to lattice 4s orbitals creates a lobe representation of the Ti $4p_\perp$ orbital. Similarly, in-plane 4p orbitals are approximated by combinations of 4s orbitals on other nuclei.

[3]W. Kolos, Theor. Chim. Acta 51, 219 (1979).

[4]A more detailed analysis of bonding including comparisons with small-cluster results on H_2-Ti_7 will be published.

[5]G. F. Wedler and H. Strothenk, Z. Phys. Chem. (Frankfurt am Main) 48, 86 (1966).

[6]M. A. von Hove, G. Ertl, K. Cristmann, R. J. Behm, and W. H. Weinberg, Solid State Commun. 28, 373 (1978).

[7]P. J. Feibelman and D. R. Hamann, Phys. Rev. B 21, 1385 (1980).

[8]The same result has been found in *ab initio* calculations of H-atom adsorption on Ni clusters, T. H. Upton, W. A. Goddard, III, and C. F. Melius, J. Vac. Sci. Technol. 16, 531 (1979).

Dynamics of the Iron-Containing Core in Crystals of the Iron-Storage Protein, Ferritin, through Mössbauer Spectroscopy

S. G. Cohen,[a] E. R. Bauminger, I. Nowik, and S. Ofer
Racah Institute of Physics, The Hebrew University, Jerusalem, Israel

and

J. Yariv
Department of Biophysics, Weizmann Institute, Rehovot, Israel

(Received 19 January 1981)

^{57}Fe γ-ray resonance-absorption spectra in crystals of the iron-storage protein, ferritin, display above 265 °K, in addition to a normal quadrupole doublet, wide Lorentzian wings extending to velocities of ± 2 cm/sec. The results are interpreted in terms of the dynamics of the iron-containing core of the protein, undergoing bounded diffusive motion within a "cage," characterized by a jump probability per unit time of about 0.5×10^{8} sec^{-1} and a mean-square displacement of about 4.8×10^{-2} Å^2 at 298 °K.

PACS numbers: 87.15.By, 33.45.+x, 63.90.+t

We report here on measurements of ^{57}Fe γ-ray resonance absorption in crystals of the iron-storage protein, ferritin.[1] The spectra obtained above 265 °K are of a striking and unusual shape. In addition to a relatively narrow part, characteristic of the Mössbauer effect as usually observed in solids, the spectra display very broad wings extending to velocities up to ± 2 cm/sec. Upon freezing, the broad wings disappear. The results are interpreted in terms of the dynamics of the iron-containing core of the protein undergoing bounded diffusive motion within a "cage."

Ferritin is the well-known soluble iron-storage protein widely found in man, animals, plants, and fungi and is composed of a protein and an iron-containing inorganic material. The protein part is composed of 24 identical subunits and has the shape of a spherical shell of 120-Å outer diameter and 70-Å inner diameter. The iron inorganic compound of approximate formula $(FeOOH)_8$-$(FeO\cdot OPO_3H_2)$ is contained in the inner cavity.[2,3] The occupancy of the inner cavity by iron is variable from zero to full corresponding to a maximum of about 4000 iron atoms per molecule.

The protein can be crystallized as cubic crystals, which contain about 50% water by weight. Absorbers I and II consisted of a suspension of single crystals of horse-spleen ferritin in an agar gel (2% agar in an aqueous solution of pH 7.0 with 0.05*M* *tris*-acetate buffer and containing

4% cadmium sulfate). In both samples I and II there was a distribution in crystal size. In sample I most of the crystals were between 50 and 100 μm in linear dimension and were crystallized from samples of protein rendered approximately homogeneous in iron occupancy by centrifugation[4]—the protein containing about 1000 atoms of Fe per protein molecule. The total amount of iron was about 7.8 mg. In sample II the crystals were somewhat bigger, and the average iron content was about 2000 atoms per cell. In sample III the crystal size was much more homogeneous and clustered around 30 μm; the crystals remained in contact with aqueous mother liquor. No essential difference among the spectral shapes obtained in the various samples at a given temperature was observed. In the following, we shall present results for sample I.

Figure 1(a) shows a typical absorption spectrum in sample I at 278 °K, measured with a narrow-line recoil-free ^{57}Co source, displayed on a scale of velocity extending to ± 3 cm/sec. Figure 1(b) shows the central part of the spectrum on an expanded scale extending to velocities of about 3 mm/sec. The spectrum thus consists in part of a central "peak" displaying a quadrupole doublet characteristic of the Mössbauer spectrum of ferritin at room temperature, and showing essential features of the recoil-free resonance absorption expected in a solid. There are in addition, however, very wide wings extending to velocities of about 2 cm/sec, which cannot be ascribed to hyperfine interactions and must be due to motional broadening. As shown in Fig. 1(a), the spectrum can be fitted as a sum of a narrow quadrupole doublet and a wide Lorentzian line with width of about 1.3 cm/sec. Measurements were carried out at several temperatures above 265 °K, and later in frozen crystals. The line shapes obtained at 268 °K, 278 °K, 298 °K, and 318 °K were very similar, showing the same structure. No appreciable change in the width of the wide component with temperature was observed in this range. Both the total area under the resonance absorption spectra and the area under the narrow line decrease significantly with increasing temperature (Fig. 2), while the ratios of intensity of the narrow to wide components (which is about 0.6 at 298 °K) decrease by about 25% in the same range. Below freezing temperatures, there occurs a dramatic change in spectral shape and only a narrow normal quadrupole doublet is seen, as in Fig. 1(c), representing data obtained at 258 °K.

The wide component of the spectra observed above 265 °K is similar to the type of resonance absorption observed in fine particles suspended or dissolved in very viscous liquids.[5,6] For example, the spectrum of ferritin dissolved in concentrated sucrose solution at a viscosity of 10 P is a pure Lorentzian of width about 3 cm/sec, and can be analyzed in terms of diffusion theory.[7] One can thus formally break up the spectrum of Fig. 1(a) into a narrow recoil-free part characteristic of a solid and a broad part which is "liquidlike."

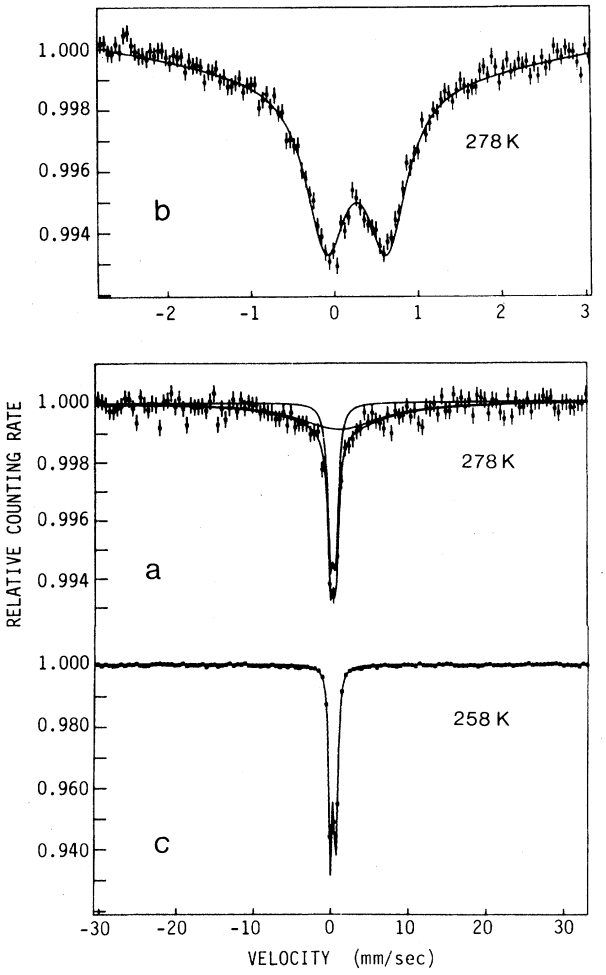

FIG. 1. γ-ray resonance absorption in crystals of ferritin. (a) Crystals at 278 °K. The spectrum is composed of a narrow quadrupole doublet and a broad line. (b) Crystals at 278 °K, in an expanded scale of velocities, showing the central region of the spectrum. (c) Frozen crystals at 258 °K.

We do not believe, however, that an explanation in terms of an inhomogeneous model containing two kinds of iron is tenable. The similar temperature dependence of the intensity of the two com-

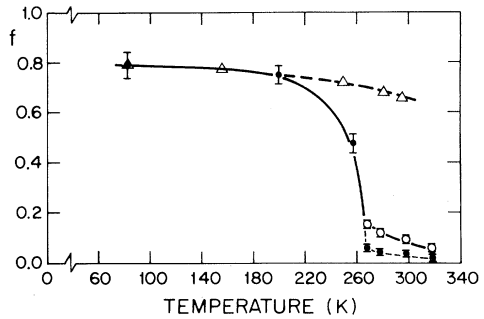

FIG. 2. Recoil-free fractions in ferritin crystals and in lyophilized ferritin. Full dots correspond to recoil-free fractions (f values) of ferritin crystals. Above 265 °K, the f values are derived from the areas of the narrow subspectra. Open circles correspond to the vibrational recoil-free fractions above 265 °K [f_v in Eq. (1)] derived from the sum of the areas of the narrow and wide subspectra of ferritin crystals. Triangles correspond to recoil-free fractions of lyophilized ferritin.

ponents and the similarity of the spectra obtained in samples containing very different concentrations of iron in the cores argue against such an assumption. We therefore prefer to interpret the observed spectra as arising from a localized or bounded diffusive motion of the iron-containing core of the protein, of which each iron atom is a part. The possibility of a limited motion in a "cage," yielding a spectral shape of the type observed here, was envisaged very early by Dicke[8] and predicted by Krivoglaz and Repetskii.[9] Experimental evidence for such processes has recently been found for an ^{57}Fe impurity in electron-irradiated aluminum[10] and in metal-hydrogen systems.[11,12] Very recently, Parak *et al.*[13] have suggested that the motion of Fe atoms in iron proteins might give rise to the kind of composite spectrum observed here, because of conformational fluctuations in the protein.[14,15]

In the present analysis we assume that the motion of the iron atom may be resolved into a vibrational motion and a diffusive motion. For the diffusive motion we assume the simplest kind of bounded motion in three dimensions, characterized by a constant jump probability per unit time, k. Namely, we assume that the center of gravity of a rigid core jumps with a fixed k between the corners of a tetrahedron of edge d. Then, in accordance with Parak *et al.*[13] and the detailed calculations of Blăsius, Preston, and Gonser,[11] the resonance absorption cross section can be written as the sum of two Lorentzian terms, of width Γ and $\Gamma + 8\hbar k$, respectively,

$$\sigma = f_v \sigma_0 (\tfrac{1}{2}\Gamma)\left[\frac{\frac{1}{2}\Gamma(\frac{1}{4}+\frac{3}{4}Z)}{(E-E_0)^2+(\frac{1}{2}\Gamma)^2}+\frac{[(\frac{1}{2}\Gamma)+4\hbar k](\frac{3}{4}-\frac{3}{4}Z)}{(E-E_0)^2+[(\frac{1}{2}\Gamma)+4\hbar k]^2}\right], \quad (1)$$

where $Z = \sin(2\pi d/\lambda)/(2\pi d/\lambda)$, λ is the wavelength of the γ ray, and f_v is the recoil-free fraction associated with the thermal vibrations of the iron atoms. In the harmonic approximation, $f_v = \exp(-\langle x_v^2\rangle/\lambda\!\!\!^{-2})$, where $\langle x_v^2\rangle$ is the vibrational mean-square displacement of the iron atom and $\lambda\!\!\!^{-} = \lambda/2\pi$. In this model the width of the broad spectrum directly yields the jump probability k per unit time. Thus at 298 °K, k is about 1.0×10^8 sec^{-1}. Since the width, and therefore, the jump rate k, do not change significantly with temperature, we conclude that there is no evidence for a large activation energy in the jump process and put an upper limit of about 1.5 kJ/mol for such an activation energy.[13] In accordance with Eq. (1), the sum of the areas of the narrow and wide subspectra is given by $A = \pi(\Gamma/2)\sigma_0 f_v$, and the ratio of the areas of the narrow and wide components, respectively, is given by $R = (\frac{1}{4}+\frac{3}{4}Z)/(\frac{3}{4}-\frac{3}{4}Z)$. From the experimental value of this ratio of areas, the value of Z and, hence, the value of d may be derived. The mean-square displacement of the core associated with the diffusive motion, $\langle x_j^2\rangle$, averaged over a long time compared to the lifetime of the excited nuclear state $\tau_N = 1.4\times 10^{-7}$ sec, is given approximately by

$$\langle x_j^2\rangle = 3d^2/8[1+(4k\tau_N)^{-1}] \sim 3d^2/8. \quad (2)$$

The values of $\langle x_j^2\rangle$ derived with use of Eq. (2) are given in Table I. The values of $\langle x_j^2\rangle$ derived under the assumption of jumps of the center of gravity of the core between the corners of an octahedron or of a cube,[11] or even more general models of diffusion, do not differ significantly from those given in Table I.

The value of the recoil-free fraction related to the narrow subspectrum of ferritin crystals above 265 °K is given, according to Eq. (1), by the expression

$$f = f_v f_j, \quad (3)$$

where $f_j = \frac{1}{4}+\frac{3}{4}Z$. The values of f_v and the associated $\langle x_v^2\rangle$ above 265 °K were derived from the experimental values of the sum of the areas of the narrow and wide absorption subspectrum of

TABLE I. Core jump probabilities per unit time, k, and mean-square amplitudes of iron atoms in ferritin crystals and in lyophilized ferritin above 265 °K. The $\langle x_j^2 \rangle$ are associated with the diffusive motion of the iron atoms in ferritin crystals. The $\langle x_v^2 \rangle$ are associated with the total vibrational motion of the iron atoms in ferritin crystals. The $\langle x_l^2 \rangle$ correspond to iron atoms in lyophilized ferritin.

Temperature (°K)	k (10^8 sec^{-1})	$\langle x_j^2 \rangle$ (10^{-2} Å^2)	$\langle x_v^2 \rangle$ (10^{-2} Å^2)	$\langle x_l^2 \rangle$ (10^{-2} Å^2)
268	0.8 ± 0.1	4.5 ± 0.3	3.5 ± 0.5	0.65
278	1.2 ± 0.1	4.6 ± 0.5	3.9 ± 1.0	0.7
298	1.2 ± 0.1	4.8 ± 0.8	4.5 ± 1.0	0.8
318	1.0 ± 0.1	5.2 ± 0.8	5.4 ± 1.0	0.8

the ferritin crystals. The values of $\langle x_v^2 \rangle$ are given in Table I.

To the extent that the type of phenomenon reported here is a common feature of protein crystals, we would like to point out the relevance to recent studies of protein structural dynamics through temperature-dependent x-ray diffraction.[15] It has been shown that by combining x-ray diffraction with Mössbauer measurements of recoil-free fractions in protein crystals, one can estimate the contribution to the mean-square displacement resulting from lattice disorder (the latter contributes only to the x-ray measurement).[15,16]

At this state of knowledge, the nature of the coupling between the iron core and the apoprotein shell is not clear. If the coupling is strong, it may well be that the core is driven by, and possibly reflects, the diffusive internal motions of the protein, perhaps associated with fluctuating conformational states. An interesting possibility is that the observed diffusional jumping motion is associated with the relative motion of the protein subunits. We note, however, that part or even all the diffusive motion of the core could arise as a consequence of the diffusive motion of the whole protein in the lattice.

Indeed, on very general grounds, one would expect the occurrence of low-frequency motions in unfrozen protein crystals. The water in the interstices of the lattice should, through the dissipative forces, strongly damp the relatively high-frequency transverse phonons present in normal solid crystals (and undoubtedly present in the frozen crystals), while leaving the longitudinal phonons relatively unaffected. The density of states of transverse phonons will thus be very different from that in a normal crystal and the phonon spectrum should be pushed down to low frequencies. In the limit of high damping a "noise" type of spectrum might prevail.

We remark that the Mössbauer spectra observed above 265 °K in ferritin crystals bear a remarkable similarity to the spectra observed above 265 °K in packed cells of E. coli,[17] mycoplasma capricolum,[18] and chicken-embryo fibroblasts.[19] An understanding of the dynamics in ferritin crystals as a model system might help towards understanding the dynamics of membrane-bound storage iron in a truly *in vivo* situation.

At the stage of final revision of this manuscript we wish to mention two more recent results. We have observed diffusive motions similar to those described above in crystals of the much-studied protein metmyoglobin above 235 °K, as have Mayo, Palak, and Mössbauer[20] in crystals of CO liganded human hemoglobin.

The authors would like to thank P. Debrunner, Hans Frauenfelder, and F. Parak for valuable discussions. One of us (S.G.C.) is indebted to the hospitality of the Johnson Research Foundation and the Department of Physics of the University of Pennsylvania, where part of the manuscript was written.

[a]Present address: Johnson Research Foundation and Department of Physics, University of Pennsylvania, Philadelphia, Pa. 19174.

[1]R. R. Crichton, Struct. Bonding (Berlin) 17, 68 (1973).

[2]S. Granick and P. F. Hahn, J. Biol. Chem. 155, 661 (1944).

[3]S. M. Heald, E. A. Stern, B. Bunker, E. M. Holt, and S. L. Holt, J. Am. Chem. Soc. 101, 67 (1979).

[4]P. M. Harrison, J. Mol. Biol. 1, 69 (1959).

[5]P. P. Craig and K. Sutin, Phys. Rev. Lett. 11, 460 (1963).
[6]K. S. Singwi and A. Sjölander, Phys. Rev. 120, 1092 (1960).
[7]E. R. Bauminger, S. G. Cohen, S. Ofer, and J. Yariv, to be published.
[8]R. H. Dicke, Phys. Rev. 89, 472 (1953).
[9]M. A. Krivoglaz and S. P. Repetskii, Fiz. Tverd. Tela (Leningrad) 8, 2908 (1966) [Sov. Phys. Solid State 8, 2325 (1967)].
[10]W. Petry, G. Vogl, and W. Mansel, Phys. Rev. Lett. 45, 1862 (1980).
[11]A. Bläsius, R. S. Preston, and U. Gonser, Z. Phys. Chem. Neue Folge 115, 187 (1979).
[12]F. Pröbst, F. E. Wagner, and M. Karger, J. Phys. F 10, 2081 (1980).
[13]F. Parak, E. N. Prolov, R. L. Mössbauer, and V. I. Goldanskii, J. Mol. Biol. 145, 825 (1981).
[14]H. Keller and P. G. Debrunner, Phys. Rev. Lett. 45, 68 (1980).
[15]H. Frauenfelder, G. A. Petsko, and O. Tsernoglou, Nature 280, 558 (1979).
[16]F. Parak and H. Formanek, Acta Crystallogr., Sect. A: 27, 573 (1971).
[17]E. R. Bauminger, S. G. Cohen, E. Giberman, I. Nowik, S. Ofer, J. Yariv, M. M. Werber, and M. Mevarech, J. Phys. (Paris), Colloq. 37, C6-227 (1976).
[18]E. R. Bauminger, S. G. Cohen, S. Ofer, and S. Rottem, unpublished data.
[19]E. R. Bauminger, S. G. Cohen, S. Ofer, and U. Bachrach, to be published.
[20]K. H. Mayo, F. Palak, and R. Mössbauer, to be published.