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FIRST-ORDER PHASE TRANSITION IN PROTEIN DYNAMICS OF FERRITIN

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Abstract

Detailed Mössbauer spectra of ^{57}Fe in the iron storage protein, ferritin, in the temperature range between 250 and 280 K reveal a first-order phase transition with a thermal hysteresis loop of 7 K width. While the temperature is raised from 90 K to 271 K, Mössbauer spectra composed of a narrow line quadrupole doublet, typical for solids, are observed. Above this temperature, each spectrum is composed of the narrow line subspectrum and a broad line subspectrum whose relative intensity increases with temperature. The intensity of the narrow line subspectrum decreases by a factor of five at the critical temperature and thus shows a large increase in the mean square displacements at T_{up} = 271 K. While decreasing the temperature, the bounded diffusive motions, expressed in the spectra by the coexistence of the narrow and broad lines, survive down to T_{down} = 264 K, where again the spectral shapes and areas undergo a discontinuous jump. The narrow line subspectrum increases in intensity and the broad line subspectrum disappears. These phenomena may be understood in terms of supercooling of the water in the free channels and in the cavity of the ferritin molecule.

1. Introduction

Solly Cohen and Shimon Ofer have devoted their last years of research to the subject of protein dynamics studied by Mössbauer spectroscopy [1–10]. They were the first to observe experimentally the appearance of broad line Mössbauer subspectra in proteins above a critical temperature. They were also among the first to understand the theory and the underlying physical mechanisms leading to the observed phenomena [8–19]. The dynamic properties of ferritin have been studied in the past, also by Mössbauer spectroscopy, mainly by Solly, Shimon and colleagues.

Ferritin is the iron storage protein in animals and plants. Iron typically accounts for about 20% of its molecular mass. If the core is completely filled with iron, it can

accommodate up to 4500 iron atoms within a single ferritin molecule, and the iron then accounts for over 30% of the molecular weight. The ferritin molecule consists of a roughly spherical protein shell with an outer diameter of 125 Å and an inner diameter of about 80 Å. The protein shell is composed of 24 sub-units. The 80 Å diameter cavity inside the shell might be filled to different degrees with crystalline particles, consisting mainly of ferrihydrite, namely, ferric oxyhydroxide, with some inorganic phosphate adsorbed on the iron core crystallites. Two types of channels lead through the protein shell. There are six equivalent hydrophobic channels, which are about 12 Å long and 3–3 Å wide, and there are eight shorter hydrophilic funnel-shaped channels, also about 3–4 Å wide, which can bind metal ions. The inner surface of the cavity is lined with loose hydrophilic residues. Thus there is water within the channels and inside the cavity [20]. If the ferritin molecule is disrupted, iron cores can still be seen attached to remnants of the concave side of the protein shell [21]. This and neutron scattering studies [22] strongly indicate that the iron cores are linked to the protein. Previous Mössbauer studies of ^{57}Fe in ferritin have exhibited the now well-established phenomenon, appearing also in many other proteins [1–7] and polymers [8], where above a critical temperature broad absorption lines appear in the spectra. In all cases this phenomenon has been explained in terms of bound diffusion of the iron nuclei, associated with the melting of new degrees of freedom of the huge molecule serving host to the iron nucleus. At low temperatures, well below the freezing point of water, all motion is frozen and the protein behaves like a solid. As the temperature is raised, the water melts and the protein is free to move between its different conformational states. This motion is reflected in the Mössbauer spectra of the iron, which is attached to the protein. In all these cases, except ferritin, this transition occurs smoothly, with no discontinuities in any of the physical parameters and with no thermal hysteresis.

A first-order phase transition is characterized by a discontinuity in entropy and associated physical parameters at the critical temperature. In many cases, first-order phase transitions also display thermal hysteresis phenomena. The present detailed studies prove that the critical transition in ferritin, unlike that in the other proteins, is definitely of first-order character. It exhibits a thermal hysteresis loop of 7 K width. These observations can be understood in terms of available theories for protein dynamics and assuming supercooling of the water in the free spaces inside the ferritin molecule.

2. Materials and methods

The measurements were performed on reconstituted horse spleen ferritin crystals containing 56 molecules of ^{57}Fe and 3600 molecules of natural iron. The uptake of iron by ferritin has been described in detail [23]. For the present measurements, apoferritin was reconstituted in a 0.02M HEPES buffer, *p*H = 7.0, in the presence of 4mM KIO_3 and 16mM $Na_2S_2O_3$ to facilitate efficient core formation.

A constant acceleration conventional Mössbauer drive with a 100 mCurie ^{57}Fe:Rh source and a Harwell proportional counter were used. The absorbers were placed in a cryostat and the temperature was stabilized to within 0.1 K. The experimental spectra were analyzed by computer fits with bound diffusion theoretical spectra [10] or simply as the sum of two subspectra, which have identical isomer shift and quadrupole interaction parameters, and differ in their respective line widths and intensities. The spectra were measured in the temperature range between 250 and 280 K at 2 K intervals.

3. Experimental results and discussion

The spectra obtained near the critical temperature in a narrow velocity range are shown in fig. 1 and those obtained in a wide velocity range are shown in fig. 2. The spectra exhibit a sharp discontinuity at a critical temperature. This temperature is different when the temperature is raised or decreased. Below a critical temperature, which is 271 K when the temperature is raised (T_{up}) and 264 K when the temperature is decreased (T_{down}), the Mössbauer spectra are composed of a narrow line quadrupole doublet. Above the critical temperature, broad line subspectra appear.

The analyses of the spectra in the narrow velocity range yield accurately the temperature dependence of the spectral area of the narrow lines (fig. 3a). The analyses of the spectra in the wide velocity range yield the temperature dependence of the total spectral area (fig. 3b), and the relative intensities of the broad line subspectra and the narrow line subspectra (fig. 3c). The relative intensity of the broad line subspectrum increases with temperature and is more than five times the area of the narrow line subspectrum at 295 K. The narrow lines decrease in intensity by a factor of six at the critical temperature. The overall area decreases by a factor of three at this temperature.

In fig. 3, the sharp character of the phase transition and the thermal hysteresis loop is seen. In fig. 4a and fig. 4b, the width of lines of the narrow line subspectrum and of the broad subspectrum are displayed. One observes that even the width of the narrow lines changes sharply at the phase transition. The temperature dependence of the isomer shift and quadrupole interaction are displayed in fig. 4c and fig. 4d. No discontinuity is observed in these two parameters.

The fact that the isomer shift and quadrupole interaction do not change at the phase transition proves that the local chemical environment of the iron is not affected by the phase transition. The phase transition does not occur in the iron compound of the ferritin molecule. It affects, however, the freedom of motion of the iron in the core, expressed in the widths and intensities of the Mössbauer absorption lines. As the broad line subspectra appearing above the critical temperature are associated with new molecular degrees of freedom [10], it is clear that the entropy of the system is changing discontinuously at this temperature.

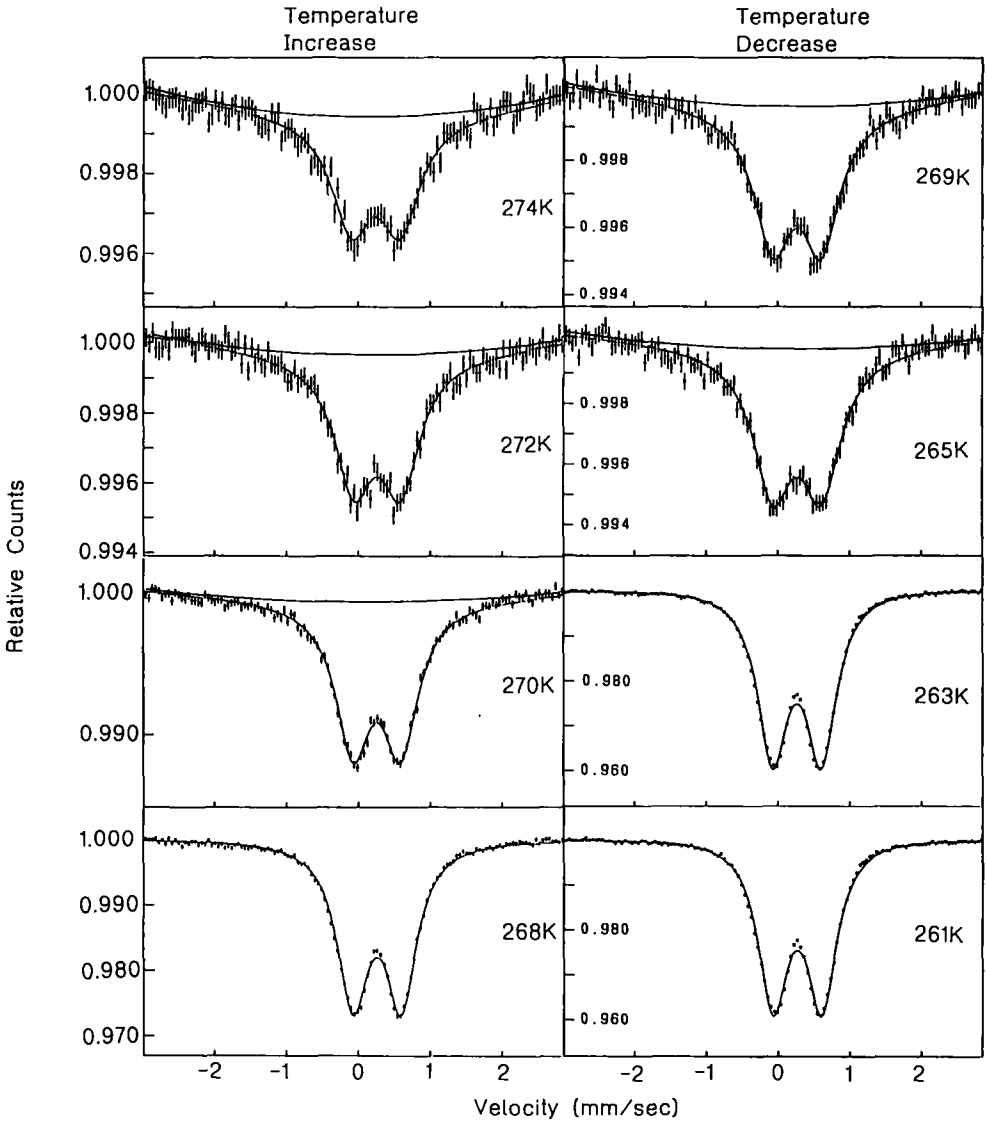

Fig. 1. Mössbauer spectra of ^{57}Fe in ferritin in a narrow velocity range, while the temperature is increased (left), and decreased (right).

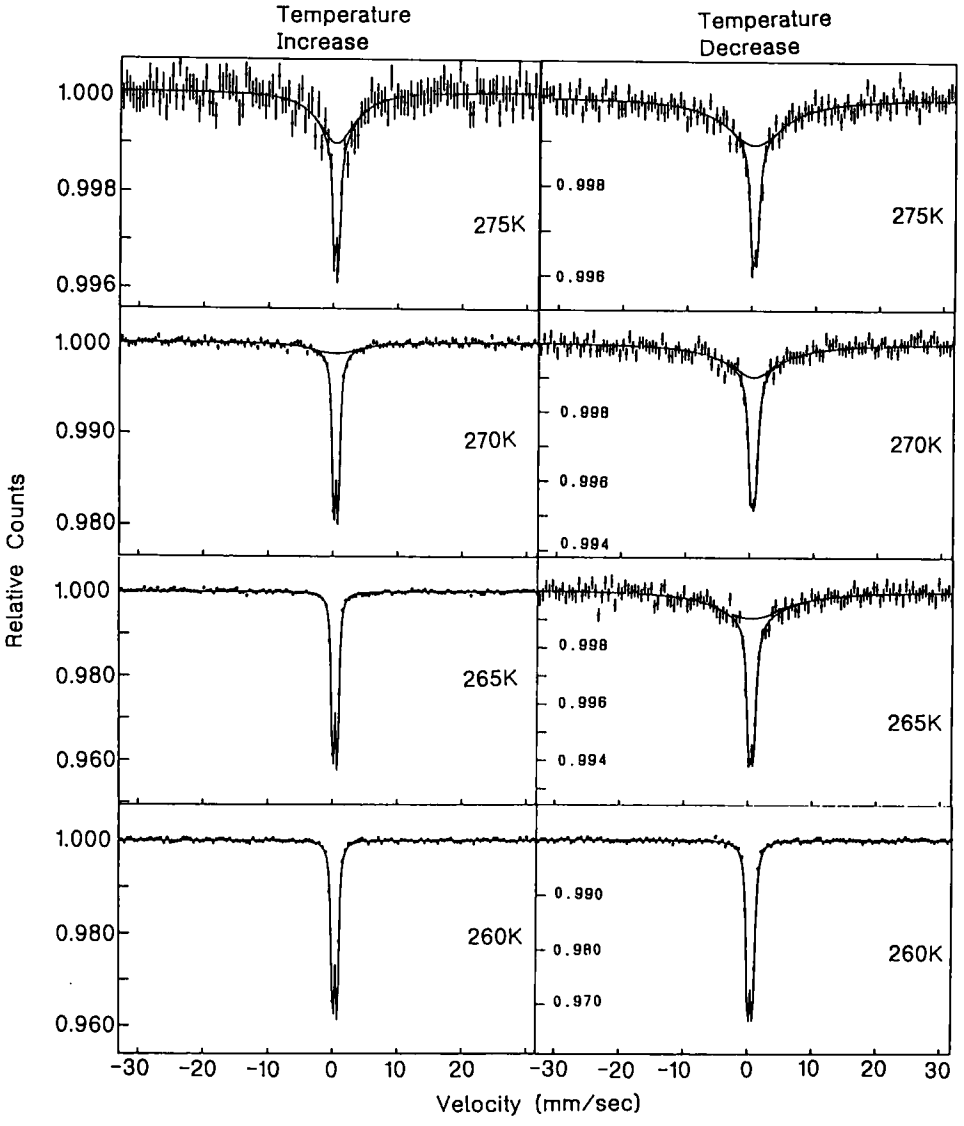

Fig. 2. Mössbauer spectra of ^{57}Fe in ferritin in a wide velocity range, while temperature is increased (left), and decreased (right). The wide velocity range allows the observation of the full broad line subspectrum.

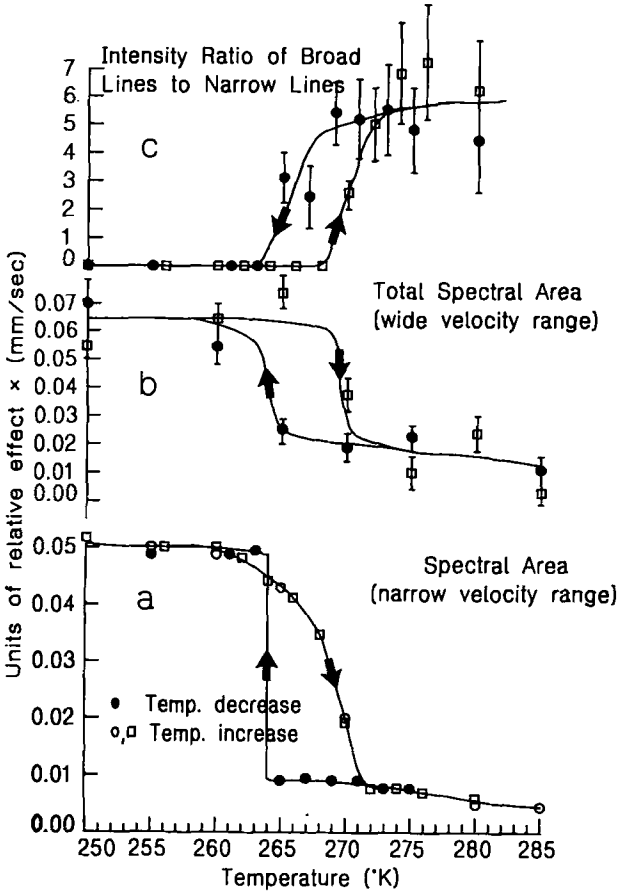

Fig. 3. Temperature dependence of the spectral area of the narrow velocity range spectrum (a), of the total Mössbauer spectral area from the wide velocity spectrum (b), and of the intensity ratio of the broad line spectral area to the narrow line spectra area (c).

A major question is why in other proteins [1–7] and polymers [8] the transition is gradual over about 10 K, whereas in ferritin it is very sharp and discontinuous. The answer probably lies in the way the iron is attached to the protein. In the other proteins investigated, each iron atom is attached directly to the protein, and as such it participates in the protein's motion. The water present in these protein crystals is probably adsorbed in small groups, loosing its properties as free water. Thus, in myoglobin the critical transition above which broad Mössbauer absorption lines appear is 235 K, far below the freezing point of water. In ferritin, only a few of the outer atoms of the iron core are directly attached to the protein shell, whereas the bulk of the

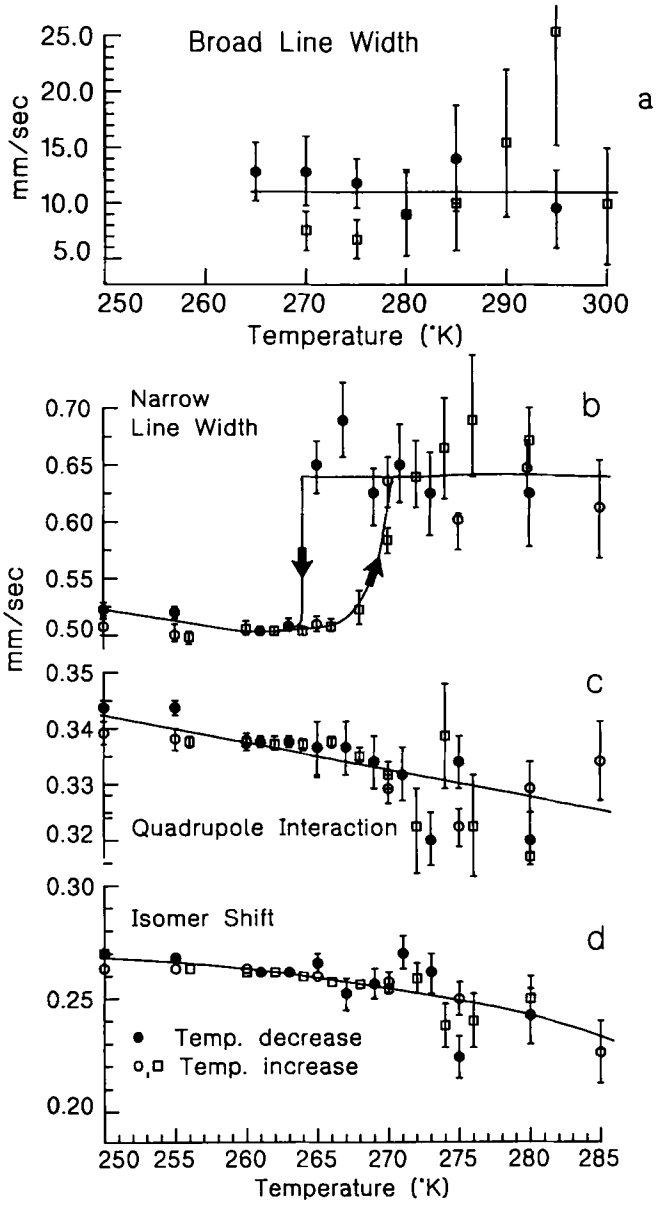

Fig. 4. Temperature dependence of the width of the broad line subspectrum (a), of the narrow line subspectrum (b), of the quadrupole interaction (c), and of the isomer shift (d).

iron atoms are attached to each other and only in an indirect way to the protein. The water is present in the protein cavity and in channels leading to the cavity containing the iron core. This water enclosing the iron core behaves almost as free water with a first-order melting phase transition at 271 K and a supercooled glass transition at 264 K. Finally, it is worthwhile pointing out the difference in the sharpness of the phase transition when the temperature is increased or decreased (fig. 3a and fig. 4b). While increasing the temperature, the melting point may have a distrubution in values depending on the location of the water molecules. On the other hand, when decreasing the temperature, the metastable supercooled state of the water undergoes a sudden cooperative transition to a glass state.

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