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Preliminary X-ray diffraction studies of the tetragonal form of native horse-spleen apoferritin

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Abstract

Horse-spleen ferritin is known to crystallize in three different space groups, cubic $F432$, orthorhombic $P2_12_12$ and tetragonal $P42_12$, but only the cubic form has been fully investigated. Crystals of the tetragonal form of apoferritin have been obtained, by the vapour-diffusion technique, which diffract beyond 3.0 Å. The unit-cell dimensions are $a = b = 146.63$, $c = 152.94$ Å. The orientation of the non-crystallographic symmetry axes of the apoferritin molecule (24 subunits of 174 amino acids each, arranged in a 432 point symmetry rhombododecahedron) has been determined by a self-rotation Patterson function. The asymmetric unit is made of six subunits and was positioned by molecular replacement.

1. Introduction

Ferritin was first isolated from mammalian spleen and liver by Laufberger (1937). Since then, it has been found in most living organisms, such as bacteria, fungi, plants, molluscs and vertebrates. Its universal character, as well as its fascinating ability to reversibly store and release iron ions to the living cells, incited many studies (Theil, 1987; Crichton, 1990; Harrison *et al.*, 1991). At present, more than 35 sequences of ferritins are fully or partially known (Andrews *et al.*, 1992). Despite a sequence homology sometimes lower than 20%, the X-ray structure determination of several of these proteins showed in invariance in their secondary, tertiary and quaternary structures. Apoferritin molecules are made of 24 subunits, each of which consists of a four-helix bundle (*A–D*) and a fifth shorter helix (*E*) at the C-terminus. The 24 bundles pack together in a 432 symmetry and form a hollow shell, whose inner cavity can host a ferrihydrite mineral core composed of up to 4500 iron ions. The 432 symmetry of the molecule leads to the formation of the following.

Eight hydrophilic channels along the threefold axes; several experimental results suggested that these channels could be the entry route of iron ions into the ferritin cavity (Wade *et al.*, 1991; Treffry *et al.*, 1993; Strange, Morante, Stefanini, Chiancone & Desideri, 1993).

Twelve grooved structures, along the twofold axes: these are the location of haem molecules in bacterioferritins [Frolow, Kalb (Gilboa) & Yariv, 1994] and are able to host porphyrins in horse-spleen apoferritin (Précigoux *et al.*, 1994) or betaines in amphibian red-cell L ferritin (Trikha, Theil & Allewell, 1995). Glutamic acids residues of helix *B*, which border these inner cavities, are believed to participate in the nucleation of the ferrihydrite mineral core (Rice, Ford, White, Smith & Harrison, 1983).

Six channels along the fourfold axes, the function of which has not yet been identified.

Crystal structures of eukaryotic apoferritins published so far (Trikha *et al.*, 1995; Rice *et al.*, 1983; Clegg, Stansfield, Bourne & Harrison, 1980; Lawson *et al.*, 1991) show that these proteins generally only crystallize in the cubic space group $F432$, except for horse-spleen apoferritin which also crystallizes in the tetragonal $P42_12$ space group, and its iron-loaded analogue in the orthorhombic $P2_12_12$ space group. These latter crystal forms were obtained some years ago (Harrison, 1963; Hoy, Harrison & Hoare, 1974) but it is most likely that their crystal quality (resolution 6 Å) compared to that of cubic crystals (2.7 Å) could allow only a preliminary structure investigation (space-group determination, crystal packing and orientation of the molecule in the unit cell).

Tetragonal crystals of horse-spleen apoferritin that diffract beyond 3.0 Å were obtained. The structure of this crystal form should be of particular interest because, in contrast to the cubic form, the point symmetry of the molecule does not correspond to that of the crystal. In this paper, we show that the asymmetric unit is made of six subunits, and that the twofold and threefold axes on which the putative active sites of the molecule are located, are non-crystallographic symmetry axes.

2. Crystallization, X-ray data collection and processing

Commercial horse-spleen apoferritin (Sigma) was further purified on a gel-filtration column (superfine Sephacryl S-300 column) (Yang, Matsubara, Yamaki, Ebina & Nagayama, 1994). Eluted fractions corresponding to the monomers were pooled and concentrated up to 25 mg ml^{-1}. The protein sample was extensively dialysed against water. Crystals grew at room temperature by the hanging-drop vapour-diffusion method from solutions containing cadmium sulfate and ammonium sulfate (1 to 4, and 1 to 10 equivalents, respectively). While cubic icosahedral crystals appeared readily in some drops, plate-shaped crystals of the tetragonal form only nucleated after 6 to 7 weeks, then grew to a maximum size of 0.50 × 0.30 × 0.25 mm.

Diffraction data were collected at room temperature on a MAR Research image-plate detector using Cu $K\alpha$ radiation from an Enraf–Nonius rotating-anode generator. The sharpness of the reflections (average spot width of 0.5° in φ) allowed collection of 90° of data in 1.2° frames of 30 min duration, without inducing overlapping of reflections at higher resolution (3.0 Å). All images were processed using the *XDS* package (Kabsch, 1988*a*,*b*). Significant radiation damage, occurred after 60 frames had been collected ($R_{sym} > 15\%$). A data set of 102 542 observations was reduced to 28 775 unique reflections with a completeness of 85.7%, and an R_{sym} of 10.6%. 68% of the unique reflections have $I > 2\sigma(I)$, and the completeness of the last resolution shell (3.2–3.0 Å) is 65.5%.

3. Solution of the tetragonal crystal structure

The orientation of the 432 point-group symmety elements of the molecule, relative to the unit-cell axes, was obtained by self-

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rotation-function map calculations using the *GLRF* program (Tong & Rossmann, 1990). One of the fourfold axes of the molecule coincides with the tetragonal c axis. However, the other fourfold axes, which lie in the (a,b) plane were found to make an angle of 22.3° with the unit-cell axis a (see Fig. 1 for details). A similar calculation performed previously (Hoy *et al.*, 1974) led to a remarkably close value of 17.5%, if one considers the much smaller amount of reflections that these authors had taken into account in their calculation (reflections of the $(h, k, 0)$ plane belonging only to the 10–6 Å resolution range). Our result prompts us to consider that the apoferritin molecules are located on the fourfold crystallographic axes, that six subunits comprise the asymmetric unit, and the unit cell contains two apoferritin molecules.

The structure solution was obtained by molecular replacement with the *AMoRe* program (Navaza, 1994). Atomic coordinates were taken from structure IHRS (Précigoux *et al.*, 1994) in the Brookhaven Protein Data Bank (Bernstein *et al.*, 1977; Abola, Bernstein, Bryant, Koetzle & Weng, 1987) and a model of six subunits was built: it consists of a pair of trimers related to each another by a twofold non-crystallographic molecular axis, the subunits of each trimer being related to one another by a threefold non-crystallographic symmetry molecular axis. A cross-rotation function search was performed with reflections in the range 10.0–3.5 Å, using 17 356 large terms, and a cutoff radius of 30 Å. The rotation function displayed several equivalent solutions (correlation factor = 21.5, peak height 8.5σ above mean density) which were subsequently discriminated by a translation-function calculation. The best solution of the translation function yielded, after fast rigid-body refinement, a 33σ peak height (correlation factor = 72.9, R factor = 0.32), with the centre of the apoferritin molecule at the Wyckoff position $x_c = 0.0$, $y_c = 0.5$, $z_c = 0.2588$. An initial packing-energy calculation using *X-PLOR* (Brünger, Kuriyan & Karplus, 1987), including all atoms, did not reveal any bad intramolecular and intermolecular contacts. The particular position of the centre of the apoferritin molecules in the unit cell leads to a pseudo body-centered lattice (Fig. 2), as deduced previously from crystal packing considerations (Hoy, Harrison & Hoare, 1974). Intermolecular contacts are different from those observed in the face-centered cubic crystal form. A Fourier difference map including all non-H atoms was performed using *X-PLOR*, with reflections in the range 8–3.0 Å, $[F_{obs} > 2\sigma(F_{obs})]$, for which a reliability factor $R = 0.31$ was obtained. High density peaks (greater than 8 r.m.s.) were observed between apoferritin molecules (Fig. 3), close to residues Glu11 and Thr10 of subunit 2 of molecule 1 and Glu11 and Gln120 of subunit 3 of a molecule related by

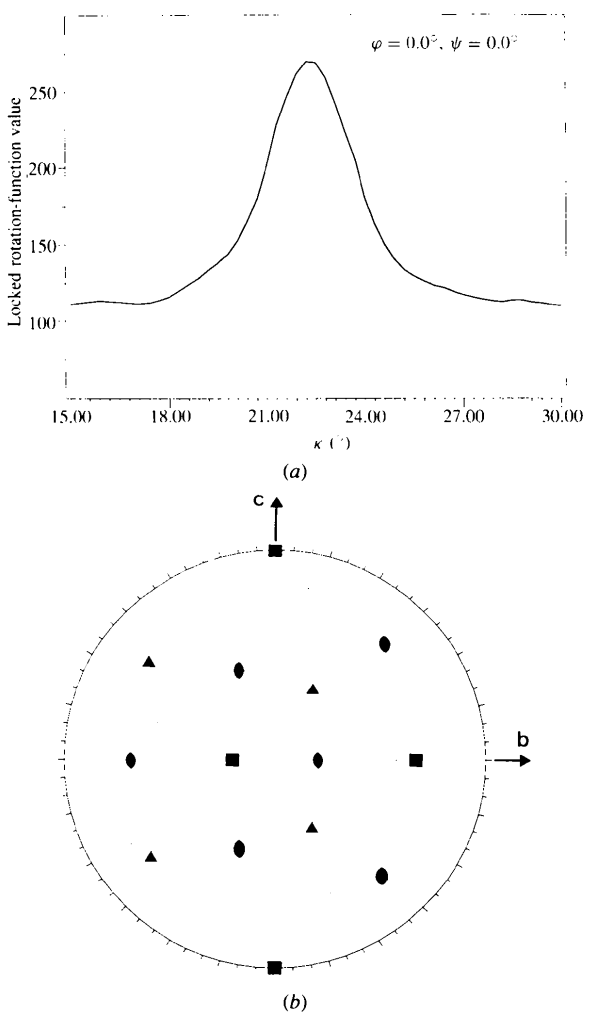

Fig. 1. (*a*) Locked self-rotation function including 432 point-symmetry elements of the apoferritin molecule, as a function of κ. A sharp maximum is observed at $\varphi = 0.0°$, $\psi = 0.0°$, $\kappa = 22.3°$. Calculation was performed using 17 194 reflections and 2480 large terms (resolution 10.0–3.5 Å), a radius of integration of 80.0 Å. Maximum is at 3σ above background. (*b*) Stereographic projection of the 432 symmetry elements of the apoferritin molecule as positioned relative to the unit-cell axes.

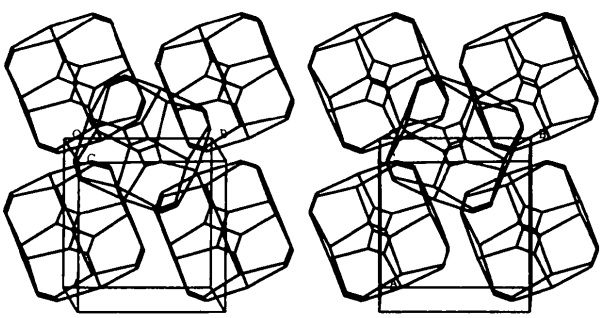

Fig. 2. Stereoscopic view of the pseudo body-centered crystal packing. Apoferritin molecules are represented by truncated rhombic dodecahedra.

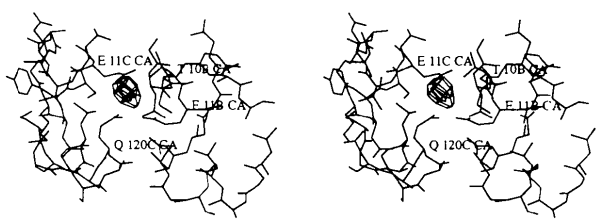

Fig. 3. Stereoscopic view of the $F_o - F_c$ electron-density map at 6σ and 8σ levels in the intermolecular region between asymmetric units 1 and $(1 - y, 1 - x, 1 - z)$. The high density peak is attributed to the presence of a Cd atom, bound to residues Glu11, Thr10 of subunit 2, and residues Glu11, Gln120 of subunit 3.

$(1-y, 1-x, 1-z)$. These peaks are due to the presence of Cd atoms, which bind apoferritin molecules together in the crystal lattice. These cadmium-binding sites involve different residues from those of the cubic structure.

Refinement of the struture is in progress. New tetragonal crystals, obtained from the latest crystallization experiments, diffract to beyond 2.6 Å, in exposures of 60 min per frame. Data-collection experiments on these crystals using synchrotron radiation at LURE, are scheduled and should give better data.

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