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Crystallization and preliminary X-ray analysis of an orthorhombic form of horse-spleen apoferritin

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Abstract

Horse-spleen apoferritin crystallizes in two different space groups: cubic $F432$ and tetragonal $P42_12$ while its iron-containing analogue is known to present a cubic and an orthorhombic form. Up to now, only the structure of the cubic form has been fully investigated by X-ray diffraction, although some information concerning the molecular packing of the two other forms was deduced from analysis of X-ray photographs. While growing cubic crystals of horse-spleen apoferritin with Pt-mesoporphyrin IX, we obtained one crystal, with a diffraction limit of 2.4 Å, belonging to the orthorhombic $P2_12_12$ space group, with unit-cell dimensions $a = 181.6$, $b = 128.9$, $c = 128.9$ Å. The orientation of the non-crystallographic axes of the molecule was determined by self-rotation Patterson function and the structure was determined by the molecular-replacement method. The asymmetric unit consists of half an apoferritin molecule. Refinement of the structure is in progress, some preliminary results of the molecular packing are given.

1. Introduction

The iron-storage molecule ferritin, which is widely encountered in animals and plants, plays a central role in cellular iron metabolism. Its ability to reversibly uptake and release Fe atoms has been and remains extensively studied by several techniques such as X-ray diffraction, spectroscopic and spectrometric measurements in order to understand better the mechanisms of iron uptake and release (for a review see Theil, 1987; Crichton, 1990; Harrison *et al.*, 1991). Each molecule consists of a protein shell (apoferritin) of nearly 80 Å internal diameter comprising 24 subunits in which iron is deposited as a mineralized core. Each subunit is a bundle of four (*A*, *B*, *C*, *D*) α-helices with a short fifth (*E*) α-helix at the C terminus and a loop connecting the antiparallel helix pairs *A*–*B* and *C*–*D*.

All mammalian apoferritins, for which three-dimensional structures have thus far been determined [horse spleen (Clegg, Stansfield, Bourne & Harrison, 1980), human H and rat liver (Lawson *et al.*, 1991), recombinant rat L (Thomas *et al.*, 1988), bullfrog L (Trikha *et al.*, 1994; Trikha, Theil & Allewell, 1995] crystallize in the $F432$ space group. The asymmetric unit always contains a single subunit. Nevertheless, horse spleen apoferritin, which is one of the most investigated apoferritins, is known, since the earliest days of its structural characterization, to also present a tetragonal form while its iron-containing analogue crystallizes in an orthorhombic space group. The structures of these two non-cubic forms were partially examined some years ago: the space-group determination, the crystal packing and the orientation of the molecules in the unit cell were interpreted from analysis of X-ray photographs (Harrison *et al.*, 1991; Hoy, Harrison & Hoare, 1974; Harrison, 1959, 1963). It is only very recently that preliminary X-ray results of the tetragonal form allowed us to solve the structure (Granier, Gallois, Dautant, Langlois d'Estaintot & Précigoux, 1996) beyond 3.0 Å. In this paper, evidence is given that horse-spleen apoferritin, like its iron-containing analogue, also crystallizes in an orthorhombic form. Taking advantage of the recent achievement of a first apoferritin crystal of this new form co-crystallized with Pt-mesoporphyrin IX, we report here crystallization and preliminary results of the structure.

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) (Yang, Matsubara, Yamaki, Ebina & Nagayama, 1994). Equilibration then elution were performed with sodium acetate buffer 50 m*M* at pH 5.3. Eluted fractions corresponding to the monomers were concentrated up to 20 mg ml^{-1}. The protein sample was extensively dialyzed against water with one equivalent of Pt-mesoporphyrin IX. Crystals grew at room temperature by the hanging-drop vapour-diffusion method from solution containing cadmium sulfate and ammonium sulfate (1/4 to 1/10 equivalents). Cubic icosahedral crystals appeared readily, as well as a single orthorhombic rod-shaped crystal

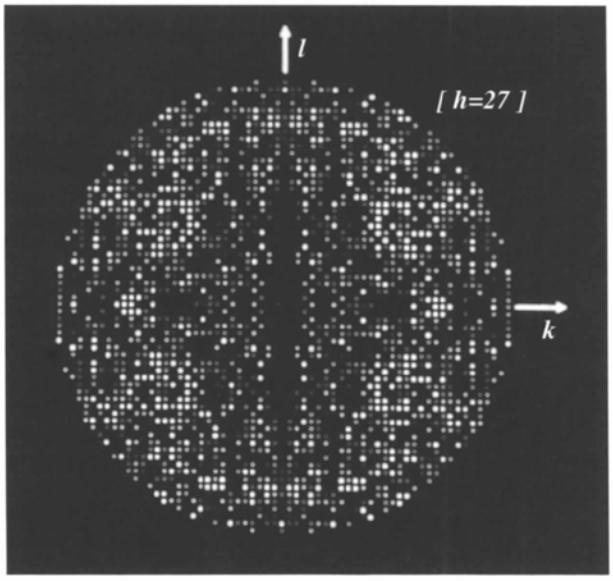


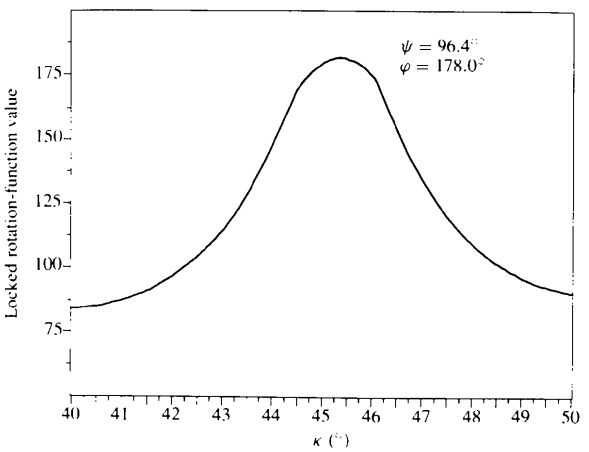
Fig.1. Pseudo precession photograph of the 27*kl* zone, constructed with the *PRECESS* program of the *PHASES*95 package (Furey & Swaminathan, 1996) and generated at 3.5 Å resolution from the image-plate detector data.

which grew alone in one drop after 4 to 5 weeks, then grew to a maximum size of 1.06 × 0.17 × 0.17 mm.

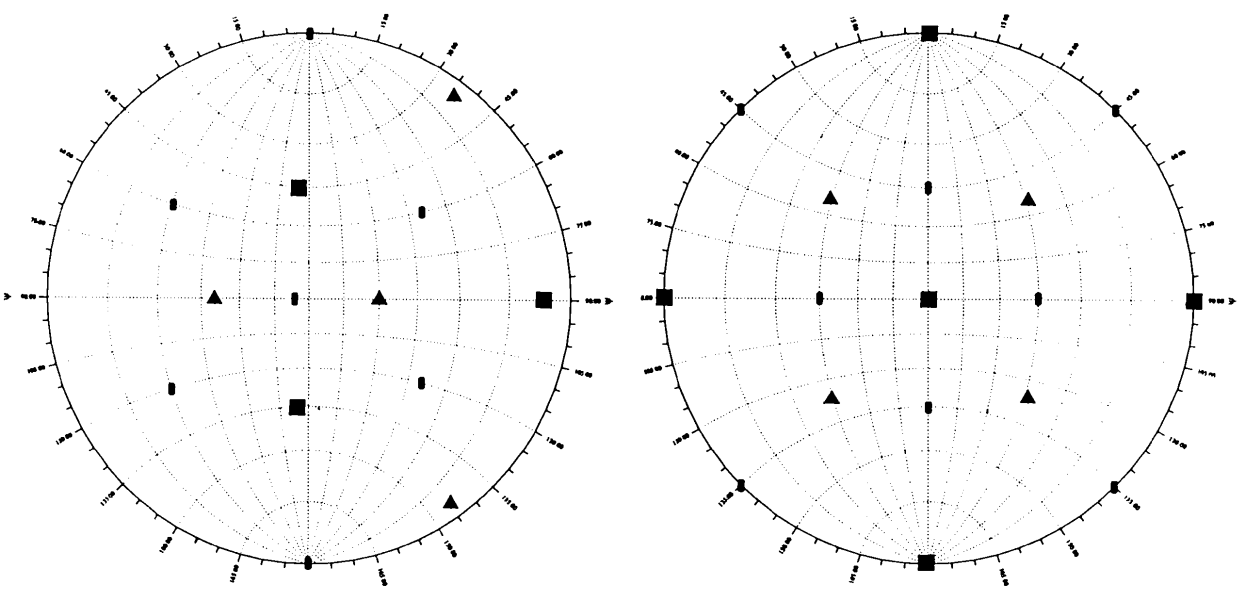
Diffraction data were collected from this crystal at 277 K on Station DW32 at LURE (Laboratoire d'Utilisation du Rayonnement Electromagnétique, Orsay, France) using a 180 mm MAR Research image-plate scanner (crystal-to-detector distance set to 220 mm). The wavelength of the incident X-ray beam was 0.901 Å. A total of 35 frames (exposure time per frame 60 s) were collected over 42° in steps of 1.2° to a resolution of 2.4 Å before appearance of significant radiation damage.

Although the cell dimensions and measurement of crystal dimensions hint at a tetragonal space group, the absence of any fourfold symmetry in (1 0 0)* reciprocal planes rules out such a possibility (Fig. 1). Also did the rather large R_{sym} value of 0.254 obtained when data processing was performed in a tetragonal space group ($R_{sym} = \sum_h \sum_i |I_{ih} - \langle I_h \rangle| / \sum_h \sum_i I_{ih}$ where $\langle I_h \rangle$ is the mean intensity for i observations of reflection h). Pseudo precession photographs indicated the crystal system was orthorhombic based upon the *mm* symmetry of the 0*kl* zones and their respective upper levels.

All images were processed using the *MOSFLM* software (Leslie, Brick & Wonacott, 1986), in an orthorhombic primitive unit cell with dimensions $a = 181.62$, $b = 128.88$ and $c = 128.88$ Å. Further data analysis was performed with *ROTAVATA/AGROVATA* programs of the *CCP*4 package (Collaborative Computational Project, Number 4, 1994). The full recorded data set is 56% complete between 22.0 to 2.46 Å. A total of 170 289 reflections (average reflection width: 0.6°) were collected, and these reduced to 61 103 independent reflections with an R_{sym} value of 0.07. A total of 83.8% of all



(*a*)



(*b*)

Fig. 2. (*a*) The locked self-rotation function, *versus* κ. A sharp maximum is observed at $\varphi = 178°$, $\psi = 96.4°$ and $\kappa = 45.35°$. Calculation was performed using 1502 large terms (resolution range 10.0–4.0 Å), a radius of integration of 80.0 Å. Maximum is at 2.5σ above background. (*b*) Stereographic projections of the symmetry axes of the apoferritin molecule within the orthorhombic unit cell reference frame compared with the standard projection of the 432 symmetry point group. In both cases, the *a* and *c* axes of the unit cell are displayed along the horizontal and vertical axes respectively.

measured reflections had $I > 3\sigma(I)$. In the resolution shell from 2.58 to 2.46 Å, 43% of the total expected reflections were observed and 66.8% of these had $I > 3\sigma(I)$.

3. Structure solution of the orthorhombic crystal

Self-rotation functions at $\kappa = 180$, 120 and 90°, using the program *GLRF* (Tong & Rossmann, 1990), allowed us to check the molecular symmetry and to establish the orientation of the non-crystallographic symmetry axes. Fig. 2(*a*) shows the resulting locked self-rotation function, including the 432 point-group symmetry, from which a peak maximum is obtained at $\varphi \simeq 178.0°$, $\psi \simeq 96.4°$ and $\kappa \simeq 45.35°$, while Fig. 2(*b*) reports the stereographic projections of the symmetry axes of the apoferritin molecule within the orthorhombic unit-cell reference frame and the standard projection of the 432 symmetry point group. From these results, the orientation of the molecule in the orthorhombic unit cell may be deduced first by a rotation of ~45.0° around the *a* axis followed by a second one of ~6.0° around the *c* direction.

The structure was solved by molecular replacement method using the program *AMoRe* (Navaza, 1994) with, as a starting model, half an apoferritin molecule built from the 1HRS entry (Précigoux *et al.*, 1994) of the Brookhaven Portein Data Bank (Bernstein *et al.*, 1977). The orthorhombic unit-cell volume was consistent with an asymmetric unit made up of 12 independent subunits and therefore the considered model led to two apoferritin molecules per unit cell. The rotation function, calculated from 53.6% [19 868 reflections with $F_o > 0.4$ mean (F_o)] of a complete data set between 8 and 3.5 Å and with a cut-off radius of 30 Å, displayed several equivalent solutions (correlation factor = 23.6 and 8σ peak height above the mean value). The translation search solved the space-group ambiguity as $P2_12_12$. The best solution of the translation function after rigid-body refinement, corresponding to a 33σ peak height (correlation factor = 71, factor = 0.30), predicted the centre of the molecule to be ($x_c = 0$, $y_c = 0.5$, $z_c = 0.2499$) and clearly indicates that the asymmetric unit contains half an apoferritin molecule lying on the Wyckoff special position 0, $\frac{1}{2}$, z close to $\frac{1}{4}$. If one describes, in a first approximation, the apoferritin molecules as spheres, the best molecular packing will obey a compact arrangement. This is exactly what is observed in the structure. The molecular packing verifies a pseudo tetragonal body-centered lattice as shown by the particular Wyckoff position of the centre of the molecule. This may explain why not only the cell dimensions but also the crystal dimensions hint at a tetragonal space group. The orthorhombic symmetry is only evident when the exact symmetry of the molecule is considered.

Despite the lack of completeness, the structure refinement was initiated using the program *X-PLOR* (Brünger, Kuriyan & Karplus, 1987). A total of 51 649 reflections [$F_o > 4\sigma(F_o)$] in the resolution range 8–3 Å were considered in order to refine the overall orientation of the molecule and the non crystallographic symmetries, leading to an *R* factor value of 0.27.

Difference ($F_o - F_c$) Fourier maps (Fig. 3*a*) clearly reveal well defined residual densities (with heights more than seven times the r.m.s. deviation) that can be easily assigned to five independent heavy atoms. Each peak location corresponds to one of the metallic sites linking adjacent protein shells in the cubic crystal and the amino acids involved in the so-formed metallic bridges are the same (Asp80 and Gln82) as those found in the cubic structure. Nevertheless, in the orthorhombic form, no metal ion is observed between adjacent molecules in the *b* direction (Fig. 3*b*). This results from a significant increase in the distance between the symmetry-related carboxylate groups of Asp80 and Gln82, due to the observed rotation of ~ 6° around the *c* direction. However, at this stage of the refinement, we cannot conclude on the exact nature of these heavy atoms.

For this data set, structure refinement as well as Patterson anomalous difference map calculations (Platinum being the only possible anomalous scatterer at the experimental wavelength) are in progress. Since the beginning of this study, new crystals of this orthorhombic form have been obtained which will allow possible improvement in data collection.

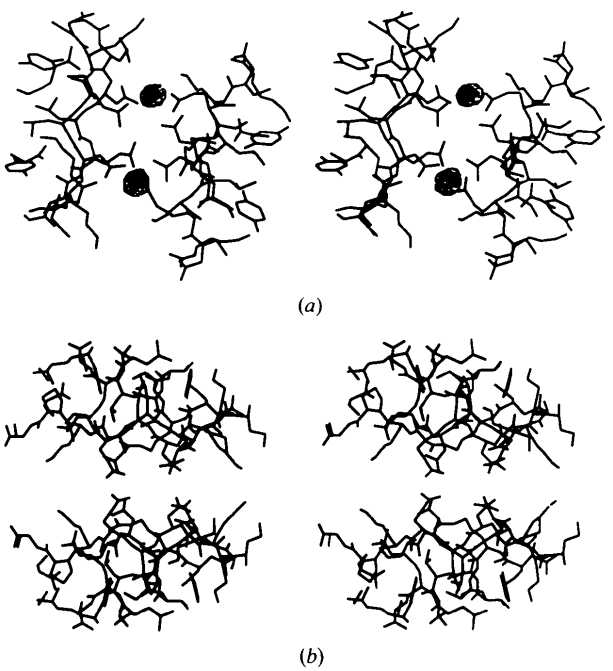


Fig. 3. Stereoscopic views of the $F_o - F_c$ electron-density maps at 5σ and 7σ levels in the intermolecular regions. (*a*) The high density peaks, between all adjacent molecules except in the *b* direction, are attributed to the presence of metal ions binding the symmetry-related residues Asp80 and Gln82; (*b*) along the *b* direction, no metallic site is observed.

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