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Crystallization and preliminary X-ray crystallographic analysis of the unusual ferritin from *Listeria innocua*

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Single crystals of ferritin extracted from *Listeria innocua* have been obtained by the vapour-diffusion method using PEG 1000 as precipitant. The crystals are orthorhombic, space group $P2_12_12_1$, with unit-cell dimensions $a = 87.7$, $b = 137.5$, $c = 173.1$ Å. The crystals diffract to 2.9 Å resolution on a rotating-anode X-ray source and to 2.35 Å resolution on a synchrotron X-ray source. The asymmetric unit contains one molecule formed by 12 subunits, corresponding to a packing density of 2.41 $Å^3\ Da^{-1}$

1. Introduction

Ferritin, the iron-storage protein, is distributed ubiquitously among living species. The three-dimensional structure of the apoferritin moiety is highly conserved and consists of 24 subunits, arranged in 432 symmetry, forming a hollow shell which can store up to 4500 Fe atoms as a ferrihydrite inorganic complex (Clegg *et al.*, 1980). The subunits are folded as four-helix bundles (helices *A*–*D*) with a fifth short helix (helix *E*) at about 60° to the bundle axis. Ferritin molecules isolated from vertebrates are heteropolymers composed of two types of subunit (H and L) with distinct functional properties (Arosio *et al.*, 1978). H chains catalyse Fe(II) oxidation at the so-called ferroxidase centre, whereas L chains assist in the formation and growth of the iron core (Harrison & Arosio, 1996). Ferritins from plants and Gram-negative bacteria are homopolymers of H-type chains, which contain the characteristic ferroxidase-centre residues (Frolow *et al.*, 1994).

A protein isolated recently from the Gram-positive bacterium *L. innocua* appears to be an iron-storage protein (or ferritin) since it can sequester up to 500 Fe atoms per molecule *in vitro* (Bozzi *et al.*, 1997), yet it displays unique structure–function relationships. The protein shell represents the first exception to the 24-mer structure common to all other ferritins; on the basis of solution and preliminary X-ray studies, it is a dodecameric homopolymer of about 240 kDa molecular mass, formed by the assembly of subunits which have a molecular mass of 18050 Da by calculation from the amino-acid sequence (Bozzi *et al.*, 1997; Stefanini *et al.*, 1997). The *L. innocua* amino-acid sequence does not show similarities to other ferritins, with the exception of a region which corresponds to the carboxylate-rich iron core nucleation site of the mammalian L chains. It lacks the ferroxidase-centre residues associated with the catalysis of Fe(II) oxidation, but behaves as an authentic iron-storage protein. The secondary-structure analysis (Garnier *et al.*, 1978) predicts the four-helix bundle fold characteristic of eukaryotic and prokaryotic ferritins without the C-terminal E helix.

Interestingly, the *L. innocua* ferritin sequence shows a relatively high similarity (~30%) to the DNA-binding proteins of the Dps family, a finding which demonstrates the proposed evolutionary link between the ferritin and Dps superfamilies (Peña & Bullerjahn, 1995). The recently published X-ray crystal structure of a Dps protein from *Escherichia coli*, a dodecamer, demonstrates that the structural similarity between *Listeria* ferritin and the Dps proteins extends to the quaternary assembly (Grant *et al.*, 1998).

2. Materials and methods

Ferritin from *L. innocua* was purified according to Bozzi *et al.* (1997). The cells were disrupted in a French press. The supernatant obtained after centrifugation was heated to 343 K and rapidly cooled. After a preliminary purification step by ammonium sulfate precipitation [80%(*w*/*v*)], the DNA was removed using a 1%(*w*/*v*) solution of streptomycin sulfate. The protein was further purified by fast protein liquid chromatography (FPLC) using a mono-Q column (Pharmacia Biotec Inc.).

Ferritin samples were concentrated to about 7 mg ml^{-1}. Crystallization was achieved at 293 K by the sitting-drop vapour-diffusion technique (McPherson, 1990). In the final conditions, the reservoir solution contained 0.1 *M* MES in the pH range 5.7–6.2 and 19–25% PEG 1000. A volume of 4 μl of the protein

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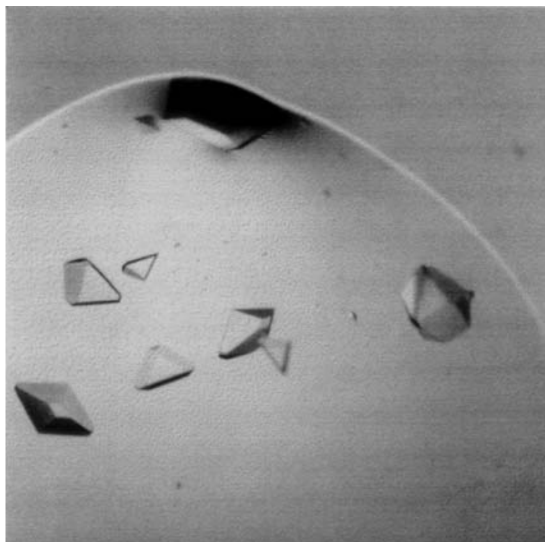

Figure 1
Crystals of *L. innocua* ferritin. The largest crystal is 0.6 mm in its longest dimension.

samples was mixed with an equal amount of the reservoir solution and allowed to equilibrate. Crystals grew in 2–4 d to about 0.4 × 0.3 × 0.2 mm (Fig. 1).

The preliminary X-ray data were collected as 1.2° oscillation frames on an R-AXIS II image-plate detector mounted on a Rigaku rotating-anode generator operating at 50 kV and 100 mA. Data frames were processed with *DENZO* and scaled with *SCALEPACK* (Otwinowski, 1986). The best data set was collected as 0.5° oscillation frames on the BW7B beamline at the European Molecular Biology Laboratory Outstation, DESY, Hamburg, Germany. The wavelength was set to 0.8373 Å. The data were collected at 100 K from a frozen crystal. Cryofreezing was accomplished by addition of PEG 400. A crystal of ferritin from *L. innocua* was placed in 200 µl of mother liquor plus 50 µl of PEG 400. A fibre loop (Hampton Research, Laguna Hills, California) was used to fish the crystal out of the cryosolvent and place it in the nitrogen-gas cold stream at 100 K.

3. Results and discussion

The crystals (Fig. 1) are not stable in the X-ray beam at room temperature and diffract to a resolution of 3 Å. To improve the quality of the data and avoid crystal decay, data have been collected at 100 K using an Oxford Cryosystems Cryostream. The data collected at the DESY synchrotron source in Hamburg at 100 K extend to a resolution of 2.35 Å. The autoindexing procedure performed with *DENZO* indicates that the crystals belong to the orthorhombic space group $P2_12_12_1$, with unit-cell dimensions a = 87.7, b = 137.5 and c = 173.1 Å. The data scaling gave an $R_{\rm merge}$ value of 7.7% for 80150 indexed reflections. The multiplicity is 4.1 and the data set is 91.3% complete to 2.35 Å resolution. The percentage of data with $I > 3\sigma(I)$ is 80.57%.

A value of V_M = 2.41 Å^3 Da^{-1} has been calculated according to Matthews (1968), assuming that there are four asymmetric units in a unit cell and that each asymmetric unit is formed by one ferritin molecule containing 12 chains of M_r = 18.05 kDa. This value, which is within the normal range for protein crystals, is consistent with the ultracentrifugation data obtained by Bozzi *et al.* (1997), which assigned an M_r of about 240 kDa to the *L. innocua* ferritin molecule.

Listeria ferritin is the only known member of the ferritin family that assembles as a dodecamer. Its molecular architecture may be envisaged as resembling that of the *E. coli* Dps dodecamer, in view of the similarity between the amino-acid sequences of the constituent polypeptide chains in the N-terminal and C-terminal regions of the four-helix bundle involved in subunit packing around the threefold axis (Bozzi *et al.*, 1997; Grant *et al.*, 1998). We plan to solve the three-dimensional structure using the molecular-replacement method with the coordinates of the Dps structure (not yet ready for distribution) and/or heavy-atom derivatives. We have begun a derivative search. Tb(III), which binds to the proposed iron-nucleation site (Stefanini *et al.*, 1999), was tried and appears promising. The other standard heavy-atom compounds of Hg, Pt, Au, Pb and Cd will be tried next. Knowledge of the *Listeria* ferritin X-ray structure will shed light on how the diversity of function with respect to Dps proteins is achieved.

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