Acta Crystallographica Section D
Biological Crystallography
ISSN 0907-4449

Crystallization and preliminary X-ray diffraction data of mouse L-chain apoferritin crystals

Thierry Granier,[a]* Bernard Gallois,[a] Béatrice Langlois d'Estaintot,[a] Alain Dautant,[a] Gérard Comberton,[a] Jean-Marc Mellado,[a] Carole Beaumont,[b] Paolo Santambrogio,[c] Paolo Arosio[c] and Gilles Precigoux[a]

[a]Unité de Biophysique Structurale, UMR CNRS 5471, Université Bordeaux I, Bâtiment B8, Avenue des Facultés, 33405 Talence CEDEX, France, [b]INSERM U409, Faculté de Médecine Xavier Bichat, 16 Rue Henri Huchard, 75870 Paris CEDEX 18, France, and [c]Unit of Protein Engineering, Dibit, San Raffaele Scientific Institute, Via Olgettina 58, 20132 Milano, Italy

Correspondence e-mail: t.granier@ubs.u-bordeaux.fr

Received 14 October 1999
Accepted 7 February 2000

Crystals of recombinant mouse L-chain apoferritin were obtained by the hanging-drop technique using ammonium sulfate as precipitant. Two crystal forms were observed in the same drop. The crystals belong to either the $P2$ monoclinic or to the $P42_12$ tetragonal space group. The monoclinic crystals diffracted to beyond 2.4 Å resolution but were systematically twinned, while the tetragonal crystals diffracted to beyond 2.9 Å. These crystallization conditions in the absence of metal salts should facilitate the study of the interaction between L-chain ferritins and heavy metals, particularly the iron core.

1. Introduction

Ferritin is a well known universal iron-storage protein which has been extensively studied. However, many aspects of the structure–function relationship of this protein have not yet been elucidated (for a review, see Harrison & Arosio, 1996; Harrison *et al.*, 1998; Chasteen & Harrison, 1999). Ferritin is a multimeric protein consisting of 24 subunits. Each subunit (15–20 kDa) consists of a four (*A*, *B*, *C*, *D*) α-helix bundle, to which a fifth small *E* α-helix is attached at the C-terminus end. The 24 subunits assemble in a 432 point symmetry; such an arrangement leads to the formation of a spherical multimer (diameter 120–130 Å), with an inner cavity of about 80 Å diameter. The cavity is able to host up to 4500 Fe atoms stored as hydrous ferric phosphate.

Natural ferritins isolated from vertebrates are heteropolymers formed of various types of subunits, named *H*, *L* or *M*, with different sequences, whereas bacterial and plant ferritins have been shown to be homopolymers of H-type chain, the subunit associated with ferroxidase activity.

Several structures of ferritins from different organisms have been solved: seven of them have had their atomic coordinate files deposited in the Protein Data Bank (Bernstein *et al.*, 1977): *Escherichia coli* bacterioferritin (1bcf, Frolow *et al.*, 1994; 1bfr, Dautant *et al.*, 1998), bullfrog L- and M-chain ferritins (1rcd, Trikha *et al.*, 1995; 1mfr, Ha *et al.*, 1997), horse L-chain ferritin (1dat, Gallois *et al.*, 1997; 1aew, Hempstead *et al.*, 1997) and human H-chain ferritin (2fha, Hempstead *et al.*, 1997).

Although the most-studied vertebrate ferritins are from horse, human and bullfrog, interest in mouse ferritins is increasing owing to the development of mouse cellular and animal models for the study of iron metabolism. The production of recombinant mouse H and L ferritins has been described and H/L recombinant heteropolymers have been obtained by coexpressing the two chains in *E. coli* (Rucker *et al.*, 1997). In addition, mouse transfectant cells overexpressing ferritin H chain showed for the first time that this protein has an active role in the control of the labile iron pool (Picard *et al.*, 1998). Although the mouse L-chain three-dimensional structure can be modelled on that of the horse L-chain (1aew) which has >85% sequence identity, it has the property, along with rat L-chain ferritin, of exhibiting an eight amino-acid insertion PAQTGAPQ in the exposed loop connecting helices *D* and *E*. The structure of this sequence is unresolved and it was found to be highly disordered in previous X-ray diffraction studies of rat L-chain ferritin (Lawson, 1990). However, there are reasons to believe that this sequence may be important for protein folding and assembly, since mutational studies on mammalian ferritins showed that the sequence of the *DE* loop has direct effects on protein conformation, assembly and solubility (Jappelli *et al.*, 1992; Jappelli & Cesareni, 1996, 1998).

Another problem is that vertebrate ferritins are usually crystallized from solutions containing metal ions (Ca^{2+}, Cd^{2+} *etc.*), which in many cases facilitate crystal lattice intermolecular interactions. These ions bind to the various ferritin metal-binding sites, including those involved in iron uptake (Lawson *et al.*, 1991; Harrison *et al.*, 1989; Granier *et al.*, 1998). To our knowledge, the unique case reported so far of direct evidence of iron-binding sites concerns recombinant *E. coli* ferritin crystals (Hempstead *et al.*, 1994) which were soaked with ammonium ferrous sulfate. The determi-

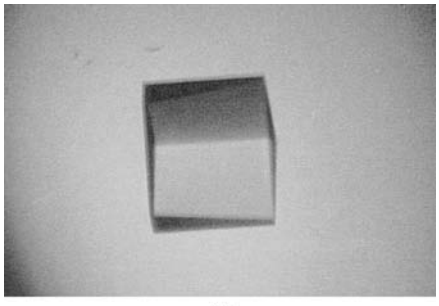
Table 1
Data-collection parameters and characteristics.

Values in parentheses correspond to the last resolution shell.

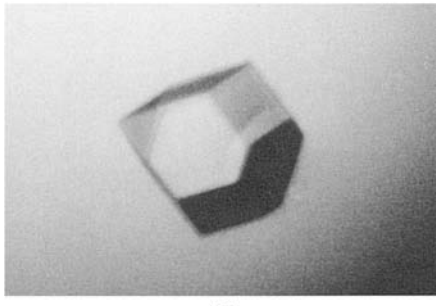
	*P*2	$P42_12$
Crystal-to-film distance (mm)	200	230
Oscillation range (°)	152.8	70.0
$\Delta\varphi$ (°) rotation range per frame	1.0	1.0
Exposure time per frame (min)	30	20
Resolution range (Å)	40.0–2.46	40.0–2.90
Last shell (Å)	2.52–2.46	3.06–2.90
No. of measured reflections	391699 (49210)	195578 (26760)
No. of unique reflections	221827 (29202)	34608 (5041)
Completeness	94.4 (94.4)	97.4 (98.7)
Multiplicity	1.8 (1.7)	5.7 (5.3)
$R_{\rm meas}$†	0.108 (0.257)	0.155 (0.414)
$R_{\rm sym}$†	0.077 (0.182)	0.140 (0.372)
PCV†	0.111 (0.270)	0.193 (0.513)
$\langle I/\sigma(I)\rangle$†	8.1 (4.1)	5.1 (2.0)

† See Diederichs & Karplus (1997) for definitions.

nation of crystallization conditions in the absence of metal ions would greatly facilitate the identification and structural characterization of the ferritin physiological iron-binding sites and of the iron core. Therefore, it was of interest to search for crystallization conditions which do not use metallic salts in order to further study crystals of ferritin loaded with iron. We report the crystallization conditions of recombinant L-chain mouse ferritin from ammonium sulfate solutions in the absence of metal ions. Two crystal forms were obtained, which diffracted to beyond 2.5 and 2.9 Å resolution.



(*a*)



(*b*)

Figure 1
Two crystal forms, monoclinic (*a*) and tetragonal (*b*), developed in the same drop.

2. Protein expression and purification

The cDNA for mouse ferritin L-chain was PCR amplified from the plasmid pMLF27 (Beaumont *et al.*, 1989), inserting restriction sites *Nde*I and *Bam*HI at the termini. The DNA fragment was subcloned into pDS20pTrp vector digested with *Nde*I and *Bam*HI. In the resulting plasmid, the Trp promoter directs the synthesis of the full mouse L-ferritin sequence (Beaumont *et al.*, 1989) with an Ala in position 122 in place of Thr. Transformed *E. coli* strain B cells were grown at 310 K in M9 broth for 7 h. The cells were harvested and disrupted by sonication; the soluble homogenates were heated at 348 K for 10 min, precipitated with ammonium sulfate (520 g l^{-1}) and treated with DNAase and RNAase. The final purification steps consisted of gel filtration on an S200 column (Pharmacia Biotech) followed by ion-exchange chromatography on a Hi-Trap Q column (Pharmacia Biotech). The yield was generally 7 mg of purified ferritin per litre of culture. Sample purification was assessed by SDS–PAGE under denaturing and non-denaturing conditions. The protein concentration was determined with BCA reagent (Pierce Chemical) using bovine serum albumin as a standard. The ferritin has an Ala in place of the reported Thr in position 122 (Beaumont *et al.*, 1989), a substitution arising from a Thr→Ala transition possibly caused by a PCR error. The substitution did not affect protein assembly, stability or ferritin iron uptake functionality (not shown). The subunit molecular weight is 20 641 Da.

3. Crystallization, X-ray data collection and processing

Crystallizations were performed using the hanging-drop vapour-diffusion method in Linbro plates. The first trials were carried out with the commercially available Grid Screen A/S kit (Hampton Research), testing different concentrations of ammonium sulfate (AS) solutions *versus* pH. Concentrations and pH conditions were refined. The best crystals were obtained by equilibration of an 8 µl drop, consisting of equal volumes of a 3.5 mg ml^{-1} solution of mouse L ferritin in 50 m*M* Tris buffer pH 7.4 and of reservoir solution containing 140 m*M* ammonium sulfate, 160 m*M* sodium citrate pH 4.15, 3 m*M* sodium azide, against 1 ml of the above-mentioned reservoir solution at 293 K. Two crystal forms appeared in the same drop. The first one appeared within two weeks. The crystals have a trigonal prismatic shape and grow to final dimensions of 0.4 × 0.3 × 0.2 mm. The second form appears later, after three to four months. The crystals are smaller (0.2 × 0.2 × 0.3 mm) and have a more complex morphology (Fig. 1).

X-ray diffraction data were collected using X-rays (Cu $K\alpha$, λ = 1.5418 Å) produced by an Enraf–Nonius FR571 rotating-anode generator operating at 40 kV and 50 mA.

Data collections were performed on a 300 mm MAR Research image-plate diffractometer. Data were processed with the program *MOSFLM* (Leslie *et al.*, 1986) and were further scaled with the program *SCALA* (Collaborative Computational Project, Number 4, 1994). The data-collection statistics are shown in Table 1. Indexing the first crystal form led to a reduced cell with unit-cell parameters a = 147.55, b = 148.26, c = 148.28 Å, α = 90.0, β = 90.0, γ = 90.0°. Despite the cubic like unit cell, space-group determination did not yield any higher symmetry than monoclinic *P*2. Data were further processed with this space group. An examination of the intensity-distribution statistics of centric and acentric reflections showed that crystals of this first form are likely to be twinned ($\langle I^2\rangle/\langle I\rangle^2$ = 1.53), with a calculated twin fraction of $\alpha \simeq 0.5$ (Yeates, 1997). In the present case, twinning by pseudo-merohedry (*i.e.* 180° rotation about the a or c axis) would be favoured by the β value of 90°, inducing a complete overlap of reflections corresponding to each twin domain. So far,

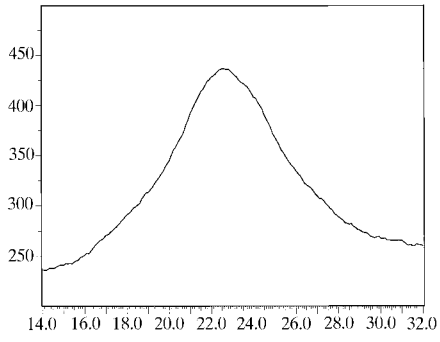


Figure 2
Locked self-rotation function value including 432 point-symmetry elements of the apoferritin molecule, as a function of κ (°). A sharp maximum is observed at φ = 90, ψ = 90, κ = 22.5°. The calculation was performed using 17 023 reflections and 2145 large terms (resolution range 15–3.5 Å) and a radius of integration of 40 Å. The maximum is at 6σ above the background.

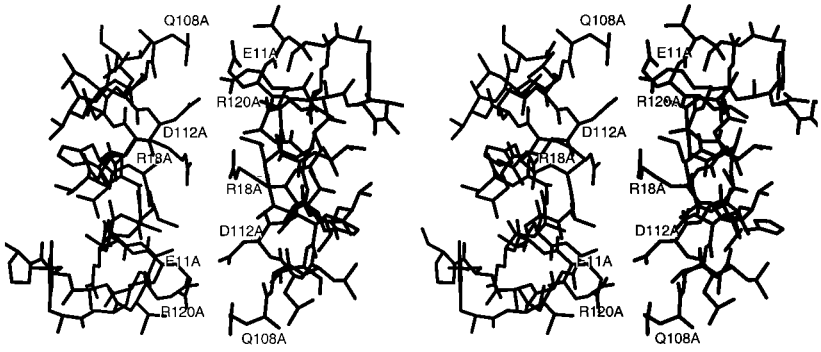


Figure 3
Stereoscopic view of one of the intermolecular contacts between asymmetric units (x, y, z) and $(1 - y, 1 - x, 1 - z)$.

Patterson self-rotation and cross-rotation function map calculations have not yielded satisfying solutions.

For the second crystal form, the space group was determined to be $P42_12$, with unit-cell parameters $a = 136.42$, $c = 167.88$ Å, and the structure determination is briefly decribed below.

4. Structure investigation

The structure determination of the tetragonal form was carried out following the same procedure as for the tetragonal structure of horse spleen apoferritin crystals (Granier *et al.*, 1996): the orientation of the 432 point-group symmetry elements of the ferritin molecule relative to the unit-cell axes was obtained by Patterson self-rotation function map calculation using the program *GLRF* (Tong & Rossmann, 1990). The crystal structure appears to be very similar to that of the tetragonal form of horse spleen ferritin crystals, despite a noticeable difference in the magnitude of the unit-cell parameters ($a = 146.6$, $c = 152.9$ Å in the latter case); the asymmetrical unit is a hexamer and the unit cell contains two ferritin molecules. One of the fourfold molecular axes coincides with the tetragonal c axis and the other fourfold molecular axes are found to make an angle of 22.5° with respect to the unit-cell axes a or b (see Fig. 2). The structure was obtained by molecular replacement using the program *AMoRe* (Navaza, 1994); the atomic coordinates of the model were generated starting from coordinates of the structure 1dat (Gallois *et al.*, 1997). The best solution of the cross-rotation function followed by a translation-function search yields a 33.8σ peak height (correlation factor = 76.6, R factor = 0.316), with the centre of the molecule at the Wickoff position ($x_c = 0.0$, $y_c = 0.50$, $z_c = 0.2451$). The intermolecular contacts involve similar hydrophilic residues as in the horse spleen ferritin isomorphous structure (see Fig. 3), *i.e.* Thr10, Glu11, Arg18 and Asp112, with the exception of a Gln→Arg substitution at position 120. The main difference arises from the fact that in the present structure no cadmium ion is involved in these contacts. Likewise, no significant electron density is observed along the threefold molecular axes, which is the location of metal-binding sites for metals such as calcium, cadmium and terbium in other ferritin structures.

References

Beaumont, C., Dugast, I., Renaudie, F., Souroujon, M. & Grandchamp, B. (1989). *J. Biol. Chem.* **264**, 7498–7504.

Bernstein, F. C., Koetzle, T. F., Williams, G. J., Meyer, E. E. Jr, Brice, M. D., Rodgers, J. R., Kennard, O., Shimanouchi, T. & Tasumi, M. (1977). *J. Mol. Biol.* **112**, 535–542.

Chasteen, N. D. & Harrison, P. M. (1999). *J. Struct. Biol.* **126**, 182–194.

Collaborative Computational Project, Number 4 (1994). *Acta Cryst.* D**50**, 760–763.

Dautant, A., Meyer, J.-B., Yariv, J., Précigoux, G., Sweet, R. M., Kalb, A. J. & Frolow, F. (1998). *Acta Cryst.* D**54**, 16–24.

Diederichs, K. & Karplus, P. A. (1997). *Nature Struct. Biol.* **4**, 269–275.

Frolow, F., Kalb, A. J. & Yariv, J. (1994). *Nature Struct. Biol.* **1**, 453–460.

Gallois, B., Langlois d'Estaintot, B., Michaux, M.-A., Dautant, A., Granier, T., Précigoux, G., Soruco, J.-A., Roland, F., Chavas-Alba, O., Herbas, A. & Crichton, R. R. (1997). *J. Biol. Inorg. Chem.* **2**, 360–367.

Granier, T., Comberton, G., Gallois, B., Langlois d'Estaintot, B., Dautant, A., Crichton, R. R. & Précigoux, G. (1998). *Proteins Struct. Funct. Genet.* **31**, 477–485.

Granier, T., Gallois, B., Dautant, A., Langlois d'Estaintot, B. & Précigoux, G. (1996). *Acta Cryst.* D**52**, 594–596.

Ha, Y., Theil, E. C. & Allewell, N. M. (1997). *Acta Cryst.* D**53**, 513–523.

Harrison, P. M. & Arosio, P. (1996). *Biochim. Biophys. Acta*, **1275**, 161–203.

Harrison, P. M., Artymiuk, P. J., Ford, G. C., Lawson, D. M., Smith, J. M. A., Treffry, A. & White, J. L. (1989). *Biomineralization: Chemical and Biochemical Perspectives*, edited by S. Mann, J. Webb & R. J. P. Williams, pp. 257–294. Weinheim, Germany: VCH.

Harrison, P. M., Hempstead, P. D., Artymiuk, P. J. & Andrews, S. C. (1998). *Metal Ions Biol. Syst.* **35**, 435–477.

Hempstead, P. D., Hudson, A. J., Artymiuk, P. J., Andrews, S. C., Banfield, M. J., Guest, J. R. & Harrison, P. M. (1994). *FEBS Lett.* **350**, 258–62.

Hempstead, P. D., Yewdall, S. J., Fernie, A. R., Lawson, D. M., Artymiuk, P. J., Rice, D. W., Ford, G. C. & Harrison, P. M. (1997). *J. Mol. Biol.* **268**, 424–448.

Jappelli, R. & Cesareni, G. (1996). *FEBS Lett.* **394**, 311–315.

Jappelli, R. & Cesareni, G. (1998). *Biochem. Biophys. Res. Commun.* **250**, 342–346.

Jappelli, R., Luzzago, A., Tataseo, P., Pernice, I. & Cesareni, G. (1992). *J. Mol. Biol.* **227**, 532–543.

Lawson, D. M. (1990). PhD thesis, University of Sheffield, England.

Lawson, D. M., Artymiuk, P. J., Yewdall, S. J., Smith, J. M., Livingstone, J. C., Treffry, A., Luzzago, A., Levi, S., Arosio, P., Cesareni, G., Thomas, C. D., Shaw, W. V. & Harrison, P. M. (1991). *Nature (London)*, **349**, 541–544.

Leslie, A. G., Brick, P. & Wonacott, A. J. (1986). *CCP4 Newslett.* **18**, 33–39.

Navaza, J. (1994). *Acta Cryst.* A**50**, 157–163.

Picard, V., Epsztejn, S., Santambrogio, P., Cabantchik, Z. I. & Beaumont, C. (1998). *J. Biol. Chem.* **273**, 15382–15386.

Rucker, P., Torti, F. M. & Torti, S. V. (1997). *Protein Eng.* **10**, 967–973.

Tong, L. & Rossmann, M. G. (1990). *Acta Cryst.* A**46**, 783–792.

Trikha, J., Theil, E. C. & Allewell, N. M. (1995). *J. Mol. Biol.* **248**, 949–967.

Yeates, T. O. (1997). *Methods Enzymol.* **276**, 344–358.