Crystallographic data for horse heart ferritin

Martino Bolognesi, Giuseppina Gatti, Sonia Levi*, Elena Gabri* and Paolo Arosio*

Department of Genetics and Microbiology, Section of Crystallography, University of Pavia, Via Taramelli 16, I-27100 Pavia and **Department of Biomedical Science and Technology, University of Milano, Via Olgettina 60, I-20132 Milano, Italy*

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Horse heart and spleen ferritins were subjected to crystallization experiments with polyethylene glycols. Crystals suitable for preliminary X-ray diffraction experiments were obtained from polyethylene glycol (6000 average M_r), under controlled conditions of pH and ionic strength. Heart ferritin crystallizes in space group F432, with unit cell edges $a = b = c = 183$ Å, with a single subunit in the crystallographic asymmetric unit.

Ferritin *Isoferritin* *Protein crystal* *Iron protein* *Iron metabolism*

1. INTRODUCTION

Ferritin is an iron storage protein widely distributed in nature [1]. The functional molecule is an oligomer composed of 24 subunits which form a hollow spherical shell, with an inner cavity, about 80 Å across, which can accommodate various amounts of hydrated ferric oxide phosphate [2]. Several physico-chemical investigations show that ferritin is heterogeneous, being composed of some 20 isoferritins, whose distribution changes in different tissues and according to different physiological states [3,4]. The heterogeneity of the ferritin molecule is presently interpreted as due to the presence of two subunit types which build up the oligomeric molecule in different proportions [3,5]. The two subunits, called H and L after their different M_r values, show significant primary and secondary structure differences [5], and appear to be different gene products [6]. The two chains seem to have different functions, since the lighter L subunit is more abundant in iron-rich tissues (i.e., spleen and liver), while the heavier H subunit is predominant in tissues with no major iron storage function such as heart [4]. In addition, the L and H subunit-rich isoferritins (those from spleen and heart, respectively) differ in their tryptic peptide maps [7], immunological reactivities [8], surface charge distributions [8], spectroscopic properties and molecular dimensions [5].

Horse spleen ferritin was first crystallized several years ago, from cadmium sulfate solutions [10] in the cubic space group F432. X-ray diffraction studies on this crystalline form have unraveled the three-dimensional structure of spleen apoferritin at a resolution of 2.8 Å [11]. Crystallographic studies on H subunit-rich ferritins started only recently, and have shown that spleen and heart ferritins can be selectively crystallized employing the two precipitating agents cadmium sulfate and 2-methyl-2,4-pentanediol (MPD), respectively [12].

We report here on crystallization experiments with polyethylene glycols (PEG) which allowed growing of single crystals of horse heart ferritin suitable for preliminary investigations; crystallographic data are reported.

2. MATERIALS AND METHODS

Ferritin from horse heart and spleen was purified as in [3]. Spleen ferritin was not treated with cadmium sulfate before crystallization ex-

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periments. All preparations used appeared to be pure, as judged by electrophoretic analyses. Apoferritin was prepared by chemical reduction with dithionite [5]; protein concentration was detected as in [13] and isoelectric focusing analyses were performed on 0.5 mm thick polyacrylamide gels, as in [3]. Crystallization experiments were performed at 4°C and at room temperature by means of vapour diffusion techniques [14]. Tris and phosphate buffers, the presence of divalent cations, protein concentration, and size of droplets were tested in different experiments. The crystals employed for diffraction experiments were grown by equilibrating 15 μl droplets, containing 10 mg/ml protein, 6% (w/v) polyethylene glycol, 80 mM Tris–HCl (pH 7.4), against 2–4 ml reservoirs of 15% (w/v) polyethylene glycol in the same buffer (pH 7.4) at room temperature. The crystallization experiments were left undisturbed for 1 month or longer; when precipitation appeared to be complete they were opened. The crystals were thoroughly washed and examined by means of conventional precession photographs, using as X-ray source an Elliott GX 20 rotating anode generator, run at 1.6 kW. The focal spot dimensions were 0.2 × 2.0 mm.

3. RESULTS AND DISCUSSION

Polyethylene glycols of average M_r 4000 and 6000 were successful in crystallizing the protein. With both precipitating agents it was possible to grow crystals or crystalline aggregates in a broad range of pH (6–8), but only careful control of the ionic strength and buffering media allowed us to obtain characteristic octahedral crystals (fig.1). In particular it was found that in the presence of 15% (w/v) PEG the yield of the crystallization ex-

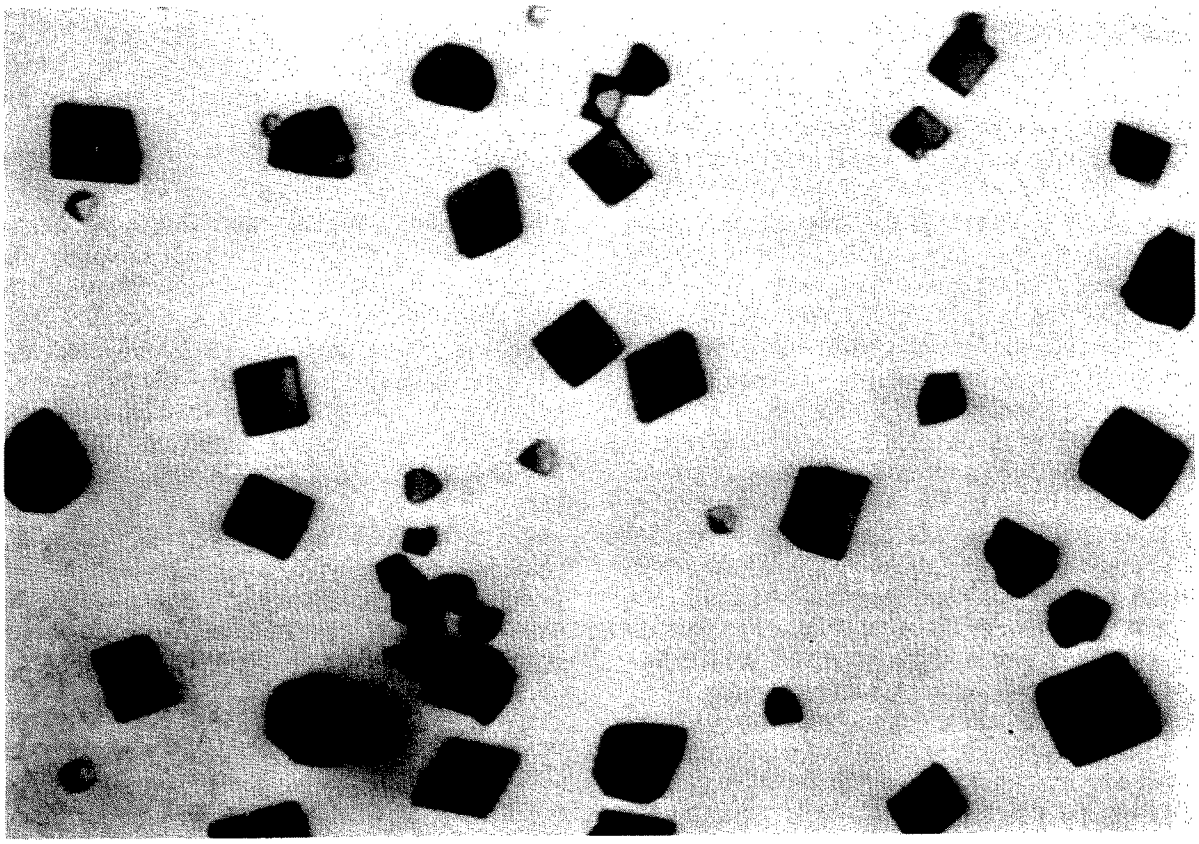

Fig.1. Octahedral crystals of horse heart ferritin grown from PEG solutions.

periments increased significantly if the concentration of the Tris buffer was above 0.08 M. In fact the crystals obtained were shown to be soluble at lower ionic strengths even in the presence of 20% PEG. Under the conditions described in section 2 heart holoferritin was repeatedly crystallized as cubic octahedra (about 0.15 mm per edge), which were isotropic under the polarizing microscope.

Owing to the contained dimensions of the crystals the precession photographs taken showed a limited number of reflections (fig.2). On the basis of the symmetry and of the systematic absences, and considering that the crystals, because of their optical properties, must belong to a cubic space group, it was possible to show that equine heart holoferritin crystallizes in space group F432, with unit cell edges $a = b = c = 182.9 \pm 1.5$ Å. If one considers the packing density values allowed for protein crystals [15] and assumes an M_r of 21000 for the subunit of heart ferritin [3], one can easily show that the unit cell contains 4 full ferritin molecules, and that the crystallographic asymmetric unit contains a single subunit. The corresponding packing density parameter v_M is 3.03 Å^3/dalton and the solvent content of the crystal is 59%. The molecular symmetry of the ferritin oligomer (24 subunits arranged in 432 point symmetry) therefore coincides with the crystallographic symmetry. This situation is entirely analogous to that observed in spleen ferritin crystals, grown from $CdSO_4$ solutions [11].

It is worth pointing out that in preliminary experiments we were able to grow crystals of equine heart apoferritin and of spleen holoferritin under conditions similar to those described above (the behavior of spleen ferritin towards crystallization

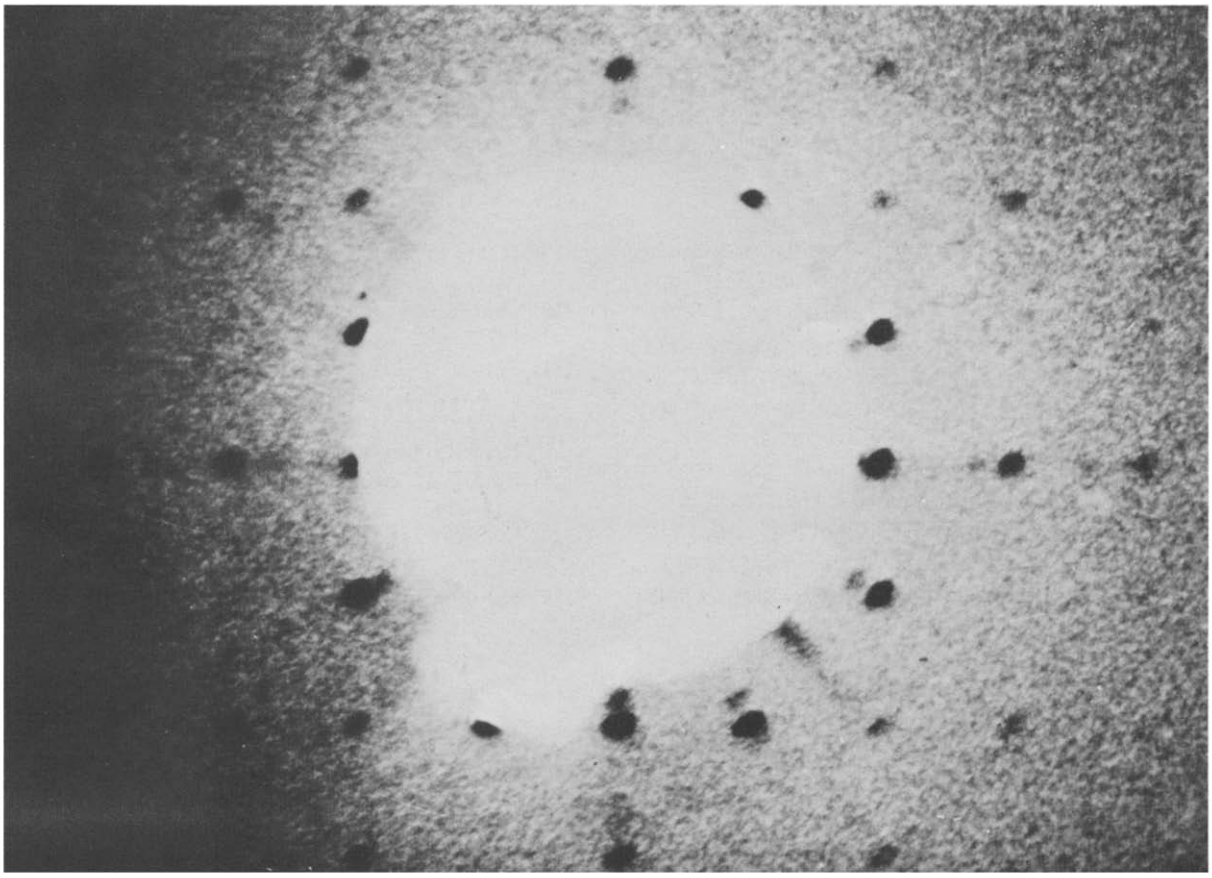

Fig.2. Low angle precession photograph of heart ferritin crystals: (*hk*0) level, 3° precession angle. *a* and *b* axes are approximately at 45° to the horizontal direction.

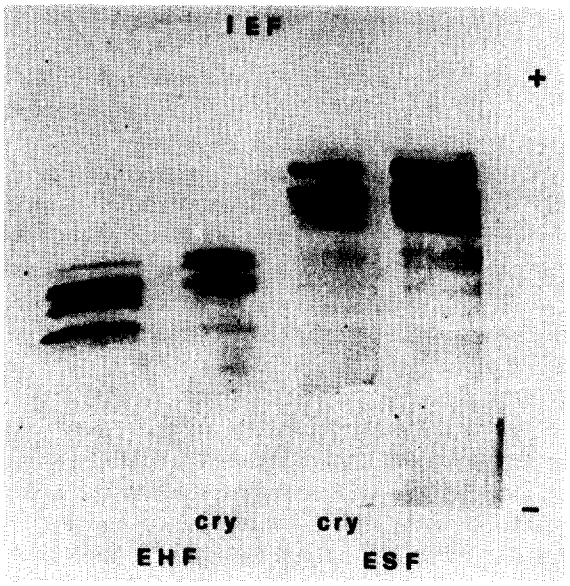

Fig.3. Isoelectrofocusing analysis (pH 4–6) of equine heart (EHF) and spleen ferritin (ESF) recovered from the crystals grown from PEG solutions (cry), compared with the original samples. Spleen crystals contain the same isoferritin population as the mother liquor, while the crystals of the heart ferritin are enriched in the more acidic components.

was distinguishable from that of the heart protein: spleen ferritin was less soluble in PEG solutions and easily formed amorphous precipitates). Both crystalline forms had octahedral habitus and were isotropic in polarized light. No X-ray diffraction has yet been observed however, owing to the limited size of the crystals (~0.05 mm across).

Crystals obtained from heart and spleen preparations were separated from their mother liquors, thoroughly washed, dissolved and their isoferritin composition analyzed (fig.3). The spleen ferritin had the same isoelectrofocusing profile in the crystallized and uncrystallized samples while crystals grown from the heart protein were enriched in the more acidic isoferritins. This finding indicates that the crystallizing agent PEG, unlike cadmium sulfate and MPD, does not select between acidic and basic isoferritins [12].

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