Structural homology between mouse liver and horse spleen ferritins

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Mouse liver ferritin is composed almost exclusively of polypeptide chains similar in molecular mass (22 kDa) to that characteristic of the major chain (H) found in heart ferritin isolated from human, horse or rat. In these species the predominant polypeptide of liver (L) is smaller (about 20 kDa). Here we show that mouse liver and horse spleen ferritins and apoferritins exhibit extensive structural homology as judged by the similarity in the diffraction patterns of their crystals grown from cadmium sulphate solutions. Implications of this finding are discussed.

Mouse liver ferritin *Horse spleen ferritin* *Structural homology*

1. INTRODUCTION

The iron-storage protein ferritin is composed of 24 subunits which enclose an iron-containing mineral core [1]. Although its subunits are structurally equivalent [1] and structural homology between ferritins from several species [2,3] has been described, there are numerous reports that ferritins are composed of two polypeptide chains of different sizes as judged by their migration in polyacrylamide gels containing SDS [4–8]. The relative proportions of these two chains differ with tissue and iron loading [4–8]. Under normal iron status the heavier chain, designated H, predominates in heart, whereas the lighter L-chain is the predominant form in liver and spleen. This has been observed in human [4,6,8], horse [4] and rat [4,5,7]. Within a single tissue, ferritins seem to be composed of mixtures of molecules that vary in H:L ratio and p*I*. The heteropolymer hypothesis has recently received support from the finding of at least two different sequences in human spleen and liver ferritin [9–11] and also in rat liver ferritin [12,13]. The L-chains of horse spleen ferritin [14] and human liver [10] and spleen [12] ferritins exhibit 83–95% identity in a sequence of 174 amino acid residues corresponding to molecular masses of nearly 20 kDa. The cDNA sequence [11], corresponding to a second human liver ferritin chain (presumed to be H), corresponds to 189 amino acids ($M_r \approx 22000$) which could account well for the more slowly moving band seen on SDS–polyacrylamide gels. This sequence shows only 55% identity with the human liver L-chain amino acid sequence [10]. The cDNA sequence [12] found for rat liver ferritin L-chain gives 83–89% identity in amino acid residues with human and horse L-chains except for an insertion of 8 residues that raises the molecular mass to nearly 21 kDa. Thus, although only three examples are so far known, there seems to be a family of L-chain sequences which is distinct from that of H chains.

Mouse liver ferritin [15] appears not to fit the pattern seen in horse, human and rat liver proteins. It consists almost exclusively of a chain of nominal M_r 22000 and only a trace of a 19 kDa chain as measured in SDS–polyacrylamide gels. This polypeptide distribution is found in the livers of iron-loaded males [15], of normal mixed litters [16] and, in this study, in livers of normal females. Here, we report preliminary X-ray diffraction data

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from single crystals of mouse liver ferritin and apoferritin grown from solutions containing $CdSO_4$.

2. MATERIALS AND METHODS

Mouse liver ferritin was prepared from normal female mice essentially as in [16]. Apoferritin was prepared from ferritin as in [7]. The ferritin preparation was checked for purity by electrophoresis in 5% polyacrylamide gels and electrophoresis in gradient gels containing SDS essentially as in [4]. Subunit composition and molecular masses were also determined by SDS–polyacrylamide gels. The subunit composition was re-examined after crystallisation. Crystallisation was achieved by the addition of $CdSO_4$ (to about 40 mM) to a solution of ferritin or apoferritin at a concentration of about 10 mg/ml protein. Precession photographs were obtained with Cu K_α radiation (λ = 1.5418 Å), 75 mm crystal-film distance, with a Philips PW1130 generator as X-ray source (40 kV, 30 mA).

3. RESULTS

Normal female mouse liver ferritin gave the same major subunit band (nominal $M_r \approx 22000$) and a trace of a lighter ($M_r \approx 19000$) band as reported previously for normal mixed litters [16] and for iron-loaded male mice [15]. The major band migrated close to the H-chain of human liver ferritin. The subunit composition was unaltered by crystallisation from $CdSO_4$ solutions.

Crystals of mouse liver ferritin and apoferritin are octahedra of a very similar appearance to crystals of horse spleen holo- and apo-ferritin. Large single crystals (0.3 mm) were used for precession photography. Mouse liver ferritin and apoferritin were found to be isomorphous with each other and with the horse spleen proteins, which are also isomorphous irrespective of their iron content [1,17]. The crystals are cubic with unit cell side 184.8 ± 1.0 Å and space group F432. Intensity distributions of both species are so similar that very close structural homology must be inferred (fig.1).

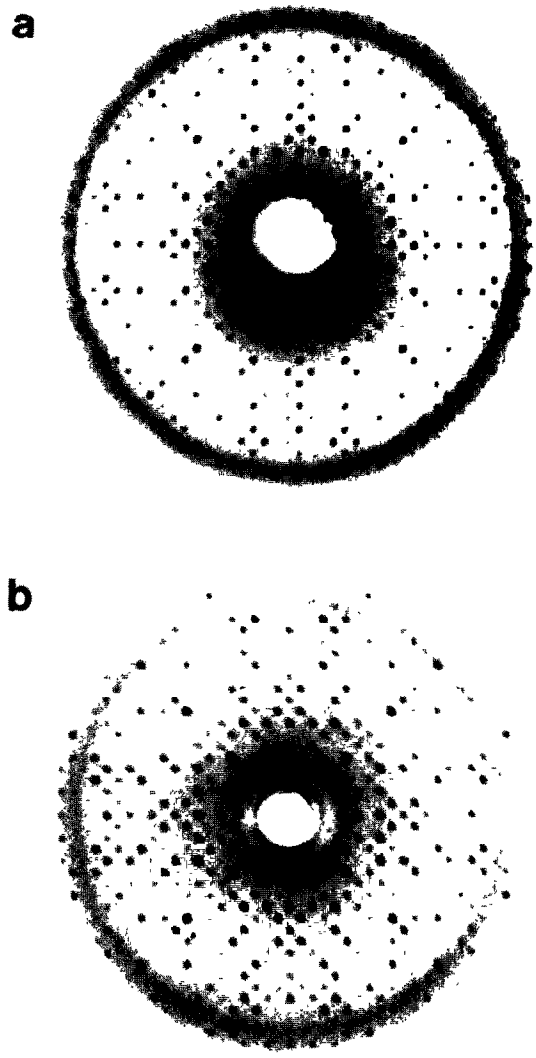

Fig.1. 7° precession photographs of (a) mouse liver apoferritin and (b) horse spleen apoferritin at a nominal resolution of 6.6 Å taken in the [100] direction. Note the identity of cell dimensions and symmetry and marked similarity in X-ray intensities implying close structural homology in the two types of apoferritin. Subunit conformation and quaternary structure must both be very similar.

4. DISCUSSION

It is of considerable interest that mouse liver ferritin is isostructural with horse spleen ferritin despite a polypeptide chain that is at least 10%

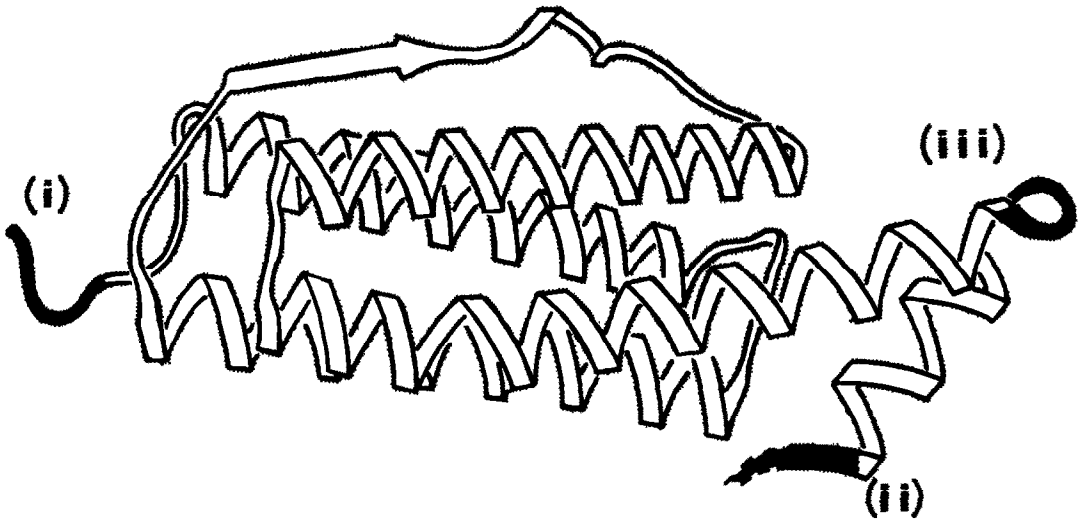

Fig.2. Schematic diagram of a horse spleen apoferritin subunit indicating where insertions in the sequence may occur without disrupting the quaternary structure of the apoferritin shell. Sites of known insertions are (i) N-terminus and (ii) C-terminus (in human liver H-chain [11]) and (iii) DE-turn (in rat liver L-chain [3,13]). Other insertions may be possible in some of the other non-helical regions although not near the centre of loop L, which is involved in intermolecular bridges within the crystal. It is not inconceivable that insertions of a few residues could occur in several positions.

larger. We have no information about the sequence but our results imply that the insertion of about 17 residues required to account for the difference in molecular mass does not affect to any significant extent either the subunit fold, the numerous intersubunit interactions within the multisubunit protein shell or the crystal packing of the molecules. There are several places where such insertions could be placed (fig.2). These are at the N- or C-termini which lie respectively on the outside and inside surface of the apoferritin shell (such insertions are found in the cDNA sequence of human (presumed H) ferritin [11]), at an interhelical turn between the D and E helices which lies on the outside of the molecule (the position of the 8-residue insertion in the rat liver sequence [3,12]) or possibly in the AB or CD turns between the A and B or C and D helices of the subunit conformation, although both these turns take part in intramolecular subunit packing. An insertion in the central region of the long BC loop, which provides ligands for Cd^{2+} bridges between molecules in the crystal [18] is unlikely since this would probably alter the crystal packing, although very short insertions at either end of the BC-loop may be possible. The existence of one or more insertions on the outside of the molecule is suggested by the apparently larger hydrodynamic volume of mouse liver compared to horse spleen ferritin as judged by gel exclusion behaviour [15] even though the intermolecular crystal packing diameters are the same.

It is of relevance to this discussion that rat liver ferritin which contains about 40% of the large 'H' chain [4,5,7] also gives crystals which are isomorphous with those of horse spleen and mouse liver proteins [2]. This implies that H chains have the same conformation as L chains and can be substituted for them within the protein shell. This is further supported by analysis of sequence substitutions between human H chain and horse L chain in relation to the 3-dimensional structure. This indicates that many of the substitutions fall on the molecular surface, that internal substitutions are mainly conservative in character and that intersubunit interactions are conserved or substituted conservatively (unpublished).

Crystallisation of mouse liver ferritin contrasts with the failure of H-rich horse heart ferritin to crystallise from $CdSO_4$ solutions [19]. This failure to crystallise may result, however, from sequence

substitutions preventing formation of the intermolecular Cd^{2+} bridges which occur in horse spleen apoferritin crystals [18] rather than from differences in chain length. Such a sequence change (Lys for Gln 82 [9,10]) may explain why the human proteins crystallise less readily than horse spleen apoferritin from $CdSO_4$ solutions. Hence, changes in crystallizability may be due to amino acid substitutions in regions of intermolecular contact rather than to differences in conformation.

An interesting question, but one to which we have yet no answer, is whether the mouse liver major chain, which is 'H-like' in size, is also 'H-like' in sequence, i.e., to which of the human sequences is it more homologous? Thus, chain size and sequence type may not always correlate and assignment of chain type as 'H' or 'L' on the basis of size alone may not be valid. Equally intriguing is the alternative that the mouse, unlike horse, human and rat, has a ferritin in its liver composed almost exclusively of 'heart-type' subunits. It is clear that more sequences are required before these questions can be resolved.

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