Electron microscopic studies of human haemosiderin and ferritin

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SUMMARY Ferritin and haemosiderin were isolated from fresh frozen human spleens that had been removed from patients with secondary iron overload due to multiple transfusions. Haemosiderin was solubilised by a novel technique that maintains its integrity. Unstained preparations of haemosiderin and ferritin were visualised and quantitative measurements made of the volumes of iron core. The mean diameter of the ferritin core (6.4 nm) was larger than that of haemosiderin (5.7 nm). In addition, haemosiderin, in contrast to ferritin, showed a large number of cores of less than 5 nm in diameter. Negatively stained preparations of haemosiderin and ferritin were visualised, confirming the small core size of the haemosiderin. The protein shell of haemosiderin, unlike that of ferritin, was thinner and irregular. These findings are consistent with the suggestion that haemosiderin is derived from ferritin by partial proteolysis and partial solubilisation of the iron core, presumably by lysosomal action.

Haemosiderin is a complex of iron oxyhydroxide microcrystals with protein, phosphate, some metal ions, and, perhaps, lipids.^{1,2} It is the principal protein that stores iron in conditions of iron overload such as β thalassaemia and may be responsible for the severe damage to tissue that accompanies such syndromes.³ Its lysosomal location, radioactive iron pulse-chase experiments,⁴ and close physiochemical similarities with both haemosiderin crystallites and ferritin crystallites⁵ has led to the view that it is a breakdown product of ferritin, but there is no direct evidence for this.

In contrast to ferritin, in which the individual crystallites (termed cores) are enclosed and solubilised by a protein shell, haemosiderin cores form an insoluble aggregate, which makes them difficult to examine morphologically. We have used a method that has recently been developed to solubilise and disaggregate haemosiderin² and will therefore facilitate such studies. Negative staining techniques were used to look for possible remnants of the ferritin protein shell in the purified preparations of haemosiderin.

Material and methods

ISOLATION OF HAEMOSIDERIN AND FERRITIN

Haemosiderin and ferritin were isolated from spleens overloaded with iron from patients who had received multiple transfusions for β thalassaemia and frozen fresh to -20°C . Haemosiderin was isolated by centrifugation through concentrated potassium iodide solutions² and was solubilised in 0.02M tetramethylammonium hydroxide, pH 12.3, by gentle stirring for 45 minutes at room temperature. Ferritin was purified according to the method of Huebers *et al.*⁶

ELECTRON MICROSCOPY

Samples of solubilised haemosiderin were applied directly to mesh 400 copper grids coated with carbon (Athene Ltd, United Kingdom) and viewed under a Philips EM 300 microscope operating at 100 KV. Samples of haemosiderin for negative staining were applied as a sonicated suspension in water and then stained with potassium phosphotungstate, pH 6.5, for 30 seconds, dried in air, and viewed under the electron microscope. Ferritin was applied to grids as an aqueous solution then viewed directly or, after negative staining, with potassium phosphotungstate, pH 6.5. Diameters of

particles of 100 to 150 cores for every sample were measured from micrographs. The volume of the particles was estimated, assuming that the shape was spherical.

Results and discussion

Fig. 1 shows an electron micrograph of a section of human spleen loaded with iron and stained with uranyl acetate and lead citrate. The figure shows the morphological definition of haemosiderin as large, densely packed aggregates of iron cores. The insert at high power shows that these aggregates were bounded by a membrane, presumably lysosomal.⁷ Individual ferritin cores were seen throughout the cytoplasm.

To permit closer inspection of the cores of haemosiderin, granules were isolated, solubilised, and examined unstained (Fig. 2). Comparison with ferritin (Fig. 3) showed clearly that the cores of haemosiderin were smaller and more irregular in shape, confirming the results of Fischbach *et al.*⁸ This observation is not an artefact of the method for solubilisation: inspection of a suspension of haemosiderin in water showed mostly large aggregates, but well separated cores could be clearly seen (Fig. 4). Fig. 5 shows the distributions of core size estimated from unstained electron micrographs of ferritin and haemosiderin.

The diameter of average volume (assuming spherical shape) for haemosiderin was about 5.7 nm, compared with 6.4 nm for ferritin, indicating that the cores of haemosiderin were, on average, 70% of the volume of ferritin cores. The distribution of the core size of haemosiderin was broader and lacking as clear a cut off (above 7.0 nm) as ferritin. There were many cores in haemosiderin below 5 nm in diameter, whereas there were very few such cores in ferritin, which gave the distribution of particle size in haemosiderin a strong asymmetric and almost bimodal appearance. Such a wide distribution could result from progressive partial chemical degradation of ferritin cores to form haemosiderin, but with a continuous addition of undegraded ferritin providing the larger cores. This, of course, implies that iron is being released from haemosiderin in the lysosome in the form of soluble complexes, which could therefore implicate haemosiderin in the peroxidation of iron catalysed lipid observed in iron overload⁹⁻¹¹ and possibly in the disruption of lysosomes after autolysis.^{12,13}

We have shown that the cores of haemosiderin are smaller and more aggregated than those of ferritin. Both of these features may be a direct result of degradation of the protein shell of ferritin as this would allow greater access for reducing or chelating agents and would also cause self association of cores. Negative staining of haemosiderin shows incomplete

Fig. 1 Haemosiderin granules seen as aggregates of cores packed together to varying degrees within section of human spleen loaded with iron and stained with uranyl acetate and lead citrate. $\times 7980$. Insert of high power shows that individual cores of ferritin (diameter ≈ 7 nm) (arrowed) are scattered through cytoplasm of spleen.

Fig. 2 Haemosiderin applied to grid as solution in tetramethylammonium hydroxide. $\times 255\ 000$.

Fig. 3 Ferritin, isolated from same spleen as haemosiderin in Fig. 2, applied as an aqueous solution to grid. $\times 255\ 000$.

Fig. 4 Haemosiderin applied to grid as water suspension. $\times 255\ 000$.

Fig. 5 Distributions of frequency and volume of haemosiderin in tetramethylammonium hydroxide solution (filled circles) and ferritin (open circles).

Fig. 6 Negatively stained haemosiderin. Variable presence of non-staining material around cores is indicated by arrows. $\times 255\ 000$.

Fig. 7 Negatively stained ferritin showing uniform, complete non-staining material around iron cores. Iron cores also have larger diameter than those of haemosiderin shown in Fig. 6. $\times 255\ 000$.

rings of protein around the cores (Fig. 6) compared with the complete protein shell of ferritin (Fig. 7), which agrees with other evidence that this protein is derived from ferritin.²

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