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. 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. Its lysosomal location, radioactive iron pulse-chase experiments, and close physiochemical similarities with both haemosiderin crystallites and ferritin crystallites 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 haemosiderin 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 solubilised by centrifugation through concentrated potassium iodide solutions and was solubilised in 0.02 M 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.

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 K. Samples of haemosiderin for negative staining were applied as a sonicated suspension in water and then stained with potassium phos-photungstate, 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 phos-photungstate, 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 spher- 
ical 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 peroxida-
tion of iron catalysed lipid observed in iron over-
load10-11 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. x 7980. Insert of high power shows that individual 
cores of ferritin (diameter a 7 nm) (arrowed) are scattered through cytoplasm of 
spleen.
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**Fig. 2** Haemosiderin applied to grid as solution in tetramethylammonium hydroxide. × 255 000.

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

**Fig. 4** Haemosiderin applied to grid as water suspension. × 255 000.

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


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