Volume 234, number 1, 69–72 FEB 06003 July 1988

Structural specificity of haemosiderin iron cores in iron-overload diseases

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Received 26 February 1988; revised version received 27 April 1988

Haemosiderin iron cores isolated from patients with secondary haemochromatosis have a goethite-like (α-FeOOH) crystal structure whereas those from patients with primary haemochromatosis are amorphous Fe (III) oxide. Haemosiderin cores isolated from normal human spleen are crystalline ferrihydrite ($5Fe_2O_3 \cdot 9H_2O$). The disease-specific structures are significantly different from the ferrihydrite structure of associated ferritin cores. The results are important in understanding the biological processing of iron in pathological states and in the clinical treatment of iron-overload diseases.

Hemosiderin; Iron-overload; Iron-core structure

1. INTRODUCTION

The iron storage proteins, ferritin and haemosiderin, consist of a non-haem iron complex associated with multiple polypeptides. Whereas both the iron complex and the protein constituents of ferritin have been extensively studied [1,2], the chemical and structural nature of haemosiderin remains to be determined even though this protein is of fundamental importance in various iron-overload diseases [3]. We report here, for the first time, the structural identification of the haemosiderin iron cores isolated from patients with primary (PH) (idiopathic haemochromatosis) and secondary haemochromatosis (SH) due to β-thalassemia treated by multiple transfusions. Our results show that the structure of the haemosiderin iron complexes is disease-specific and different from the ferrihydrite ($5Fe_2O_3 \cdot 9H_2O$) structure of associated ferritin cores. These results are important in understanding the biochemical mechanisms of iron processing in pathological states and in the clinical treatment of these diseases.

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2. EXPERIMENTAL

Haemosiderin was isolated from two patient samples of PH iron-overloaded liver and from the livers or spleens of three patients with SH using methods described [5]. Ferritin was isolated from these tissues by conventional procedures [6]. Electron microscopy and selected area electron diffraction were undertaken using a Jeol 2000FX transmission electron microscope operating at 200 keV. ^{57}Fe Mössbauer spectra were recorded on a conventional constant acceleration spectrometer using a ^{57}Co in rhodium source. Protein and iron concentrations were determined by the method of Lowry et al. [12] and by flameless atomic absorption spectroscopy, respectively. Peptide analysis was undertaken using SDS-polyacrylamide gel electrophoresis (SDS-PAGE). The solubility of the iron cores in the presence of oxalate was determined by dialysis of matched Fe concentrations (80 μmol/l) against 0.2 M ammonium oxalate/oxalic acid at pH 3.0 and 25°C. The rate of Fe dissolution was determined by assaying the iron in the dialysate after various times by electrothermal atomic absorption spectroscopy.

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00145793/88/$3.50

3. RESULTS

Iron/protein wt/wt ratios in the haemosiderins were 0.43 (PH) and 0.40 (SH). Peptide analysis of the haemosiderins by SDS-PAGE showed major bands of approx. 20 kDa (PH) and 15 kDa (SH). Although the 20 kDa band was similar to that observed for the ferritin peptides, the ultrastructure of the PH-derived haemosiderin, as shown by electron microscopy of the unstained protein, was clearly different from the discrete cores of ferritin isolated from the same tissue. The haemosiderin cores were of low electron density, extensively aggregated and irregular in morphology (fig.1a). In contrast, haemosiderin cores isolated from both the liver and spleen of SH patients were well-defined angular particles (fig.1b) which were less aggregated than the PH-derived material but less discrete than the associated ferritin cores.

The structural nature of the haemosiderin and ferritin cores was determined by electron diffraction and ^{57}Fe Mössbauer spectroscopy. Ferritin cores from all the samples studied showed ferrihydrite electron diffraction patterns (PH ferritin cores were generally less crystalline). Diffraction patterns of PH-derived liver haemosiderin showed only minimal evidence for long-range order; the one distinct diffraction line at 2.49 Å and two broadened lines centred around 2.12 Å and 1.53 Å (table 1) indicated that the material was primarily amorphous Fe(III) oxide with some partial ordering based on the ferrihydrite structure. In contrast, the electron diffraction patterns of both liver and spleen SH haemosiderin showed eight or nine diffraction lines. Although many of these lines could be assigned to either ferrihydrite or goethite (α-FeOOH), the diffraction lines at ~4.2 and 2.7 Å provided unequivocal evidence for the presence of goethite. However, some of the weaker goethite lines were absent, indicating that the SH haemosiderin was not perfectly ordered stoichiometric goethite but a defect microcrystalline phase.

The ^{57}Fe Mössbauer spectra of liver PH haemosiderin showed a predominant central doublet down to 4.2 K with substantial magnetically split six-line components only occurring below this temperature. This spectral behaviour combined with the isomer shift and quadrupole splitting parameters at 0.50 and 0.67 $m \cdot ms^{-1}$, respectively, is characteristic of a magnetically disordered high spin Fe(III) material and is consistent with the electron diffraction results. The distinct difference between the Mössbauer spectra of PH- and SH-derived haemosiderins can be seen in fig.2, which shows their 4.2 K spectra. The SH haemosiderin from both liver and spleen showed only a six-line magnetically ordered high spin Fe(III) spectral component at this temperature compared with the

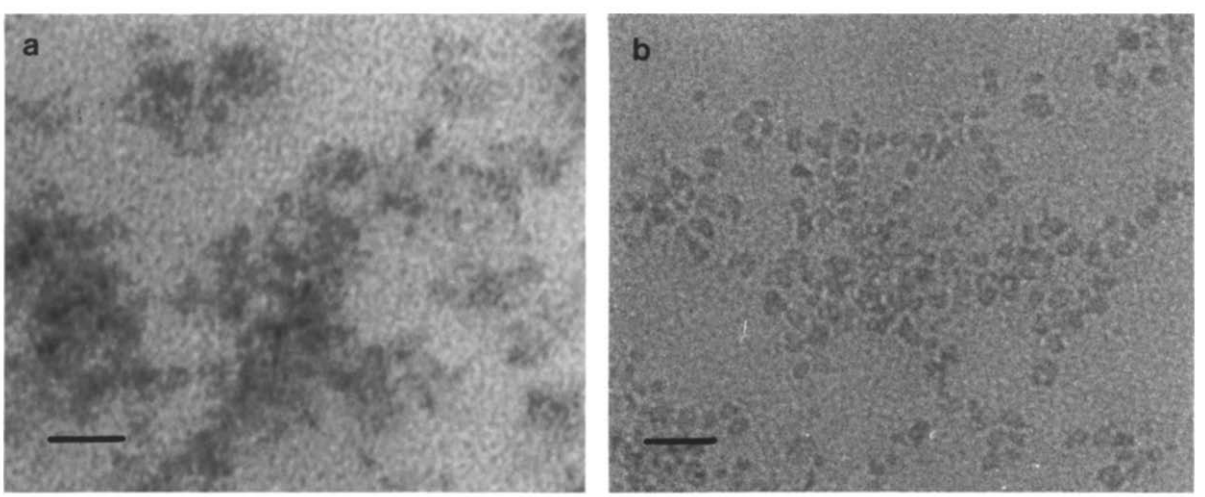

Fig.1. Transmission electron micrographs of (a) primary haemochromatosis liver haemosiderin (mean particle size = 5.8 nm; range, 5.36–6.31 nm) and (b) secondary haemochromatosis spleen haemosiderin (mean particle size = 5.6 nm; range, 4.33–6.50 nm). Scale bars = 20 nm.

Table 1

Electron diffraction data (d spacings (Å) and relative intensities) for haemosiderin isolated from iron-overloaded tissue

Primary haemochromatosis in liver	Secondary haemochromatosis			Ferritin (liver/spleen)
	Liver	Spleen		
	4.13 M	4.25 M	G	
	2.65 M	2.68 M	G	
2.49 S F	2.45 S	2.45 S	G/F	2.47 S F
	2.22 S	2.20 S	G/F	2.22 S F
2.12 B F	1.97 W	1.99 W	G/F	1.93 M F
	1.72 S	1.71 S	G/F	1.71 W F
		1.54 W	G	
1.53 B F	1.49 W	1.47 W	G/F	1.48 W F
	1.46 S	1.43 S	G	

Intensities: S, strong; M, medium; W, weak; B, broad band. Assignments made on the basis of known standard d spacings; G, goethite; F, ferrihydrite. Data for ferritin are shown for comparison

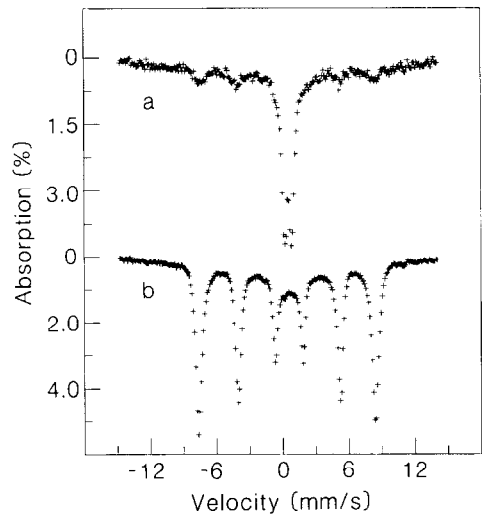

Fig.2. ^{57}Fe Mössbauer spectra recorded at 4.2 K of (a) primary haemochromatosis liver haemosiderin and (b) secondary haemochromatosis liver haemosiderin.

predominant doublet of the PH sample. The temperature dependence of the SH haemosiderin spectra (data not shown) was characteristic of a single Fe-containing phase consisting of small, magnetically ordered particles. This behaviour was qualitatively identical to that observed for the ferritin samples but the temperature at which the doublet and sextet components were equal in spectral intensity was approximately 65 K for the SH haemosiderin compared with 40 K for human ferritin [4], 28 K for horse haemosiderin [14] and 23 K for rat haemosiderin [7].

Dialysis of the protein cores against oxalate gave the following order in decreasing solubility: PH haemosiderin > PH ferritin > SH ferritin > SH haemosiderin (fig.3). Oxalate buffer was used as it is a standard analytical method for the preferential dissolution of mineralogical ferrihydrite over goethite and other iron oxides [8]. Similar results were obtained for dissolution experiments using clinically important chelators such as desferrioxamine (O'Connell, M., unpublished data).

The structural specificity of haemosiderin iron cores in PH and SH has important biochemical and biomedical implications. The general consensus in the literature is that haemosiderin is derived from ferritin by lysosomal degradation of its protein shell. For example, both horse, normal human (Dickson, D.P.E., unpublished data) [9,14] and rat [7,13] haemosiderins have structures and blocking temperatures consistent with ferrihydrite particles of smaller size than ferritin cores. However, our results indicate that this direct relationship does not apply to human haemosiderin and ferritin cores derived from iron-overloaded tissue. PH-derived liver haemosiderin arises from the uptake and storage of iron by the hepatocytes under conditions of enhanced absorption of dietary iron. The disordered nature of these cores indicates rapid intracellular iron precipitation presumably due to high local iron levels; consistent with this hypothesis is the poorly ordered ferrihydrite cores of the associated liver ferritin, and the presence of amorphous Fe(III) deposits in rat myocardial cells subjected to high iron concentrations in the growth media [10]. The major peptide band at 20 kDa and the extensive aggregation of the cores as viewed in the electron microscope suggest that although the protein is ferritin-like it is disorganised around and between the mineral particles. One possibility is that iron uptake by the hepatocytes is at such a high rate that non-specific deposition takes place external to apoferritin resulting in extensive protein-mineral aggregates.

The goethite-like structure of both liver and spleen SH haemosiderin cores is more ther-

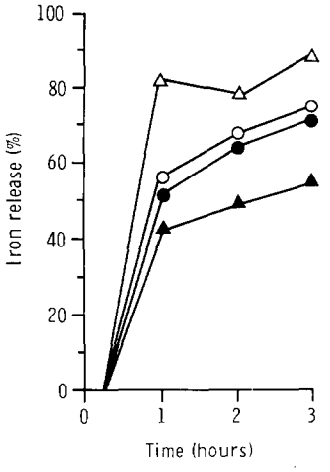

Fig.3. Dissolution of iron from iron-storage proteins. Primary haemochromatosis haemosiderin and ferritin (△,○) and secondary haemochromatosis haemosiderin and ferritin (▲,●), respectively.

modynamically stable than the ferrihydrite cores of ferritin, which suggests that the formation of these haemosiderin complexes takes place under conditions of slow crystallization, for example, from the slow oxidation of aqueous Fe(II) ions arising from low Fe(II) and O_2 concentrations, complexation, and low lysosomal pH. Alternatively, the slow degradation of Fe(III) complexes could favour goethite precipitation. Significantly, the in situ phase transformation of ferritin cores is not possible because the structures of goethite and ferrihydrite are not closely related. If ferritin is to be implicated, therefore, in the formation of SH haemosiderin it must be via chemical modification of the ferrihydrite cores. Since the source of iron in transfusional iron overload is from effete erythrocytes, the rate of processing of this iron by the reticuloendothelial (RE) system is likely to be relatively slow when compared with the dietary iron-overload of PH. Thus there is the possibility of normal ferritin uptake of iron followed by protein degradation (as shown by the 15 kDa band in SH haemosiderin) and (partial) dissolution of the ferritin cores in the lysosomes of the RE cells. The slow reprecipitation of the soluble iron within the lysosomes rather than mobilization via transferrin may reflect the saturation of this protein in the iron-overloaded system.

Finally, our results predict that the more crystalline goethite-like deposits of SH haemosiderin will be less labile than the corresponding deposits in PH and will therefore have a lower intrinsic toxicity as shown, for example, in the ability to mediate lipid peroxidation in the presence of ascorbate [11]. Also, the lower solubility of SH haemosiderin cores implies that they will be less readily removed by chelation therapy than predicted on the basis of ferrihydrite models. Furthermore, the results explain the anomalous magnetic properties of haemosiderin cores isolated from patients with SH [4].

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