Biol Metals (1988) 1:33–42

BIOLOGY OF METALS

Studies on haemosiderin and ferritin from iron-loaded rat liver

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Summary. Haemosiderin has been isolated from siderosomes and ferritin from the cytosol of livers of rats iron-loaded by intraperitoneal injections of iron-dextran. Siderosomal haermosiderin, like ferritin, was shown by electron diffraction to contain iron mainly in the form of small particles of ferrihydrite ($5Fe_2O_3 \cdot 9H_2O$), with average particle diameter of 5.36 ± 1.31 nm (SD), less than that of ferritin iron-cores (6.14 ± 1.18 nm). Mössbauer spectra of both iron-storage complexes are also similar, except that the blocking temperature, T_B, for haemosiderin (23 K) is lower than that of ferritin (35 K). These values are consistent with their differences in particle volumes assuming identical magnetic anisotropy constants. Measurements of P/Fe ratios by electron probe microanalysis showed the presence of phosphorus in rat liver haemosiderin, but much of it was lost on extensive dialysis. The presence of peptides reacting with anti-ferritin antisera and the similarities in the structures of their iron components are consistent with the view that rat liver haemosiderin arises by degradation of ferritin polypeptides, but its peptide pattern is different from that found in human β-thalassaemia haemosiderin. The blocking temperature, 35 K, for rat liver ferritin is near to that reported, 40 K, for human β-thalassaemia spleen ferritin. However, the haemosiderin isolated from this tissue, in contrast to that from rat liver, had a T_B higher than that of ferritin. The iron availability of haemosiderins from rat liver and human β-thalassaemic spleen to a hydroxypyridinone chelator also differed. That from rat liver was equal to or greater, and that from human spleen was markedly less, than the iron availability from either of the associated ferritins, which were equivalent. The differences in properties of the two types of haemosiderin may reflect their origins from primary or secondary iron overload and differences in the duration of the overload.

Key words: Haemosiderin — Ferritin — Iron — Rat liver

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Introduction

Iron is stored in the liver, spleen and other organs in two forms, ferritin and haemosiderin (Wixom et al. 1980; Theil 1987). Both contain ferric iron as small, crystalline, electron microscopically visible mineral particles (Iancu 1983; Richter 1984; Mann et al. 1986) composed of a hydrous ferric oxide often associated with inorganic phosphate. In ferritin these particles are deposited as the mineral ferrihydrite ($5Fe_2O_3 \cdot 9H_2O$) inside a well-defined protein shell comprising 24 polypeptide chains (Ford et al. 1984; Harrison et al. 1987). Ferritin is thus a water-soluble molecule. Haemosiderin, in contrast, is insoluble in aqueous media (McKay and Fineberg 1964a), although it can be solubilized by treatment with detergent (Weir et al. 1984). It is found as massive aggregates of electron-dense particles often surrounded by membrane in a body known as a siderosome (an iron-laden lysosome). The average particle size of human haemosiderin isolated from β-thalassaemia spleen is smaller than that of ferritin "iron-cores" from the same tissue (Mann et al. 1986). A number of organic constituents are found associated with the haemosiderin iron mineral (McKay and Fineberg 1964b; Weir et al. 1984). In human β-thalassaemia spleen haemosiderin these constituents included peptides considered to have been

derived from ferritin (Weir et al. 1984). It has been concluded from these observations, and from the similarities in the X-ray and electron diffraction patterns and the electron microscopic appearance of their iron components, that haemosiderin, whether from horse (Fischbach et al. 1971) or human (Mann et al. 1986) spleen, is a degradation product of ferritin. There have been two observations, however, that are inconsistent with this hypothesis. Both haemosiderins show Mössbauer spectra, which, as the temperature is lowered, change from a quadrupole doublet spectrum to a magnetically split sextet spectrum, but the temperature range over which this change occurs is higher for haemosiderin from human thalassaemic spleen than for ferritin from the same tissue (Bell et al. 1984). The theory of superparamagnetism predicts that for materials with the same atomic structure but smaller particle size, the doublet-to-sextet transition occurs at a lower temperature. Secondly iron is released less readily from β-thalassaemia haemosiderin than from ferritin when the two are treated with ascorbate (O'Connell et al. 1985, 1986). Both these observations suggest that human β-thalassaemia haemosiderin iron differs in structure and composition from the ferrihydrite mineral of ferritin. This raises the possibility that human β-thalassaemia haemosiderin is not derived directly from ferritin or that it has been altered after its separation from ferritin molecules.

In the work described here we have used a rat model, iron-loaded by intraperitoneal injection of iron-dextran, to investigate the origin of haemosiderin and have carried out the first detailed study of haemosiderin isolated directly from siderosomes. Our data are consistent with the view that haemosiderin, formed under experimental conditions, is derived from ferritin. Rat liver haemosiderin differs in some of its physical and chemical properties from those reported for haemosiderin isolated from human thalassaemia spleen. Hence the term "haemosiderin" (originally given to indicate its origin from blood) should not be used without specifying the tissue or cell types from which it is isolated and the form of iron-loading from which it has arisen.

Materials and methods

Materials. Iron-dextran (Imferon) was obtained from Fisons Ltd (Leicester, UK). Haemosiderin from human β-thalassaemic spleen was a gift from Prof. T. J. Peters (Clinical Research Centre, Harrow) and 1,2-dimethyl-3-hydroxy-pyridin-4-one a gift from Prof. R. C. Hider (King's College, London). Peptides used for production of antisera were kindly synthesised by Dr. J. E. Fox of Birmingham University. All other chemcials were of the highest grade available.

Animals. Rats were female Wistars weighing 190–210 g. They were fed ad libitum except that prior to slaughter they were fasted overnight with access to water. Each rat was loaded with a single intraperitoneal injection of 1.2 ml iron-dextran (60 mg iron).

Isolation of rat liver siderosomes. Animals were killed in groups of three 14–28 days after iron-loading and siderosomes purified from homogenized livers by centrifugation through discontinuous sucrose gradients as described by Richter (1984).

Purification of haemosiderin and ferritin. Siderosomes were subjected to osmotic shock and ferritin and haemosiderin purified from the soluble and insoluble fractions respectively as described in Richter (1984). After a purification step involving centrifugation through 4.1 M KI (McKay and Fineberg 1964a), haemosiderin was suspended in water and repelleted. It was then sonicated and dialysed against distilled water. Freeze-dried haemosiderin was dissolved by sonication in 10 mM tetramethyl ammonium hydroxide plus 1% (wt/vol) Nonidet-P40 (Weir et al. 1984). Ferritin was purified from homogenized tissue as described in Andrews et al. (1987a).

Electron microscopy. Unstained ferritin, solubilized haemosiderin and iron dextran were air-dried onto carbon-coated Formvar films supported by copper mesh electron microscopy grids and examined in a Jeol 100CX analytical electron microscope operated at 100 keV. Approximately 70 iron-cores were measured for particle size determinations in low-magnification transmission electron microscope (TEM) micrographs. Dense areas were used for selected area diffraction in order to maximize intensities.

Mössbauer spectroscopy. ^{57}Fe Mössbauer spectra were obtained with a constant acceleration-mode spectrometer calibrated by means of the accurately known room temperature spectrum of metallic iron. The source was 10 mCi ^{57}Co in rhodium, used at room temperature. The data were collected by means of a 1024-channel analyser operating in the time mode. An Oxford Instruments continuous-flow helium cryostat was used to control temperatures over the range 5 K to room temperature.

P/Fe ratios by electron probe microanalysis. Aliquots (25 µl) of each of the samples were dried on carbonized pioloform films mounted on 70-mesh copper grids and analysed in a Philips EM400 analytical electron microscope operating at 80 kV with a LaB_6 electron source and equipped with a Tracor Northern-type TN2000 X-ray microanalyser. Multiple point analyses were performed at random over the grid squares essentially as decribed in (Cleton et al. 1986). Peaks in the recorded spectra were identified by the available peak identification program. Net-intensity values for iron and phosphorus were obtained by the introduction of 400-eV-wide regions around the identified peaks and calculation by the energy filter program of the TN2000 computer software. Iron/phosphorus mass ratios were calculated from net intensities by the peak-ratio program and converted to atomic ratios.

Protein and iron determination. Protein concentration was determined by the method of Lowry and iron as the Fe(II)-bipyridine complex as described by Drysdale and Munro (1965).

Gel electrophoresis. Electrophoresis of haemosiderin was carried out by 20% sodium dodecyl sulphate/polyacrylamide gel electrophoresis (SDS-PAGE) using double the concentration of Tris in the separating gel and the running buffer as described by Fling et al. (1986). Samples for immunoblots were applied to 2.4-cm-wide wells.

Immunoblotting. After electrophoresis each well was individually transferred electrophoretically to nitrocellulose membranes and the membranes blocked according to Blake et al. (1984). Each membrane was then cut into 4-mm-wide strips which were incubated with different antisera. The immunoblots were developed using anti-(rabbit IgG) conjugated to alkaline phosphatase.

Mobilization of iron(III). Ferritins and solubilized haemosiderin were incubated at constant concentration of iron (450 μM) with a 15-fold molar excess of 1,2-dimethyl-3-hydroxypyridin-4-one n 0.1 M Hepes buffer, pH 7.0, at 37° C. Three 1-ml samples were removed at 5-h and 24-h and immediately centrifuged through Amicon Centriflo ultrafiltration cones to separate the iron(III)-chelate complex. Measured volumes of ultrafiltrates were boiled in sealed Eppendorf tubes with excess of acidic sodium sulphite to reduce the iron, together with 2,2′-bipyridine; after cooling, the absorbance of the Fe(II)-bipyridine complexes were measured at 520 nm. Results were expressed as percentages of original iron mobilized. The cytosolic rat liver ferritin used had approximately 3000 Fe atoms/molecule and the human β-thalassaemic spleen ferritin approximately 3400 Fe atoms/molecule.

Production of antisera. Antisera to rat liver ferritin were produced in rabbits as described by Treffry et al. (1984). Synthetic peptides used for immunization, taken from specified regions of the amino acid sequences of rat and human ferritins, were coupled to bovine serum albumin (BSA) either with glutaraldehyde (Walter et al. 1980) or *m*-maleimidobenzoyl-*N*-hydroxysuccinimide ester (Liu et al. 1979). Rabbits were injected with 1 mg BSA-peptide in Freund's complete adjuvant followed at 14-day intervals by two further injections of 1 mg peptide-BSA in 50% incomplete Freund's adjuvant. Rabbits were bled 1 week after the third injection and at monthly intervals thereafter following booster injections.

Results

Electron microscopy and electron diffraction

Purified rat liver ferritin and siderosomal haemosiderin (solubilized according to Weir et al. 1984), when spread onto grids and examined by electron microscopy, were very similar to preparations previously isolated from horse spleen (Fischbach et al. 1971) or human spleen (Mann et al. 1986). The ferritin iron-cores appeared as discrete, electron-dense particles, whereas the haemosiderin particles, even though solubilized with detergent, were more clumped and difficult to resolve from one another. Particle size distributions are shown in Fig. 1. The average diameters found are 5.36 ± 1.31 nm (SD) for haemosiderin and 6.14 ± 1.18 nm for ferritin iron-cores. Assuming spherical particles, the average volumes are 80.6 nm^3 and 121 nm^3 for rat liver haemosiderin and ferritin, respectively. The injected iron-dextran could easily be distinguished electron microscopically from ferritin and haemosiderin. This was also shown by electron diffraction (Table 1). The iron-dextran gave diffraction lines at interplanar spacings similar to those of β-FeOOH as previously reported by Towe (1981). The diffraction lines of rat liver haemosiderin and ferritin are similar to those of ferrihydrite and again like those of ferritin and haemosiderin from horse (Fischbach et al. 1971) and human (Mann et al. 1986) spleen, although the iron-cores were less well ordered than those from the other two species and also than those of cytosolic ferritin from control rats.

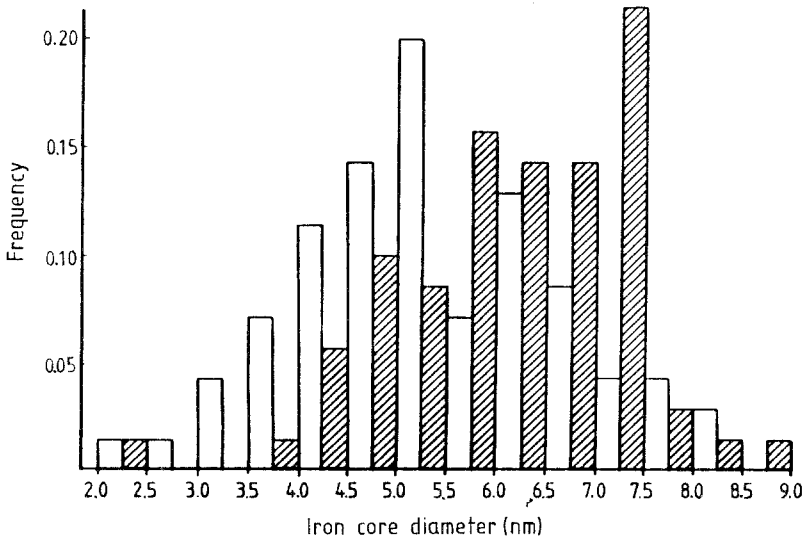

Fig. 1. Particle size distributions for siderosomal haemosiderin (□) and cytosolic ferritin (▨) from iron-loaded rat liver

Table 1. Interplanar spacings derived from electron diffraction for ferritin from normal and iron-loaded rat livers, rat liver siderosomal haemosiderin and iron-dextran

Iron-dextran (Imferon)		Reference β-FeOOH		Normal ferritin		Iron-loaded ferritin		Siderosomal haemosiderin		Reference ferrihydrite	
d_0 (nm)	I_0	d_0 (nm)	I_0	d_0 (nm)	I_0	d_0 (nm)	I_0	d_0 (nm)	I_0	d_0 (nm)	I_0
0.338	S	0.330	S								
				0.259	S	Uncertain		0.250	S	0.254	S
				0.222	S	0.224	S	0.229	M	0.224	S
0.210	MS	0.210									
				0.202	S	0.197	M	0.200	M	0.198	M
0.174	W	0.174	W								
				0.179	M	Uncertain		0.173	W	0.173	W
				0.153	W	0.150	W	0.153	M	0.152	MW
				0.149	S	0.146	S	0.144	M	0.147	S

d_0 = observed d-spacing; uncertain = not clear above background, diffuse; I_0 = observed intensity, visually estimated (S, strong; M, medium; W, weak). Results for samples labelled "reference" were obtained from Towe (1981)

Mössbauer spectroscopy

Mössbauer spectra of rat liver haemosiderin, ferritin and iron-dextran are shown at several temperatures in Fig. 2 and hyperfine parameters obtained from these spectra are given in Table 2. The latter show that the iron environments in the three complexes are closely similar. All three complexes also give a six-line magnetic spectrum at low temperatures and a gradual transition to quadruple doublet spectra as the temperature is raised. At intermediate temperatures the two types of spectrum coexist. The transition temperature or blocking temperature, T_B, is the temperature at which the six-line spectrum is replaced by the doublet spectrum and is defined by

$$KV = kT_B$$

where V is the particle volume, K the magnetic anisotropy constant and k is the Boltzmann constant. Where there is a range of particle volumes, the transition takes place over a wider temperature range and the average T_B is then conveniently defined as the temperature at which the sextet and doublet components are of equal intensity (St. Pierre et al. 1986; Mann et al. 1987). For substances having the same magnetic anisotropy constant T_B is proportional to volume. T_B values for haemosiderin and ferritin from iron-loaded rat livers were found to be 23 K and 35 K respectively. The ratio of these values, namely 0.714, is close to the ratio of volumes, 0.666, derived from the electron microscope measurements. This suggests that the anisotropy constants are the same and this would be expected for materials of the same crystal structure (as shown by the electron diffraction patterns) provided no shape or other magnetic distortions contributed to K.

Phosphorus/iron ratios

The compositions of ferritin iron-cores in terms of the ratios of phosphorus or phosphate to iron reported in the literature are quite variable (Treffry et al. 1987). In the present study phosphate-buffered saline (PBS) was used in the isolation of fer-

Table 2. Mössbauer hyperfine parmeters for rat liver ferritin and siderosomal haemosiderin and for iron-dextran at the temperatures indicated

Compound	Magnetic hyperfine field (T)	Isomer shift/Fe metal (mm s^{-1})	Quadrupole splitting (mm s^{-1})
Ferritin	0.41 ± 0.01 (300 K)	0.70 ± 0.02 (300 K)	49.1 ± 0.5 (14 K)
Haemosiderin	0.39 ± 0.01 (300 K)	0.65 ± 0.02 (300 K)	48.9 ± 0.5 (14 K)
Iron-dextran	0.39 ± 0.01 (300 K)	0.67 ± 0.02 (300 K)	48.8 ± 0.5 (5 K)

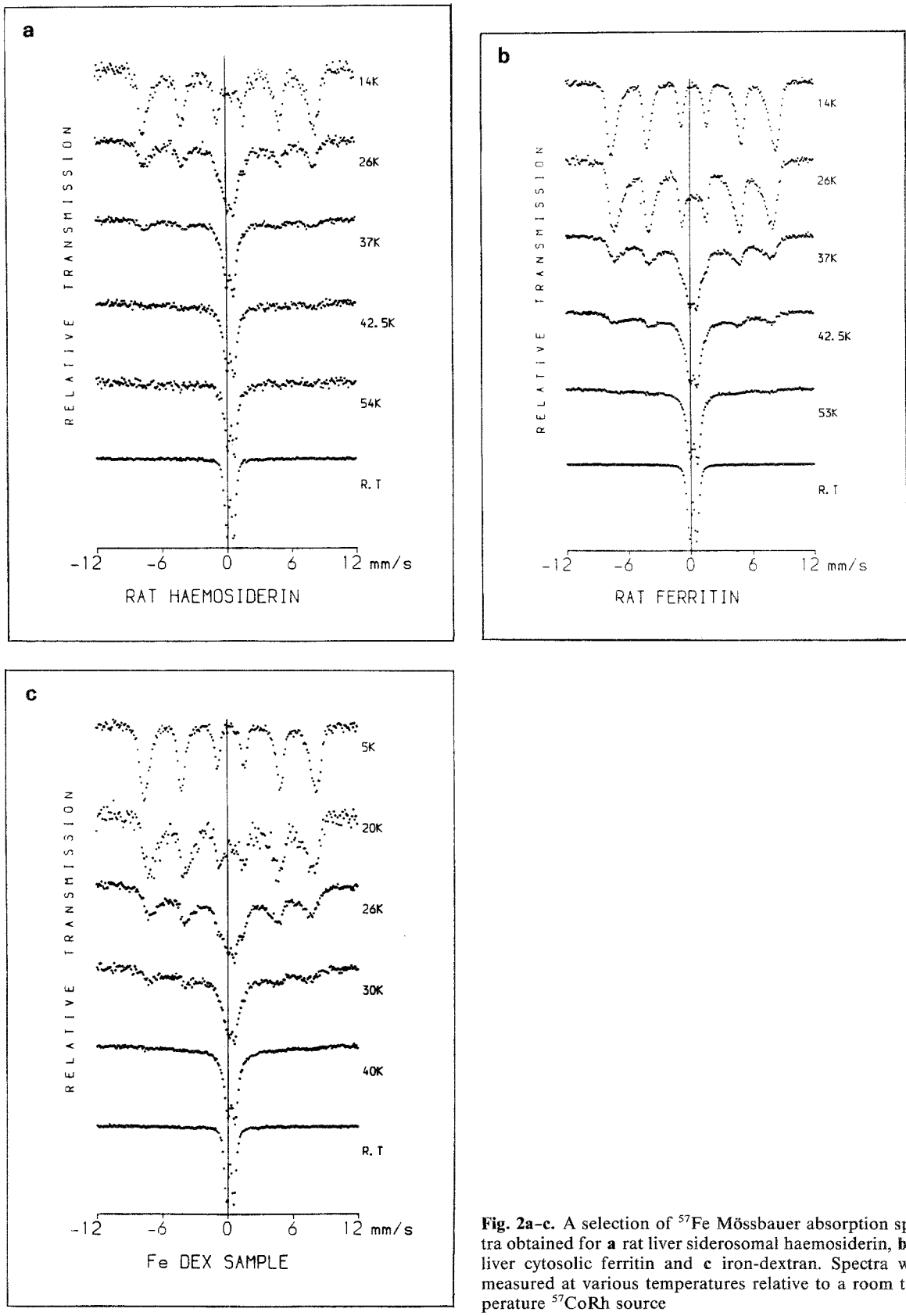

Fig. 2a–c. A selection of ^{57}Fe Mössbauer absorption spectra obtained for **a** rat liver siderosomal haemosiderin, **b** rat liver cytosolic ferritin and **c** iron-dextran. Spectra were measured at various temperatures relative to a room temperature ^{57}CoRh source

Table 3. Phosphorus/iron atomic ratios of iron-loaded rat liver ferritin and haemosiderin measured by electron probe microanalysis

Substance		P/Fe ratios after dialysis for			
		24 h		5 days	
Cytosolic ferritin		1:7.0±1.1	(13)	1:36.3±21.1	(14)
Siderosomal ferritin		1:7.8±1.4	(10)	no P detected	(5)
Haemosiderin	insoluble clumps	1:8.1±1.3	(5)	1:16	(1)
	suspension	1:3.1±0.44	(12)	1:28.3±16.4	(8)

The samples were examined after short (24 h) and longer (5 days) periods of dialysis against distilled water. Phosphorus was near the limits of detectability in several but not all of the latter samples, leading to high overall standard deviations. The numbers of determinations are given in *parentheses*

ritin (as is frequently done) but not of haemosiderin. Samples were examined after a 24-h dialysis and again after dialysis for several days against distilled water, in both cases with frequent changes of water. Phosphorus/iron ratios measured by electron probe microanalysis (EPMA) are given in Table 3. In this table we include data for soluble siderosomal ferritin isolated from siderosomes, but the amount of this ferritin was insufficient for Mössbauer studies. After sonication, part of the haemosiderin was brought into suspension, so two samples were examined, the suspension and the insoluble clumps. EPMA data for the latter are considered to be less reliable because of the difficulty of spreading them uniformly on the electron microscope grids. Following extensive dialysis, the amounts of phosphate were near the limit of detectability in cytosolic and siderosomal ferritin and in the haemosiderin suspension. We cannot say whether these very low values, or those obtained after short dialysis, represent the in vivo values. Significant amounts of phosphorus were found in both 24-h dialysis haemosiderin samples, however, and these may indicate in vivo levels. It is conceivable that the larger P/Fe ratio (1:3.1) found in the suspension fraction (which gave very consistent measurements) may have been associated with particles of smaller than average size and high surface phosphate may even have aided their suspension. We cannot, however, be certain that the difference between the two fractions is significant. What is clear is that considerable amounts of phosphorus (relative to iron) were lost from this haemosiderin during dialysis. That this did not happen to any extent with the insoluble fraction may have been because the aggregates were too large for efficient dialysis. In two other samples we examined, both of which had been pelleted and resuspended in water but not further dialysed, we obtained P/Fe ratios of 1:16.1±1.7 (13) and 1:10.8±1.0 (10). The second of these ratios was for a sample that had been repelleted, freeze-dried and solubilized with detergent.

Iron mobilization from haemosiderin and ferritin

Table 4 shows the percentage of iron(III) removed from rat liver and human β-thalassaemia spleen ferritin and detergent-solubilized haemosiderins by the action of the chelator 1,2-dimethyl-3-hydroxypyridine-4-one [which has a high affinity for Fe(III), $k_1 = 35.6$] after incubation for 5 h and 24 h at pH 7.2. A clear difference can be seen between the siderosomal haemosiderin isolated from iron-dextran-injected rat and the splenic haemosiderin from a thalassaemia patient. The re-

Table 4. Comparison of percentage iron(III) release values from rat and human haemosiderin and ferritin with 1,2-dimethyl-3-hydroxypyridin-4-one at pH 7.2 and 37°C

Time (h)	Ferritin		Haemosiderin	
	Rat	Human	Rat	Human
5	27.10±2.91	27.68±5.73	24.04±1.73	10.58±0.81
24	38.53±2.94	37.69±6.68	48.17±2.87	19.87±0.81

Results are the average of triplicate determinations, with root-mean-square (r.m.s.) deviation, expressed as percentage of initial iron

latively low availability to chelator of the human haemosiderin as compared to human spleen ferritin contrasts with the percentage iron released from rat liver haemosiderin which was equal to or greater than that from rat liver ferritin. There were no significant differences in iron-release values when rat and human ferritins were compared. Horse ferritin behaved similarly (data not shown).

Haemosiderin peptides

Immuno- and gold-staining patterns obtained from SDS-PAGE blots for rat liver siderosomal haemosiderin and ferritin and for cytosolic ferritin are shown in Fig. 3. Cytosolic ferritin gave a single approximately 20-kDa band, staining strongly with gold and with anti-(liver ferritin), that must correspond mainly to the L subunit (Arosio et al. 1978). As expected from previous work (Andrews et al. 1987a) siderosomal ferritin gave the L subunit band and a major approximately 17-kDa band. This band, which arises from a nick in the L subunit between residues 163 and 164 of the 182-amino-acid sequence (Andrews et al. 1987b), also stained with antisera to peptide H3, but not with antisera to peptides HL or H4. Peptide H3 comprises residues 116–125 that are identical in rat and human subunits. Peptides HL and H4 correspond respectively to sequences 83–91 and 151–162 of the human L subunit. The peptide patterns of rat haemosiderin were more complex. Anti-(rat ferritin) antiserum showed the presence of L subunit, F subunit and a band at approximately 7 kDa, confirming the presence of ferritin within the insoluble haemosiderin. Gold-staining showed this 7-kDa peptide, small amounts of F subunit and L subunit, several bands of relatively high molecular mass, and a major band at about 18 kDa not present in the soluble ferritins. This band stained intensively with HL and H4 antisera, as did the band at about 7 kDa and one at about 11 kDa.

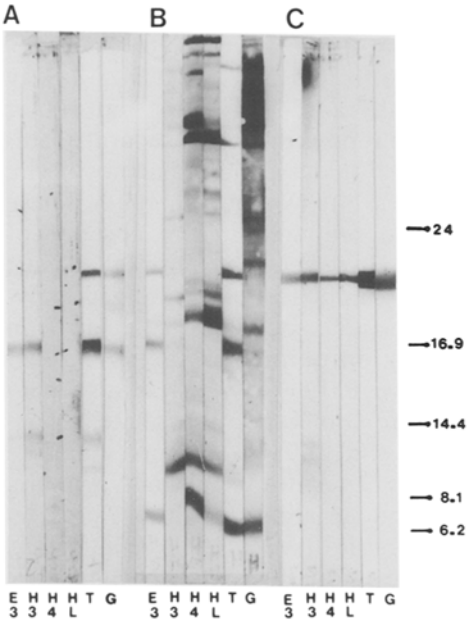

Fig. 3A–C. SDS-gel electrophoresis patterns of peptides in iron-loaded rat liver ferritins and haemosiderin. **A** Siderosomal ferritin; **B** haemosiderin; **C** cytosolic ferritin.
After electrophoresis the peptides were transferred to nitrocellulose membranes and reacted with the following: *G* gold stain; *T* rat liver ferritin antiserum; *H* antisera to peptides from human liver ferritin; *E* antiserum to peptides from horse spleen ferritin; *3, 4* and *L* stand for peptides from regions of the ferritin molecule respectively near the 3-fold or 4-fold channels or within the long inter-helical loop

Discussion

The finding that rat liver siderosomal haemosiderin can be distinguished from iron-dextran shows that we are not looking at unprocessed injected material which may have been engulfed by pinocytosis. In an earlier study, Richter (1959) reported that 2–3 weeks after a single intraperitoneal injection in mice of iron-dextran (50 mg Fe/mouse) most of the aggregated iron in macrophages and endothelial cells was indistinguishable from indigenous haemosiderin and that in liver parenchymal cells iron-dextran particles were also scarce and much of the iron was located in siderosomes. In a later study with rats (male animals injected intraperitoneally with either 25 or 50 mg Fe as iron-dextran) Richter (1984) reported that most of the material present in siderosomes, judged to be ferritin by its ultrastructural appearance and immunofluorescence behaviour, was water-insoluble. Although, in principle, the iron of iron-dextran could have been dissolved and converted to ferrihydrite without prior incorporation into ferritin molecules, our finding of ferritin subunits and peptides within siderosomal haemosiderin supports the hypothesis that haemosiderin arises by degradation of ferritin protein shells within the siderosome. It is not clear, however, why the average iron-core size in haemosiderin should be significantly less than that of cytosolic ferritin. It is possible that partial dissolution of the cores occurs during ferritin degradation or during isolation of the siderosomes. The solubilized haemosiderin spread onto elec-

tron microscope grids could conceivably have contained smaller than average particles, but the particle size is consistent with the Mössbauer blocking temperature of bulk haemosiderin. The average core volumes of haemosiderin and cytosolic ferritins are also in the same ratio as the average numbers of iron atoms per molecule found for siderosomal and cytosolic ferritin, namely 2170 ± 450 and 3050 ± 70 (Andrews et al. 1987a). This could mean that haemosiderin was derived directly from siderosomal ferritin or that both were independently processed to the same extent from cytosolic ferritin after it had entered the siderosomes.

Our results indicate significant differences in the properties of haemosiderin from rat liver and from β-thalassaemia patients, as judged by their Mössbauer blocking temperatures and iron availability. These differences had to some extent been anticipated by Rimbert et al. (1985) who had reported differences in the 77-K Mössbauer spectra of the livers of rats which had been iron-loaded by excessive absorption from carbonyl iron added to the diet and of patients with β-thalassaemia major who were regularly transfused. They suggested that the difference was due to the physiological nature of the iron overload, which was primary or secondary in origin, respectively. Their interpretation of the sextet spectrum, which could be seen at 77 K in the human haemosiderin, was that it resulted from the presence of an additional iron compound (called X). In contrast, the view of Bell et al. (1984) is that the haemosiderin they isolated from spleen had a higher T_B due to a larger magnetic anisotropy constant than ferritin, since the particle sizes observed in Mann et al. (1986) were 4.5–5.0 nm for haemosiderin and 5.5–6.0 nm for ferritin from thalassaemic spleen. If, however, haemosiderin contains ferritin iron-cores denuded of much of their protein and of smaller particle size, then haemosiderin iron should be more available than that of ferritin. In other experiments, we have found that ferritin iron-cores released from protein by treatment with 67% (vol/vol) acetic acid/water, followed by pepsin digestion at pH 2.4, yield as much or more iron than ferritin to a variety of hydroxypyridinone chelators at pH values in the range 5.2–8.2 (Brady et al. 1987). Here we observe that rat, but not human β-thalassaemic haemosiderin, shows this behaviour. The low availability to chelator of human haemosiderin iron parallels a similar effect with ascorbic acid and with 1,10-phenanthroline (O'Connell et al. 1985); it has led to the view that haemosiderin is a safer form of storage iron than that in ferritin. This does not seem to be the case with rat liver haemosiderin, which appears from this study to differ physically and chemically from human β-thalassaemia haemosiderin. The anomalous properties of human β-thalassaemia haemosiderin have recently been explained by analysis of electron diffraction patterns (Ward et al. 1988). The data clearly show that its mineral particles have a structure similar to goethite (α-FeOOH). The greater structural order in this mineral compared with ferrihydrite accounts both for the high T_B and for the low solubility of β-thalassaemia haemosiderin compared with ferritin. The differences in the types of haemosiderin may arise from differences in the way iron is delivered and deposited and the length of time in storage, both of which may also be related to tissue or cell types. In the present work we studied a mixed population of siderosomes that were present in all cell types, although they were particularly concentrated in Kupffer cells (Andrews 1986). The total or relative numbers in the various cell types of the liver was not determined.

The P/Fe ratios found for haemosiderin and ferritin (Table 3) suggest that such measurements should be viewed with caution. The values for the haemosiderin samples after short dialysis are similar to ratios reported for horse spleen haemosiderin, which range from 1:2.8 to 1:11.2 (McKay and Finberg 1964b) and similar to, but rather greater than, those reported for two separate samples of human β-thalassaemia haemosiderin, namely 1:6.0 and 1:14.9 (Weir et al. 1984). These samples were also prepared in the absence of phosphate buffer. However, the finding that most of the phosphorus can be lost on prolonged dialysis indicates a loose association with the iron complex, probably on particle surfaces. Another sample which gave P/Fe = 1:16.1 was also analysed chemically for inorganic phosphate, as described in Treffry et al. (1987), but none could be detected. Isolation of ferritin in PBS may explain, to some extent, the variability of these ratios reported in the literature (see Treffry et al. 1987). Very recently exceptionally high phosphate/Fe ratios of about 1:2 (associated with relatively high bound aluminium) have been found for ferritins isolated from human brain, liver and horse spleen (Fleming and Joshi 1987). However, these samples were also prepared in PBS and the ratios found may not reflect those of the indigenous molecules.

There are significant differences in peptide patterns between rat liver and human β-thalassaemia spleen haemosiderins. In the latter, traces

of a band corresponding to intact subunits were observed but the major component had a mass of 14.5 kDa (Weir et al. 1984). Immunoblots suggest this has arisen by scission of the L subunit at about 40 residues from its N-terminus (M. J. O'Connell, R. J. Ward, H. Baum, A. Treffry and T. J. Peters, unpublished work). A peptide of this size is not seen in rat liver siderosomal haemosiderin. The major gold- and immuno-staining peptides (that are smaller than intact subunits) are at about 18 kDa and 7 kDa. This may reflect differences in the amino acid sequences of the ferritin chains in the two species and hence in the sites of attack of radical species or degradative enzymes responsible for their breakdown. The immunoreactivity of the 18-kDa peptide to native rat liver ferritin antiserum is low. This could indicate that a major antigenic site, perhaps near the N-terminus of the molecule, has been removed but this would not explain its low reactivity to H3 antiserum. It reacts strongly to HL antiserum, raised to the inter-helical loop region of L subunits (residues 83–91) where rat and human L subunits differ by only two amino acid residues, and this suggests that it may be a ferritin L-chain peptide. If so, then it could not be a breakdown product of the smaller 17-kDa F subunit. This may mean either that the F subunits of siderosomal ferritin (making up 69% of the total; Andrews et al. 1987) are more readily degraded than the 18-kDa peptide, or that cytosolic ferritin is the source of this peptide and that siderosomal ferritin represents a different, partial degradation step.

Acknowledgements. We thank the Science and Engineering Research Council for a studentship (to S.C.A.) and the Wellcome Trust and the Nuffield Foundation for financial support.

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Received March 5, 1988