Variation in the Distribution of Two Human Heart Ferritin Species

ISOFERRITIN PROFILE AND SUBUNIT COMPOSITION IN NORMAL AND IRON-OVERLOADED SUBJECTS

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(Received 25 July 1977)

On polyacrylamide slab electrophoresis two ferritin species, termed fast and slow, were present in three control and four iron-loaded human hearts. The ratio of the fast to slow species varied between hearts and correlated with the distribution of isoferritins, which had pI values ranging from 4.8 to 5.6. The acidic isoferritins were prominent in all hearts, but one control and the four iron-loaded hearts contained, in addition, basic isoferritins. All ferritins were composed of two subunits, an acidic H type and a more basic HL type, which was the main subunit in normal liver. Hearts with the highest proportion of acidic isoferritin contained the highest proportion of the H subunit.

Ferritin, a predominantly intracellular protein and the main form of storage iron in animals, is found in highest concentration in liver and spleen. It is also present in other organs, such as heart and kidney, that normally have no iron-storage function (Linder & Munro, 1973), and in these tissues it may have a regulatory or protective role (Harrison, 1977). Ferritin molecules also appear to demonstrate structural heterogeneity, for, although the protein exists mainly in the monomeric form, stable oligomers are detectable in tissues. Furthermore monomeric ferritin from a single tissue may be resolved into multiple components by isoelectric focusing. This heterogeneity is thought to be due to groups of molecules with different surface charge called isoferritins (Drysdale, 1974). The structural basis for isoferritins is not known. Drysdale *et al.* (1977) have suggested that in man they are hybrid molecules derived from at least two subunits of different primary structure, which vary in their relative proportions through the isoferritin spectrum. Another possibility is that there is a progressive change in surface charge of ferritin molecules through the isoferritin spectrum due to post-translational modification of a single subunit type (Harrison, 1977).

The distribution of human tissue isoferritins, which have isoelectric points between 4.8 and 5.6, is reported to differ between various organs, so that the more basic isoferritins are found in liver and the more acidic in heart (Powell *et al.*, 1975a). This characteristic distribution may be changed in iron overload with the loss of the acidic isoferritins from heart and the concomitant appearance of basic liver-like isoferritins (Powell *et al.*, 1974), although this has not been proved by subunit analysis.

In the present studies we investigated the electrophoretic mobilities, isoferritin profile and subunit composition of heart ferritins from four iron-loaded and three control subjects, and found evidence that iron overload in man is not invariably associated with the loss of acidic isoferritins from heart. Furthermore there appears to be a variation in the isoferritin profile and subunit composition, even in normal individuals.

Materials and Methods

Human material

Heart muscle was obtained at post-mortem between 8 and 10 h after death from seven adult males. The bodies were refrigerated at 4°C before post-mortem, and the tissues on removal were stored at -25°C until use. Three subjects (preparations A, B and C) had normal iron stores, as assessed by the absence of stainable iron from liver sections (Walker *et al.*, 1971), two (preparations D and E) had chronic renal failure and secondary iron overload (grade-3 and -4 siderosis respectively in hepatic biopsy sections according to Scheuer *et al.*, 1962) and two (preparations F and G) had untreated idiopathic haemochromatosis and grade-4 siderosis. Normal liver was also obtained.

Ferritin was prepared by the method of Drysdale & Munro (1965), but omitting the ion-exchange chromatography and including as a final step gel chromatography (gel bed 90 cm × 2.5 cm) on Sepharose 6B (Pharmacia Fine Chemicals, Uppsala, Sweden) with phosphate-buffered saline, pH 7.2 (120 mM-NaCl in 15 mM-sodium phosphate buffer) as the eluting buffer. Monomeric ferritin was taken

for further study (Niitsu & Listowsky, 1973) and concentrated by ultrafiltration (Amicon Corp., Lexington, MA, U.S.A.). The purity of each preparation was assessed by polyacrylamide-gel electrophoresis, with staining for both protein and non-haem iron as described by Powell *et al.* (1974).

Chemicals

Biochemical reagents were from Hopkin and Williams, Chadwell Heath, Essex, U.K. Polyacrylamide and *NN'*-methylenebisacrylamide were from Eastman Organic Chemicals, Rochester, NY, U.S.A. Ammonium persulphate and *NNN'*-tetramethyl-1,2-diaminoethane were obtained from Sigma (London) Chemical Co., Kingston-on-Thames, Surrey, U.K. Carrier ampholytes (pH 3.5–10) were from LKB Produkter AB, Bromma, Sweden.

Methods

The protein concentration of ferritin solutions was measured by the method of Lowry *et al.* (1951), with fat-free human serum albumin as a standard. Ferritin proteins were kept at 4°C in deionized water made 0.05% for NaN₃ at concentrations of 500 µg/ml–2.5 mg/ml.

Polyacrylamide-gel electrophoresis. Gels (3 mm × 130 mm × 180 mm) containing 4.8% (w/v) acrylamide and 0.2% (w/v) methylenebisacrylamide were cast and run in a vertical slab-gel apparatus (Shandon Southern Instruments, Camberley, Surrey, U.K.). Gel buffer was 76 mM-Tris/citrate, pH 8.9, and electrode buffer 82 mM-Tris/borate, pH 8.3, containing 3.4 mM-EDTA (disodium salt). Bromophenol Blue was the tracking dye. Samples were electrophoresed at 10°C according to the manufacturer's instructions. Polyacrylamide-gel electrophoresis of ferritin subunits was performed in gels containing 6.25 M-urea, pH 3.2, by the method of Panyim & Chalkley (1969). Samples were run at 10°C in gel rods (7 mm × 80 mm) containing 15% (w/v) acrylamide and 0.1% (w/v) methylenebisacrylamide in a Shandon Disc Electrophoresis apparatus at 4 mA per gel for 6 h. Methyl Green was the tracking dye.

Gel electrofocusing. This was performed in flat bed gels (1.5 mm × 120 mm × 250 mm) containing 4% (w/v) acrylamide, 0.16% (w/v) methylenebisacrylamide and 2% (w/v) carrier ampholytes (pH 3.5–10) in an LKB Multiphor. Samples were run at 4°C according to the manufacturer's instructions.

The pH gradient in the gel-electrofocusing experiments was determined as described by Vesterberg (1972).

Preparation of ferritin subunits. Ferritin was dissociated in 67% (v/v) acetic acid at 0°C for 1 h, and the resulting subunits (Harrison & Gregory, 1968) were dialysed overnight against 0.9 M-acetic acid/6 M-urea. This mixture was then applied directly to the denaturing gels for electrophoresis.

Gels were stained for protein with 0.025% Coomassie Blue R in methanol/acetic acid/water (3:1:8, by vol.) for 12 h and 19°C and cleared in repeated changes of the solvent. Stained gels were quantified by densitometric scanning (Vitatron Instruments, Dieren, The Netherlands), and peak areas read directly from the chart recorder linked to an integrator.

Results and Discussion

The heart ferritins from the three control subjects and the four iron-loaded subjects migrated as two distinct species on electrophoresis in polyacrylamide slab gels. The slow species had similar mobility to monomeric ferritin prepared from normal liver (Plate 1a). There was a marked variation in the relative proportion of the two ferritins in the three normal preparations, the ratio of fast to slow species being 5.66, 1.0 and 0.43 for preparations A, B and C respectively (Table 1). In the iron-loaded subjects the ratios were 0.66, 1.22, 2.33 and 3.0 for preparations D, E, F and G respectively (Table 1), indicating a more even distribution of the two ferritin species. Although the presence in human heart of two ferritin species that are separable by electrophoresis has been noted by others (Arora *et al.*, 1970; Powell *et al.*,

Table 1. *Relative proportions and ratios of two ferritin species and subunits in heart and liver ferritins*
Gels were quantified by densitometric scanning. The percentage of slow and fast ferritin species (Plate 1a) and H and HL subunits (Plate 2) in each preparation was calculated from peak areas.

Preparation	Fast species	Slow species	Ratio fast/slow	Subunit H	Subunit HL	Ratio H/HL
A	85	15	5.66	90	10	9.0
B	50	50	1.0	35	65	0.54
C	30	70	0.43	20	80	0.25
D	40	60	0.66	30	70	0.43
E	55	45	1.22	45	55	0.82
F	70	30	2.33	55	45	1.22
G	75	25	3.0	60	40	1.50
Liver	—	100	—	5	95	0.05

EXPLANATION OF PLATE I(a)

Electrophoresis in polyacrylamide gel slab to show the presence of two ferritin species (fast and slow) in various proportions in human heart

Electrophoresis was performed in 5% polyacrylamide gels at pH8.9 in a discontinuous buffer system as described in the text. Heart ferritin (10–15 μ g; A–G) in 100 μ l of water made 10% for sucrose and 0.1% for Bromophenol Blue was loaded. Monomeric ferritin from normal liver was loaded (20 μ g and 10 μ g) in the first and last slots respectively. (A), (B) and (C) were from controls; (D), (E), (F) and (G) were from iron-loaded subjects. The liver ferritin and heart (D) contain a small amount of dimeric ferritin. The slab was stained with Coomassie Blue.

EXPLANATION OF PLATE I(b)

Isoelectric focusing of heart and liver ferritins in polyacrylamide gel slabs

Isoelectric focusing was performed in 4% polyacrylamide gel slabs in the pH range 3.5–10 as described in the text. Ferritin (10 μ g in water) was loaded at the cathodic side of the slab. The most basic and acidic isoferritins shown had pI values of 5.6 and 4.8 respectively. The gel was stained with Coomassie Blue.

EXPLANATION OF PLATE 2

Electrophoresis of ferritin subunits under denaturing conditions to show the increasing proportion of the H subunit in preparations (B)–(G)

Subunits were electrophoresed in 15% polyacrylamide gels containing 6.25M-urea at pH 3.2 by the method of Panyim & Chalkley (1969). Ferritin subunits were produced as described in the text and loaded in 6M-urea/0.9M-acetic acid; 10–12 μ g of protein was used for each gel. Gels were stained with Coomassie Blue.

1975a), there is only one previous study in which the ferritins of different human hearts were compared. Munro *et al.* (1975) reported that one normal heart contained both slow and fast species, whereas in a second heart there was the fast-migrating species only, a variability which we observed in the present three control hearts. The cause of this variation is not clear, for, although ferritin is an iron-inducible protein and the iron status of an individual influences the concentration of ferritin protein in tissues (Linder & Munro, 1973), in the present control subjects there was no evidence of increased body iron stores. Factors in addition to iron overload may influence the distribution of ferritin in heart, as in rats there is reported to be a sex difference, with the fast ferritin predominating in females and the slow in males. Furthermore the acute administration of iron does not change the relative proportion of the two ferritins in either sex (Vulimiri *et al.*, 1977). In our study, however, the subjects were all adult males. The lack of a reproducible pattern in the controls makes it difficult to determine whether there was any significant difference in the distribution of the two ferritin species in iron-overloaded subjects. However, in three of the four subjects studied the fast ferritin species predominated.

When examined by isoelectric focusing in polyacrylamide gels, all the ferritin preparations consisted of multiple components (Plate 1b). The most acidic and basic of these isoferritins had isoelectric points of 4.8 and 5.6 respectively. Isoferritins are now recognized to be stable entities that are not affected by storage at -25°C or the isolation procedure (Drysdale *et al.*, 1977). In addition, as the time between death and collection of tissues was similar in every case, the variable isoferritin patterns observed are unlikely to be due to change *post mortem*. In the three control preparations the appearance of basic or liver-type isoferritins corresponded to an increasing proportion of the slow ferritin species (Plate 1b) on polyacrylamide-gel electrophoresis. Thus the isoferritins in normal hearts are not confined to the acidic part of the spectrum. Furthermore preparation C contained virtually the whole spectrum of isoferritins, and this contrasts with the previous report by Powell *et al.* (1975a) that isoferritins in normal heart are predominantly acidic with only small variations between different subjects.

Acidic ferritins were demonstrated by isoelectric focusing in the four preparations from iron-loaded subjects, and these were the most prominent isoferritins in three of the four preparations. These findings do not agree with those of Powell *et al.* (1974, 1975b), who reported that with long-standing iron overload there was a loss of acidic isoferritins from heart. In our study there was no consistent pattern of isoferritin distribution, even in iron overload, and it is thus possible that the effects of iron overload are

superimposed on the same type of variable isoferritin pattern as seen in the normal subjects.

Dissociation of human tissue ferritins with 67% acetic acid results in two polypeptide chains designated HL and H, which differ in surface charge (Adelman *et al.*, 1975) and size (Drysdale *et al.*, 1977). The L-type subunit reported by Adelman *et al.* (1975), which is said to be a component of the most basic isoferritins of liver, is unlikely to be a true ferritin subunit, as reduction with dithiothreitol altered its electrophoretic properties in their system and caused it co-migrate with the HL subunit.

We found that normal liver ferritin contained one main subunit type, HL, with very small amounts of an H subunit. All the heart ferritins contained both the HL and H subunits but in various proportions (Plate 2, Table 1). The presence of dithiothreitol in our system did not change the relative proportions of the subunits or their mobilities. With increasing relative amounts of the fast ferritin species and acidic isoferritins the proportion of the H subunit also increased. Differences in the subunit composition of the same tissue ferritin from different individuals has not been previously reported, although Arosio *et al.* (1976) have shown that an increasing proportion of an H-type subunit corresponds to an increasing proportion of acidic isoferritins in liver, liver infiltrated with tumour, heart and HeLa cells. In our study, the highest proportion of an H-type subunit was found in preparations A (a control) and E and F from subjects with untreated idiopathic haemochromatosis, whose tissues were extensively iron-loaded. Thus ferritin molecules containing a high proportion of the acidic subunit may be isolated from heart tissues with a wide range of iron loading.

In conclusion, our results suggest that the distribution of the slow and fast ferritin species, as shown by a number of techniques in human heart, is not as well defined as previously reported. This is reflected in varying isoferritin profiles and subunit composition. Although the iron status of an individual is important in determining the amount of ferritin in certain iron-storage tissues, other factors, which are presently unknown, may also influence the distribution of the two ferritin species in heart.

We are grateful to the Nuffield Foundation and King's College Hospital Research Committee for support and to Linda Rimmer for editorial assistance.

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