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THE IRON CONTENT OF HUMAN LIVER AND SPLEEN ISOFERRITINS CORRELATES WITH THEIR ISOELECTRIC POINT AND SUBUNIT COMPOSITION

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SUMMARY

The six isoferritins in normal human liver and spleen with pIs between 5.7 and 5.2 were fractionated according to iron content by sucrose density gradient centrifugation. The pIs and subunit composition of the isoferritins in fractions from the gradients were determined by gel electrofocussing and electrophoresis in acid/urea polyacrylamide gels respectively. Fractions with the lowest iron content contained the two basic isoferritins, which were homopolymers of the L subunit and had pIs of 5.7 and 5.6. Increasing iron content correlated with decreasing isoelectric point and an increasing substitution of the H subunit in the isoferritin shell. The two isoferritins with highest iron content consisted of 92% and 8% respectively of the L and H subunits and had pIs of 5.3 and 5.2.

INTRODUCTION

Ferritin, the main form of soluble storage iron in animal tissues, consists of a protein component apoferritin of m.w. 445,000 associated with variable amounts of trivalent iron. Apoferritin is composed of 24 sub-units arranged as a hollow sphere and functions by catalysing the conversion of ferrous to ferric iron and storing the product. Not all ferritin molecules in a tissue are equally loaded with iron and within any one specimen molecules with iron content varying from 0 to 4,500 atoms may be identified (1,2). There seem to be two possible reasons for this. Firstly, ferritin synthesis is stimulated by iron with apoferritin the initial product (3). These iron-free molecules subsequently take up iron over the next 48 to 72 hours (4) and the synthesis and turnover of ferritin in a tissue results in the characteristic distribution of iron. Secondly, ferritin is structurally heterogeneous and on isoelectric focussing forms a series of stable isomers or isoferritins (5). Evidence from in vitro experiments suggests that isoferritins vary both in their iron content and ability to

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take up iron (6). Whether these differences in isoferritin metabolism relate to differences in their structure is uncertain. In this study we report a correlation between the iron content of human tissue isoferritins and their structure in terms of isoelectric point and sub-unit composition.

MATERIALS AND METHODS

The liver and spleen were obtained at autopsy from a 56-year-old male who died of a myocardial infarction. Total liver iron (7) 1.4 ug/mg protein, was within the normal range of 0.2 ug - 2.2 ug iron/mg protein. Ferritin was prepared by the method of Drysdale and Munro (8) modified as previously described (9), and the purity confirmed by polyacrylamide gel electrophoresis.

Ferritin protein was measured by the method of Lowry et al (10) with fat free human serum albumin as standard. Ferritin iron and total liver iron were measured by atomic absorption spectrophotometry using a Perkin Elmer instrument with a heated graphite furnace and automatic sample loader. Ferritin solutions appropriately diluted were injected into the furnace without previous treatment.

Discontinuous gradients were layered with 3 ml aliquots of 40%, 30%, 20% and 10% (w/v) sucrose in 15 mM sodium phosphate buffer containing 120 mM NaCl pH 7.2. 1 ml of liver and spleen ferritin at concentrations of 1.7 mg/ml and 1.9 mg/ml respectively were centrifuged on these gradients in a 3 x 23 ml swing-out rotor on an MSE Superspeed 65 centrifuge at 80,000 xg for 100 mins at 4°C. Thirteen 1 ml fractions were collected from the top of the gradients and dialysed against 15 mM sodium phosphate buffer containing 120 mM NaCl at pH 7.2 to remove the sucrose. Protein and iron estimations were then performed and fractions selected for gel electrofocussing and sub-unit analysis on the basis of their iron/protein ratios.

Gel electrofocussing was performed in 4% (w/v) acrylamide slabs (1.5 x 120 x 250 mm) containing 2% carrier ampholytes (LKB) pH range 3.5 - 9.5 as described by Bomford et al (9). The pH gradients achieved in the slabs were determined according to Vesterberg (11).

Sub-units were prepared by dissociating ferritin in 67% (v/v) acetic acidic at 0°C for 1 hour (12), with subsequent dialysis against 0.9 M acetic acid made 6M for urea. Electrophoresis was performed in 15% (w/v) acrylamide gel rods (7 x 80 mM) containing 6.25 M urea at pH 3.2 (13).

RESULTS

Four fractions with iron/protein ratios varying from 0.01 to 0.18 in the case of liver ferritin and 0.01 to 0.26 in the case of spleen ferritin (Table 1) were subjected to gel electrofocussing (Fig 1). The fractions from the top of the gradients containing molecules with the lowest iron content, were composed predominantly of the two basic isoferritins in the spectrum. With increasing molecular iron content the isoelectric

TABLE 1: The iron/protein ratios (ug iron/ug protein) of fractions of liver and spleen ferritin from sucrose density grandients. These fractions were run on electrofocussing gels (Fig. 1) as described in Material and Methods.

Fraction No.	Liver	Fraction No.	Spleen
1	0.01	1	0.01
2	0.04	2	0.03
4	0.10	5	0.17
6	0.18	7	0.26

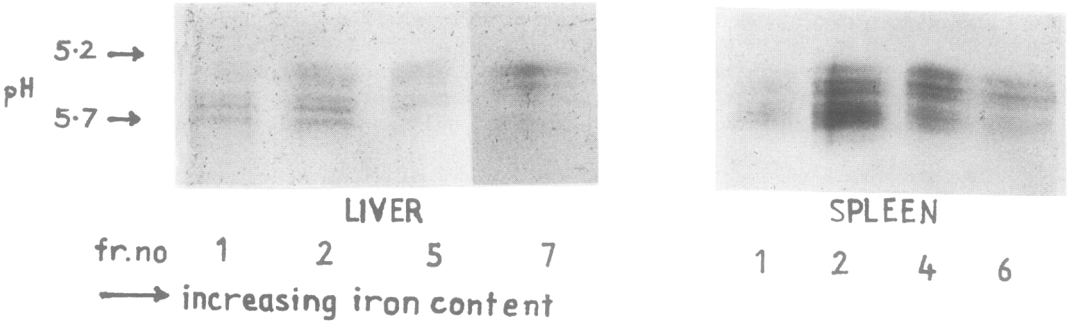

Fig. 1 The distribution of isoferritins in four fractions with increasing iron/protein ratios from liver and spleen density gradients. Gel electrofocussing was performed in acrylamide slabs in the pH range 3.5 - 9.5 and staining was with Coomassie Blue R.

point of the isoferritins fell so that fractions 6 and 7 consisted almost entirely of the two most acidic isoferritins in the spectrum. The unfractionated liver and spleen ferritins consisted of six isoferritins with isoelectric points between 5.2 and 5.7 and a comparison of these profiles with the isoferritins in each fraction confirmed the association between isoelectric point and iron content. Separation of isoferritins according to iron content allowed the selection of 3 fractions containing, with only a minor degree of contamination from adjacent isoferritins, 2 basic, 2 intermediate and 2 acidic isoferritins.

These appeared to be stable entities with no redistribution after 2 months of storage at $4^{o}C$ and pH 7.2 in 15 mM sodium phosphate buffer containing 120 mM NaCl made 0.05% for sodium azide. Accordingly these fractions were used for subunit analysis.

Subunit analysis

It has previously been shown that treatment of human tissue ferritins with 67% acetic acid liberates 2 subunit types with different surface charge but similar m.w. (approx. 20,000) separable by acid/urea gel electrophoresis and termed L (basic) and H (acidic).

The relative proportion of the two subunits correlates with the isoelectric point of an isoferritin so that the H type subunit predominates in the more acidic isoferritins of heart (9,14) and hela cells (14) and the L type in the basic isoferritins found in liver and spleen (9,14).

The results of electrophoresis in acid/urea gels of subunits obtained from fractions 1,4 and 6 of the liver gradient indicate (Fig. 2) that the two basic isoferritins in fraction 1 (gel a) contained no detectable H subunit and were thus homopolymers of the L subunit. In agreement with the two subunit model of ferritin structure the isoferritins in fractions 4 (gel b) and 6 (gel c) were heteropolymers containing an increasing proportion of the H subunit (Table 2). Similar results (gels not shown) were obtained with spleen ferritin (Table 2). As the percentage of the H subunit in the liver isoferritins was small a fourth gel (gel d, Fig 2) has been included to indicate the position of the two subunits in this gel system. For this a preparation of human heart ferritin which was known (9) to contain 30% and 70% respectively of the H and L subunits was electrophoresed under identical conditions.

DISCUSSION

We have demonstrated a clear correlation between increasing iron content and decreasing isoelectric point in the six isoferritins of human

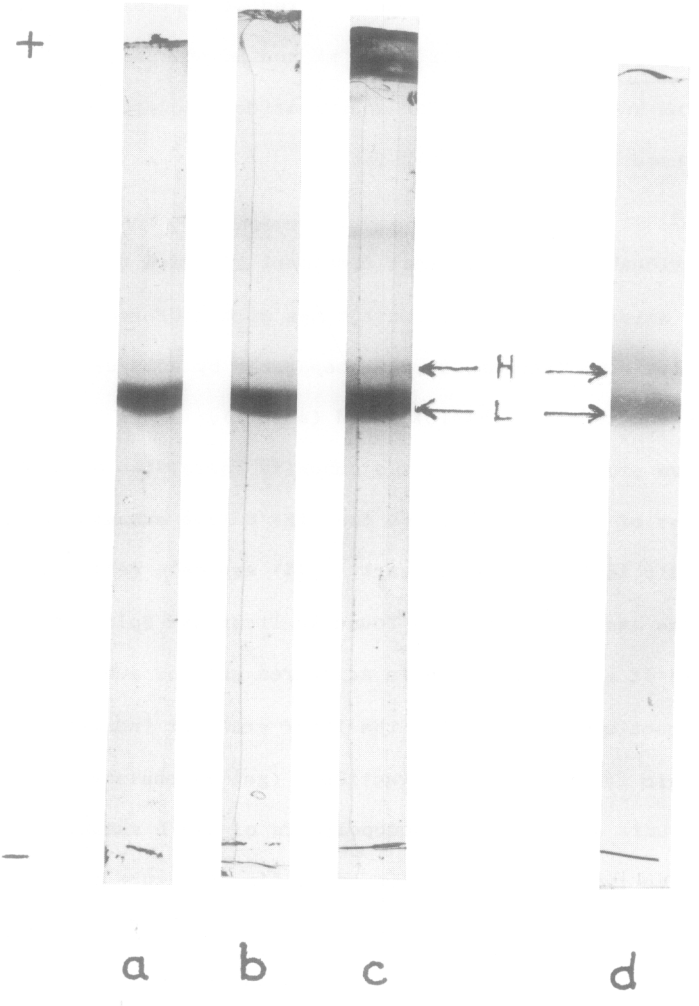

Fig. 2 Gels a, b, and c contain subunits from fractions 1, 4, and 6 respectively of the liver gradient electrophoresed under denaturing conditions in 6.25M urea at pH 3.2. 10 ug of protein were loaded and gels stained with Coomassie Blue R. The percentage proportion of the H and L subunits was determined by scanning stained gels near 570 nm (see Table 2). Gel d containing subunits from human heart ferritin electrophoresed under identical conditions was included to show the position of the two subunits in this gel system. The position of the methyl green tracking dye was marked with fine wire.

liver and spleen. Subunit analysis indicated that the relative proportion of the L and H subunits varied in the predicted manner (5) through the isoferritin spectrum of both tissues so that increasing iron content also

TABLE 2: Percentage proportion of the L and H subunits in liver and spleen density gradient fractions. Subunits were produced by incubating sample with 67% acetic acid at 0°C for 1 hour and separated in the denaturing gel system (Fig. 2) described in Material and Methods. Stained gels were scanned near 570 nm.

Fraction No.	Liver Subunit L	: H	Fraction No.	Spleen Subunit L	: H
1	100%	-	1	100%	-
4	96%	4%	5	95%	5%
6	92%	8%	7	92%	8%

correlated with an increasing proportion of the H subunit in the isoferritin shell. Natural apoferritin or ferritin of very low iron content from both tissues in agreement with previous reports (6,15,16) had a restricted isoferritin profile composed of the two most basic isoferritins in the spectrum. The iron content of the remaining four isoferritins increased in an approximately linear fashion with decreasing isoelectric point.

These data may be interpreted in several ways. The variable iron content of the isoferritins could be responsible for the observed differences in their surface charge although on the basis of albeit indirect evidence this is unlikely as (a) iron is situated within the protein shell (17), (b) the removal of iron from ferritin by chemical reduction results in an apoferritin preparation that has very similar banding patterns in electrofocussing gels to the iron rich protein (18,19), and (c) evidence is accumulating to suggest that it is the subunit composition of an isoferritin that determines its position in the isoferritin spectrum (5,9,14).

An alternative explanation may be that the structure of an isoferritin in some way determines its iron content. Iron uptake probably occurs in two stages (20), an initial binding and oxidation of ferrous iron, catalysed by apoferritin (21,22), with the subsequent formation of a

nucleus of ferric iron, on the surface of which further oxidation and deposition of iron occurs. It is the first stage that is most likely to be affected by conformational changes in the protein with changes in the efficiency of the catalytic site as a possible consequence. Indeed, Harrison and her colleagues have recently reported that with horse spleen ferritin fractionated according to iron content by serial precipitation with ammonium sulphate and reduced with sodium dithionite, the fractions that originally contained most iron were the most catalytically active in the initial phase of iron uptake (6).

Our findings also complement those of Wagstaff et al (23) who report that rates of iron uptake into human liver isoferritins fractionated by ion exchange chromatography increase with decreasing isoelectric point. If the ability to acquire iron is enhanced by the H subunit, then heart isoferritins which contain a high proportion of this subunit should have higher rates of iron uptake than liver isoferritins. This appears to be the case as Wagstaff et al also found the initial rates of uptake to be steeper with heart isoferritins and the progress curves hyperbolic as opposed to sigmoidal progress curves obtained with liver. This suggests that the relationship between surface charge and subunit composition on one hand and the ability to acquire iron on the other, extends across the whole range of human tissue isoferritins.

The synthesis of two iron free isoferritins by liver and spleen which process and store large amounts of iron seems paradoxical. Even in iron overload when ferritin synthesis is increased the relative proportion of iron-free to iron-loaded isoferritins is maintained (24), and the iron free species do not store significant amounts of iron. In the short term, however, they may have a regulatory or protective role to maintain intracellular iron levels within critical limits.

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