Binding of human serum ferritin to concanavalin A

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Summary

1. A high proportion of the ferritin in normal serum binds to concanavalin A. Binding is prevented by the addition of α-D-methylglucoside to the reaction mixture.

2. Ferritin in extracts of normal heart, liver and spleen or serum ferritin from patients with massive hepatic necrosis does not bind to concanavalin A.

3. Isoelectric focusing of preparations of serum ferritin from patients with primary haemochromatosis shows that the ferritin fraction binding to concanavalin A consists, predominantly, of the more acidic isoferritins.

4. These findings suggest that carbohydrate residues may be added to ferritin during its secretion into the plasma. Glycosylation may account for the heterogeneity of serum ferritin on isoelectric focusing.

5. Direct release of intracellular ferritin from damaged tissue may be indicated by an increase in the proportion of circulating ferritin which does not bind to concanavalin A. Such an increase has been found in sera from patients with iron overload.

Key words: concanavalin A, ferritin, serum ferritin, glycosylation, iron overload, isoelectric focusing, liver damage.

Abbreviation: ConA, concanavalin A.

Introduction

The concentration of ferritin in human serum is closely related to storage iron concentrations in the body (Worwood, 1977), but little is known about the origin of circulating ferritin or the way in which it enters or leaves the plasma. In normal subjects serum ferritin appears to be largely derived from cells of the reticuloendothelial system (Jacobs & Worwood, 1975). However, high concentrations of serum ferritin are found in patients with liver damage (Prieto, Barry & Sherlock, 1975). Most plasma proteins are glycosylated during secretion into the plasma and such a process may be important in determining the properties of circulating ferritin. Little is known about the carbohydrate content of human ferritin, although small amounts of neutral sugar are present in crystalline ferritin from horse spleen (Shinjyo, Abe & Musada, 1975; Cynkin & Knowlton, 1977). We report here studies on the interaction of serum ferritin with concanavalin A, which provide evidence for glycosylation of the circulating protein.

Methods

Sources of ferritin

Normal heart liver and spleen were obtained post mortem and stored at −20°C. Extracts were prepared by homogenizing thawed tissue in distilled water (1 g/10 ml of water) and insoluble material was removed by centrifugation at 15 000 g for 30 min. In addition, purified spleen ferritin was prepared from iron-loaded spleen as described previously (Worwood, Aherne, Dawkins & Jacobs, 1975). Serum for the chromatographic studies was obtained from patients with idiopathic haemochromatosis being treated by phlebotomy.
Binding of ferritin to concanavalin A

Binding of tissue ferritins was studied by chromatography on columns (30 mm x 10 mm) of concanavalin A (ConA)–Sepharose 4B (Pharmacia Great Britain Ltd, London W5 5SS) prepared in 2 ml syringes and washed with acetate buffer (sodium acetate solution, 50 mmol/l, adjusted to pH 6.0 with acetic acid, and containing sodium chloride, 500 mmol/l, and CaCl₂, MgSO₄ and MnSO₄ all at 1 mmol/l). Tissue extracts containing a maximum of 10 mg of protein or serum (0.5 ml) were diluted and dialysed against acetate buffer and passed through the column at a rate of 20 ml/h. Preliminary studies showed that the columns were able to bind a constant proportion of protein from up to 0.6 ml of serum. In some cases tissue extracts were incubated at 37°C for 1 h with 0.4 ml of human serum of low ferritin content (<10 μg/l) before being diluted with acetate buffer and passed through the column. Elution was continued with the same buffer until no more protein was removed from the column. The column was then washed (second eluate) with the same buffer containing α-D-methylglucoside (50 mmol/l) (Sigma London Chemical Co. Ltd, Poole, Dorset, U.K.) until no more protein could be removed from the column. Ferritin concentrations were determined by immunoradiometric assay (Jones & Worwood, 1978) with anti-(spleen ferritin) and spleen ferritin standards, except for the heart extracts for which heart antibodies and standards were employed.

Binding of serum ferritin was also measured by incubation of serum with ConA–Sepharose 4B and separation of bound and free ferritin by centrifugation. A suspension of ConA–Sepharose 4B was washed three times in five times its own volume of buffer A. This consisted of barbitone sodium (50 mmol/l) adjusted to pH 8.0 with HCl (5 mol/l), and containing sodium chloride (500 mmol/l) and sodium azide (3.1 mmol/l). The volume of the suspension was adjusted to twice that of the packed volume of Sepharose. Serum (0.2 ml) was added to 0.5, 1.0 or 1.5 ml of ConA–Sepharose 4B suspension and the total volume was made up to 2.0 ml with buffer A. After mixing on a roller-mixer for 2 h at room temperature the suspension was centrifuged at 3000 rev./min for 15 min. The ferritin content of the supernatant was determined by immunoradiometric assay with antibodies to spleen ferritin and spleen ferritin standards (Jones & Worwood, 1978) after diluting five times in albumin containing buffer B. This consisted of barbitone sodium (50 mmol/l), sodium chloride (100 mmol/l), bovine serum albumin (5 g/l) and sodium azide (3.1 mmol/l), and was adjusted to pH 8.0 with HCl (5 mol/l). The standard spleen ferritin for the assay was diluted in the following buffer: 1:10 dilution of normal rabbit serum in buffer A further diluted (1:5) in buffer B. If further dilution of the supernatant was necessary this was carried out with the same buffer as that used to dilute the standard ferritin.

Isoelectric focusing was used to determine which components of serum ferritin bind to concanavalin A. Serum (500 ml) was obtained from a patient undergoing phlebotomy for treatment of haemochromatosis and ferritin was partially purified by heat treatment (Worwood, Dawkins, Wagstaff & Jacobs, 1976). After the anion-exchange chromatography step ferritin was collected, concentrated and dialysed against acetate buffer, pH 6, before being passed through a ConA–Sepharose 4B column. The eluates were concentrated and subjected to isoelectric focusing in polyacrylamide gel with preparative system described by Worwood et al. (1976). The gels were sliced into 5 mm sections and amphotolytes were eluted in 2 ml of boiled, distilled water for pH determination. After addition of a further 10 ml of phosphate buffer (50 mmol/l), pH 7.4, and overnight incubation, the ferritin concentrations in the eluates were determined by immunoradiometric assay with anti-(spleen ferritin) and spleen ferritin standards (Jones & Worwood, 1978).

Results

No binding to ConA–Sepharose 4B was observed for ferritin in extracts of normal tissue with or without previous incubation with serum (Table 1). In contrast a significant proportion of the ferritin in serum samples from patients with haemochromatosis bound to the column and could be eluted by washing the column with buffer containing α-D-methylglucoside. Binding of ferritin in normal serum was measured by incubating serum with ConA–Sepharose and separating bound and free ferritin by centrifugation. No binding was found when purified spleen ferritin, added to serum from a patient with iron-deficiency anaemia, was incubated with concanavalin A, but a high proportion of the ferritin in normal serum bound to the lectin (Figs. 1 and 2). Ferritin from normal serum did not bind when α-D-methylglucoside (50 mmol/l) was included in the incubation mixture or
TABLE 1. Chromatography of ferritin in tissue extracts and serum on concanavalin A-Sepharose 4B

Each figure refers to a single experiment. The sera were obtained from two patients with idiopathic haemochromatosis during treatment by venesection.

<table>
<thead>
<tr>
<th>Tissue extract or serum</th>
<th>Ferritin loaded on column (µg)</th>
<th>Ferritin in eluate 1 (% of total eluted)</th>
<th>Ferritin in eluate 2 (% of total eluted)</th>
<th>Total ferritin eluted from column (% of ferritin loaded)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>133</td>
<td>99</td>
<td>1</td>
<td>84</td>
</tr>
<tr>
<td>Spleen</td>
<td>174</td>
<td>99</td>
<td>1</td>
<td>99</td>
</tr>
<tr>
<td>Heart</td>
<td>13.4</td>
<td>99</td>
<td>1</td>
<td>134</td>
</tr>
<tr>
<td>Liver and serum</td>
<td>1.50</td>
<td>97</td>
<td>3</td>
<td>116</td>
</tr>
<tr>
<td>Spleen and serum</td>
<td>1.74</td>
<td>96</td>
<td>4</td>
<td>90</td>
</tr>
<tr>
<td>Heart and serum</td>
<td>1.05</td>
<td>91</td>
<td>9</td>
<td>100</td>
</tr>
<tr>
<td>Serum (haemochromatosis: 1030 µg of ferritin/l)</td>
<td>0.44</td>
<td>58</td>
<td>42</td>
<td>96</td>
</tr>
<tr>
<td>Serum (haemochromatosis: 1950 µg of ferritin/l)</td>
<td>1.15</td>
<td>70</td>
<td>30</td>
<td>87</td>
</tr>
</tbody>
</table>

FIG. 1. Binding of spleen ferritin and normal serum ferritin to concanavalin A. Bound and free ferritin were separated by centrifugation. Experimental details are given in the text. The spleen ferritin added to serum of ferritin content <10 µg/l as determined by immunoradiometric assay with anti-(spleen ferritin). Results are the means of five experiments ± 1 SD. □, Serum ferritin from six normal subjects. Results are the means ± 1 SD.

when ConA-Sepharose 4B was replaced by Sepharose 4B. Ferritin in sera from patients with clinical and biochemical evidence of massive hepatic necrosis did not bind to concanavalin A. Reduced binding was found in sera from patients with idiopathic haemochromatosis or iron overload resulting from multiple blood transfusions.

Isoelectric focusing of eluates from the ConA-Sepharose column showed that only the most basic isoferritins in serum passed through unbound (Fig. 3). The more acidic isoferritins bound to ConA and were eluted with α-D-methylglucoside.
Discussion

Our results show that both in normal serum and serum from patients with idiopathic haemochromatosis there is a high proportion of ferritin which binds to concanavalin A but that the fraction bound in patients with haemochromatosis is lower. We have also confirmed a previous observation that in serum from patients with haemochromatosis it is predominantly the more acidic isoferitins which bind (Worwood, Wagstaff, Jones, Dawkins & Jacobs, 1977). We assume that this is also the case in normal serum, which has been shown to contain a wide range of isoferritins on isoelectric focusing (Halliday, McKeering, Tweedale & Powell, 1977). Much of the heterogeneity of tissue ferritin, demonstrable by isoelectric focusing or ion-exchange chromatography, appears to be due to the presence of two types of subunit (Drysdale, 1977), although post-translational modification may also be a contributing factor. There are marked immunological differences between the most acidic and the most basic tissue isoferitins (Jones & Worwood, 1978), but these differences are not observed in serum isoferitins from patients with iron overload. These isoferitins, both more basic and acidic, are immunologically similar to the more basic ferritins of liver and spleen rather than the acidic ferritin of heart (Worwood et al., 1977). In addition, the correlation between isoelectric point and ion-exchange affinity, which has been described for tissue isoferitins, does not apply to serum isoferitins, which all have a low affinity on anion-exchange chromatography (Worwood et al., 1977).

It is possible that the heterogeneity of serum ferritin on isoelectric focusing is largely due to glycosylation rather than variation in subunit composition. Our results suggest that carbohydrate residues may be added during secretion of the protein after synthesis on polysomes associated with endoplasmic reticulum. Synthesis of ferritin on membrane-bound polysomes of rat liver has been demonstrated (Puro & Richter, 1971) but the significance of this finding remains unknown. Glycosylation of ferritin during its secretion from cells would not only modify the properties of the circulating protein but may also be an important factor controlling the rate of removal of ferritin from the plasma. Binding of ferritin to concanavalin A may provide a way of distinguishing between ferritin secreted from reticuloendothelial cells and ferritin released from the cytoplasm of damaged cells, particularly hepatic parenchymal cells. This may have clinical use in assessing the contribution made by tissue damage to the elevated serum ferritin concentrations found in patients with iron overload or malignancy.

Acknowledgment

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References


Glycosylated serum ferritin


