SHORT COMMUNICATION

Sialic acid and the microheterogeneity of human serum ferritin

S. J. CRAGG, M. WAGSTAFF AND M. WORWOOD
Department of Haematology, Welsh National School of Medicine, Cardiff, Wales, U.K.

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Summary

1. Ferritin has been partially purified from the serum of patients with idiopathic haemochromatosis.
2. Incubation with neuraminidase of this partially purified serum ferritin eliminated much of the microheterogeneity of the protein so that only ferritin of isoelectric point approximately 5.8 was present.
3. There was no change in the total amount of ferritin present (measured immunologically) or in the percentage of ferritin binding to concanavalin A.
4. Incubation of liver, spleen or heart ferritin with neuraminidase did not change the isoelectric focusing patterns.

Key words: heart ferritin, human serum ferritin, isoelectric focusing, microheterogeneity, neuraminidase, sialic acid.

Introduction

The protein ferritin provides an intracellular iron store and is synthesized in response to iron administration in many types of cell (Harrison, Hoare, Hoy & Macara, 1974). Ferritin from any tissue consists of a number of isoforms, and there is a relationship between isoelectric point (pI) and the proportions of two subunit types, H and L, for isoforms (Arosio, Adelman & Drysdale, 1978). Iron content, anion-exchange affinity and immunological properties are also related to pI (Wagstaff, Worwood & Jacobs, 1978).

Small amounts of ferritin circulate in the plasma and concentrations are related to the level of iron stores in the body (Worwood, 1979). High concentrations of serum ferritin are found in patients with liver disease or cancer as well as in patients with iron overload, but little is known about the way in which ferritin enters or leaves the plasma. Many proteins are glycosylated during secretion into the plasma and evidence that ferritin may also be a glycoprotein was provided by studies on the binding of serum ferritin to concanavalin A (Worwood, Cragg, Wagstaff & Jacobs, 1979). The serum isoforms which bind to concanavalin A are relatively acidic (Worwood et al., 1979), but their anion-exchange affinity and immunological properties are different from isoforms of the same pI from heart and other tissues (Worwood, Wagstaff, Jones, Dawkins & Jacobs, 1977). Tissue ferritins appear to contain little carbohydrate (Shinjyo, Abe & Masuda, 1975) and do not bind to concanavalin A (Worwood et al., 1979). We now provide evidence that much of the heterogeneity of serum ferritin, demonstrable by isoelectric focusing, is due to the presence of sialic acid residues and not to variation in subunit composition.

Methods

Ferritin preparations

Purified, iron-rich ferritin was prepared by the method of Worwood, Aherne, Dawkins & Jacobs (1975) from spleen obtained at operation from a
patient with thalassaemia and from normal liver and heart obtained post mortem. Ferritin was also extracted from heart muscle by heating an homogenate (1:4) of the tissue in distilled water at 70°C for 10 min, cooling, centrifuging at 10 000 g for 30 min and retaining the supernatant. Serum was collected from three patients undergoing regular venesection for treatment of idiopathic haemochromatosis. Ferritin was partially purified by affinity chromatography (Worwood, Dawkins, Wagstaff & Jacobs, 1976). After elution from the immunoadsorbent column with potassium thiocyanate (3 mol/l) the partially purified ferritin was immediately dialysed against phosphate buffer (50 mmol/l, pH 7.4). All the ferritin preparations were dialysed against acetate buffer (sodium acetate solution, 50 mmol/l, containing sodium chloride, 150 mmol/l, and calcium chloride, 1 mmol/l, adjusted to pH 5.5 with acetic acid).

Neuraminidase treatment

Neuraminidase from *Vibrio cholerae* was obtained from Calbiochem-Behring Corporation, La Jolla, California, U.S.A. The preparation was supplied in acetate buffer, pH 5.5, and contained 500 units/ml. The tissue or serum ferritin preparation (0.9 ml) was incubated at 37°C for 18 h with 100 µl of neuraminidase solution in acetate buffer with one drop of chloroform added as a preservative. A control incubation contained 100 µl of acetate buffer instead of neuraminidase. The serum ferritin preparations contained about 3 mg of protein/ml and about 200 µg of ferritin/ml (as determined by the immunoradiometric assay for spleen ferritin described by Jones & Worwood, 1978). Free sialic acid was estimated in the incubation mixtures by the thiobarbituric acid assay (Spiro, 1966). Total sialic acid was determined in the same way after hydrolysis of the protein sample in sulphuric acid (100 mmol/l) at 80°C for 1 h.

Preparative isoelectric focusing

Ferritin preparations, incubated with or without neuraminidase, were subjected to isoelectric focusing in polyacrylamide gel cylinders as described by Wagstaff et al. (1978). A mixture of Ampholines was used (67%, pH range 5–8, and 33%, pH range 4–6; LKB Instruments, South Croydon, Surrey, U.K.). The gels were cut into 5 mm sections, eluted in boiled distilled water before pH determination and then in buffer before ferritin assay as described by Worwood et al. (1979). Ferritin concentrations were measured by immunoradiometric assay (Jones & Worwood, 1978) with anti-(spleen ferritin). Heart ferritin concentrations were also measured with anti-(heart ferritin) and heart ferritin standards.

Binding to concanavalin A

Binding was measured by the method of Worwood et al. (1979), in which free and bound ferritin are separated by centrifugation. The buffer solution used to dilute the standard ferritin for immunoradiometric assay did not contain rabbit serum.

Results

Initially, incubations were carried out in buffer containing calcium chloride at 20 mmol/l. This caused precipitation of purified heart ferritin solutions at concentrations of about 0.5 mg of protein/ml. In the presence of calcium chloride at 1 mmol/l the appearance of an additional ferritin band at a pH of about 6.5 was noted after isoelectric focusing of purified heart and spleen ferritin. However, no changes in the focusing pattern were caused by neuraminidase. No precipitation or changes in focusing patterns due to calcium were observed with the partially pure preparations of serum or heart ferritin.

Sialic acid determinations on the partially purified serum ferritin preparations showed that a mean of 85% of the total sialic acid was released on incubation with neuraminidase (seven preparations). No release of sialic acid was detected in the control samples or in the purified tissue ferritin samples.

Isoelectric focusing showed that spleen and liver ferritins gave single, broad bands with pH values about 5.6 and heart ferritin (Fig. 1) gave a single band in the range pH 4.9–5.3. Incubation with neuraminidase did not change the total amount of ferritin present (measured immunologically) and the focusing patterns were not altered. It should be noted that this technique of slicing the gels and detecting ferritin immunologically does not make it possible to separate heart ferritin into its component isoferritins in the range pH 4.7–5.3. Serum ferritin gave two bands on isoelectric focusing; a more acidic band in the range pH 4.9–5.1 and the major band in the range pH 5.7–5.8. The more acidic band was eliminated by incubation with neuraminidase (Fig. 1) but there was no change in the total amount of ferritin present (measured
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immunologically). Similar results were obtained with five preparations of serum ferritin from three patients.

Tissue ferritins incubated with or without neuraminidase did not bind to concanavalin A. A mean value of 64% (from five measurements) of serum ferritin bound to concanavalin A, and after treatment with neuraminidase the binding remained unchanged with a mean value of 62%.

Discussion

These studies were carried out with partially purified preparations of serum ferritin. Although the greater content of sialic acid in such preparations allowed the measurement of sialic acid release it is not possible to be certain that ferritin was modified, only that sialic acid was released from serum proteins in general. However, the effect on the isoelectric point of serum ferritin suggests that sialic acid was removed from the more acidic isoferitins. The elimination of these isoferitins by incubation with neuraminidase and the demonstration that the acidic isoferitins are immunologically more similar to spleen than to acidic heart ferritin (Worwood et al., 1977) suggests that the microheterogeneity of serum ferritin results from glycosylation and not variation in subunit composition. The factors influencing the binding of a glycoprotein to concanavalin A are complex but Kornfeld & Ferris (1975) found that the removal of sialic acid residues had little effect on the reaction of glycopeptides with concanavalin A. It is not therefore surprising that neuraminidase treatment did not reduce the binding of serum ferritin to concanavalin A.

An important function of sialic acid concerns the survival of plasma proteins in the circulation. Removal of sialic acid exposes galactose residues, which act as a signal for the rapid removal and degradation of the sialic acid-deficient molecules by the liver (Ashwell & Morell, 1974). Halliday, Mack & Powell (1979) injected labelled ferritin intravenously into rats and found that the clearance rate of rat ferritin which bound to concanavalin A was significantly slower than that for ferritin which did not bind. We have studied serum ferritin from patients with idiopathic haemochromatosis but a high proportion of normal serum binds to concanavalin A (Worwood et al., 1979) and acidic isoferitins are also present in normal serum (Halliday, McKeering, Tweedale & Powell, 1977). Thus the presence of sialic acid explains much of microheterogeneity of serum ferritin and it may control the clearance of ferritin from the plasma.
References