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Ferritin—A General Metal Detoxicant

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ABSTRACT

Binding of nonferrous metal ions to ferritin was compared to that of the phosphate-free or phosphate containing synthetic iron cores. The Scatchard plots for the synthetic cores reveal a high affinity site for Cd, Zn, Be, and Al, with K_D in the range 10^{-5}–10^{-7} *M*. Preloading the cores with phosphate increased the number of metal ions bound without altering the K_D. The metal ions with smaller ionic radii (Be, Al) were bound in larger numbers than those with larger ionic radii (Cd, Zn).

Ferritin isolated from soybean (*Glycina max*), horse spleen, and rat liver bound the metal ions in amounts larger than predicted from their iron core. Whereas the iron cores and their nonferrous metal ion complexes were insoluble, those in the protein shell remained in solution. Thus apoferritin precipitated with lower concentrations of aluminum than did holoferritin. Also, Al bound to apoferritin reduced the rate of iron loading into the protein.

Index Entries: Ferritin; metals; toxicity.

INTRODUCTION

Feritin is a ubiquitous, constitutive protein of Mr 480,000. It is composed of 24 subunits. The subunits form a protein shell that sequesters in its central cavity up to 4500 g atoms of iron as Fe(III) hydroxyphosphate per molecule of protein (*1,2*).

In addition, ferritin binds other divalent and trivalent metal ions in vitro as well as in vivo, albeit in lesser amount (*3,4*). Holoferritin bound a

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much higher number of the various metal ions than was predicted by the specific metal binding sites of the apoprotein. This implicated the iron cores in metal binding. We, therefore, initiated the study of the metal binding properties of the iron core itself.

Preparation of artificial iron polymers using citrate results in a highly defined complex that in many respects mimics the core of ferritin. These polymers contain approximately 1200 iron atoms linked by hydroxy and oxy bridges (*5*). In the present study, these citrate-iron polymers were used as a model to investigate the binding of Cd(II), Zn(II), Be(II), and Al(III) to holoferritin. As the presence of inorganic phosphate in the ferritin core has been implicated in these interactions (*6*) we examined polymers that were loaded with phosphate to more closely resemble the iron core found in ferritin. These results are then compared to the nonferrous metal-binding of ferritin from mammalian and plant sources.

MATERIALS AND METHODS

Holoferritin and apoferritin from horse spleen were purchased from Sigma and further purified as before (*3*). Ferritin from soya beans and human brain was isolated as described before (*7,8*). All metals were obtained from Baker Chemicals as their chloride, sulfate, or nitrate salts, and used without further purification. Metal concentrations were determined with flameless atomic absorption spectrophotometer (AAS). Synthetic iron cores, prepared as described earlier (*5*) were dialyzed exhaustively against 0.1 *M* 4-morpholine ethane sulfonic acid (Mes), pH 6.0, for 7–10 d to remove excess citrate. Dialysis against 0.05 *M* $NaPO_4$, pH 7.0 was then carried out for 12–14 h, at which time extensive precipitation of the polymer was evident. The sample was again dialyzed exhaustively against MES buffer for 3–4 d to remove excess phosphate, at which time the accreted polymer was used for metal binding studies. Bound phosphate was measured by the method of Ames (*9*), and iron by AAS after dissolution in 50% HNO_3 (ULTREX), followed by dilution. The binding analyses of the metals to synthetic cores were performed with polymers that were preloaded with phosphate, in order to more closely approximate the in vivo situation with ferritin. To quantitate the actual binding of phosphate to the core, inorganic phosphate spiked with ^{32}Pi (New England Nuclear) was incubated with the cores that had been dialyzed to remove excess citrate, the iron polymer pelleted by centrifugation, and the amount of free phosphate in the supernatant determined by scintillation counting. From these values, a Scatchard plot of the binding of the phosphate anion to the synthetic cores was obtained.

Metal Binding

Metal stocks (in 1 m*M* HCl) were diluted into 0.1 *M* MES, pH 6.0. Metal content was quantified by AAS. Soyaferritin as isolated contained

approximately 1200 Fe atoms per protein molecule. For phytoferritin stoichiometry, the Centrifree system by Amicon was employed. The cartridges were first centrifuged with 1 mL of MES buffer to remove glycerin, azide, and residuals from the filter. This rinse step eliminated false high blanks of metal bound. In a separate test tube (borosilicate), was added MES buffer, metal ion in MES buffer, and finally an aliquot of protein in MES (total volume, 1 mL). After 5 min at 23°C, the sample was transferred to the prewashed Centrifree cartridge, and centrifuged (5 min, 12,000 rpm in a clinical fixed angle rotor). The filtrate was immediately analyzed for the metal. Controls were treated as described, minus the protein.

For metal binding to synthetic cores, 1.5 mL Eppendorf microfuge tubes were used. As the synthetic cores were insoluble in the buffer used (MES, pH 6.0), simple centrifugation was sufficient for separation of bound and free metal. To the premeasured volume of MES buffer was added an aliquot of the metal prepared as above, followed by addition of the core suspension (final volume was 1 mL). After 5 min at room temperature the samples were centrifuged (2 min 12,000 rpm) in a Brinkman Eppendorf centrifuge. The supernatant was immediately analyzed as above. Controls were treated as described, minus the cores.

The Scatchard plots of the data obtained as above were used to calculate the K_D for different metal ions bound to the core or to the proteins.

The rate of incorporation of Fe was measured as described by Treffry and Harrison (*10*).

RESULTS AND DISCUSSION

The Scatchard plots of the binding of nonferrous metals to soyaferritin show that all of the metals tested (Cd, Zn, Be, and Al) bound in significant amounts to the plant protein. Figure 1 shows such plots for Cd and Zn. As seen, there is evidence of at least one class of high-affinity binding sites (solid line) and a second class of rather low-affinity sites (dashed line). This was true for all the four metals tested. Qualitatively similar plots were obtained with synthetic cores as well as with animal ferritins (data not shown). The dissociation constants calculated from such slopes varied from $1 \times 10^{-5} M$ (Al to the Core) to 8×10^{-7} (Cd to the horse spleen ferritin).

The synthetic cores bound inorganic phosphate very tightly (K_D $2.5 \times 10^{-9} M$), omission of the phosphate from the cores reduced the number of binding sites for the metal ions on the core, but did not significantly alter the dissociation constant.

It appears, therefore, that ferritin from plant and animal sources is well-adapted to function as a general metal detoxicant. The nonferrous metal binding capabilities of ferritin, at least for high stoichiometries, seem principally attributable to the holoprotein and, therefore, under-

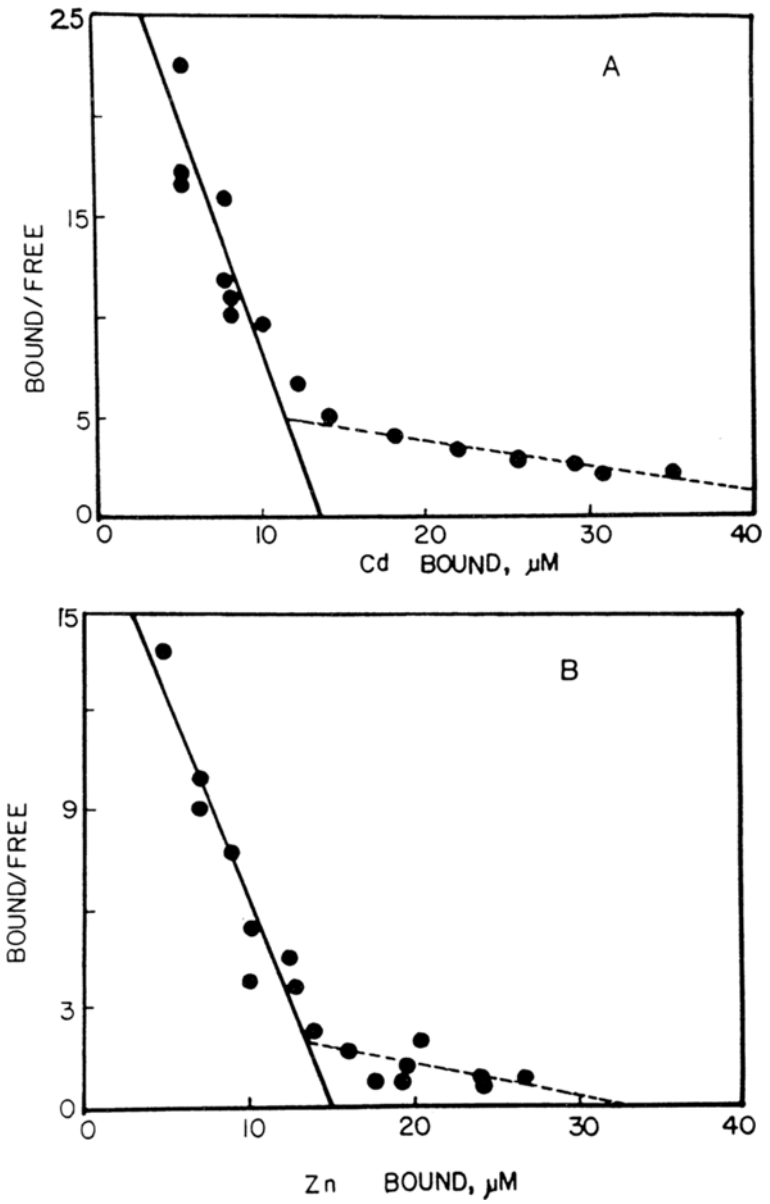

Fig. 1. Scatchard plots of nonferrous metal binding to soya ferritin containing 1200 iron atoms per molecule of protein. Assays were conducted in 0.1 *M* Mes., pH = 6.0. The appropriate metal was incubated at various concentrations with the protein for 5 min at 25°, followed by separation via centrifugation through a Centrifree cartridge. Metal concentrations were measured via atomic absorption, and corrected for background binding. Binding constants were calculated. For solid line, (A) cadmium, $K_D = 5.62 \times 10^{-7} M$ (B) Zinc, $K_D = 8.01 \times 10^{-7} M$. For dotted line

A; $Y = -0.14 \times + 6.8$, $K_D = 7.14 \times 10^{-6} M$

B; $Y = -0.0915 \times + 3.129$, $K_D = 10.9 \times 10^{-6} M$

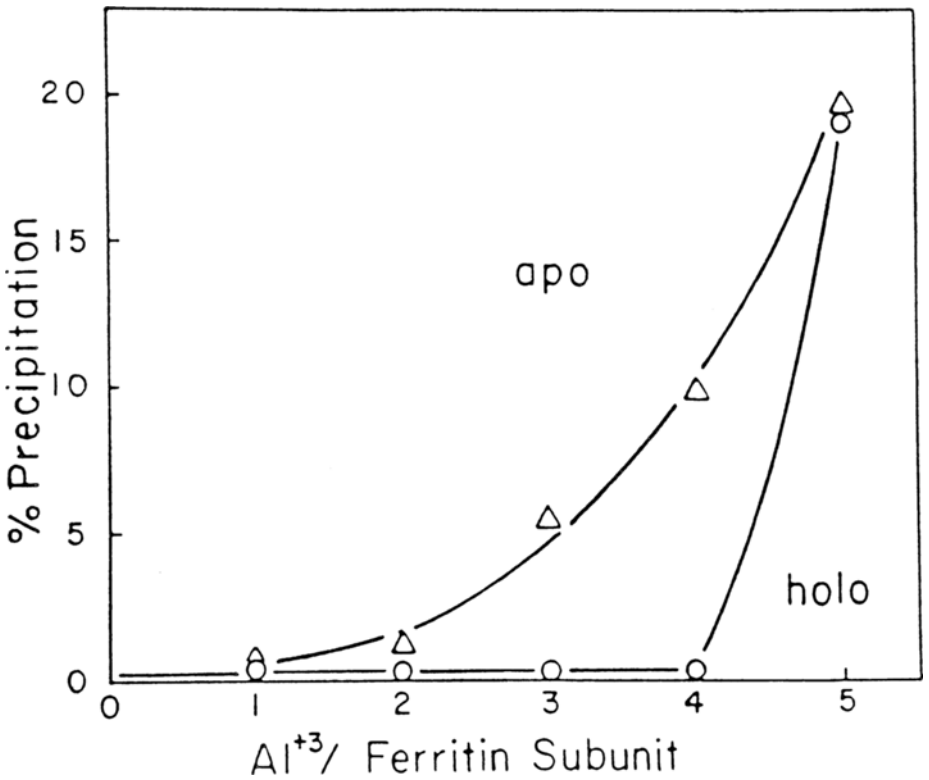

Fig. 2. Precipitation of apo and holoferritin by aluminum chloride. 500 μg of demetallo - apo and holo ferritin (horse spleen) in 20 m*M* HEPES pH 7.0 were incubated with $AlCl_3$ in acid washed microcentrifuge tubes at several Al^{+3}/ferritin subunit ratios. After 5 mins the tubes were spun at 8500 xg and the supernatants assayed for protein.

scores the leading role of the iron core in this process. Not only do the synthetic cores bind the test metals with affinities nearly identical to those of ferritin, the preference of metals in terms of stoichiometries also follows the order found in the protein. The smaller metals, Be and Al (ionic radii of 0.44 A° and 0.51 A°, respectively), are bound in much higher numbers than are Zn or Cd (ionic radii of 0.7 A° and 0.97 A°, respectively). Only the absolute numbers of metals bound are altered, with the synthetic preparation binding fewer metals in every case examined.

It is noteworthy that these insoluble iron cores, when in the protein shell of ferritin, remain soluble and also permit the holoferritin to bind larger amounts of nonferrous metal ions without the precipitation of the protein-core complex. This is illustrated with horse spleen ferritin in Fig. 2. As seen, apoferritin began to precipitate when it had bound only 1 g atom of Al(III) per subunit, whereas holoferritin remained in solution even after binding 4 g atoms of Al per subunit.

The first discovered and the well-established primary function of ferritin is to detoxify, store, and transport iron in a bioavailable form. The

structure of the protein shell permits accumulation of unusually large quantities of ferric hydroxyphosphate, which in turn binds large quantities of other metal ions. In vitro data suggests that these nonferrous cations such as Zn and Al reduce the ability of the protein to bind iron. The unique structure of ferritin permits it to execute the multifunctions mentioned earlier.

All of these are either directly or indirectly related to the binding or release of iron from the iron core. Therefore, in addition to affecting iron binding to ferritin, nonferrous metal ions may conceivably affect ferritin's other physiological functions (*11*). This remains to be established.

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