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Iron transfer from ferritin to transferrin

Effect of serum factors

Yi Jin and Robert R. Crichton

Unité de Biochimie, Université Catholique de Louvain, Place Louis Pasteur, 1, B-1348 Louvain-la-Neuve, Belgium

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The transfer of iron from ^{59}Fe-labelled human spleen ferritin to human apotransferrin occurs in the absence of either reducing or chelating agents. The reaction is first order with respect to ferritin and zero order to apotransferrin. The transfer is enhanced by low-M_r substances from human serum such as ascorbate, citrate, bicarbonate and lactate. A mixture of the four molecules at their normal physiological concentrations can increase the iron exchange to the same extent as that observed with an ultrafiltrate of serum. A pathway of intracellular iron mobilization is considered.

Transferrin; Ferritin; Iron transfer; Iron incorporation; Serum ultrafiltrate

1. INTRODUCTION

Iron plays an essential role in a large number of biological functions. In the serum and the extravascular fluids, it is transported by transferrin, a β-globulin consisting of a single polypeptide chain of M_r ~80000 [1,2] with two specific metal-binding sites. The iron-storage protein ferritin maintains iron in a soluble, non-toxic and bioavailable form within cells. The structure of horse spleen apoferritin consists of 24 symmetrically related subunits forming a near-spherical hollow shell. The central cavity is occupied by iron deposited in small crystalline particles essentially as an inorganic ferric oxyhydroxide polymer with some phosphate [3].

The uptake of iron from serum transferrin by a number of cell types, both normal and transformed, follows a similar pathway (review [4]). After binding to a specific receptor at the plasma membrane the transferrin-receptor complex is internalized within an endosome and at some stage during the passage of the complex from the endosome to the compartment of uncoupling of receptor and ligand (CURL) the iron is released as a consequence of the energy-associated pumping of protons into the vesicle (and presumably of other factors such as complexation of Fe^{3+} by biological chelators). The apotransferrin remains firmly bound to its receptor and is recycled back to the plasma membrane where it dissociates from the receptor at the neutral pH of the extracellular fluid. The iron released within the cell is rapidly incorporated into cytosol ferritin and into mitochondria [5,6].

While we know much about the uptake of transferrin iron by cells, little is known about the mechanisms by which intracellular iron is mobilised from ferritin. In normal human subjects some 40 mg tissue iron is mobilized per day [7]. 80% of this is accounted for by the recycling of iron derived from the catabolism of effete erythrocytes by the macrophages of the reticuloendothelial system

Correspondence address: R.R. Crichton, Unité de Biochimie, Université Catholique de Louvain, Place Louis Pasteur, 1, B-1348 Louvain-la-Neuve, Belgium

Abbreviations: Mes, 4-morpholineethanesulfonic acid; Mops, 4-morpholinepropanesulfonic acid

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to the erythroid bone marrow for hemoglobin synthesis. Although apotransferrin receptors have been found recently on rat macrophages [8], the presence of apotransferrin does not appear to be essential for the excretion of iron by these cells [9]. It thus appears that the inverse 'transferrin cycle' [10,11] would probably not be appropriate to explain the mechanism of intracellular iron mobilization.

We report here experiments designed to quantify the iron transfer from human spleen ferritin to human apotransferrin influenced by serum ultrafiltrate and by some low-M_r substances at their normal physiological concentrations in blood.

2. MATERIALS AND METHODS

Human apotransferrin was purchased from Behring Diagnostics (La Jolla, CA). ^{59}Fe was supplied as ferrous ammonium sulfate (crystalline) by the Radiochemical Centre (Amersham, England).

Human spleen ferritin was isolated and purified essentially by the methods of Granick [12] and Penders et al. [13] modified by Crichton et al. [14] and Wustefeld-Nelis [15]. The purity of the protein was controlled by polyacrylamide gel electrophoresis in 0.1 M Tris-borate buffer at pH 8.6 [14]. Apoferritin was prepared by sequential dialysis of human spleen ferritin against 1% (v/v) thioglycolic acid in 0.25 M sodium acetate buffer, pH 5.5 (4 times), 0.5% NH_4HCO_3 (3 times), 1 mM Mops buffer, pH 7.0 (2 times), 50 mM EDTA buffer, pH 6.2 (1 time), and 1 mM Mops buffer, pH 7.0 (4 times).

^{59}Fe-labelled reconstituted ferritin was prepared by the method established in this laboratory in which the yield of iron incorporation into apoferritin was optimized. 1 vol. acidic $FeSO_4$ solution (pH 3.0) was added to 1 vol. apoferritin (6 mg/ml) buffered by 1 vol. of 0.5 M Hepes and 0.5 M Mes, pH 7.0. The molar ratio Fe/apoferritin was maintained between 200 and 800. After incubation overnight, the reconstituted ferritin was filtered and dialysed against 1 mM Mops buffer, pH 7.0 (2 times), prior to determination of both iron and protein concentration.

An ultrafiltrate of serum (or plasma) was prepared by ultrafiltration of the serum (or plasma) through a Diaflo PM-10 filter (Amicon, Oosterhout, Netherlands) with a cut-off of M_r 10000 in an ultrafiltration cell.

Serum ultrafiltrate, chelate, ascorbic acid and other solutions were adjusted to pH 7.5 before addition to each reaction. All reactions were carried out in 0.05 M KCl/0.05 M Hepes, pH 7.5, and incubation performed at room temperature. At desired time intervals, 0.1 ml aliquots were taken for counting, and 1 ml aliquots were passed through a 1.5 × 6.0 cm column of Bio-Rad AGI-X4 anion-exchange resin (Bio-Rad, Richmond, CA). The column was previously equilibrated with 0.05 M KCl/0.05 M Hepes at pH 7.5; elution was carried out with the same buffer. About 7 ml eluate was collected and used for scintillation counting. Control experiments showed that transferrin was eluted exclusively with 0.05 M KCl/0.05 M Hepes buffer. Ferritin was then eluted from the column with 12 ml buffer containing 0.5 M KCl.

3. RESULTS

3.1. *[^{59}Fe]Ferritin*

By using the optimalised conditions described previously, ^{59}Fe was incorporated into human spleen apoferritin. About 70% of the initial radioactivity was detected in the ^{59}Fe-reconstituted ferritin. The product of reconstitution was analysed as described by Bryce and Crichton [16]. It was similar to native ferritin according to electrophoresis. Duplicate polyacrylamide gels were stained respectively for iron and protein. All of the protein migrated with the iron.

3.2. *Separation of ferritin and transferrin*

The mixture of the two proteins was rapidly separated by ion-exchange chromatography using Bio-Rad AGI-X4 resin. Fig.1A illustrates the separation of [^{59}Fe]ferritin from apotransferrin immediately after mixing the two proteins. After 20 h of incubation, as shown in fig.1B, the apotransferrin had bound ^{59}Fe.

3.3. *Iron transfer in the absence of reducing and chelating agent*

The exchange of iron from ^{59}Fe-reconstituted ferritin to apotransferrin in 0.05 M KCl/0.05 M Hepes buffer, pH 7.5, at 20°C as a function of ferritin concentration is shown in fig.2. For ferritin

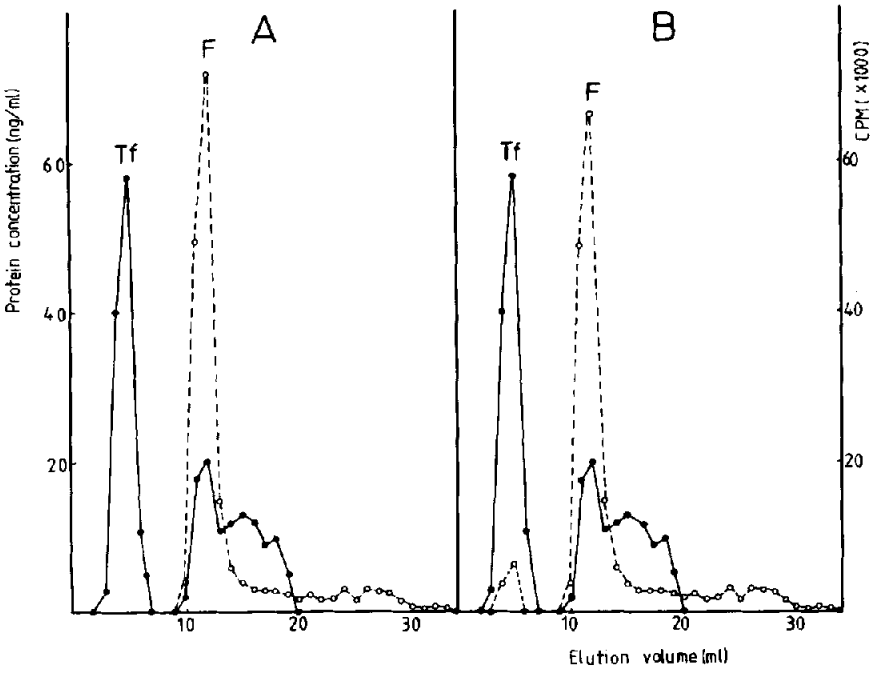

Fig.1. Elution profiles of a mixture containing 0.2 mg apotransferrin and 0.2 mg [^{59}Fe]ferritin. (A) Chromatography was carried out immediately after mixing the two proteins; (B) chromatography was carried out 20 h after mixing the two proteins: (●) protein concentration (mg/ml); (○) radioactivity (cpm × 10^3); Tf, transferrin; F, ferritin.

labelled with an average of 800 iron atoms per protein, the reaction followed first-order kinetics. When ferritin was incubated with varying amounts of apotransferrin, iron was taken up at a rate which appears to be independent of transferrin concentration (table 1).

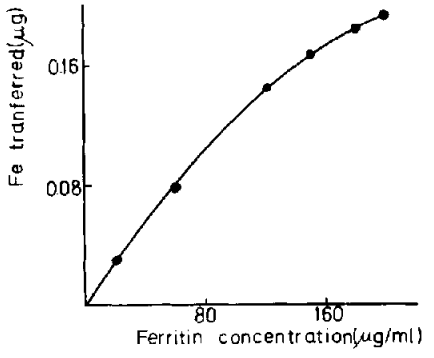

Fig.2. Iron transfer from [^{59}Fe]ferritin to apotransferrin (400 μg/ml) after 21.5 h as a function of ferritin concentration.

3.4. *Effect of serum ultrafiltrate on iron transfer*

When 50% (v/v) of the rat or human serum ultrafiltrate was incubated with [^{59}Fe]ferritin and apotransferrin, the rate of iron uptake was significantly enhanced (fig.3). After 100 h incubation, about 80 and 60% respectively more iron was transferred from ferritin to apotransferrin than the iron transferred in the absence of any mediating agents.

Table 1

Effect of apotransferrin concentration on iron transfer from [^{59}Fe]ferritin to apotransferrin

[Ferritin] (μg/ml):		45	45	90	90
[Apotransferrin] (μg/ml):		200	400	200	400
10^3 × μg Fe	6 h	21.8	24.7	44.5	44.1
transferred to	27 h	51.0	57.2	108.8	108.2
apotransferrin	50 h	80.1	83.3	154.2	145.2
	78 h	98.6	100.6	186.8	187.5
	120 h	117.4	120.3	215.3	220.0

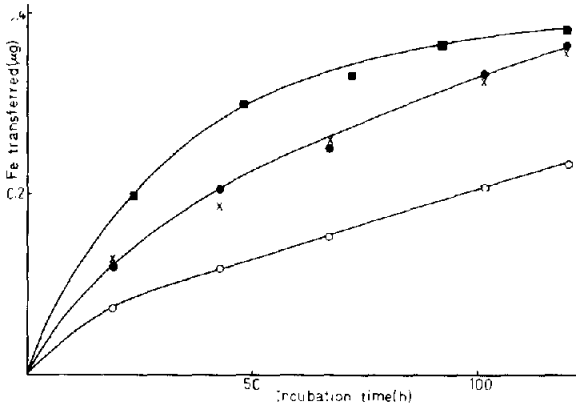

Fig.3. Iron transfer from reconstituted human spleen ferritin (90 μg/ml, 800 Fe/molecule) to human apotransferrin (400 μg/ml) in the presence of 50% (v/v) (●) human serum ultrafiltrate, (■) rat serum ultrafiltrate; (×) mixture of bicarbonate, ascorbate, citrate and lactate; (○) in the absence of any mediators.

3.5. *Influence of low-*M_r *serum components on iron transfer*

In order to determine the biochemical nature of the factors in serum ultrafiltrate responsible for the activation of iron transfer, we studied the influence of some substances present in human blood. Fig.4 illustrates the effects of ascorbate, citrate, lactate and bicarbonate which all enhance the rate of iron transfer from ferritin to apotransferrin in the range of their physiological concentration.

The action of ascorbate may be accounted for by reduction of iron in the ferritin to Fe^{2+} which can be released from the protein and be bound to the apotransferrin molecule. The citrate molecule may be able to penetrate into the ferritin core and form an Fe^{3+}-citrate complex. Apotransferrin could then take up iron from this complex. Carbonate has been shown to play a critical role in the iron-binding function of transferrin [18,19]. The carbonate anion is exchangeable with bicarbonate free in solution. Transferrin does not bind iron in the absence of carbonate or a synergistic anion. As our incubation medium was always in equilibrium with the atmospheric CO_2, there was an almost constant bicarbonate concentration that was necessary for the formation of the ternary Fe-transferrin-carbonate complex. When the incubation medium contained an increasing amount of bicarbonate or lactate, the iron uptake by apotransferrin was slightly enhanced (fig.4C,D).

To study the effect of the ensemble of the reductor, chelate and synergistic anions, we have prepared a solution containing 0.07 mM ascorbate, 0.161 mM citrate, 24.8 mM bicarbonate and 1.3 mM lactate in 0.05 M KCl/0.05 M Hepes, pH 7.5. 50% (v/v) of the mixture was incubated with [^{59}Fe]ferritin and apotransferrin as previously described for serum ultrafiltrate. The result (fig.3,X) indicated that the combination of these four molecules was comparable to human serum ultrafiltrate in the ability to increase the rate of iron transfer from ferritin to apotransferrin.

4. DISCUSSION

The present experiments show that iron exchange from reconstituted ferritin to apotransferrin takes place in the absence of any reducing and chelating agent. No additional bicarbonate and other synergistic anions are necessary for the reaction. The atmospheric CO_2 that is in equilibrium with the incubation medium provides enough bicarbonate anions that are essential for the iron binding capacity of the transferrin [20].

When the incubation medium contains serum ultrafiltrate the rate of iron transfer to apotransferrin was significantly enhanced. This increase is due to the presence of low-M_r substances in the human blood. These are: the reducer ascorbate, the iron chelate citrate, the bicarbonate and the synergistic anion lactate. The combination of the four agents at their normal physiological concentration in human blood is able to play the same role as the human serum ultrafiltrate in the ability to enhance the rate of iron uptake by transferrin.

Ferritin is localized within the cell and transferrin in the circulation. At the cellular level, there is no evidence that these two proteins come into contact. The reaction of iron exchange between them in vitro seems to have little physiological meaning. The only possibility that this reaction could take place in vivo would be iron release from serum ferritin to transferrin. Saito et al. [9] have observed release of iron in a ferritin-like form by cultured rat macrophage after ingestion of ^{59}Fe-labelled erythrocytes. They have also found that much of the initial labelled iron was bound to apotransfer-

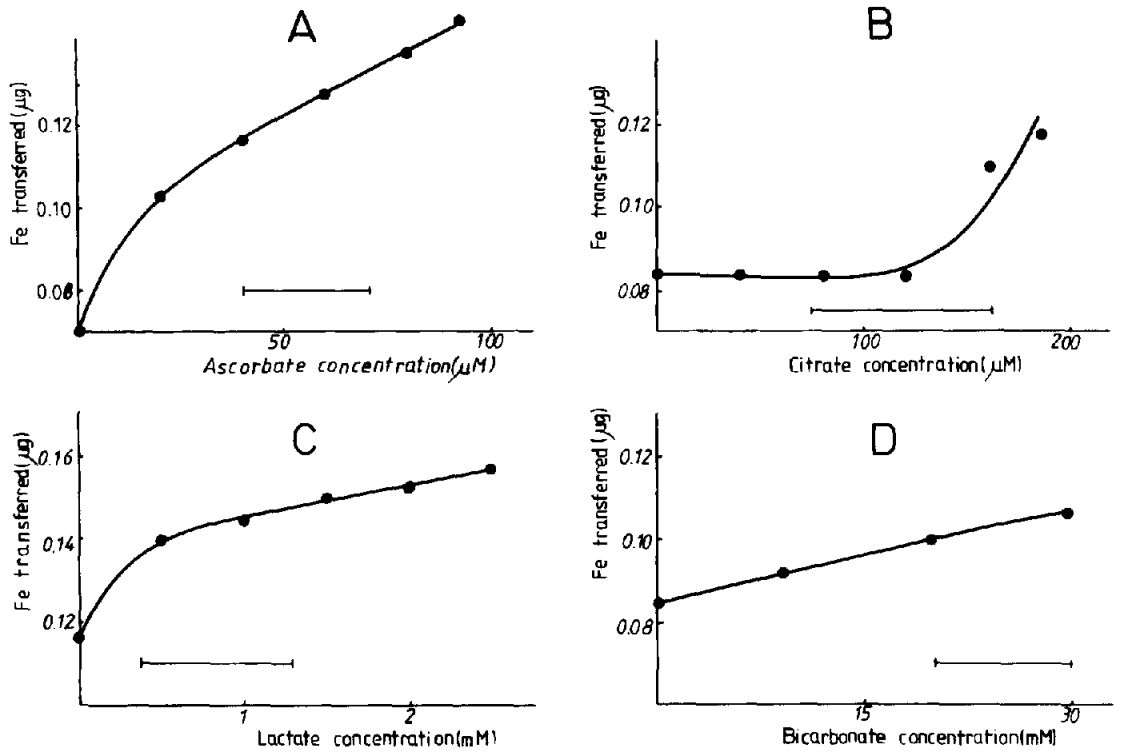

Fig.4. Influence of some low-M_r substances on the iron transfer from ^{59}Fe-reconstituted ferritin (90 μg/ml, 800 Fe/molecule) to apotransferrin (400 μg/ml) for different incubation times. (A) 10 h with ascorbate, (B) 24 h with citrate, (C) 23 h with lactate, (D) 20 h with bicarbonate. (——) Respective physiological concentrations of each substance in human blood plasma [17].

rin in the incubation medium. The effect of serum ultrafiltrate on iron transfer suggests that the secreted ferritin and apotransferrin that come into contact in the blood would be able to exchange their iron easily.

Consequently, we might consider the possibility of a pathway for mobilization of intracellular iron. When iron is excess in the cell, the biosynthesis of ferritin will be induced and iron will be released by secretion of ferritin to the circulatory system. In the blood, the iron of the secreted ferritin could be taken up by the apotransferrin and then transported elsewhere. Such a possible mechanism is under further investigation.

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