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IRON TRANSFER FROM TRANSFERRIN TO FERRITIN MEDIATED BY PYROPHOSPHATE

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SUMMARY

There is no significant iron exchange from transferrin to ferritin in the absence of reducing and chelating agents. Pyrophosphate can release iron from transferrin and can be isolated as a ferric pyrophosphate complex by ion exchange chromatography. We have established that pyrophosphate alone can mediate iron exchange from transferrin to ferritin. Under these conditions, iron is incorporated directly into ferritin as Fe(III).

INTRODUCTION

The mechanism by which iron is transferred from transferrin to ferritin is unknown. Whatever the cellular process involved (cell membrane and/or internalization phenomena), it is obvious that the first step implies iron release from transferrin. It has been shown that polyphosphate compounds such as ATP, GTP, 2,3-DPG and pyrophosphate can release iron from transferrin both in the presence and in the absence of a suitable chelator (1-6).
It is generally accepted that the deposition of iron in ferritin involves the oxidation of ferrous iron to ferric, catalysed by the protein with molecular oxygen as electron acceptor, followed by hydrolysis and deposition of the ferric oxyhydroxide in the protein shell (7). However it has been shown (8,9) that ferritin is capable of binding small amounts of Fe(III) (up to 200 atoms/molecule), which are incorporated into the micelle.

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function seems to be to (i) remove iron from transferrin (ii) form a ferrous chelate intermediate and (iii) maintain iron in the ferrous state for deposition in ferritin. Harris studied the iron transfer between the two proteins in free solution without any separation by a membrane (11). He observed that, in the presence of citrate, ATP or ascorbate, there is iron exchange between ferritin and transferrin, but the net flow was always from ferritin to transferrin. A significant iron exchange also occured in the absence of any chelator.

Pyrophosphate is by far the best chelating agent that removes iron from transferrin in the presence or absence of an iron acceptor. Based on the experimental model of Harris, we report here studies on the iron exchange between ^{59}Fe(III)-transferrin and ferritin in the presence of pyrophosphate under various conditions.

MATERIAL AND METHODS

Human apotransferrin was obtained from Behringwerke AG, Marburg, GDR. (^{59}Fe) transferrin was prepared as in (12). To remove unbound ^{59}Fe, the iron-transferrin solution was dialysed for 24h against 20mM $NaHCO_3$ at pH 8.0. Human spleen ferritin was isolated and purified as described in (13).

All reagents used were of analytical grade and solutions were made with iron-free distilled water. The iron content in ferritin was estimated by plasma torch (J.Y. 48 of Jobin-Yvon, France).

100 % saturated (^{59}Fe) transferrin (0.2 mg protein/ml) and ferritin containing 1,300 Fe atoms/molecule (0.02 mg/ml) were incubated at 37° C in a medium containing 225mM sucrose, 10mM Hepes/KOH buffer, pH 7.4 and 10mM KCl. Further additions were as shown in the figure legends. At appropriate time intervals, 50 µl aliquots were taken as counting standards and 1 ml aliquots were passed through a 1.5 x 6.0 cm column of Bio-Rad AG1-X4 anion-exchange resin. The column was equilibrated with 0.05M KCl/0.05M Hepes at pH 7.5 ; elution was with the Hepes buffer, first with 6 ml containing 0.50M KCl, then with 6 ml containing 0.1M KCl, then with 4 ml containing 0.5M KCl and finally with 16 ml containing 1.0M KCl. Transferrin was eluted exclusively with 0.05M KCl; ferritin with 0.1M KCl and the iron-pyrophosphate complex with 1.0M KCl. Fractions of 1 ml were collected. In each fraction ^{59}Fe radioactivity and absorbance at 280nm were determined. ^{59}Fe radioactivity was measured in a well-type scintillation counter, type BF 5300 (Berthold, Benelux Analytical Instruments, S.A., Vilvorde, Belgium). Spectrophotometric studies were made with DW-2A Aminco spectrophotometer (Beun-de Ronde, Amsterdam, Holland) or Beckman model 24 spectrophotometer (Analis, Namur, Belgium). The range of ^{59}Fe recovery was about 90-98 % of the total counts applied to the column.

RESULTS

Control experiments showed that, in the absence of a chelator or reducing agent, transfer of iron from transferrin to ferritin was insignificant. After 48h and 72h only 6 % and 9 % of ^{59}Fe, respectively, was found on the ferritin fraction. In the presence of pyrophosphate the iron was rapidly removed from (^{59}Fe) transferrin and bound to pyrophosphate.

The addition of pyrophosphate to $^{59}FeCl_3$, at pH 7.4, resulted in formation of a pyrophosphate-ferric iron complex, which was rapidly reduced by several reducing agents (ascorbate, dithionite, glutathione, cysteine, NADH) ; the Fe^{2+} can be quantitated as the Fe^{2+}-bipyridyl complex.

The effect of pyrophosphate concentration on iron removal from (^{59}Fe) transferrin as a function of time is shown in Figure 1. In all incubations, after 24h, more than 80 % of the iron formed a complex with pyrophosphate but virtually none of the ^{59}Fe from transferrin was bound to ferritin in the range of 1.0-0.5mM pyrophosphate. With 0.5mM pyrophosphate some iron was present in the ferritin fraction.

Therefore, in order to study the effect of pyrophosphate on the transfer of iron from transferrin to ferritin, it was necessary to decrease the pyrophosphate concentration. Figure 2 shows the results obtained after 24h incubation

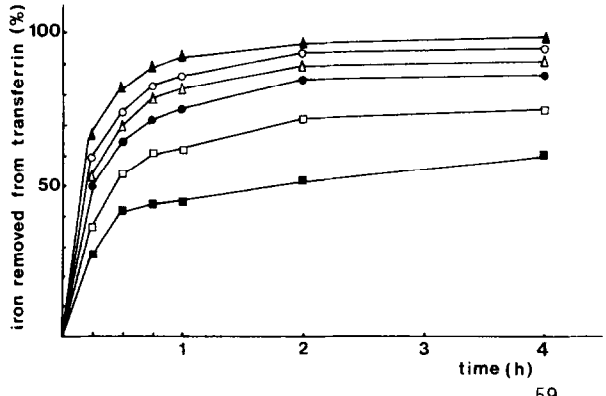

Figure 1 : The time course of the removal of iron from (^{59}Fe) transferrin in the presence of pyrophosphate. Concentrations employed are (■) 0.5mM ; (□) 1.0mM : (●) 2.0mM ; (△) 3.0mM ; (○) 4mM ; (▲) 5mM.

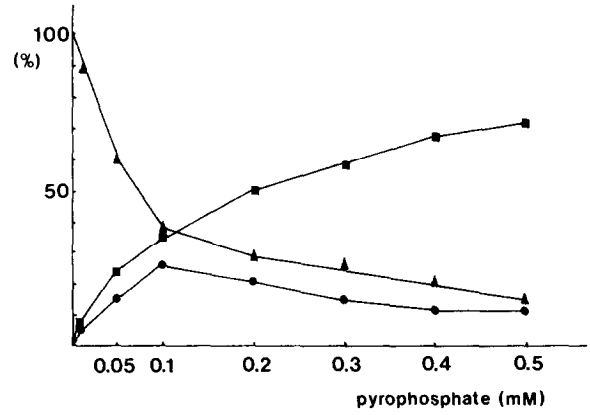

Figure 2 : The removal of ^{59}Fe from transferrin and binding to pyrophosphate and ferritin in the variable concentrations of pyrophosphate. The incubation time was 24h. At the ordinate is expressed the percentage of radioactivity present in the transferrin (▲), pyrophosphate (■) and ferritin (●) fractions.

expressed as percentage of iron bound to transferrin and of iron bound to pyrophosphate or ferritin, as a function of pyrophosphate concentration in the range of 0.01-0.5mM. It is apparent that 0.1mM pyrophosphate was most effective and after 24h, 26 % of ^{59}Fe was found in ferritin.

Figure 3 shows the results of 0.1mM pyrophosphate-mediated transfer of iron between (^{59}Fe) transferrin and ferritin as a function of time. ^{59}Fe uptake by ferritin proceeds linearly up to 24h and then slows down considerably. In contrast, the binding of ^{59}Fe by pyrophosphate increases rapidly during 2h, slows down up to 12h, then decreases and reaches a plateau. These results establish that the transfer of iron from (^{59}Fe) transferrin to ferritin is mediated by pyrophosphate. Approximately 30 % of the ^{59}Fe is associated with ferritin after 48h resulting in the deposition of 33 iron atoms/molecule of ferritin. When the Fe^{3+}-pyrophosphate complex, which is formed after incubation of 0.1mM pyrophosphate with 0.01mM $^{59}FeCl_3$, is used as source of iron, 18.5 % of the ^{59}Fe was found in the ferritin fraction after 24h. This corresponds to 41 iron atoms/molecule. This observation is in good agreement with the hypothesis that pyrophosphate acts as mediator for the iron transfer from transferrin to ferritin.

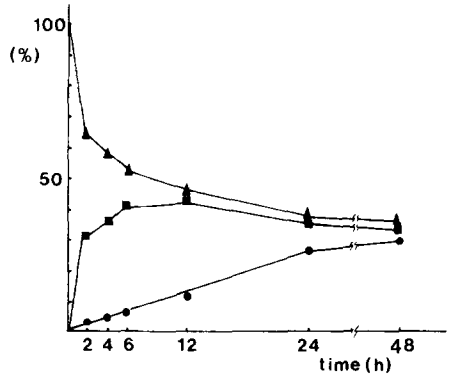

Figure 3 : The removal of ^{59}Fe from transferrin and binding to ferritin and pyrophosphate as a function of time, in the presence of 0.1mM pyrophosphate. At the ordinate is expressed the percentage of radioactivity present in the transferrin (▲), pyrophosphate (■) and ferritin (●) fractions.

DISCUSSION

In the present study we have confirmed that in the absence of reducing and chelating agents there is no significant iron exchange from transferrin to ferritin (10, 11). We have also confirmed (2, 5) that pyrophosphate can release iron from transferrin and have established that the iron can be isolated as a ferric pyrophosphate complex by ion-exchange chromatography. It is well established that pyrophosphate can form a stable, multiligand complex with ferric iron (14) and a less stable complex with ferrous iron. The pyrophosphate-Fe(III) complex can be readily reduced and the iron recovered essentially quantitatively as the Fe(II) - bipyridyl complex. Similar results were also found with dithionite and bathophenanthroline (5).

That pyrophosphate alone can mediate iron exchange from transferrin to ferritin is most clearly apparent at low pyrophosphate concentrations (figure 2 and 3). We interpret the results of figure 2 in terms of an increased affinity of polyvalent pyrophosphate-iron complexes for iron such that iron can be more readily assimilated by ferritin when the complexation of the metal by the pyrophosphate ligand is in its initial stages (where M is the metal iron and L the ligand) :

$$M + L \longrightarrow ML + L \longrightarrow ML_2 + L \longrightarrow ML_3 \ldots\ldots$$
$$\downarrow\uparrow$$
$$+ \text{ ferritin}$$
$$\downarrow\uparrow$$
$$\text{ferritin } M + L$$

We conclude that, in a physiological context, some transferrin iron might be incorporated directly into ferritin as Fe(III) in an analogous manner to the mechanism proposed for iron uptake by ferritin from ferric citrate (9). Although the amount of iron involved is small, it none the less represents one quarter of the transferrin iron engaged in the experiment at the plateau value observed after 24h of incubation. One final cautionary remark seems appropriate. It is not clear where in the cell transferrin and ferritin could come in contact. One possibility is at the plasma membrane after binding of transferrin to its receptor. Thus we cannot be sure at the present state of our knowledge of the physiological significance of the interesting findings reported here.

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