DIFFERENTIAL EFFECTS OF METAL-BINDING AGENTS ON THE UPTAKE OF IRON FROM TRANSFERRIN BY ISOLATED RAT LIVER MITOCHONDRIA

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1. Introduction

In eucaryotic cells the enzymes connected with the biosynthesis of haem are distributed between the mitochondria and the cytosol. The final insertion of ferrous iron into protoporphyrin is catalyzed by ferrochelatase, an enzyme reported to be firmly bound to the matrix side of the mitochondrial inner membrane [1]. It means that iron has to pass across the mitochondrial membranes, where its passage may be regulated by specific carriers.

It has been shown that mitochondria isolated from different tissues and species transfer iron to the matrix space. Several complexes have been used as donor compounds: ferric adenine nucleotide complexes [2]; ferric/ferrous 8-hydroxyquinoline complex [3]; and ferric sucrose complex [4–10]. However, it seemed important to investigate a more physiological substrate. It has been suggested that transferrin molecules may enter the cell by the process of endocytosis and release its iron at sites on the mitochondria [11–13]. The transferrin–iron complex has been used as the donor compound and it has been shown that isolated rat liver mitochondria accumulate iron from transferrin [14,15]. The mechanism by which isolated mitochondria release iron from transferrin is unknown.

The transferrin–iron complex is extremely stable under physiological conditions and no measurable iron exchange between the transferrin and the transferrin and ferritin was observed [16]. Transferrin binds one molecule of HCO_3^- together with each atom of iron [17]. A number of chelating agents have been shown to be effective anion substitutes in the transferrin–iron complex and these compounds facilitate the exchange of transferrin-bound carbonate. Most of the anions able to substitute for HCO_3^- do not labilize transferrin iron, others affect iron binding, too, accelering the intermolecular iron exchange [18]. Recently it has been suggested that some organic phosphates may play a role in iron release from transferrin or in the intracellular transport of iron. ATP, ADP and pyrophosphate accelerate the exchange of carbonate of the transferrin–iron–carbonate complex [19]. GTP, 2,3-diphosphoglycerate, ATP and ADP produce the most rapid exchange of iron between the two types of transferrin [20]. The uptake of transferrin-bound iron by immature erythroid cells was closely correlated with the intracellular concentration of ATP [21,22].

This paper reports the effect of chelating agents and phosphate compounds on the uptake of iron from transferrin by isolated rat liver mitochondria.

2. Materials and methods

2.1. *Accumulation of [^{59}Fe]transferrin*

Rat liver mitochondria were prepared in 250 mM sucrose containing 10 mM Tris–HCl buffer, pH 7.4, at conc. 15.0–20.0 mg protein/ml, essentially as in [23]. [^{59}Fe]Transferrin was prepared as in [12]. To remove unbound ^{59}Fe, the iron–transferrin solution was dialyzed for 24 h against 20 mM $NaHCO_3$. Protein was estimated using the biuret test. The mitochondria were incubated at 37°C for 30 min in a medium containing 225 mM sucrose, 10 mM Tris–HCl buffer, pH 7.4, 5 mM $MgCl_2$, 10 mM KCl and 5 mM

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succinate in vol. 1 ml. Further additions or omissions and the concentration of iron and transferrin were as shown in the figure legends. After incubation the mitochondria were centrifuged in a high speed centrifuge type 310 (Unipan, Warsaw). The pellet was washed twice with ice-cold 250 mM sucrose with 10 mM Tris–HCl buffer, pH 7.4 and ^{59}Fe radioactivity was measured in the well-type scintillation counter type PT 62 (Polon, Warsaw). All experiments were performed in triplicate.

2.2. *Dialysis experiments*

After incubation the samples were dialyzed against 200 ml 250 mM sucrose adjusted to pH 7.4 at 4°C. At the indicated time intervals the sacs were removed, rinsed with water and placed in the scintillation counter for the determination of radioactivity. In the control experiments the mitochondria were omitted from the incubation mixture.

3. Results

3.1. *Effect of EDTA, EGTA, citrate, pyruvate, succinate and glutathione on the uptake of iron from transferrin*

The concentration of EDTA and EGTA in the incubation mixture was from 10 μM to 5 mM. In this range the total iron accumulation was inhibited more than 50% (fig.1A). The concentration of citrate, pyruvate and glutathione in the incubation mixture was from 0.1–10 mM. As seen in fig.1B, iron accumulation was inhibited by citrate, but neither pyruvate nor glutathione had any effect on the iron uptake. All the studies on mitochondrial iron accumulation were performed with mitochondria respiring on 5 mM succinate in the absence of P_i. Higher concentrations of succinate gave a slight progressive inhibition of the iron uptake (fig.1C). Iron incorporation was stimulated by Mg^{2+} (fig.1C). In the standard incubation medium (see section 2) 5 mM Mg^{2+} was used.

3.2. *Effect of phosphate compounds on the uptake of iron from transferrin*

Several phosphate compounds were tested for their ability to influence iron accumulation. The concentration of phosphate compounds in the incubation mixture was from 0.1–5 mM. As seen in fig.1D, iron accumulation was stimulated by ATP, ADP and PP_i; AMP had no effect. The most active compound was PP_i. For example 2.5 mM PP_i enhanced iron uptake by 75%. ATP and ADP were less active and in the same concentration stimulated iron accumulation by 50% and 25%, respectively (fig.1D). Preincubation with atractyloside (50 μM) did not abolish the effect of ATP (table 1). Atractyloside alone had virtually no influence on iron accumulation. When incubation was carried out in the presence both ATP and EDTA the ATP-stimulatory effect was abolished and iron accumulation was inhibited (table 1).

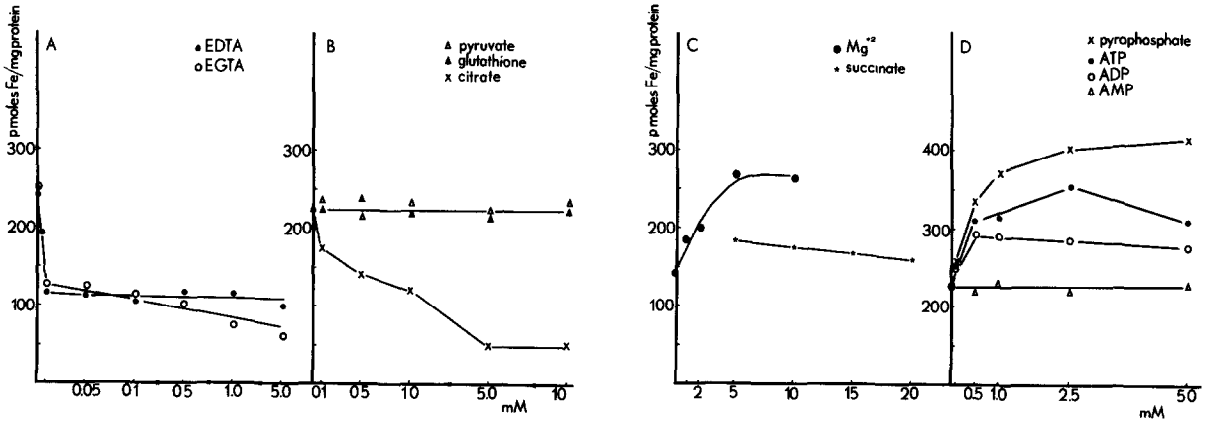

Fig.1. Effect of chelating agents, phosphate compounds and Mg^{2+} on the uptake of iron from [^{59}Fe]transferrin. The final concentrations of iron and transferrin in the reaction mixture were 1.835 μM and 1.125 μM, respectively. Protein concentration was 1.93 mg/ml.

Table 1
Influence of atractyloside and EDTA on the ATP-stimulatory effect

Treatment ATP (mM)	–	0.1	0.5	1.0	2.5	5.0
(pmol Fe/ mg protein)	185.5	259.5	293.9	306.3	289.2	256.3
Treatment ATP + atractyloside (50 μM)						
(pmol Fe/ mg protein)	185.7	259.9	284.3	297.8	281.5	237.0
Treatment ATP + EDTA (0.5 mM)						
(pmol Fe/ mg protein)	90.6	89.8	112.3	106.8	103.2	104.9

3.3. *Dialysis experiments*

Experiments were carried out as described in section 2 to see whether the incubation with mitochondria influenced the removal of iron from [^{59}Fe]transferrin by chelating agents (fig.2). The iron–transferrin complex could not dialyze through Visking tubing. The radioactivity of control bags was decreased to 90% after 7 days of dialysis in agreement with the decay of ^{59}Fe. ATP and ADP had no effect when added to the incubation mixture both in the absence and in the presence of mitochondria. Different results were obtained in the presence of EDTA and citrate. It is clear that under the conditions described above these chelating agents have the capacity of releasing iron from transferrin. When dialysis was carried out in the presence of mitochondria the removal of iron from transferrin was inhibited (fig.2B).

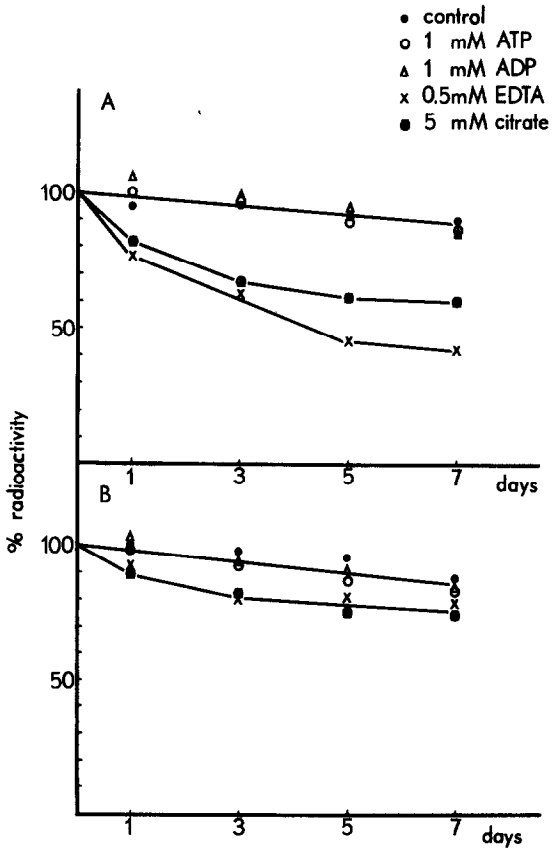

Fig.2. The time course of the removal of iron from [^{59}Fe]transferrin by various chelating agents. The final concentrations of iron and transferrin in the reaction mixture were 1.835 μM and 1.125 μM, respectively. Figure 2 represents results of dialysis carried out in the absence of mitochondria (fig.2A) and in the presence of mitochondria (fig.2B). Protein concentration was 2.07 mg/ml.

4. Discussion

The results obtained in the present study support the observations that isolated rat liver mitochondria accumulate iron from transferrin. The process is markedly influenced by chelating agents and phosphate compounds.

Analysis of the influence of the chelating agents on iron accumulation reveals two stages to this process:

1. Release of iron from the complex probably after interaction of transferrin with an other iron-binding molecule.
2. Incorporation of the released iron into the mitochondria.

The inhibitory effect of EDTA, EGTA and citrate on iron accumulation can be explained as a chelating effect after dissociation of transferrin. These compounds can by themselves release iron from transferrin by substituting for HCO_3^-, but in consideration of the short time of incubation, this mechanism seems less likely [24]. The stimulation of iron uptake by ATP, ADP and PP_i supports the hypothesis that cellular phosphate compounds may facilitate the release of iron from transferrin [20]. The mechanism of the action of these compounds is not clear. It is possible that they interact with the transferrin molecule to produce a conformational change which allows the release of iron. It was found that these compounds can not substitute for HCO_3^- in the ternary complex [19,20]. In view that atractyloside had not effect the adenine nucleotide translocator must not play a role in iron accumulation by mitochondria, and iron is not transferred in form of a complex with the nucleotides.

It can be concluded that the rates of iron exchange between transferrin and mitochondria in the presence of a chelating agent depend partly upon the affinity for iron (steric effects) as well as upon the stability constant. EDTA, EGTA and citrate have relatively high affinities for iron and form stable complexes with the released iron, which were washed out during centrifugation. Consistent with this interpretation are the results of dialysis experiments. When dialysis was carried out in the presence of mitochondria, a new equilibrium state was probably established and the exchange of iron between transferrin and the chelating agent was inhibited. ATP and ADP have relatively low affinities for iron and under the conditions of dialysis have not the capacity of releasing iron, but they can mediate and stimulate the exchange of iron between transferrin and mitochondria. The ATP-stimulatory effect was abolished by the EDTA chelating effect. The results observed for AMP, pyruvate and glutathione are consistent with observation [20] for exchange of iron between the two types of transferrin.

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