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Iron transport into erythroid cells by the Na^+/Mg^{2+} antiport

Lee M. Stonell, Donna L. Savigni, Evan H. Morgan *

Department of Physiology, The University of Western Australia, Nedlands, Western Australia 6907, Australia

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Abstract

Rabbit erythroid cells can take up non-transferrin-bound iron by a high-affinity and a low-affinity transport mechanism (Hodgson et al. (1995) J. Cell. Physiol. 162, 181–190). The latter process, which is present in mature erythrocytes as well as reticulocytes, was investigated in this study using rabbit reticulocytes and erythrocytes. Iron uptake was optimal in isotonic KCl (pH 7.0), was shown to be much greater for Fe(II) than Fe(III), to be saturable with a K_m value of approx. 15 μM Fe(II), temperature-dependent and inhibited by inhibitors of cell energy metabolism, by Na^+ and many divalent cations and by several known inhibitors of membrane cation transport mechanisms. Uptake was more rapid with rabbit than with rat or human erythrocytes. The Fe(II) transport process was much more sensitive to inhibition by Mg^{2+} than by Ca^{2+} and the inhibition by both Mg^{2+} and Na^+ was of competitive type. Cells depleted of intracellular Mg^{2+} by the use of the ionophore A23187 had low rates of Fe(II) uptake. High rates of uptake could be achieved by replenishment of intracellular Mg^{2+}, and the Mg^{2+}-dependent uptake of Fe(II) was inhibited by the same reagents which reduced the uptake by untreated cells. Many features of the Fe(II) transport process are very similar to those of the previously described Na^+/Mg^{2+} antiport. These features, plus the stimulation of Fe(II) uptake by intracellular Mg^{2+} and inhibition by extracellular Mg^{2+} or Na^+, strongly suggest that the iron is transported into the cells by the antiport.

Keywords: Iron transport; Sodium/magnesium antiport; Erythrocyte

1. Introduction

The normal mechanism of iron uptake by developing red blood cells involves receptor-mediated endocytosis of transferrin [1,2]. However, erythroid cells also possess the capacity to take up non-transferrin-bound ferrous iron, Fe(II) [3–5]. At least two mechanisms have been described to account for the way this form of iron crosses erythroid cell membranes [5,6]. These are a high-affinity process which is found only in immature cells such as reticulocytes and a low-affinity process which is present in mature erythrocytes as well as reticulocytes [6]. The high-affinity process has been studied in some detail [3,7–9]. It has a K_m of approx. 0.2 μM and has the characteristics of active transport. It has been proposed that this mechanism is the one by which iron released from transferrin within endosomes is transferred across the endosomal membrane into the cytosol [8,9].

Much less information is available on low-affinity iron transport. This process was first identified by the stimulation of iron uptake which was observed when NaCl in the incubation medium was replaced by KCl [5,6]. It differs from the high-affinity mechanism in several ways in addition to the affinity for iron. Iron transport becomes more prominent as the extracellular iron concentration is raised and does not saturate until a level of 40–50 μM is reached, compared with 1–2 μM for high-affinity uptake. The capacity to take up iron by the low-affinity pathway does not disappear as reticulocytes mature and is unimpaired in erythroid cells from homozygous Belgrade rats which have an abnormality of iron transport which leads to diminished uptake from transferrin or by high-affinity Fe(II) transport [6]. Also, low-affinity iron transport is inhibited by NaCl and amiloride, but is stimulated by KCl, RbCl, LiCl and CsCl [5,6]. These results have led to the hypothesis that the iron is transported into the cells by a Na^+/Fe^{2+} counter-transport mechanism [5].

The aim of the present work was to investigate the mechanism of low-affinity iron transport in erythroid cells. Initially, this was undertaken by examining the effects of incubation temperature and ATP depletion, of other metal ions and of known inhibitors of membrane transport of cations. During the course of the work it became apparent

* Corresponding author. Fax: +61 9 3801025; e-mail: ehmorgan@uniwa.uwa.edu.au.

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that extracellular Mg^{2+} is an effective inhibitor. Hence, its possible role in Fe(II) transport was investigated in some detail. The results lead to the conclusion that low-affinity Fe(II) transport occurs by the same mechanism as is responsible for the exchange of intracellular Mg^{2+} for extracellular Na^+, i.e., by the previously-identified Na^+/Mg^{2+} antiport.

2. Materials and methods

2.1. Materials

Radioactive iron ($^{59}FeCl_3$) was purchased from Dupont, Australia (Sydney, Australia) and the biochemical reagents from Sigma Chemical Co. (St. Louis, MO). Reticulocyte-rich blood was obtained from rabbits with phenylhydrazine-induced hemolytic anemia, 4–6 days after the last injection of phenylhydrazine [10]. Blood with a low reticulocyte count was from normal adult rabbits. The red blood cells were washed three times in 0.155 M NaCl and once in 0.155 M KCl and then suspended in 0.155 M KCl at a packed cell volume (PCV) of approx. 0.30. The cells from the anemic rabbits contained 45–70% reticulocytes and, from the normal rabbits, 1–3% reticulocytes. They will be referred to as reticulocytes and erythrocytes, respectively. In one set of experiments erythrocytes were also obtained from normal rats and humans by heart puncture and venepuncture, respectively, and were washed and suspended in the same way as the rabbit cells.

2.2. Measurement of iron uptake

In most experiments iron uptake was determined by incubating samples of the cell suspensions (usually 80 μl) in 2.0 ml Hepes-buffered (pH 7.0) isotonic KCl solution (290 mOsm/kg) at 37°C for 15 min in the presence of ^{59}Fe-labelled Fe(II) as in earlier work [6]. The cells were then washed once with ice-cold 5 mM EDTA in 0.15 M NaCl (pH 7.4) and twice with ice-cold 0.155 M NaCl, followed by hemolysis in 15 mM Hepes (pH 7.4) and centrifugation to separate cytosolic and stromal fractions of the cells [3,6]. These fractions were then counted for radioactivity.

The incubation conditions (KCl, pH 7.0, 15 min) were chosen after preliminary studies which showed that the rate of iron uptake was maximal in KCl (or RbCl) solution at pH 7.0 and that uptake under these conditions was linear for at least 30 min. Hence, the 15 min uptake results enable the rate of iron uptake to be calculated. The results are expressed as iron uptake in nmol per ml cells for the 15 min incubation period or as iron uptake per ml cells per min, as indicated below.

The radioactive iron solution was prepared immediately before use, by mixing ^{59}Fe ($FeCl_3$) with ^{56}Fe ($FeSO_4$) in a molar ratio of approx. 1:100, and diluting to the desired concentration with 0.27 M sucrose. An aliquot of this solution was then added to the cell incubation mixture to give the final iron concentration. In most experiments this was 20 μM, but in some experiments the concentration was varied, as indicated below. By use of the Fe(II) colorimetric reagent, α,α'-bipyridine it was shown that the iron remained in the ferrous state for the time required for the experiments and, due to the rapid self exchange of Fe(II) and Fe(III) [11], the iron would be uniformly labelled with ^{59}Fe.

Changes to the above incubation procedure were made in some of the experiments. These include alteration of the incubation temperature, or the addition of various membrane transport inhibitors or the chloride salts of a range of cations to the incubation solution. In these cases the cells were incubated with the inhibitors or salts for 10 min at room temperature before the addition of the ^{59}Fe-labelled Fe(II). Metabolic depletion was achieved by preincubation of the cells at 37°C for 30 min in the presence of 5 mM iodoacetamide and 5 mM inosine [12]. In some experiments, the intracellular Mg^{2+} concentration was changed before the cells were incubated with ^{59}Fe through the use of the ionophore A23187 and solutions containing 0 to 12 mM $MgCl_2$ [13]. In this procedure the A23187 was removed by four 10 min incubations at 37°C with bovine serum albumin (10 mg/ml) in KCl solution (pH 7.0), containing the required concentrations of $MgCl_2$.

2.3. Analytical methods

The reticulocyte count was determined by staining blood cells with new methylene blue and the packed cell volume by the microhematocrit method. Radioactivity was counted in a three-channel γ-scintillation counter (LKB 1282 Compu-Gamma). Osmolality was measured by freezing point depression using a Fiske One-Ten Osmometer (Fiske, Needham Heights, MA).

3. Results

3.1. Uptake of Fe(II) and Fe(III)

The ability of reticulocytes and erythrocytes to take up Fe(III), as well as Fe(II), was investigated by incubating the cells with increasing concentrations of ^{59}Fe-labelled Fe(II) prepared as described above, and Fe(III) prepared in a comparable way but with the $FeSO_4$ replaced by $FeCl_3$. The oxidation state of the iron in the incubation solutions was verified by the use of the colorimetric ferrous iron chelator, α,α'-bipyridine, which produced a pink coloration immediately after addition to the Fe(II) solution but not with the Fe(III) solution. The rate of iron uptake from Fe(II) was much greater than from Fe(III) and saturated as the iron concentration was raised (Fig. 1). At an iron concentration of 20 μM the rate of uptake of Fe(II)

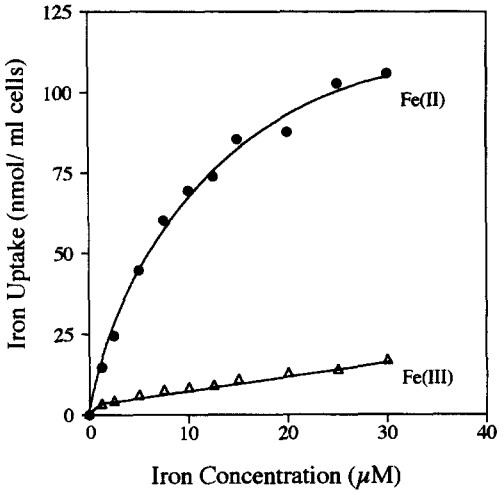

Fig. 1. Effect of iron concentration on the uptake of ferrous (●) and ferric (△) iron to the cytosol of rabbit reticulocytes. The cells were incubated for 15 min at 37°C with ^{59}Fe-labelled $FeSO_4$ or $FeCl_3$ in isotonic KCl (pH 7.0). They were then washed, hemolysed and fractionated into cytosolic and stromal fractions as described in the text. This experiment was repeated 4 other times, with similar results.

was more than 10 times as great as from Fe(III). Results of 5 determinations of Fe(II) uptake similar to that shown in Fig. 1 were used to calculate the maximal rates (V_{max}) and Michaelis-Menten constants (K_m) for iron uptake. The mean values for V_{max} were 17.8 ± 3.1 and 8.8 ± 0.8 nmol/ml cells/min (mean ± S.E.M.) for reticulocytes and erythrocytes, respectively, and the mean K_m values were 14.9 ± 1.8 and 14.4 ± 2.2 μM, respectively. It should be noted that these values differ somewhat from previous results [6]. The reason is that in the earlier experiments the incubations were performed at pH 6.5, not 7.0, which has since been found to be the optimum pH for the incubation conditions used in this study. Lower K_m values result from the use of the higher pH, indicative of a higher affinity of Fe(II) for the iron transporter at pH 7.0 than at pH 6.5.

3.2. Inhibition of iron uptake by NaCl and divalent cations

Fe(II) uptake was measured using cells which had been preincubated for 10 min at room temperature with the chloride salts of several divalent cations. All of the salts inhibited iron uptake in a concentration-dependent manner. At high concentrations they all produced at least 95% inhibition. The concentrations of the salts which produced 50% inhibition of Fe(II) uptake (IC_{50}) varied from 4 mM for Na^+ to 20 μM for Co^{2+}, Ni^{2+} and Zn^{2+} (Table 1). Very similar values were obtained for reticulocytes and erythrocytes. When Ca^{2+} and Mg^{2+} were compared, the IC_{50} value for Mg^{2+} was found to be only one-tenth that for Ca^{2+}. This raised the question whether there is an interaction between the transport of Fe(II) and Mg^{2+} in

Table 1
Inhibition of iron uptake by Na^+ and divalent cations

Cation	IC_{50} (μM)	
	Reticulocytes	Erythrocytes
Na^+	4300 ± 530	4100 ± 290
Co^{2+}	20 ± 5.8	20 ± 2.7
Ni^{2+}	20 ± 4.1	18 ± 1.9
Zn^{2+}	19 ± 3.6	25 ± 3.7
Mg^{2+}	90 ± 14	95 ± 6.9
Mn^{2+}	105 ± 17	100 ± 13
Cd^{2+}	140 ± 15	145 ± 21
Ca^{2+}	970 ± 112	975 ± 125
Sr^{2+}	2500 ± 380	2800 ± 350

The uptake of 20 μM Fe(II) by reticulocytes and erythrocytes was measured using cells which were incubated in isotonic KCl (pH 7.0) containing varying concentrations of the chloride salts of Na^+ or several divalent cations. The results are expressed as the concentrations of the cations which produced 50% inhibition (IC_{50}) of iron transport into the cytosol of the cells. Each value is the mean (± S.E.M.) of three separate determinations.

erythroid cells and led to several of the experiments described below.

3.3. Effects of cation transport inhibitors on Fe(II) uptake

Several reagents which have been shown previously to inhibit a variety of cation transport processes were studied for their effects on low-affinity Fe(II) transport. Some had no effect at the concentrations used. These included ouabain (500–1000 μM), bumetanide (10–100 μM), furosemide (30–100 μM), vanadate (10–1000 μM) and *p*-chloromercuriphenyl-sulfonic acid (10–25 μM). Others were effective inhibitors and their IC_{50} values are presented in Table 2. Iron uptake was particularly susceptible to inhibition by valinomycin, oligomycin and diethylstilbestrol, but was also blocked by relatively low concentrations of the other reagents listed in the table. Once again, the results for erythrocytes were very similar to those for reticu-

Table 2
Effect of membrane transport inhibitors on iron uptake

Inhibitor	IC_{50} (μM)	
	Reticulocytes	Erythrocytes
Valinomycin	0.12 ± 0.035	0.093 ± 0.017
Oligomycin	0.34 ± 0.024	0.52 ± 0.041
Diethylstilbestrol	1.1 ± 0.10	1.2 ± 0.10
Quercetin	6.6 ± 1.0	5.9 ± 1.1
Imipramine	7.7 ± 0.64	10.0 ± 0.71
Quinidine	67 ± 6.0	100 ± 15
Amiloride	190 ± 17	250 ± 30
N-Ethylmaleimide	200 ± 6.5	210 ± 5.9

Reticulocytes and erythrocytes were incubated with 20 μM Fe(II) in KCl (pH 7.0) in the presence of varying concentrations of the inhibitors. The results show the concentrations of the inhibitors which produced 50% inhibition (IC_{50}) of iron uptake into the cytosol of the cells (means ± S.E.M. of 3 determinations).

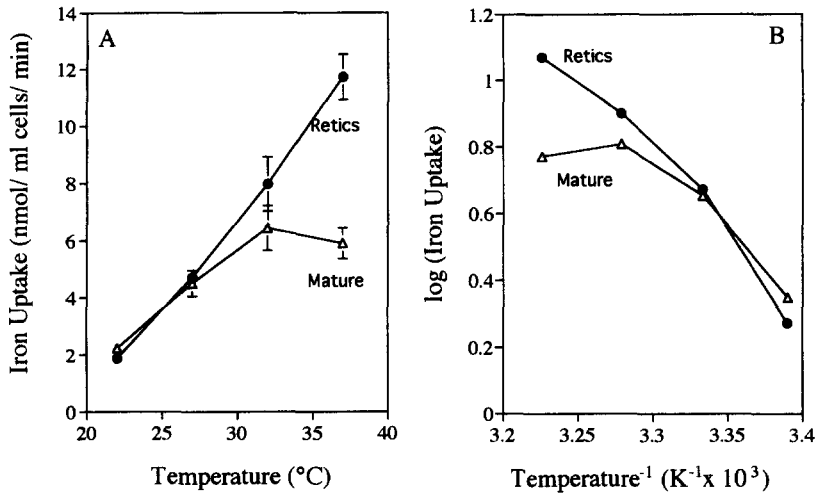

Fig. 2. Effect of incubation temperature on the uptake of Fe(II) to the cytosol of reticulocytes (Retics) and erythrocytes (Mature). The figure shows (A) the results for uptake (means ± S.E.M., $n = 3$) and (B) Arrhenius plots of the mean values. Incubation was performed in isotonic KCl (pH 7.0).

locytes, and high concentrations of the reagents produced at least 95% inhibition.

3.4. Kinetics of Fe(II) uptake

The rate of iron uptake into the cytosolic fraction of reticulocytes increased as the incubation temperature was raised from 22°C to 37°C (Fig. 2A). However, with erythrocytes the rate reached a maximum at about 32°C and fell from this value as the temperature was raised to 37°C. Arrhenius plots of these results were not linear, especially with erythrocytes (Fig. 2B) so that values of the activation energy (E_a) could not be calculated for these cells. In the case of reticulocytes the plot was almost linear between 27°C and 37°C, and the approximate value for E_a over this temperature range was calculated to be 74 kJ/mol.

3.5. Effect of ATP depletion

The method used to deplete ATP levels (incubation with iodoacetamide and inosine) has previously been shown to lower ATP concentrations of erythrocytes to less than 20% of control values [12]. This was confirmed in the present work when the mean ATP concentration of reticulocytes was lowered from the control value of 510 to 35 nmol/ml cells. These changes were accompanied by marked inhibition of Fe(II) uptake (Fig. 3). That this effect is probably due to the lowering of the ATP levels, and not to a direct toxic effect of iodoacetamide, is indicated by the observation that iron uptake was not inhibited in cells to which the iodoacetamide was added immediately before the radioactive iron (Fig. 3).

3.6. Effect of Na^+

The findings that extracellular Na^+ (Table 1) and amiloride [5,6] can inhibit Fe(II) uptake led to the suggestion that Na^+ competes with Fe(II) for uptake by a Na^+/Fe^{2+} exchange process [5]. This hypothesis was investigated by determining the effects of varying extracellular concentrations of NaCl on iron uptake from KCl solutions containing increasing concentrations of Fe(II). It was found that NaCl raised the K_m for iron uptake but had little effect on the V_{max} (Fig. 4), features which are characteristic of a competitive type of inhibition.

3.7. Uptake by rat and human erythrocytes

The rates of transmembrane cation transport, including Mg^{2+} [14] have been shown to vary considerably between

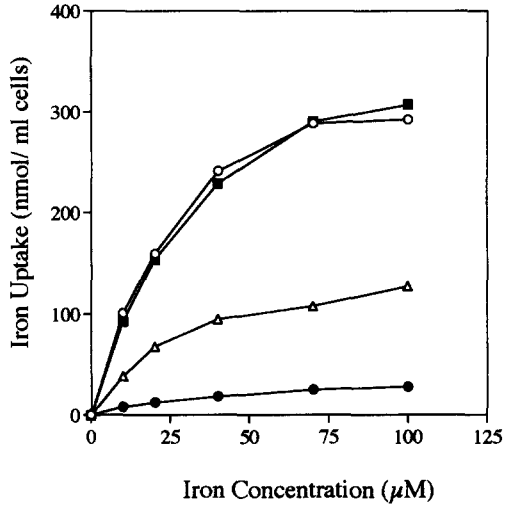

Fig. 3. Effect of preincubation with iodoacetamide and inosine on Fe(II) transport into the cytosol of reticulocytes. The cells were incubated for 15 min at 37°C in isotonic KCl (pH 7.0) with varying concentrations of Fe(II) after 30 min preincubation in the same solution containing no reagent (■), 5 mM iodoacetamide (△) or 5 mM iodoacetamide +5 mM inosine (●). Also shown is the uptake by cells to which the iodoacetamide was added at the same time as the radioactive iron (○). Similar results were obtained in a repeat experiment with reticulocytes and in two experiments with erythrocytes.

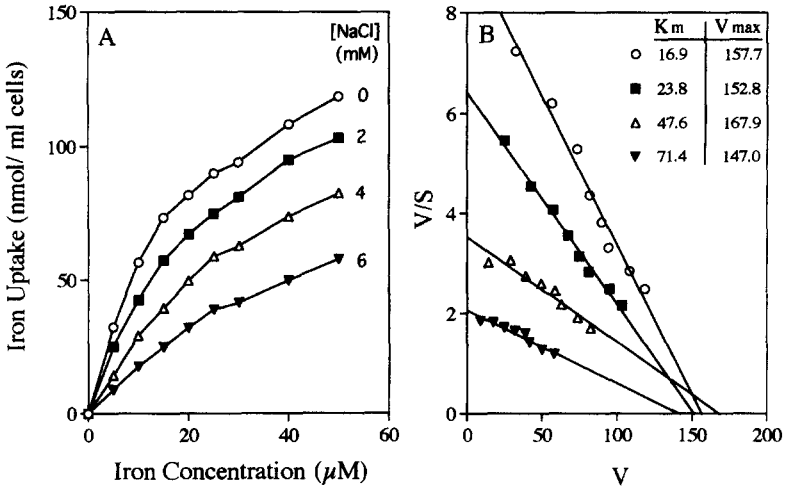

Fig. 4. Inhibition of Fe(II) transport into reticulocytes by NaCl. The cells were incubated for 15 min at 37°C in isotonic solutions of KCl (pH 7.0) plus no NaCl (○), 2 mM NaCl (■), 4 mM NaCl (△) or 6 mM NaCl (▼). The figure shows Fe uptake to the cytosol (A) and Eadie-Hofstee plots of the uptake results (B). In this and a second experiment the addition of NaCl had little effect on V_{max} (nmol/ml cells) but raised the K_m (μM) values. Similar results were obtained with erythrocytes in two other experiments of this type.

erythrocytes obtained from different animal species. In order to see whether this was the case for low-affinity Fe(II) transport and for comparison with Mg^{2+} transport, comparative studies were made between rat, human and rabbit erythrocytes. Iron uptake rates were found to be very low with human erythrocytes, high with rabbit cells, and intermediate with rat cells (Fig. 5). Also, with rat as well as rabbit cells, the rate of iron uptake increased as the KCl concentration was raised.

3.8. Role of Mg^{2+} in Fe(II) uptake

Three types of experiment were undertaken to investigate the possible interrelations between Fe(II) and Mg^{2+} transport in erythroid cells: (1) measurement of Fe(II) uptake by cells with varying intracellular Mg^{2+} concentrations, (2) determination of the effects of inhibitors on Mg^{2+}-dependent Fe(II) uptake, and (3) investigation of whether or not inhibition of Fe(II) uptake by extracellular Mg^{2+} is competitive in nature.

The effects of manipulation of the intracellular Mg^{2+} concentration on Fe(II) uptake are illustrated in Fig. 6. With both erythrocytes and reticulocytes iron uptake increased markedly as the Mg^{2+} concentration was raised from 0 to 1.0 mM and was maintained at a high level as the concentration was raised further, to 12 mM with erythrocytes but to only 2 mM with reticulocytes since hemolysis occurred with these cells at higher Mg^{2+} concentrations. A feature of the results is the high level of uptake by erythrocytes which had been treated with A23187. This was a consistent but, as yet, unexplained observation.

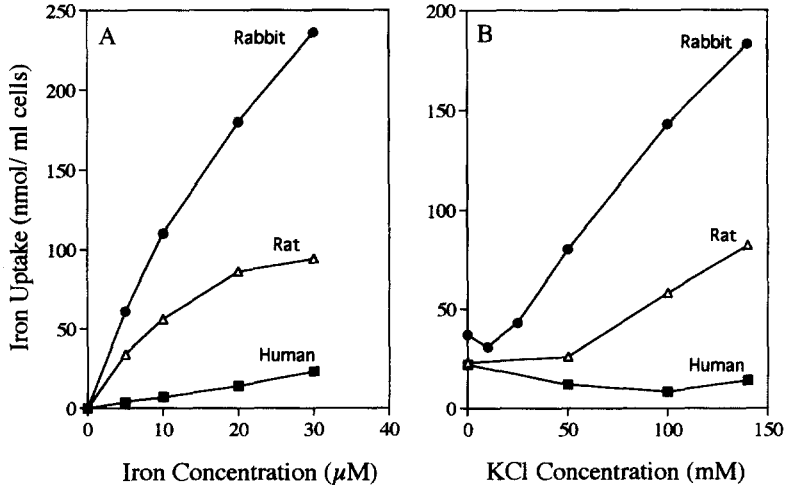

Fig. 5. Iron transport into the cytosol of rabbit (●), rat (△) and human (■) erythrocytes. The cells were incubated at 37°C for 15 min with (A) varying concentrations of Fe(II), or (B) with 20 μM Fe(II) in isotonic solutions containing varying concentrations of KCl. The latter solutions were prepared by mixing isotonic sucrose with isotonic KCl. The figure shows the result of one out of two separate experiments which gave similar results.

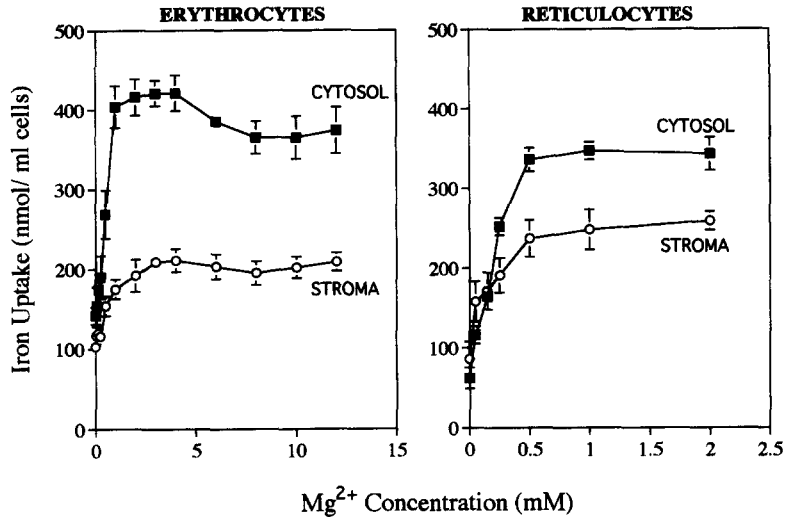

Fig. 6. Effect of concentration of Mg^{2+} on the uptake of Fe to the cytosol and stroma of erythrocytes and reticulocytes. The Mg^{2+} concentrations were varied by the use of A23187, as described in the text. The values show the extracellular Mg^{2+} concentration during loading in the presence of A23187. Each result is the mean ± S.E.M. of 3–4 measurements of Fe uptake during 15 min incubation at 37°C with 20 μM Fe(II) in isotonic KCl (pH 7.0). Concentrations of Mg^{2+} in excess of 2 mM produced hemolysis with reticulocytes, but not with erythrocytes.

Several inhibitors of Fe(II) uptake by untreated cells were tested for their capacity to block Mg^{2+}-dependent iron uptake using Mg^{2+}-depleted cells and cells in which the intracellular Mg^{2+} concentration had been adjusted to 1 mM by the A23187 exchange technique. They were all effective inhibitors of iron uptake by cells containing 1 mM Mg^{2+} but, with the exception of quercetin, had little effect on iron uptake by Mg^{2+}-depleted cells (Fig. 7).

Inhibitory extracellular concentrations of Mg^{2+} were found to increase the K_m values but to have little effect on V_{max} for iron uptake (Fig. 8). Hence, extracellular Mg^{2+} inhibits iron uptake in a competitive manner.

4. Discussion

The iron transport mechanism investigated in this study is present in erythrocytes as well as reticulocytes and in both types of cells has a K_m of approx. 15 μM. Ferrous iron is transported preferentially to ferric iron. The process

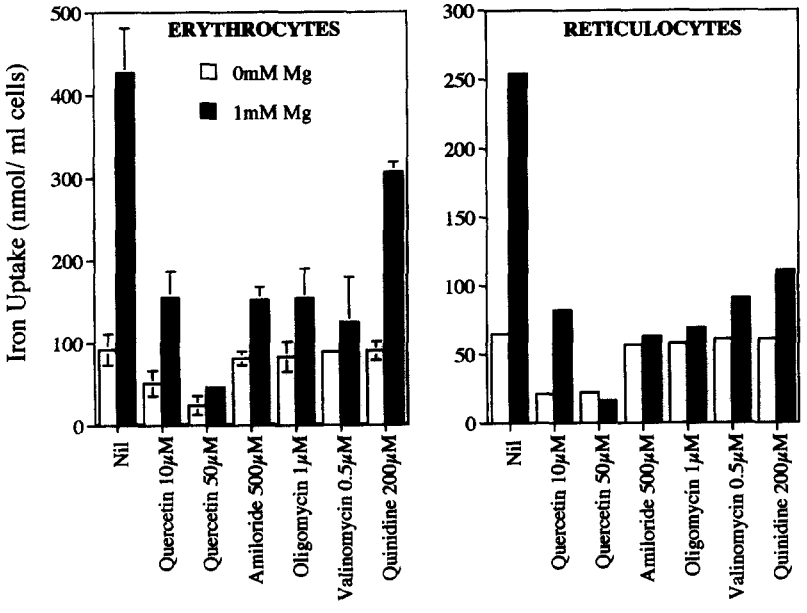

Fig. 7. Effect of several inhibitors on iron transport to the cytosol of erythrocytes and reticulocytes in which the Mg^{2+} concentrations were adjusted by the use of A23187. The Mg^{2+} concentrations shown on the figure (0 and 1 mM) are the extracellular Mg^{2+} concentrations which were present during treatment with A23187. Incubation with 20 μM Fe(II) was performed for 15 min at 37°C in isotonic KCl (pH 7.0). The values are the means ± S.E.M. of 3 determinations with erythrocytes and the means of 2 determinations with reticulocytes.

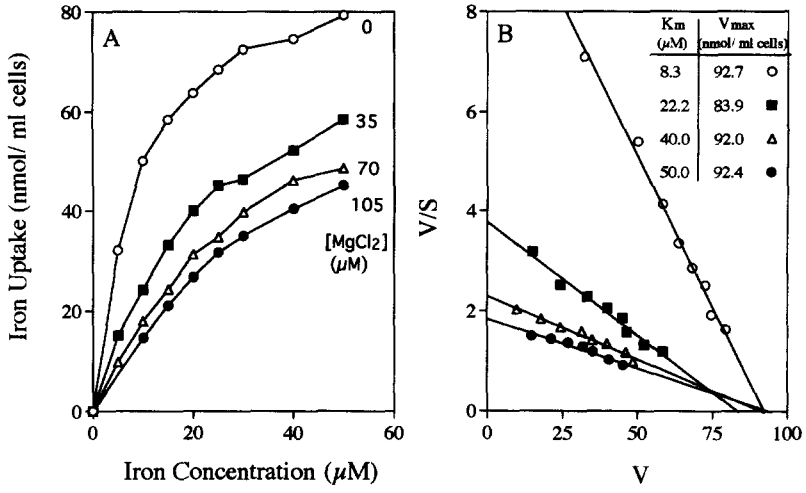

Fig. 8. Inhibition of iron uptake by $MgCl_2$. Reticulocytes were incubated with 20 μM Fe(II) at 37°C for 15 min in isotonic KCl (pH 7.0) containing zero (○), 35 μM (■), 70 μM (△) or 105 μM (●) $MgCl_2$. The figure shows uptake to the cytosol (A) and Eadie-Hofstee plots of the uptake results (B). In this and two other similar experiments the addition of $MgCl_2$ produced little change in V_{max} but caused a concentration-dependent increase in K_m (μM) values.

has a high activation energy and appears to be dependent on cellular metabolism. Overall, the evidence points to a carrier-mediated process, possibly to an active transport process.

The inhibitory effects of the other cations and of transport inhibitors provide clues to the nature of the process. In most instances at least 95% inhibition could be achieved by the use of the cations and inhibitors. This implies that only one Fe(II) transport process is involved or, if there is more than one, they have very similar properties with respect to the effects of the inhibitors. Inhibition by NaCl and amiloride indicates an involvement of Na^+ transport as has been suggested earlier [5]. The competitive nature of the Na^+-dependent inhibition makes it likely that Fe^{2+} and Na^+ compete for a common site on the transport mechanism. However, other ions in addition to Fe^{2+} and Na^+ may be involved. A role for Mg^{2+} is suggested by the greater sensitivity of Fe(II) transport to Mg^{2+} than to Ca^{2+}. The high degree of sensitivity to Co^{2+}, Ni^{2+} and Zn^{2+} and, to a lesser degree, to Mn^{2+} and Cd^{2+} are also of interest. How these ions interact with Fe(II) transport will be the subject of future investigations. At present it should be noted that the IC_{50} values for the first 3 of these ions are the same as the Fe(II) concentration which was present in the incubation solutions (20 μM).

All of the biochemical reagents which inhibited Fe(II) uptake have been shown to inhibit other ion transport processes and, in many cases, to be inhibitors of ion-transporting ATPases. However, none of them are specific in their action so that it is not possible to conclude with any confidence that Fe(II) is transported by the action of an ATPase, although the possibility must be considered. In any case it is possible to rule out P- and F-type ion motive ATPases, since Fe(II) transport was resistant to vanadate and ouabain which inhibit P-type ATPases, and F-type ATPases are not present in erythrocytes since they have no mitochondria.

Several investigators have shown that chicken, human, rat and ferret erythrocytes contain a Na^+-dependent Mg^{2+} efflux mechanism which is considered to be due to the presence of a Na^+/Mg^{2+} antiport [13–22]. The Fe(II) transport process studied in this investigation is inhibited by both extracellular Na^+ and Mg^{2+} and has many characteristics which are very similar to those of the Na^+/Mg^+ exchanger. These include inhibition by amiloride, quinidine, imipramine, Mn^{2+} and Co^{2+}; resistance to inhibition by vanadate, ouabain, bumetanide, furosemide and p-chloromercuriphenyl-sulfonic acid; and dependence on cellular ATP levels. Moreover, Na^+/Mg^{2+} exchange is more active in rat than human erythrocytes [18], as was found for Fe(II) uptake. Also, Na^+ acts as a competitive inhibitor of Fe(II) uptake. Hence, there is good reason to suggest that Fe(II) is taken into erythroid cells by the Na^+/Mg^{2+} exchanger, competing with extracellular Na^+ for a place on the transporter. A role for Mg^{2+} efflux in Fe(II) uptake is also strongly supported by the stimulatory effects of intracellular Mg^{2+} on iron uptake and the inhibition of Mg^{2+}-stimulated iron uptake by inhibitors of Na^+/Mg^{2+} exchange. In addition, Mg^{2+} was found to inhibit Fe(II) uptake in a competitive manner which suggests that the exchanger can function in the reverse direction, taking Mg^{2+} into the cells, as has been shown to be the case with Na^+/Mg^{2+} exchange under appropriate conditions [22,23].

The arguments summarized above are very strong evidence that low-affinity iron transport into erythroid cells is a newly-recognized manifestation of the function of a Na^+/Mg^{2+} antiport. The present results also illustrate several new and interesting features of this transporter. These include higher levels of activity in rabbit than in rat or human cells, higher activity in immature erythroid cells

(reticulocytes) than in mature erythrocytes, and very high sensitivity to inhibition by diethylstilbestrol, valinomycin and certain divalent cations (Co^{2+}, Ni^{2+}, Zn^{2+}). In the case of valinomycin we have evidence, to be reported elsewhere, that the inhibition is due to a competitive interaction between the valinomycin-K^+ complex and the transporter, and is not due to alterations in transmembrane flux of K^+ or potential difference. The results with Co^{2+}, Ni^{2+} and Zn^{2+} raise the question that these metals can also be carried into erythroid cells by the Na^+/Mg^{2+} transporter but, at present, there is no information on this matter.

A possible explanation for the effect of changes in intracellular Mg^{2+} concentration on iron uptake is that it results from changes in the concentration of ATP-Mg^{2+} which is the substrate for an ATP-ase involved in iron transport. Indeed, there is evidence that Mg^{2+} extrusion from human red cells by the Na^+/Mg^{2+} antiport requires an input of metabolic energy from ATP hydrolysis [17]. The present results with ATP-depleted cells suggest that the same applies to iron uptake. This conclusion is additional to and not opposed to the one reached above that low affinity iron transport occurs via the Na^+/Mg^{2+} antiport.

An important question relates to the function of the low-affinity iron transport process. It is unlikely that this is the mechanism involved in iron uptake by cells in vivo since the uptake is inhibited by Na^+, the major cation of the extracellular fluid. The function of Na^+/Mg^{2+} antiport is believed to be that of mediating Mg^{2+} efflux from the cells to maintain the optimum intracellular concentration of Mg^{2+}. Could it also function to export Fe^{2+}? Iron export is not an important function of erythroid cells, but it is important in other types of cells, particularly intestinal mucosal cells, placental trophoblast cells, macrophages and hepatocytes. Is iron efflux from the cells mediated by the same transport mechanism as described for erythroid cells in the present investigation, but working in the reverse direction? There is evidence that Mg^{2+} transport can reverse direction [22,23]. Why not Fe^{2+}? Also, Na^+-dependent Mg^{2+} efflux has been described in several other types of cells in addition to erythroid cells [24]. Hence the possibility that iron efflux from the cells mentioned above may be mediated by the same or a related transporter must be considered and should be investigated in the future. At the present time there is extremely little information on the mechanism by which iron is transported out of any type of cell.

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References

[1] Morgan, E.H. (1981) Mol. Aspects Med. 34, 1–122
[2] Morgan, E.H. and Baker, E. (1988) Ann. N.Y. Acad. Sci. 256, 65–82
[3] Morgan, E.H. (1988) Biochim. Biophys. Acta 943, 428–439
[4] Egyed, A. (1988) Br. J. Haematol. 68, 483–486
[5] Egyed, A. (1991) Biochem. J. 275, 635–638
[6] Hodgson, L.L., Quail, E.A. and Morgan, E.H. (1995) J. Cell. Physiol. 162, 181–190
[7] Qian, Z.M. and Morgan, E.H. (1991) Biochim. Biophys. Acta 1073, 456–462
[8] Qian, Z.M. and Morgan, E.H. (1992) Biochim. Biophys. Acta 1135, 35–43
[9] Quail, E.A. and Morgan, E.H. (1994) J. Cell. Physiol. 159, 238–244
[10] Hemmaplardh, D. and Morgan, E.H. (1974) Biochim. Biophys. Acta 373, 84–99
[11] Silverman, J., and Dodson, R.M. (1952) J. Phys. Chem. 56, 846–852.
[12] Lew, V.L. (1971) Biochim. Biophys. Acta 233, 827–830
[13] Günther, T., Vormann, J. and Förster, R. (1984) Biochem. Biophys. Res. Comm. 119, 124–131
[14] Günther, T. and Vormann, J. (1985) Biochem. Biophys. Res. Comm. 130, 540–545
[15] Féray, J.-C. and Garay, R. (1986) Biochim. Biophys. Acta 856, 76–84
[16] Günther, T. and Vormann, J. (1987) Biochem. Biophys. Res. Comm. 148, 1069–1074
[17] Lüdi, H. and Schatzmann, H.J. (1987) J. Physiol. 390, 367–382
[18] Féray, J.-C. and Garay, R. (1988) Nauyn-Schmiedeberg's Arch. Pharmacol. 338, 332–337
[19] Günther, T. and Vormann, J. (1989) FEBS Lett. 250, 633–637
[20] Frenkel, E.J., Graziani, M. and Schatzmann, H.J. (1989) J. Physiol. 414, 385–397
[21] Günther, T., Vormann, J. and Hölbriegel, V. (1990) Biochim. Biophys. Acta 1023, 455–461
[22] Flatman, P.W. and Smith, L. (1990) J. Physiol. 431, 11–25
[23] Günther, T. and Vormann, J. (1995) Biochim. Biophys. Acta 1234, 105–110
[24] Flatman, P.W. (1984) J. Membr. Biol. 80, 1–14