Induction of Mn^{2+}/H^+ antiport in chicken erythrocytes by intracellular Mg^{2+} and Mn^{2+}

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Chicken erythrocytes preloaded with Mg^{2+} exchange one extracellular Mn^{2+} fo two intracellular H^+. Chicken erythrocytes preloaded with Mn^{2+} alone or with Mg^{2+} plus Mn^{2+} performed efflux of Mn^{2+}, which was higher at pH 6 than at pH 7.4, indicating reversibility of Mn^{2+}/H^+ antiport. Mn^{2+}/H^+ antiport was not inhibited by 1 mM KCN plus 1 mM iodoacetic acid or 1 mM amiloride. Mn^{2+} influx was activated by anions. Mn^{2+} efflux via Mn^{2+}/H^+ antiport was inhibited by competition between H^+ and K^+.

Mn^{2+} influx; Mn^{2+} efflux; Mn^{2+}/H^+ antiport; Mg^{2+}-Mn^{2+} loading; Erythrocyte

1. INTRODUCTION

In preceding experiments we found that Mg^{2+}-loaded chicken erythrocytes took up considerable amounts of Mn^{2+}, whereas Mg^{2+}-unloaded chicken erythrocytes took up only a little Mn^{2+} [1].

Since the drastic Mn^{2+} uptake by chicken erythrocytes was not in exchange for intracellular Mg^{2+}, as was the case in rat erythrocytes [1], we investigated by which mechanism intracellular Mg^{2+} can induce Mn^{2+} uptake in chicken erythrocytes.

2. MATERIALS AND METHODS

Blood was taken by venous puncture from chicken by means of a heparinized syringe and centrifuged at 1000 × *g* for 10 min. The plasma and buffy coat were aspirated and the red cells were washed twice with 150 mM KCl.

The cells were loaded with Mg^{2+} by incubating a 10% cell suspension for 30 min at 37°C in KCl medium (in mM: 140 KCl, 50 sucrose, 5 glucose, 30 Hepes-Tris, pH 7.4) with the addition of 12 mM $MgCl_2$ and 6 μM A23187 (dissolved in dimethyl sulfoxide). For loading the cells with Mg^{2+} plus Mn^{2+}, the cells were loaded first with $MgCl_2$ for 30 min and thereafter 3 mM $MnCl_2$ were added and incubated for an additional period of 15 min. For loading the cells with Mn^{2+} alone, the cells were analogously incubated with 3 mM $MnCl_2$ in the presence of A23187.

The cation ionophore was removed by 4 incubations in KCl medium with the addition of $MgCl_2$ or $MnCl_2$ and 1% bovine serum albumin for 10 min at 37°C. Thereafter, the cells were washed twice with sucrose, KCl or NaCl medium. Sucrose medium contained (in mM): 350 sucrose, 5 glucose, 30 Hepes-Tris, pH 7.4. NaCl medium was prepared by substitution of KCl in KCl medium by 140 mM NaCl.

Mn^{2+} influx into Mg^{2+}-loaded cells or Mn^{2+} efflux from Mn^{2+}-loaded cells was measured by reincubation of a 10% cell suspension at 37°C as indicated. At the beginning of reincubation and after 30 min, 0.5 ml aliquots of the cell suspension were centrifuged for 1 min at 10000 × *g*.

For measuring intracellular Mg^{2+} and Mn^{2+} content, the sedimented cells were washed twice with 150 mM KCl and hemolyzed by adding 750 μl H_2O. 50 μl of the hemolysate were taken for determination of hemoglobin by means of the cyanmethemoglobin method. The rest was deproteinized by addition of 50 μl 75% trichloroacetic acid (TCA) and centrifuged.

Additionally, Mn^{2+} efflux from Mn^{2+}-loaded cells was determined by measuring Mn^{2+} in the reincubation medium after centrifugation of the cell suspension. Mg^{2+} and Mn^{2+} were measured by atomic absorption spectrophotometry after dilution with 10% TCA/0.175% $LaCl_3$.

H^+ excretion of chicken erythrocytes during Mn^{2+} uptake was measured directly by pH measurement of a 10% cell suspension in unbuffered KCl medium (Orion, model 701 A). For determination of total H^+ release, the same cell suspension, incubated without addition of $MnCl_2$, was titrated with 0.1 N HCl to the same decrease of pH which was measured during Mn^{2+} uptake.

When neutralized ampholine was used in the medium instead of NaCl or KCl, the same procedure was applied as described earlier [1].

3. RESULTS AND DISCUSSION

Mn^{2+} uptake by Mg^{2+}-loaded chicken erythrocytes occurred in sucrose medium which did not contain Na^+, K^+ or Cl^- ([1] and Fig. 1). Therefore, Mn^{2+} influx cannot be performed unspecifically by the (KCl)- or (Na,K,Cl)-cotransport system.

Mn^{2+} influx into Mg^{2+}-loaded chicken erythrocytes was the same in NaCl and KCl medium and 5 times higher than in sucrose medium [1]. Mn^{2+} influx in these media obeyed Michaelis-Menten kinetics; the K_m values were identical, amounting to 4 mM (Fig. 1).

From these results it can be concluded that the mechanism of Mn^{2+} uptake in all media may be the same, although V_{max} of Mn^{2+} influx was increased in NaCl and KCl medium. Fig. 2 shows that the activation of Mn^{2+} influx by KCl followed Michaelis-Menten

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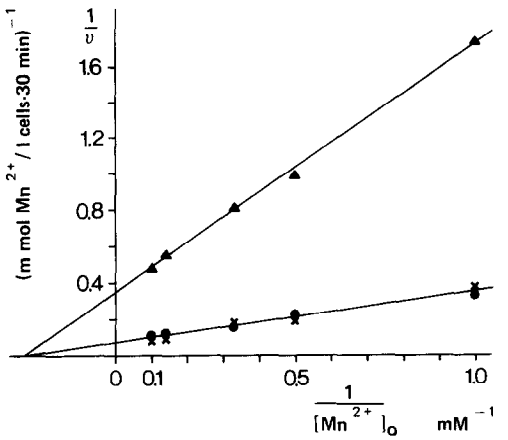

Fig. 1. Lineweaver-Burk plot of Mn^{2+} influx into Mg^{2+}-loaded chicken erythrocytes. The cells were incubated in sucrose medium (▲); NaCl medium (●) or in NaCl medium with 10 mM bicarbonate (×). Mean of 2 experiments.

kinetics; the K_m value for KCl activation amounted to 87 mM. Since ampholine-Cl activated Mn^{2+} influx (Table I), the activation must be performed by Cl^-. The activation of Mn^{2+} influx by various potassium salts (Table I) shows that the activation was unspecifically performed by anions. To evaluate the mechanism of Mn^{2+} uptake, we measured whether other cations are simultaneously released from the erythrocytes during Mn^{2+} uptake. Mn^{2+} influx into chicken erythrocytes was not accompanied by an efflux of intracellular Mg^{2+} [1], K^+ or Na^+ (data not shown). Therefore, in these cells, extracellular Mn^{2+} is not exchanged for intracellular Mg^{2+}, K^+ or Na^+. Since Zn^{2+} is taken up by human erythrocytes as a negatively charged (Zn,bicarbonate,Cl)-complex [2], we tested the effect of extracellular bicarbonate on Mn^{2+} uptake.

Mn^{2+} influx was not influenced by extracellular bicarbonate (Fig. 1) nor was it inhibited by SITS (Table II). Thus, Mn^{2+} uptake as a negatively charged bicarbonate or (bicarbonate,Cl)-complex via capnophorin, as found for Zn^{2+} [2], can also be excluded.

Table I

Activation of Mn^{2+} influx into Mg^{2+}-loaded chicken erythrocytes by various anions

Medium	Mn^{2+} influx
Sucrose	1.60
NaCl	7.30
KCl	7.66
Ampholine-Cl	7.45
KNO_3	7.53
KI	8.85
K_2SO_4	6.82

Salt concentration of the media amounted to 150 mM and 3 mM $MnCl_2$. Values in mmol/l cells × 30 min. Mean of 2 experiments

Table II

Effect of inhibitors on Mn^{2+} influx and Mn^{2+} efflux from chicken erythrocytes

Medium	Mn^{2+} influx		Mn^{2+} efflux	
	Sucrose	KCl	Sucrose	KCl
Control	2.15	9.63	4.98	2.26
Amiloride (1 mM)	2.10	9.40	5.24	2.40
Furosemide (1 mM)	2.08	9.30	5.54	2.53
SITS[a] (30 μM)	2.05	9.20	5.44	2.43
SITS (100 μM)	1.99	9.15	–	–
KCN (1 mM) + iodoacetate (1 mM)	2.12	8.38	–	–

Mn^{2+} influx was measured using Mg^{2+}-loaded cells and 3 mM $MnCl_2$ in sucrose or KCl medium. For measurement of Mn^{2+} efflux, Mn^{2+}-loaded cells were taken. Values in mmol/l cells × 30 min. Mean of 2 experiments

[a] 4-Acetamino-4′-isothiocyanatostilbene-2,2′-disulfonic acid

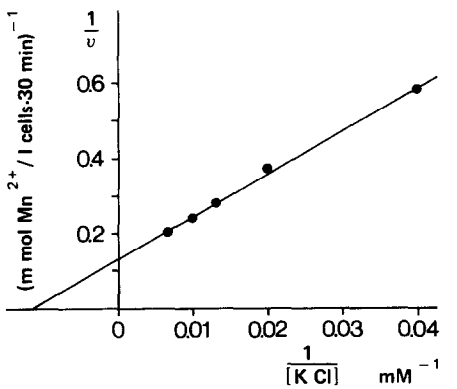

Fig. 2. Lineweaver-Burk plot of Mn^{2+} influx into Mg^{2+}-loaded chicken erythrocytes. The cells were incubated with 3 mM $MnCl_2$, sucrose medium was isoosmotically substituted by KCl, as indicated. Mn^{2+} influx at 0 mM KCl (sucrose medium) was subtracted. Mean of 2 experiments.

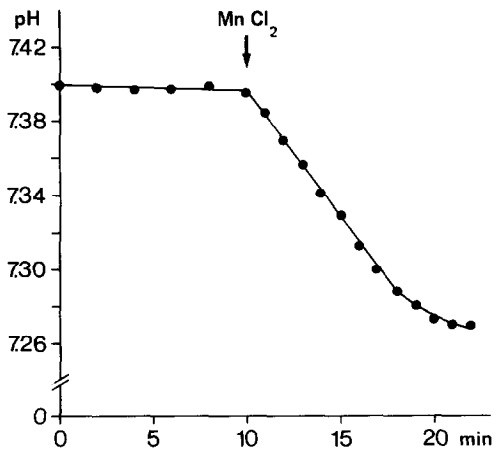

Fig. 3. Mn^{2+}-induced efflux of H^+ from Mg^{2+}-loaded chicken erythrocytes. The cells were incubated in unbuffered KCl medium, 1 mM $MnCl_2$ was added as indicated. Mean of 2 experiments.

Therefore, we investigated whether Mn^{2+} can be taken up in exchange for intracellular H^+.

When Mg^{2+}-loaded chicken erythrocytes were incubated in unbuffered 150 mM KCl, with 5 mM glucose, there was no significant alteration in pH (Fig. 3). However, after addition of 1 mM $MnCl_2$, pH in the medium dropped by 0.12 units within 10 min. At the same time, the cells had taken up 4.2 mmol Mn^{2+}/l cells. Since a part of the released H^+ was bound to the cells, we measured H^+-binding by adding 0.1 N HCl to an analogous cell suspension without addition of $MnCl_2$, until the pH of the cell suspension was reduced by 0.12 units.

This drop in pH was reached by addition of 9 mmol H^+/l cells. When comparing this amount with the uptake of Mn^{2+} under the same conditions (4.2 mmol/l cells) it can be deduced that 2 H^+ were released for 1 Mn^{2+}, indicating electroneutral Mn^{2+}/H^+ antiport. The alternative possibility that Mn^{2+} was taken up in combination with 2 OH^- is unlikely because of the high Mn^{2+} concentration needed for Mn^{2+} influx and the low OH^- concentration in the medium.

Furthermore, from the result that Mg^{2+}-unloaded cells did not perform Mn^{2+}/H^+ antiport, it can be inferred that the increase in intracellular Mg^{2+} by Mg^{2+} loading was necessary to induce this antiport.

Among the antiport systems, Na^+/H^+ antiport [3,4] and Na^+/Ca^{2+} antiport [5,6] are operating in a reversible manner, whereas Na^+/Mg^{2+} antiport from Mg^{2+}-loaded erythrocytes was asymmetric [7,8]. Therefore, we tested the reversibility of Mn^{2+}/H^+ antiport. As shown in Fig. 4, in sucrose medium there was a high rate of Mn^{2+} efflux from chicken erythrocytes

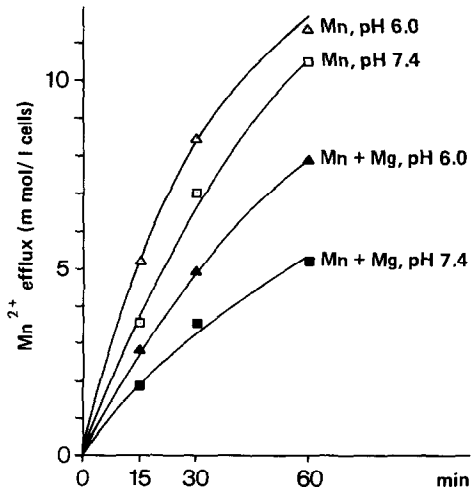

Fig. 4. Mn^{2+} efflux from preloaded chicken erythrocytes. The cells were loaded with Mn^{2+} alone (△, □) or with Mg^{2+} plus Mn^{2+} (▲, ■) and reincubated in sucrose medium at pH 6 (Hepes-Tris) (△, ▲) and pH 7.4 (Hepes-Tris) (□, ■). Mn^{2+} content amounted to 24 mmol/l cells for Mn^{2+}-loaded cells and to 21 mmol Mn^{2+}/l cells for Mn^{2+} plus Mg^{2+}-loaded cells. Mg^{2+} content of Mg^{2+} plus Mn^{2+}-loaded cells amounted to 16 mmol Mg^{2+}/l cells. Mean of 2 experiments.

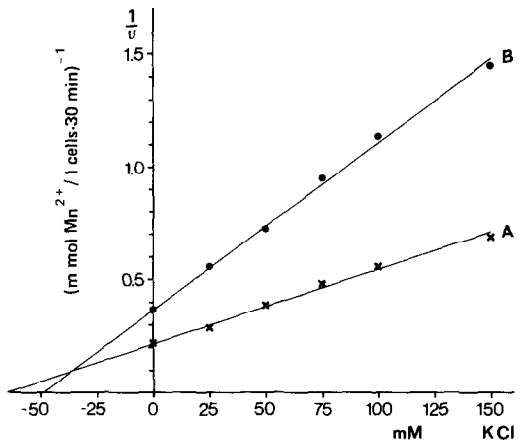

Fig. 5. Dixon plot of Mn^{2+} efflux from Mn^{2+}-loaded chicken erythrocytes. Mn^{2+} content of Mn^{2+}-loaded cells amounted to 22 mmol/l cells. Mn^{2+} efflux was measured at pH 6 (A) and pH 8 (B) in KCl medium with different KCl concentrations. KCl in KCl medium was isoosmotically substituted by sucrose. Mean of 3 experiments.

preloaded either with Mn^{2+} alone or with Mg^{2+} plus Mn^{2+}, indicating the reversibility of Mn^{2+}/H^+ antiport. In agreement with the existence of Mn^{2+}/H^+ antiport, Mn^{2+} efflux was higher at pH 6 than at pH 7.4 (Fig. 4). Therefore, the H^+ as well as Mn^{2+} gradient are driving forces in Mn^{2+}/H^+ antiport.

Since Mn^{2+} efflux was operating in cells preloaded with Mn^{2+} alone, it can be concluded that Mn^{2+}/H^+ antiport can also be induced by the increased intracellular Mn^{2+} concentration. In chicken erythrocytes preloaded with Mn^{2+} plus Mg^{2+}, Mn^{2+}/H^+ antiport was smaller than in cells preloaded with Mn^{2+} alone (Fig. 4). This result may indicate competition between intracellular Mn^{2+} and intracellular Mg^{2+} for the Mn^{2+}/H^+ antiporter, although Mg^{2+} was not transported out of the cell by this transport system.

Mn^{2+}/H^+ antiport in both directions was not inhibited by amiloride, furosemide or SITS (Table II). Thus, Mn^{2+}/H^+ antiport is different from Na^+/H^+ antiport which is inhibited by amiloride [4].

Antiport systems, such as Na^+/H^+ and Na^+/Ca^{2+} exchange, which are operating in both directions, are independent of ATP, although these transport systems are modified by ATP with respect to intracellular affinity for H^+ [4] or Ca^{2+} [5,6]. Therefore, we tested energy-dependency of Mn^{2+} influx. As also shown in Table II, Mn^{2+} influx in sucrose or KCl medium was not inhibited by 1 mM KCN plus 1 mM iodoacetic acid, indicating that Mn^{2+}/H^+ antiport was independent of ATP. Mn^{2+} influx via Mn^{2+}/H^+ antiport was activated by anions (Table I, Fig. 2). Therefore, we tested whether Mn^{2+} efflux was affected by extracellular KCl and NaCl. KCl (Table II) and NaCl inhibited Mn^{2+} efflux to the same extent, whereas ampholine-Cl had no inhibitory effect (data not shown). As can be seen from Fig. 5, Mn^{2+} efflux from

Mn^{2+}-preloaded chicken erythrocytes was competitively inhibited by KCl, K_i amounted to 35 mM. These results indicate that extracellular H^+ and K^+ compete in Mn^{2+} efflux via Mn^{2+}/H^+ antiport.

The physiological significance of Mn^{2+}/H^+ antiport in chicken erythrocytes remains to be established.

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