Journal of Bioenergetics and Biomembranes (1977) 9, 65–72

Effect of Mg^{2+} and Spermine on the Kinetics of Ca^{2+} Transport in Rat-Liver Mitochondria

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Received 26 July 1976

Abstract

Plots relating the initial rate of mitochondrial Ca^{2+} transport to the Ca^{2+} concentration (kinetic plots) have a hyperbolic shape in a Ca^{2+} concentration range of 2.5–100 µM as measured in sucrose or KCl media. In the presence of Mg^{2+} or a polyamine spermine, which both are competitive inhibitors of Ca^{2+} binding to low affinity sites at the membrane surface, the shape of the plots becomes sigmoidal. At higher concentrations of these agents linear kinetic plots are obtained as measured in a sucrose medium. In a KCl medium the sigmoidality of the kinetic plots is enhanced by an increase in the Mg^{2+} or spermine concentration. It is suggested that Mg^{2+} and spermine affect the kinetics of Ca^{2+} transport by interfering with Ca^{2+} binding to low affinity sites of the membrane surface and that the binding of Ca^{2+} to these sites is the first step of the mitochondrial Ca^{2+} transport.

Introduction

The kinetics of mitochondrial Ca^{2+} transport have been studied using various methods in different conditions [1–5]. Using low temperatures, Ca^{2+} buffers, and millipore filtration techniques some workers have reported a sigmoid shape for plots relating the initial rate of Ca^{2+} transport to the free Ca^{2+} concentration [1, 2, 4]. The sigmoidality occured at very low free Ca^{2+} concentrations (less than 1 µM). Others have obtained sigmoidal Ca^{2+} transport kinetics measuring Ca^{2+} uptake in the presence of

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KCl and $MgCl_2$ using the murexide technique [3]. However, in their work the sigmoidality occurred at much higher Ca^{2+} concentrations (about 10 μM). Different values for the Km of mitochondrial Ca^{2+} transport varying between 1 and 50 μM have also been reported [1–6]. The controversies are probably due to the use of different conditions and methods. The main problem is how fast mitochondria transport Ca^{2+} in the presence of the low free Ca^{2+} concentrations (about 1 μM) present in the cytosol, which is of importance in considering a possible role of mitochondria in regulating cytosolic Ca^{2+}. Ca^{2+} is bound to so-called low affinity sites at the outer surface of the mitochondrial membrane [7–10]. They are mainly phospholipid in nature [9, 10]. Ca^{2+} binding to these sites has been suggested to be the first step of mitochondrial Ca^{2+} transport [10].

It has recently been demonstrated theoretically that the surface properties of membranes might influence the transport kinetics of ions across the membranes [11]. Thus the aim of this work was to study the effects of Mg^{2+}, which inhibits Ca^{2+} binding to low affinity sites at the surface of the mitochondrial membrane in a competitive manner [9] and the tetravalent polyamine spermine which decreases the negative surface charge of mitochondria [12] in order to obtain information about the role of the surface binding of Ca^{2+} in the kinetics of Ca^{2+} transport.

Materials and Methods

Rat-liver mitochondria were prepared from young male rats (Sprague-Dawley) by a conventional procedure [13]. Ca^{2+} uptake was measured by using the EGTA quenching method essentially as described by Reed and Bygrave [14], in order to be able to distinguish between transport and external binding of Ca^{2+}. The mitochondria were removed from the medium by millipore filtration (pore size 0.6 μm). The $^{45}Ca^{2+}$ was counted in a Packard Tricarb scintillation spectrometer in Bray's solution (PPO, POPOP, naphthalene, ethylenglycol, dioxan, and methanol). Energy-independent Ca^{2+} binding was measured as described before [15].

Reaction media: 0.25 M sucrose, 20 mM tris–Cl, pH 7.4 (sucrose medium), and 130 mM KCl, 20 mM tris–Cl, pH 7.4 (KCl medium).
Reagents: FCCP (carbonylcyanide *p*-trifluoromethoxy phenylhydrazone) was obtained from Dr. P. G. Heytler and spermine from Fluka AG, Buchs, Switzerland. All the other reagents were commercial products.

Results

If the initial rate of mitochondrial Ca^{2+} transport, as measured in the sucrose medium, is plotted against the Ca^{2+} concentration added, a

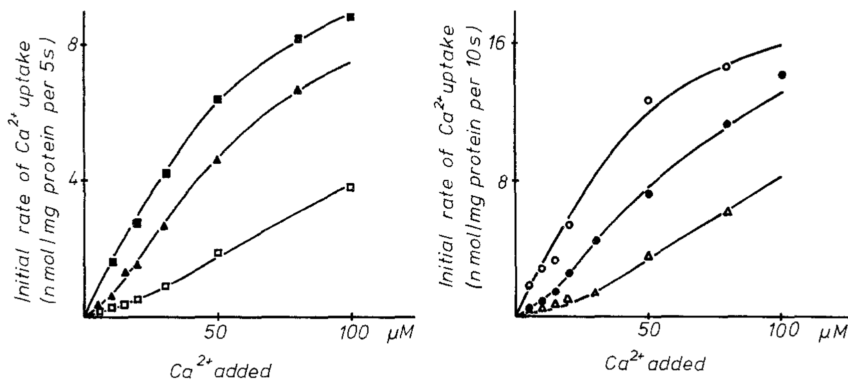

Figure 1. Effect of Mg^{2+} and spermine on the mitochondrial Ca^{2+} transport kinetics. Mitochondria (0.5 mg protein/ml) were incubated in the sucrose medium containing 10 mM succinate 5 μM rotenone and $^{45}Ca^{2+}$ labeled $CaCl_2$ (5–100 μM) at +5°C before quenching by an addition of 1 mM EGTA, (a) control (■–■), 1 mM $MgCl_2$ (▲–▲), and 5 mM $MgCl_2$ (□–□), and (b) control (O–O), 200 μM spermine (●–●), and 500 μM spermine (△–△). After quenching, the mitochondria were removed by millipore filtration and the radioactivity remaining in the filters after washing with cold medium was counted.

hyperbolic plot is obtained (Fig. 1) in the Ca^{2+} concentration range studied (5–100 μM). In the presence of Mg^{2+} (1 mM) or a polyamine spermine (200 μM) the plot becomes sigmoidal. An increase in the concentration of Mg^{2+} or spermine causes a decrease in the sigmoidality and the rising part of the plots become nearly linear. The plots seem rather biphasic than sigmoidal. At higher concentrations of Mg^{2+} and spermine linear kinetic plots are obtained (Fig. 2).

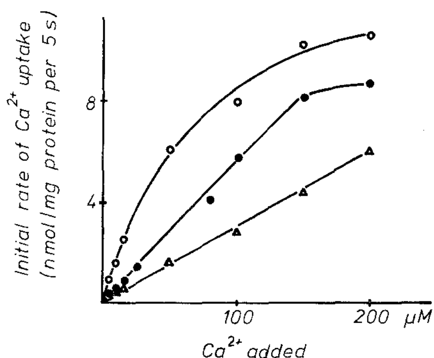

Figure 2. Effect of Mg^{2+} and spermine on mitochondrial Ca^{2+} transport kinetics. Conditions as in Fig. 1. Incubations made in the sucrose medium (O–O) containing 15 mM MgCl (△–△) or 1 mM spermine (●–●). Mitochondrial protein 0.6 mg/ml.

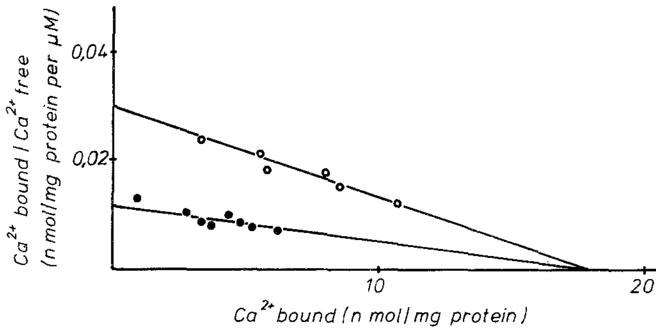

Figure 3. Scatchard plots for Ca^{2+} binding to mitochondria in the absence and presence of spermine. Mitochondria (5 mg protein/ml) were preincubated in the sucrose medium containing 7 µM FCCP (O–O) and 200 µM spermine (●–●) for 1 min. Thereafter $^{45}Ca^{2+}$ labeled $CaCl_2$ (100–800 nmol) was added and the mitochondria were removed by centrifugation after 1 min further incubation.

Because spermine decreases the negative surface charge of mitochondria [12] it also ought to inhibit Ca^{2+} binding to the low affinity sites at the membrane surface. Figure 3 shows Scatchard plots for Ca^{2+} binding to mitochondria in the absence and presence of spermine. Spermine decreases the slope of the plot, but does not affect the amount of binding sites (intercept on the abscissa). This would suggest a competitive inhibition.

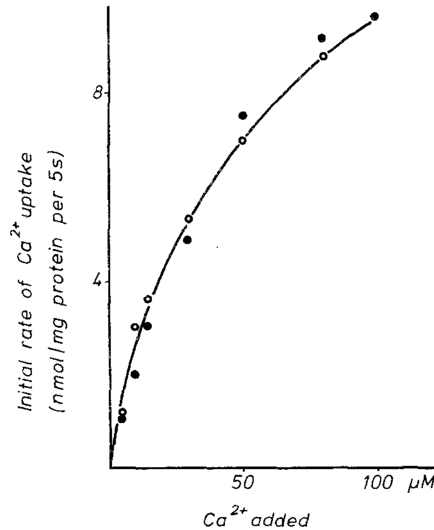

Figure 4. Kinetics of mitochondrial Ca^{2+} transport in the sucrose and KCl medium. Conditions as in Fig. 1. Incubations were made in the sucrose medium (O–O) or KCl medium (●–●). Mitochondrial protein 0.5 mg/ml.

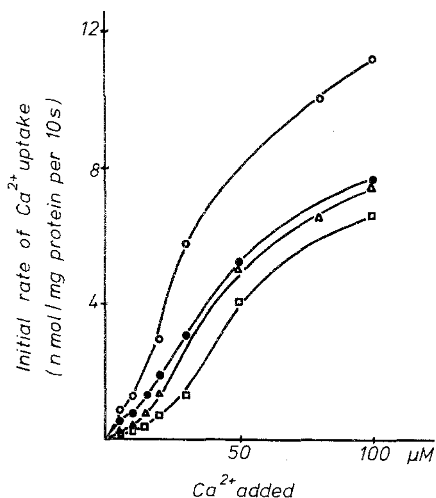

Figure 5. Effect of Mg^{2+} on mitochondrial Ca^{2+} transport. Conditions as in Fig. 1. Incubations were made in the KCl medium containing 1 mM $MgCl_2$ (○–○), 2.5 mM $MgCl_2$(●–●), 3 mM $MgCl_2$ (△–△), and 5 mM $MgCl_2$ (□–□). Mitochondrial protein 0.5 mg/ml.

K^+ has no significant effect on the kinetics of mitochondrial Ca^{2+} transport (Fig. 4). A decrease in the pH from 8 to 6.8 also does not affect the shape of the kinetic plot in the sucrose medium (not shown). In the KCl medium a sigmoidal plot is again obtained in the presence of Mg^{2+} (Fig. 5). The sigmoidality is enhanced by an increase in the Mg^{2+} concentration. A similar result is obtained in the presence of spermine (not shown).

Discussion

The results of this study show that agents known to stabilize various membrane systems including mitochondria, Mg^{2+} [16, 17], and spermine [12, 18, 19], change the kinetics of mitochondrial Ca^{2+} transport from essentially hyperbolic to sigmoidal in the Ca^{2+} concentration range studied (2.5–100 µM) as measured in a sucrose medium. Cations with one charge (K^+, H^+) have no effect on the shape of kinetic plots. However, the kinetics of mitochondrial Ca^{2+} transport seem to be sigmoidal also in sucrose [1, 2, 4] and KCl [4] media, the sigmoidality occuring at very low free Ca^{2+} concentrations (less than 1 µM). Thus it appears possible that Mg^{2+} and spermine do not actually change the kinetics from hyperbolic to sigmoidal but transfer the point at which the sigmoidality occurs to higher Ca^{2+} concentrations. Such a phenomen has been observed for the kinetics of Rb^+

uptake by yeast (*Saccaromyces cervisae*) membranes [20] in the presence of di- and trivalent cations. The authors presented a two-site carrier model to account for the sigmoidal kinetics of Rb^+ transport and suggested that the effects of the cations were due to an alteration of the surface charge rather than an interaction with the Rb^+ transport system. Two-site carrier models have also previously been proposed to account for the sigmoidal kinetics of mitochondrial Ca^{2+} transport [3, 4]. Such models could well account for the sigmoidal kinetics of mitochondrial Ca^{2+} transport in light of the results presented in this study. However, hyperbolic Ca^{2+} transport kinetics in rat-heart mitochondria, as measured with two different techniques, have been obtained, when care was taken to remove the endogenous Mg^{2+} present in the mitochondria [21]. The same workers also found sigmoidal kinetics in the presence of Mg^{2+}.

It has recently been shown [11] that the transport kinetics of ions may mimic those of cooperative mechanisms (sigmoidal kinetics), when the surface charge of the membrane is changed towards the charge of the ionic species translocated. Thus the effect of Mg^{2+} and spermine on the shape of the kinetic plots for Ca^{2+} transport could simply be a result of an interference with the surface charge of mitochondria.

In the presence of high Mg^{2+} or spermine concentrations, at which no significant interference with the respiration of mitochondria is seen (unpublished results), linear kinetic plots are obtained. It is known that linear kinetic plots are obtained if the substrate/K_m ratio decreases significantly in systems obeying Michaelis–Menten kinetics. The effects of Mg^{2+} and spermine could thus be due either to an increase in the K_m of the Ca^{2+} transport system (competitive inhibition) or a decrease in the Ca^{2+} concentration. The first alternative is unprobable because La^{3+}, which is thought to interact competitively mainly with the mitochondrial Ca^{2+} transport system [14], does not affect the kinetics (i.e., a change from hyperbolic to sigmoidal kinetics) in a similar way as Mg^{2+} or spermine [22]. It is also unprobable that two chemically unrelated compounds such as Mg^{2+} and spermine would interfere with specific Ca^{2+} transport sites in a similar way. It is also known that rat-liver mitochondria do not transport Mg^{2+}. Thus it is improbable that Mg^{2+} interacts with the mitochondrial Ca^{2+} transport system. The second alternative (decrease in Ca^{2+} concentration) could result from an inhibition of Ca^{2+} binding to the low affinity sites providing that the transport system is dependent on the Ca^{2+} bound at the outer surface of the membrane rather than on the Ca^{2+} in the outer medium. A third possible explanation for the linear kinetcis of Ca^{2+} transport could be an unmasking of a pore or channel mechanism. The saturation kinetics of Ca^{2+} transport could result from a saturation of low affinity sites with comparably higher affinity for Ca^{2+} in the vicinity of the Ca^{2+} transport

system. This binding would be inhibited by Mg^{2+} and spermine competitively. The activation energy of mitochondrial Ca^{2+} transport decreases to almost 0 kJ/mole in the presence of spermine [23], which gives further evidence in favor of a pore or channel mechanism of Ca^{2+} transport.

It is concluded that mitochondrial Ca^{2+} transport seems to occur in at least two steps. The first step is an interaction of the cation with binding sites at the membrane surface. This step is sensitive to other cations such as Mg^{2+} and spermine. The second step is the transfer of Ca^{2+} through the hydrophobic core of the membrane. This step is catalyzed either by a carrier or pore and inhibited by agents such as La^{3+} and ruthenium red [14].

It is noteworthy that both Ca^{2+} and spermine are present in the cytosol. Thus it is of interest to speculate that they might regulate the kinetics of mitochondrial Ca^{2+} transport also *in vivo*, which could be of importance in considering a possible role of mitochondria in regulating the cytosolic Ca^{2+} concentration.

Acknowledgments

This work was supported by a grant from the Sigrid Jusélius Foundation. The author wishes to thank Professor N.-E. L. Saris for discussion and criticism, Ms. Kaija Viitanen for technical assistance and Ms. Anneli Saulamo for typing the manuscript.

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