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Biochimica et Biophysica Acta 1371 (1998) 96–100

BIOCHIMICA ET BIOPHYSICA ACTA
BBA

Stimulation of the yeast mitochondrial calcium uniporter by hypotonicity and by Ruthenium Red

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Received 28 July 1997; revised 22 December 1997; accepted 13 January 1998

Abstract

The Ca^{2+} uptake by mitochondria from the yeast *Endomyces magnusii* has earlier been found to be driven by the membrane potential and to be stimulated by spermine. It thus functions in a similar fashion as the animal mitochondrial calcium uniporter. Here, it is shown that the uptake is stimulated, i.e., Ca^{2+} can be accumulated from lower $[Ca^{2+}]$, under hypotonic conditions. Ruthenium Red, an inhibitor of the animal uniporter, under certain conditions, stimulates the yeast uniporter. The mechanism of the stimulation by hypotonicity and Ruthenium Red is discussed. © 1998 Elsevier Science B.V.

Keywords: Calcium uniporter; Mitochondrion; Ruthenium Red; Swelling; (Yeast)

1. Introduction

Endomyces magnusii yeast cell mitochondria have a Ca^{2+} uptake system that resembles the calcium uniporter of animal mitochondria in many respects: Ca^{2+} uptake is driven by the membrane potential of the inner mitochondrial membrane [1]; it is stimulated by polyamines [2,3] and inhibited by Mg^{2+} [3]. This yeast uniporter when stimulated by spermine and other modulators like ADP, Ca^{2+} itself and NADH [4], is capable of high-capacity Ca^{2+} uptake. However, while the animal mitochondrial calcium uniporter is strongly and seemingly specifically inhibited by low concentrations of Ruthenium Red (RR) [5], we found earlier the yeast system to be only marginally affected. Changes in osmolarity in the hypoosmotic range changed the pH dependence of K^+ efflux in rat liver mitochondria and also reduced the rate of RR-insensitive Ca^{2+} efflux [6], while in placental mitochondria, the activity of the calcium uniporter was hardly affected [7]. In this study, we show that Ca^{2+} uptake is stimulated by hypotonic conditions in yeast mitochondria, and that under these conditions, RR had an additional stimulatory effect.

2. Materials and methods

2.1. Materials

E. magnusii cells were cultivated, and their mitochondria were isolated after treatment of the cells

Abbreviations: RR, Ruthenium Red

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PII S0005-2736(98)00004-2

with lytic enzymes from snail gut juice as described earlier [8]. Rat liver mitochondria were prepared by a conventional procedure as described in Ref. [9].

RR and spermine were from Sigma (St. Louis, MO).

2.2. Assays

The protein content of yeast mitochondria was estimated by the Coomassie Blue method [10] using bovine serum albumin as standard. Their Ca^{2+} uptake was followed with the aid of ^{45}Ca-labelled calcium salts with Millipore filtration for the separation of mitochondria from the incubation medium [11]. Incubations were carried out at room temperature in a medium containing 0.6 mannitol, 2 mM Tris–phosphate, 20 mM Tris–pyruvate, 5 mM Tris–malate, pH 7.4, 0.5 mg protein/ml, and with less mannitol for hypotonic solutions, as indicated in figure legends. Figures show typical traces of duplicate experiments deviating less than 5%. All the Ca^{2+} accumulated was released on addition of the Ca^{2+} ionophore A23187, indicating that a Ca^{2+} gradient had been formed (not shown). The time needed for uptake of half of the Ca^{2+} maximally accumulated ($t_{1/2}$) was calculated with Easy Plot for Windows, Version 2.22-5, the equation being $Y = C_{max} \cdot k \cdot xt/(1 + kt)$, where C_{max} is given in nmol/mg protein, t denotes time, and $k = 1/t_{1/2}$.

3. Results

3.1. Stimulation of Ca^{2+} uptake in yeast mitochondria by hypoosmolarity

In yeast cells, 0.6 M mannitol corresponds to isoosmolar conditions [12]. Reducing the concentration of mannitol increased the initial rate of Ca^{2+} uptake, shifting the uptake curves to the left (Fig. 1). At 15 s, the rate of uptake of 150 μM Ca^{2+} had approximately doubled when the concentration of mannitol was 0.4 M, and even slightly more with 0.25 M mannitol (Fig. 1A). The steady-state levels were approached after 30 s under hypoosmolar conditions, and more slowly under isoosmolar conditions. Thus, the time needed for taking up half of the maximal amount taken up shortened from 0.71 min in 0.6 M mannitol to 0.20 min in 0.4 M and 0.17 min in 0.25 M. The picture is similar for the uptake of 300 μM Ca^{2+} (Fig. 1B), $t_{1/2}$ being shortened from 0.61 min to 0.18 and 0.14 min, respectively.

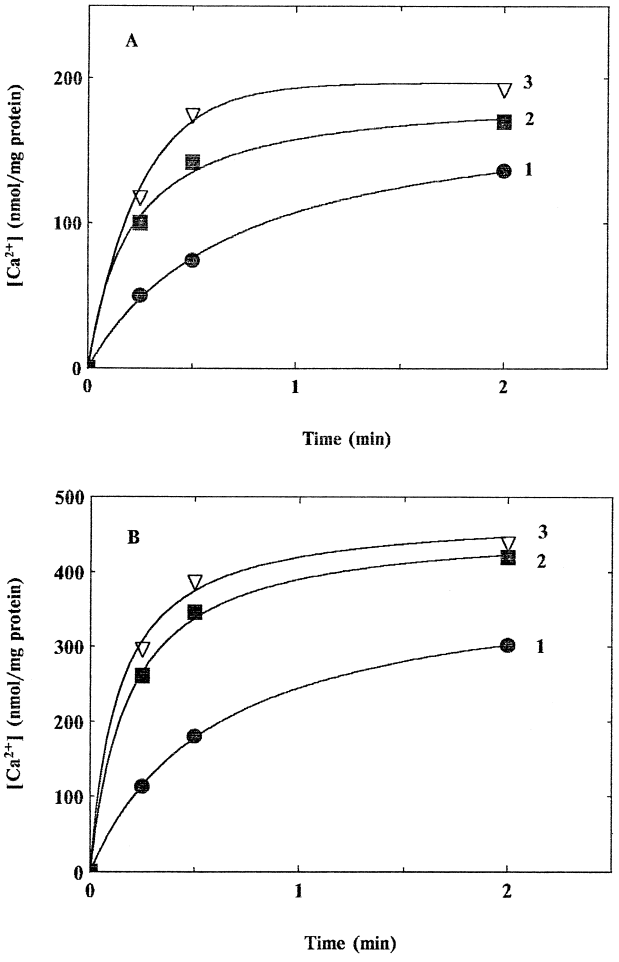

Fig. 1. Stimulation of yeast mitochondrial calcium uniporter by hypotonic conditions. (A) Ca^{2+} added was 150 μM; (B) Ca^{2+} added was 300 μM. The mannitol concentration was 0.6 M in Trace 1 (filled circles), 0.4 M in Trace 2 (filled squares, and 0.25 mM in Trace 3 (open triangles). For experimental details see Section 2.

3.2. Stimulation of Ca^{2+} uptake in yeast mitochondria by RR

RR is a well-known inhibitor of the mitochondrial calcium uniporter in animal cells [5]. However, in these yeast mitochondria, there was a clear stimulation of Ca^{2+} uptake both with 150 and 300 μM Ca^{2+} (Fig. 2). The extent of stimulation was similar to that obtained with spermine, but appeared relatively larger for both substances under isoosmolar conditions (Fig. 2A,C) than in the presence of 0.4 M

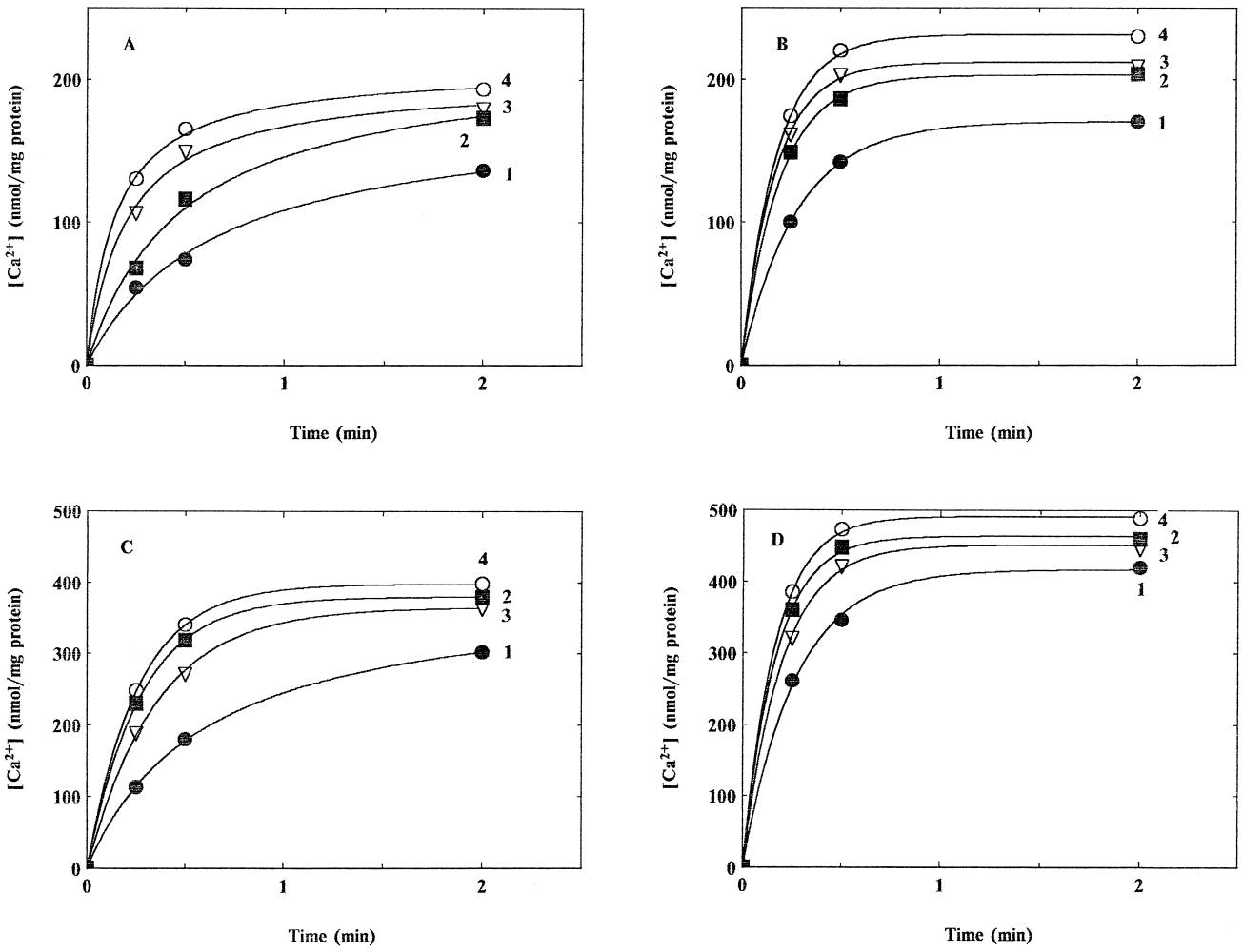

Fig. 2. Stimulation of the yeast uniporter by RR and spermine. (A) and (B) the [Ca^{2+}] was 150 μM, in C and D 300 μM; the mannitol concentration was 0.6 M in A and C, 0.4 M in B and D. Trace 1 (filled circles), control; Trace 2 (filled squares), 15 μM RR present; Trace 3 (open triangles), 25 μM spermine present; Trace 4 (open circles), both RR and spermine present. For other experimental details, see Fig. 1.

mannitol (Fig. 2B,D). This is probably because the control rates were already stimulated under hypoosmolar conditions. The stimulation of the rate of uptake was always highest in the presence of both RR and spermine, indicating at least some additive effect.

4. Discussion

4.1. The effect of hypotonicity on Ca^{2+} uptake by yeast mitochondria

In isotonic media, the yeast mitochondrial Ca^{2+} transporter mediates uptake of Ca^{2+} driven by the membrane potential as the animal mitochondrial uniporter, but has a very low activity with physiological, low Ca^{2+} concentrations, exhibiting a K_M of 100–300 μM [1]. In this study, we found that incubation in hypoosmolar solutions markedly stimulated the calcium uniporter, control traces in Figs. 1 and 2. This indicates that in the absence of activators of the yeast mitochondrial uniporter, it is effectively turned off. The osmotic swelling in hypotonic media would cause stretching and unfolding of the inner membrane that could unmask the uniporter with enhancement of its activity. Swelling in this way would affect biophysical parameters such as surface lateral pressure, interaction of membrane components, charge distribu-

tions, and surface potentials that affect cation-binding, protein conformations and channel states, as shown for the megachannel responsible for the mitochondrial permeability transition [13]. It seems likely that also the binding of modulators of the calcium uniporter like spermine [2,3] are changed, and thereby also their relative Ca^{2+} transport-activating potency (Fig. 2).

Swelling of animal mitochondria has been shown to activate some proton/cation antiporters, i.e., H^+/K^+, which is one mechanism for volume control [14]. These are also activated by Mg^{2+} depletion [15]. Hypoosmolarity also influences K^+ and Ca^{2+} effluxes in liver mitochondria [6].

4.2. The stimulation of the yeast mitochondrial calcium uniporter by RR

RR is a highly positively charged (6 positive charges) inhibitor of the animal mitochondrial calcium uniporter [5] (see Fig. 1) that in earlier studies was found not to inhibit the yeast transporter appreciably [8], nor Ca^{2+} uptake by plant mitochondria [16]. In this study, we found a clear stimulation by RR of the *E. magnusii* mitochondrial transport (Fig. 2). The stimulatory effect of RR may be related to its positive charge, and may thus act in a similar way as spermine. We also tested polylysine and Polybrene that, however, did not affect the transport markedly (data not shown). The effect of RR demonstrates a striking difference between the animal and yeast mitochondrial calcium uniporters, RR being a potent inhibitor of the former but stimulating the latter.

The specificity of RR as an inhibitor of the animal uniporter is, however, rather low. It binds to many calcium-binding proteins and may even be used as a stain for these [17]. Thus, RR inhibits other Ca^{2+}-transporters and channels at higher concentrations than needed for inhibition of the animal calcium uniporter, i.e., at a few micromolar concentrations [17,18]. The situation is complicated by the finding that RR preparations may contain varying amounts of contaminants with higher inhibitory potency than RR itself [19]. In animal mitochondria, a rapid mode of Ca^{2+} uptake has been described that appears to be of physiological significance, since the signal of cytosolic Ca^{2+} spikes may be transferred to the mitochondrial matrix by this mechanism [20]. In liver mitochondria, this mode of Ca^{2+} uptake was stimulated by lower concentrations of RR than those inhibiting the calcium uniporter [21]. The rapid mode of Ca^{2+} uptake in heart mitochondria, however, had different properties, RR being without effect, and spermine being an inhibitor [22]. These data indicate that the differences of animal and yeast calcium transporter in regard to the effects of RR are relative rather than fundamental. RR was found not to be an inhibitor of mung bean mitochondria [16].

The apparent additive stimulating effects of spermine and RR in yeast mitochondria (Fig. 1) could be due to binding to a modulator-binding sites on the Ca^{2+} uniporter. RR may be bound to the same sites. However, it is possible that the stimulation was due to a general membrane effect, i.e., a neutralisation of negative surface charges, though we saw no effects of polylysine. In this context, it is of interest that the calcium uniporter in rat liver mitochondria is stimulated slightly (ca. 10%) by low concentrations (half-maximal stimulation at 1.1–1.8 μM) of the hexammine-complexes of ruthenium and cobalt, while higher concentrations (IC_{50} 5–11 μM) were inhibitory (Drs. Ingo Rustenbeck and Sigurd Lenzen, personal communication).

Acknowledgements

This study was supported by grant Nr. 97-04-49464 from the Russian Foundation for Basic Research to Dr. Zvyagilskaya, by a grant to Dr. Saris from the Finnish Society of Sciences and Letters and by a grant from the Academy of Finland for promoting cooperation between Russian and Finnish research groups.

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