Volume 261, number 1, 139–141 FEBS 08125 February 1990

Polyamines improve Ca^{2+} transport system of the yeast mitochondria

Tatyana V. Votyakova, Elena N. Bazhenova and Renata A. Zvjagilskaya

A.N. Bach Institute of Biochemistry, Academy of Sciences of the USSR, Moscow 117071, USSR

Received 15 December 1989

Spermine at concentrations of 12–100 μM considerably activates the Ca^{2+} transport system of the *Endomyces magnusii* yeast mitochondria. As a result, in the presence of spermine the mitochondria are able to decrease extramitochondrial Ca^{2+} to the physiological level. At Ca^{2+} concentrations up to 200 μM, spermine enhances the initial rate of Ca^{2+} uptake (a half-maximal effect at 12 μM spermine). The Ca^{2+} concentrations required for half-maximal Ca^{2+} uptake rate to be achieved were 160 and 60 μM Ca^{2+} without and with spermine, respectively. Spermidine is shown to be less effective (a half-maximal effect at 50–100 μM spermidine). The polyamines do not change the parameters of energy coupling of mitochondria. The data obtained enabled the yeast mitochondria to be considered to take part in regulation of cytoplasmic and matrix Ca^{2+}.

Calcium ion; Yeast mitochondria; Spermine; Spermidine; (*Endomyces magnusii*)

1. INTRODUCTION

For a long time it was generally accepted that yeast mitochondria, in contrast to mitochondria of higher eucaryotes, have no Ca^{2+} transport system [1]. Later, yeast mitochondria were found to be capable of the energy-dependent Ca^{2+} uptake, but the rate of the process was so slow and the affinity of the system for Ca^{2+} was so low that participation of yeast mitochondria in maintenance of the homeostasis seemed to be hardly probable [2]. However, we have recently found that the energy-dependent Ca^{2+} transport in the *Endomyces magnusii* mitochondria proceeds, under certain conditions, as fast as in animal mitochondria (or even faster), though the affinity of the system for Ca^{2+} remained low (K_M^{app} = 100–300 μM) [3]. In the present paper we report the ability of spermine, a constituent of all eucaryotic cells [4–8], to alter the kinetic properties of the *End. magnusii* mitochondria Ca^{2+} transport, by increasing the initial rate of Ca^{2+} uptake and buffer capacity of the mitochondria with simultaneous increasing the Ca^{2+} affinity of the Ca^{2+} transport system.

2. MATERIALS AND METHODS

Cultivation of *End. magnusii* cells, isolation of the mitochondria, measurements of the respiration and phosphorylation activities were carried out as described in [9]. Ca^{2+} uptake by mitochondria was monitored with murexide as indicator [10]. The absorption difference of murexide (540–507 nm) was registered by using a Hitachi-557. The incubation medium contained: 0.3 M mannitol, 10 mM Tris-phosphate, 10 mM Hepes, pH 7.4, 16 mM pyruvate, 4 mM malate, 50 μM murexide, 0.5 mg mitochondrial protein/ml. The protein was determined by the Bradford method [11].

Correspondence address: T.V. Votyakova, A.N. Bach Institute of Biochemistry, Academy of Sciences of the USSR, Moscow 117071, USSR

3. RESULTS AND DISCUSSION

Fig.1A,B shows the results of the spectrophotometric measurements of Ca^{2+} uptake by the *End. magnusii* mitochondria with and without spermine. Mitochondria accumulated half the amount of the calcium added at a concentration of about 100 μM (fig.1A), which is in good agreement with our previous results [9]. The portion of absorbed Ca^{2+} rose up to 90–98% when the Ca^{2+} concentration in the medium was 250–500 μM. 50–75 μM spermine was found to cause some additional Ca^{2+} uptake by the mitochondria (fig.1B). A similar effect could be obtained only by considerably increasing the mitochondrial protein concentration [9]. All calcium ions accumulated by the mitochondria were released when ionophore A23187 was added; subsequent addition of EGTA restored the initial value of the suspension absorbance. Thus, the yeast mitochondria in the presence of low spermine concentrations could maintain extramitochondrial free Ca^{2+} concentration at a lower level, the Ca^{2+} gradient on the mitochondrial membrane being considerably higher.

Spermine also affected the initial rate of calcium uptake by the yeast mitochondria at Ca^{2+} concentrations below 200–300 μM (fig.1B, fig.2). For instance, the initial rate of the calcium uptake was enhanced 2–5 times at 150 μM of Ca^{2+} in the presence of spermine. The half-maximal effect was attained at the polyamine concentration of 12 μM, and the maximal one at 12–50 μM. It should be noted that these spermine concentrations were much lower (by a factor of 10) than those exerting a similar action on liver, kidney and brain mitochondria [4–6].

Spermidine also activated the Ca^{2+} uptake by the yeast mitochondria with the half-maximal effect at 50–100 μM (fig.2). Putrescine and cadaverine had vir-

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/90/$3.50

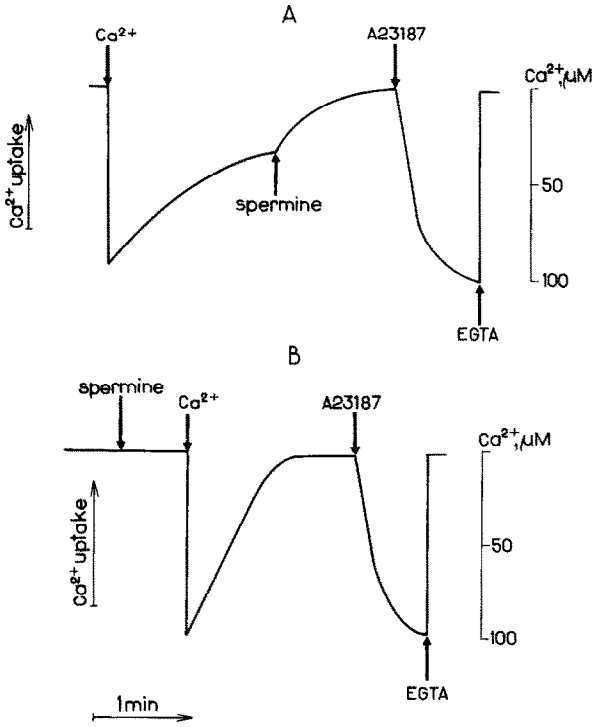

Fig.1. The Ca^{2+} uptake by aerobic *End. magnusii* mitochondria. For the incubation medium, see section 2. When indicated, 50 μM of spermine, 100 μM of Ca^{2+}, 1 μM of A23187, 200 μM of EGTA were present in the incubation medium.

tually no influence (not shown).

Thus, we found that spermine at low concentrations has a significant modulating effect on the Ca^{2+} transport system of the *End. magnusii* mitochondria. The observed phenomena were not caused by change of the energy parameters of mitochondria, since spermine had no influence either on the rate of respiration, or on the ADP/O and respiratory control values (not shown).

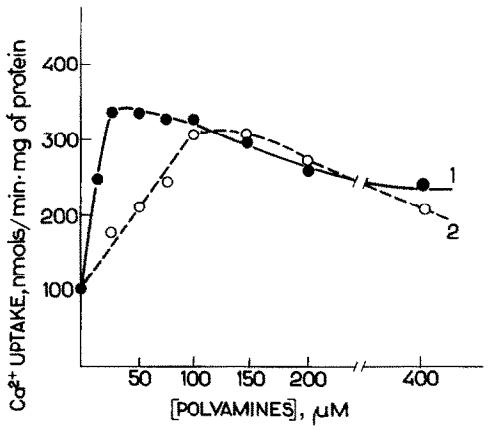

Fig.2. The dependence of the initial rate of 150 μM Ca^{2+} uptake by the mitochondria upon the spermine (1) and spermidine (2) concentration. For the incubation medium, see section 2. Initial Ca^{2+} concentration, 150 μM.

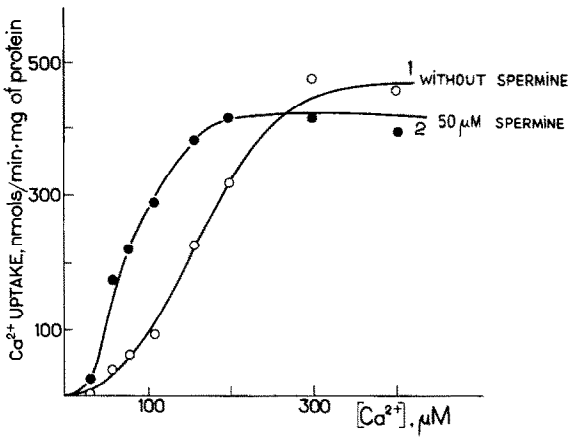

Fig.3. The dependence of the initial rate of calcium uptake by the mitochondria upon Ca^{2+} concentration in the incubation medium. For the incubation medium, see section 2.

It is possible that polyamine changes the kinetic properties of the yeast mitochondrial Ca^{2+} transport system. Fig.3 shows the dependence of the initial rate of Ca^{2+} uptake upon its concentration. The addition of 50 μM spermine resulted in a shift of the curve towards lower Ca^{2+} concentrations, with decrease of the $C_{1/2}$ value down to 60 μM. At the same time, spermine had little effect on the maximal rate of Ca^{2+} uptake. These data could be considered in terms of the increased affinity of the yeast mitochondria calcium carrier for Ca^{2+} cations. Probably, the spermine action on the Ca^{2+} carrier is mediated by changes in membrane properties (i.e. surface charge or microviscosity).

It should be noted that spermine concentrations yielding the marked effect in vitro on the kinetic parameters of the Ca^{2+} transport system were close to the physiological concentrations of free polyamine in the cell. The spermine content in the yeast was estimated to be about 0.3–1 μmol/g dry weight [8]. The greater part of spermine was bound to membranes and nucleic acids, so that the free spermine concentration in the cytoplasm was estimated to be about 10^{-4} M [7].

The above data allowed us to suggest that the yeast mitochondria may play a significant role in Ca^{2+} distribution inside the cell since they can reduce the extramitochondrial Ca^{2+} concentration to the normal physiological level that according to Eilam [12] is over the range of 6×10^{-6} to 2.3×10^{-5} M.

Acknowledgement: The authors are very much obliged to Dr Yu.N. Leikin for fruitful discussion of the work.

REFERENCES

[1] Carafoli, E., Balcovage, W.X., Lehninger, A. and Mattoon, J.R. (1970) Biochim. Biophys. Acta 205, 18–20.
[2] Balcovage, W.X., Lloyd, J.L., Mattoon, J.R. et al. (1973) Biochim. Biophys. Acta 305, 41–51.

[3] Zvjagilskaya, R.A., Leikin, Yu.A., Kozhokaru, N.L. and Kotelnikova, A.V. (1983) Doklady AN SSSR 269, 1233–1240.
[4] Karadgov, Yu.S., Kudzina, L.Yu. and Zinchenko, V.P. (1988) Biophisika 33, 1001–1005.
[5] Nicchita, C.V. and Williamson, J.R. (1984) J. Biol. Chem. 259, 12978–12983.
[6] Kroner, H. (1988) Arch. Biochem. Biophys. 267, 205–210.
[7] Bachrach, U. (1973) Function of Naturally Occurring Polyamines, Academic Press, New York.
[8] Cohn, M.N. and Tabor, C.W. (1978) J. Bacteriol. 134, 208–213.
[9] Leikin, Yu.N., Votyakova, T.V., Bazhenova, E.N. et al. (1987) Biokhimia 52, 676–682.
[10] Scarpa, A. (1972) Methods Enzymol. 24 part B, 343–351.
[11] Bradford, M.M. (1976) Anal. Biochem. 72, 248–254.
[12] Eilam, Y., Lavi, H. and Grossowicz, N. (1985) J. Gen. Microbiol. 131, 623–629.