Vol. 158, No. 1, 1989 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
January 16, 1989 Pages 287-293

POLYAMINES ARE SUFFICIENT TO DRIVE THE TRANSPORT OF THE PRECURSOR OF ORNITHINE CARBAMOYLTRANSFERASE INTO RAT LIVER MITOCHONDRIA: POSSIBLE EFFECT ON MITOCHONDRIAL MEMBRANES

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Received December 8, 1988

The polyamines spermidine, spermine and putrescine, by themselves, at physiological concentrations, induce the transport of the precursor of ornithine carbamoyltransferase into isolated rat liver mitochondria. The presence of polyamines in the transport medium results in the approach of both mitochondrial membranes, suggesting a possible role of these molecules in the transport of the precursor of ornithine carbamoyltransferase into mitochondria, by the formation and/or stabilization of mitochondrial structures involved in the transport system.

The majority of the mitochondrial proteins are nuclear encoded, synthesized in the cytoplasm as precursor polypeptides usually containing an amino-terminal extension, and subsequently imported into the organelle (1).

The biogenesis of ornithine carbamoyltransferase (OCT), a mitochondrial matrix protein of the urea cycle, shows this general model (2).

Previous studies have reported that the transport of the precursor of OCT (pOCT) requires soluble cytosolic component(s) present in the rabbit reticulocyte lysate (3, 4) or in rat liver cytosol (manuscript in preparation), and low molecular weight components such as magnesium and potassium ions (3, 4). Recently, we showed that polyamines spermidine and spermine, at physiological concentrations, stimulate the "in vitro" transport of pOCT into rat liver mitochondria, in the presence of rabbit reticulocyte lysate (5).

0006-291X/89 $1.50

In this report we present data showing that the polyamines spermidine, spermine or putrescine alone are able to drive the import of pOCT into isolated rat liver mitochondria. In addition, we present preliminary results that clearly indicate a closer approximation of the outer and inner mitochondrial membranes when these polyamines are present in the transport medium.

MATERIALS AND METHODS

Materials

[^{35}S]methionine (1,000 Ci/mmol, 15 mCi/ml) was from The Radiochemical Center, Amersham.

General

pOCT-mRNA of rat liver was transcribed and translated in a nuclease-treated rabbit reticulocyte lysate system as described in (6)."In vitro" import and processing of pOCT by isolated rat liver mitochondria, sodium dodecyl sulfate-12 % polyacrylamide gel electrophoresis, fluorography of dried gels and other methods related to this study were performed as described in (6).

Electron microscopy

Aliquots of 300 µl of mitochondria incubated in the presence or absence of polyamines were fixed in 600 µl of 0.1 M phosphate-buffered 3 % glutaraldehyde at 4 ºC. After fixation, the mitochondrial suspension was centrifuged at 10,000xg for 5 min. Fixed mitochondrial pellets were washed overnight in phosphate buffer, postfixed in 1 % phosphate-buffered osmium tetroxide at 4 ºC, for 2 hours, dehydrated in a graded series of acetone solutions and embedded in Vestopal. After polymerization, ultrathin sections of these samples were examined in a Phillips EM 300 electron microscope.

RESULTS AND DISCUSSION

In a previous study we have shown that polyamines spermidine and spermine, plus rabbit reticulocyte lysate (5) or rat liver cytosol (manuscript in preparation) stimulate the transport of pOCT into isolated rat liver mitochondria. We tested the effect of polyamines by themselves on the transport of pOCT. The results obtained (Fig. 1) indicated that both the binding and the transport of pOCT were stimulated when the incubation was performed in a transport medium consisting of 4mM spermidine in the presence or absence of reticulocyte lysate. The stimulation produced by spermidine alone was as efficient as in the presence

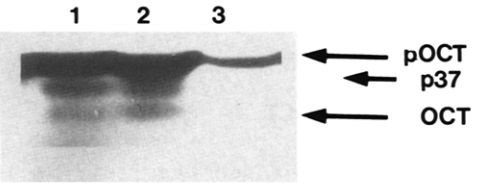

FIGURE 1. Spermidine alone stimulates pOCT binding and transport into isolated rat liver mitochondria

2 µl of the cell-free translated mixture (containing pOCT) were incubated in a final volume of 100 µl with 400 µg of mitochondrial protein, in a medium containing 5 mM Hepes buffer pH 7.4, 0.25 M sucrose, 5 mM succinate, 75 µM ADP, 1.25 mM P, and additions as follows, Lane 1: 20 µl of rabbit reticulocyte lysate (145 mg of protein/ml) and 4 mM spermidine. Lane 2: 4 mM spermidine. Lane 3: none.

Mitochondria were analyzed by SDS-polyacrylamide 12% gel electrophoresis and further fluorography.

of reticulocyte lysate, indicating that spermidine alone is sufficient for the transport of this precursor.

The additional band that appears between pOCT and the mature form, corresponds to a 37 Kd polypeptide that has also been described by other authors (7), however it is not known at present whether it is a product of an unspecific event (3) or represents an intermediate form in the transport of pOCT (8). In our transport conditions this intermediate form appears to be unspecific because it is detected only occasionally and it is not imported into mitochondria.

To determine whether the mature form had been translocated into the mitochondrial matrix, mitochondria were mildly treated with trypsin after the transport experiments (Fig. 2). This trypsin treatment resulted in total degradation of pOCT as well as of the intermediate form bound to mitochondria but mature OCT was resistant to the treatment, indicating that it had been transported into mitochondria.

Further experiments were carried out using related polyamines, spermine and putrescine, in the same transport conditions. As can be observed in Fig. 3, spermine and putrescine are also able to drive the import of pOCT into rat liver mitochondria.

The stimulatory effect produced by these polyamines on the binding and transport of pOCT was at physiological concentrations

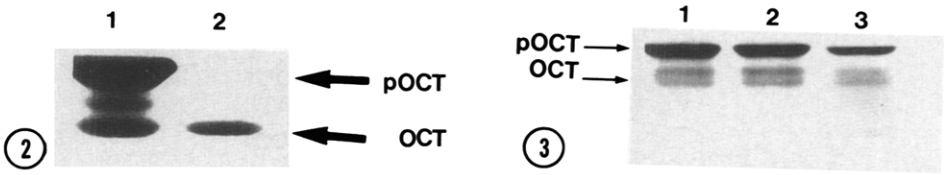

FIGURE 2. pOCT is transported into mitochondria in a trypsin-resistant manner in the presence of spermidine alone.

Lane 1: 2 µl of the cell-free translated mixture (containing pOCT) were incubated in a final volume of 100 µl with 400 µg of mitochondrial protein, in a medium containing 5 mM Hepes buffer pH 7.4, 0.25 M sucrose, 5 mM succinate, 75 µM ADP, 1.25 mM P, and 4 mM spermidine. Lane 2: as in lane 1 but at the end of the transport reaction, a trypsin treatment was performed (10 µg of trypsin/ml, 10 min at 0-4 ºC). Trypsin activity was arrested by the addition of soybean trypsin inhibitor (100 µg/ml).

Mitochondria were analyzed by SDS-polyacrylamide 12 % gel electrophoresis and further fluorography.

FIGURE 3. Spermine and putrescine alone also stimulate the binding and transport of pOCT.

2 µl of the cell-free translated mixture (containing pOCT) were incubated in a final volume of 100 µl, with 400 µg of mitochondrial protein, in a medium containing 5 mM Hepes buffer, 0.25 M sucrose, 5 mM succinate, 75 µM ADP, 1.25 mM P, and additions as follows, Lane 1: 1 mM spermine. Lane 2: 1 mM spermidine. Lane 3: 10 mM putrescine.

Mitochondria were analyzed by SDS-polyacrylamide 12 % gel electrophoresis and further fluorography.

0.1-4 mM spermidine, 0.1-1 mM spermine and 10-30 mM putrescine. At higher than physiological concentrations both processes (binding and transport) were slightly inhibited, particularly with spermine (data not shown).

In an attempt to understand the mechanism of action of the polyamines on the pOCT import into isolated rat liver mitochondria, we have studied the effect of polyamines on the ultrastructure of mitochondria when mitochondria are incubated in a transport medium containing spermidine or spermine alone, at physiological concentrations, by thin section electron microscopy. Preliminary results indicate that mitochondria incubated in the presence of polyamines (fig.4 upper) show an orthodox configuration, with the outer and inner membranes in closer proximity than in mitochondria incubated in the absence of polyamines. In the latter condition, mitochondria showed a condensed configuration and an evident separation between the two membranes (Fig. 4 lower). These observations extend previously

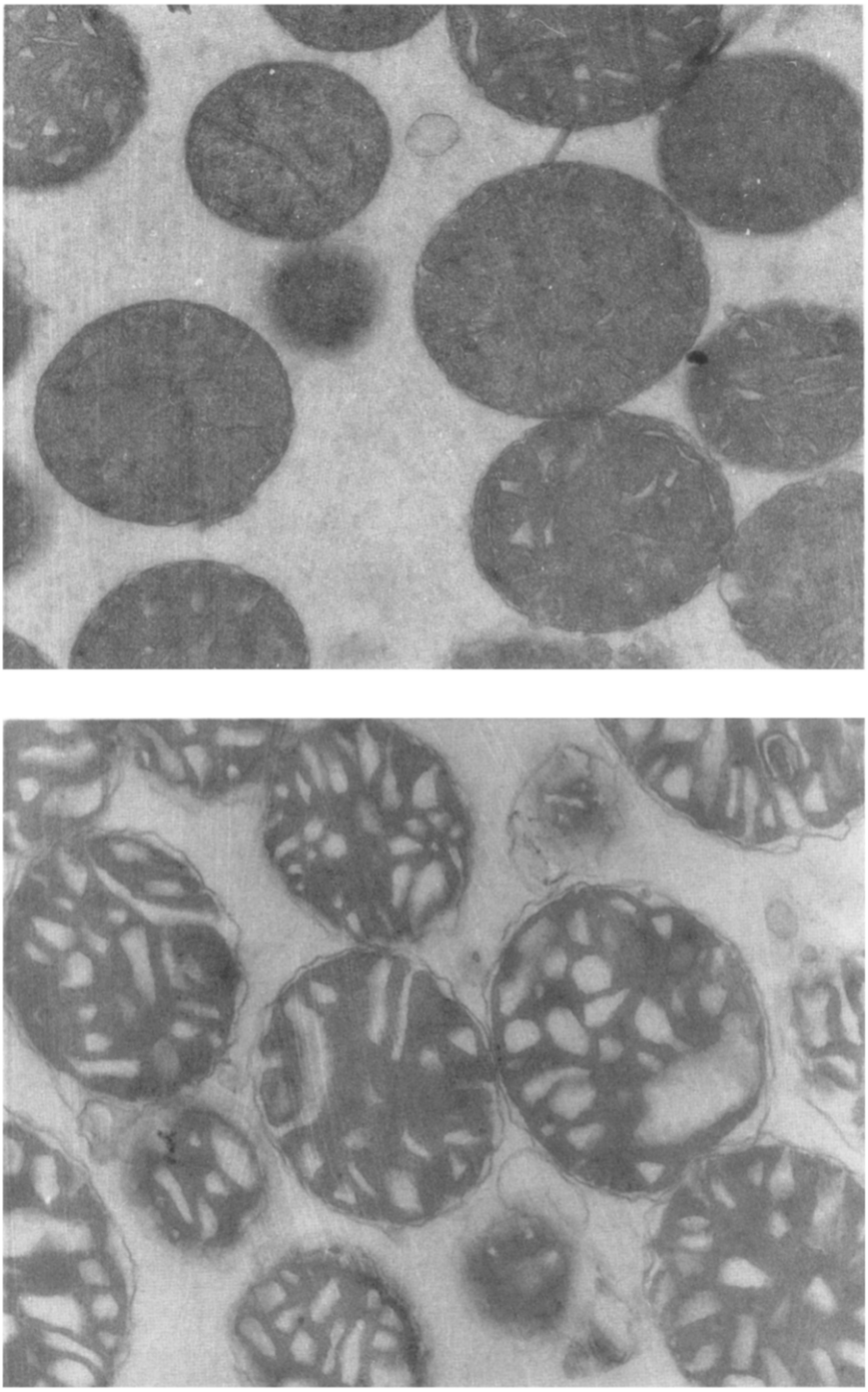

FIGURE 4. Effect of polyamines on the ultrastructure of isolated rat liver mitochondria, at physiological concentrations Electron micrographs of isolated rat liver mitochondria incubated for 30 min at 30 °C, in a transport medium containing 5 mM Hepes buffer pH 7.4, 0.25 M sucrose, 5 mM succinate, 75 µM ADP, 1.25 mM P in the absence (lower) or presence (upper) of 4 mM spermidine.

reported results (9) showing that the presence of high concentrations of 60 mM spermine in the incubation medium, produced morphological changes in isolated rat liver mitochondria, which presented an obliterated outer mitochondrial compartment and the inner and outer membranes in apparent contact with one another.

It is not known at present what the mechanism of action of polyamines on the "in vitro" transport of pOCT is. These polyamines, which occur in the millimolar range in eukaryotic cells, play an important role in the regulation of ion transport in mitochondria (10, 11) and in the stabilization of mitochondrial membranes (12) among many other processes.

Possibly polyamines act at different levels in the pOCT transport, affecting the precursor polypeptide and/or acting directly on mitochondria. These molecules may affect some mitochondrial structures related to the binding and/or translocation of pOCT, because it is known that polyamines are able to interact with components of mitochondrial membranes (proteins and phospholipids) (13). The fact that the two mitochondrial membranes are indeed closer in the presence of polyamines, suggests that the formation and/or stabilization of "contact sites" by these molecules may be responsible for their effect on the transport of pOCT, because the "contact sites" have been proposed as transport routes for the mitochondrial protein precursors (14). Another possibility is that polyamines may favour an optimal conformation of the precursor polypeptide for the transport, as has been reported for the NTPs (15). Also, the amphiphilic nature of these polyamines, similar to the signal peptide of mitochondrial precursors (16), may be related to the translocation of the precursor polypeptides across mitochondrial membranes.

These possible roles of polyamines, not only in the transport of pOCT but of other precursors with or without leading peptide, remain to be investigated, particularly their physiological "in vivo" significance.

ACKNOWLEDGMENTS

We thank Dr. M. Nguyen and Dr. G. Shore for supplying the clone pMN152. We also thank A. Montaner, E. Barber and M. J. Barber, for technical assistance. This work has been supported by the Comisión Asesora de Investigación Científica y Técnica of Spain (2386/83 0547/84), the U. S.-Spain Joint Committee for Scientific and Technological Cooperation, the Fondo de Investigaciones Sanitarias and the IIC-KUMC International Cytology Program. V. J. Miralles is now a postdoctoral fellow at the University of Medicine Dentistry of New Jersey (USA). C. González-Bosch is a "Giménez del Río" fellow and M. J. Marcote is a "Reina Doña Sofía" fellow of the I.I.C.

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