INHIBITION BY CALCIUM OF ADENINE NUCLEOTIDE TRANSLOCATION IN MITOCHONDRIA ISOLATED FROM EHRLICH ASCITES TUMOUR CELLS

Rupert F. W. THORNE and Fyfe L. BYGRAVE

Department of Biochemistry, Faculty of Science, The Australian National University, Canberra, A.C.T. 2600 Australia

Received 7 January 1974

1. Introduction

A carrier-system specifically involved in transporting AdN has been shown to exist in mitochondria isolated from a variety of eukaryotic cells. This translocase which is inhibited by atractyloside is located in the inner membrane of these organelles and operates by catalyzing an exchange of endogenous with exogenous AdN; generally ADP is exchanged at a rate greater than that of ATP (for review see ref. [1]).

It has become clear from recent work carried out, particularly in our laboratory [2–6] that low concentrations of Ca^{2+} stimulate the translocation of AdN in rat liver and heart mitochondria. We have proposed a mechanism for this which involves interaction of the Ca^{2+} with phospholipid located in the membrane environment of the AdN translocator. The stimulation is greater for ATP than for ADP and is not identifiable with that (of ATP) induced by uncouplers of oxidative phosphorylation [2, 3].

While investigating the effects of Ca^{2+} on energy-linked reactions in mitochondria from Ehrlich ascites tumour cells [7–10] we observed that quite low levels of this ion prevented the usual respiratory response to ADP, a property not seen in normal mitochondria. It occurred to us that this inhibitory action might be related in some way to an interaction of the Ca^{2+} with the AdN translocase system [9, 10].

Abbreviations: AdN = adenine nucleotide; ATP = adenosine triphosphate; ADP = adenosine diphosphate.

We have tested this proposition by examining the translocation of AdN in mitochondria isolated from Ehrlich ascites tumour cells and as shown in this communication have found that low concentrations of Ca^{2+} inhibit the entry of both ADP and ATP, a feature of the translocase which sharply contrasts to that found in rat liver mitochondria.

2. Experimental

The Ehrlich ascites tumour used as well as the details of propagation and harvesting are described elsewhere [8, 11]. Mitochondria from these cells were prepared using a Dounce homogeniser [12] and adopting minor modifications [8].

Cells were washed and homogenised in a medium containing 250 mM sucrose, 2.0 mM HEPES and 1 mM EDTA (pH 6.5). The final washings and resuspension of the mitochondria were carried out with a medium containing 150 mM sucrose, 2.0 mM HEPES and 50 mM KCl (pH 7.4). The presence of KCl in the medium greatly facilitated the handling and sedimentation properties of these mitochondria. Mitochondria from rat liver were prepared as previously described [2]. AdN levels in mitochondria were determined as previously described [2].

An aliquot of the final mitochondrial suspension was incubated with [^{3}H] ATP for 60 min at 0°C in preparation for the back-exchange experiments (for details see ref. [13]. Incubations were performed in

North-Holland Publishing Company – Amsterdam

a basic medium consisting of a 250 mM sucrose and 2.5 mM HEPES (pH 7.4). The final volume was 2 ml. To this were added approximately 4 mg mitochondria and other metabolites or components as indicated. After a 1 min preincubation the reaction was started by adding ADP or ATP. The reaction samples were removed at the predetermined times and placed into tubes containing actractyloside (final concentration 50 μM). The mitochondria were separated from the suspending medium by centrifugation for 2 min in an Eppendorf bench centrifuge. A portion of the supernatant was removed and transferred to a scintillation vial containing 10 ml of methoxy ethanol scintillation fluid, and counted to 1% standard deviation in a Beckman scintillation counter. The percent exchange was determined as indicated by the method outlined elsewhere [13].

3. Results

The tumour mitochondria used in these experiments were prepared in a manner such that they consistently exhibited acceptor control ratios of greater than 3 with succinate as substrate. We have argued in earlier reports that this property is a good reflection of the 'intactness' of mitochondria [7, 8, 14].

Data in fig. 1 show results from experiments in which the influence of added Ca^{2+} on the translocation of ATP was examined in mitochondria isolated from ascites tumour cells. It is clear that the rate of ATP translocation in the ascites mitochondria is inhibited some 50% by added Ca^{2+}. Under identical incubation conditions (i.e. 50 μM ATP, 100 nmoles Ca^{2+} per mg protein) Ca^{2+} stimulates ATP translocation in mitochondria isolated from rat liver [2–4].

Similar experiments to those described in fig. 1 were carried out with ADP in place of ATP (see fig. 2).

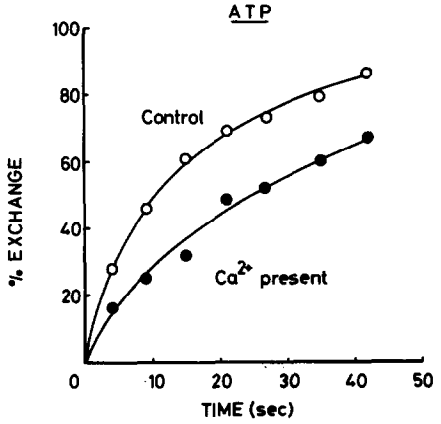

Fig. 1. Time course of the exchange of external ATP in mitochondria isolated from ascites tumour cells. The back exchange was measured as described in Experimental. ATP and Ca^{2+} (when added) were present at concentrations of 50 μM and 100 nmoles/mg protein respectively.

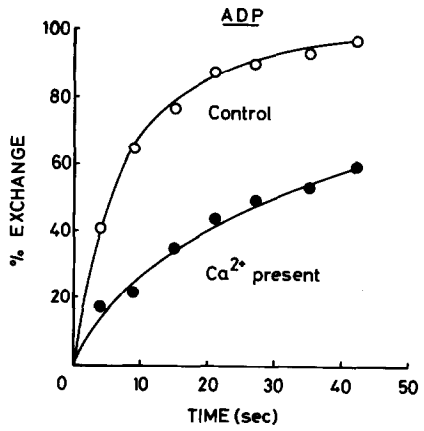

Fig. 2. Time course of the exchange of external ADP in mitochondria isolated from ascites tumour cells. Experimental conditions were as in fig. 1 except that ADP replaced ATP.

With liver mitochondria, Ca^{2+} is known to slightly stimulate ADP translocation [2–4]. However again it is clear that ADP translocation by the tumour mitochondria is particularly sensitive to the added Ca^{2+}. In the presence of 100 nmoles Ca^{2+} per mg protein the rate of translocation of 50 μM ADP is inhibited some 75%. In other experiments we have found that Ca^{2+} also inhibits the translocation of both ADP and ATP in mitochondria isolated from Yoshida hepatoma ascites (HA 130) cells but not in mitochondria isolated from the minimally-deviated Morris 5123 C hepatoma cells.

Adenine nucleotide translocation appears to differ in at least two other respects in the ascites mitochondria. The first is that although the total pool of ATP+ADP+AMP in the ascites mitochondria is approximately the same as that found in rat liver

mitochondria, the 'exchangeable' pool is considerably smaller amounting to only about 1.5 nmoles per mg protein. The other apparent difference is that in the ascites mitochondria, ATP and ADP are converted into AMP at rates considerably faster than those seen in rat liver mitochondria.

However not all of the properties of AdN translocation ascites mitochondria are uncharacteristic of the mammalian system. As will be reported in detail elsewhere, ATP translocation is stimulated by uncouplers of oxidative phosphorylation, ATP is translocated more slowly than ADP and atractyloside inhibition is enhanced by added K^+. Each of these properties characterize AdN translocation in mitochondria isolated for example from rat liver [1, 6, 15].

4. Discussion

It would appear from the data presented in this communication that the inability of ADP to stimulate respiration in tightly-coupled ascites tumour mitochondria in the presence of added Ca^{2+} [9, 10] is probably due in large part, if not entirely, to the inhibitory action this ion has on AdN translocation in these mitochondria. In this latter regard the added Ca^{2+} does not discrimminate between ADP and ATP translocation although it was observed repeatedly that ADP was more sensitive than ATP to the inhibitory action of Ca^{2+}. This inhibitory effect of Ca^{2+} on the translocase system directly contrasts with the stimulatory effect this ion has on ATP translocation, in particular, in liver and heart mitochondria [2–5].

The mechanism by which Ca^{2+} inhibits AdN translocation in the ascites tumour mitochondria is not clear at this time. Several possibilities are currently under examination one of which involves interaction of the Ca^{2+} with the lipoprotein translocator. Other work from our laboratory [5, 15] provides strong evidence for the direct involvement of phospholipid in AdN translocation and the mechanism of Ca^{2+}-stimulated ATP translocation.

Although we have examined so far mitochondria from only a limited number of tumours, it is apparent from our work that Ca^{2+} inhibition of AdN translocation does not occur in mitochondria isolated from all types of tumours. It is also relevent that ATP translocation in mitochondria isolated from blow-fly flight muscle which appear to lack high and low affinity binding sites for Ca^{2+} [16], is unaffected by the presence of added Ca^{2+} [17]. Thus the Ca^{2+} effect appears to be specific for certain species and tissues.

For some years we have advocated a vital role for Ca^{2+} in the regulation of cell metabolism [11, 18–22]. In the present work we have demonstrated that Ca^{2+}, by inhibiting the transport of ADP into mitochondria, is in a position to modify the energy metabolism of the ascites tumour cell by preventing the oxidative phosphorylation of cytoplasmic ADP. It is conceivable that this inhibitory action could contribute to the apparent inadequate respiratory energy supply thought to be a characteristic of this type of tumour [22].

Acknowledgement

F. L. Bygrave is grateful to the Australian Capital Territory Cancer Society for financial assistance in this work.

References

[1] Klingenberg, M. and Pfaff, E. (1968) Biochem. Soc. Symp. 27, 105.
[2] Spencer, T. and Bygrave, F. L. (1972) Biochem. J. 129, 355.
[3] Spencer, T. and Bygrave, F. L. (1973) J. Bioenergetics 4, 347.
[4] Spencer, T. and Bygrave, F. L. (1972) FEBS Letters 26, 225.
[5] Spencer, T. and Bygrave, F. L., Biochem. J., in press. tion.
[6] Meisner, H. Biochemistry 10 (1972) 3485.
[6] Meisner, H. (1971) Biochemistry 10, 3485.
[7] Thorne, R. F. W. and Bygrave, F. L. (1973) Biochem. Biophys. Res. Commun. 50, 294.
[8] Thorne, R. F. W. and Bygrave, F. L. (1973) Cancer Research 33, 2562.
[9] Thorne, R. F. W. and Bygrave, F. L. Nature, In Press.
[10] Thorne, R. F. W. and Bygrave, F. L. Submitted.
[11] Bygrave, F. L. (1966) Biochem. J. 101, 480.
[12] Wu, R. and Sauer, L. A. (1967) in: Methods in Enzymology (Estabrook, R. W. and Pullman, M. E., eds.) 105, Academic Press, New York.
[13] Pfaff, E. and Klingenberg, M. (1968) Eur. J. Biochem. 6, 66.

[14] Bygrave, F. L. and Lehninger, A. L. (1966) J. Biol. Chem. 241, 3894.
[15] Spencer, T. and Bygrave, F. L. In preparation.
[16] Carafoli, E. and Lehninger, A. L. (1971) Biochem. J. 122, 681.
[17] Doy, F., Daday, A. and Bygrave, F. L., unpublished observation.
[18] Bygrave, F. L. (1966) Biochem. J. 101, 488.
[19] Bygrave, F. L. (1967) Nature 214, 667.
[20] Meli, J. and Bygrave, F. L. (1972) Biochem. J. 128, 415.
[21] Bygrave, F. L. in: An introduction to bioinorganic chemistry (Williams, D. R. ed.), Butterworths, London in press.
[22] Warburg, O. (1956) Science 123, 309.