THE INFLUENCE OF LANTHANUM ON CALCIUM-STIMULATED ATP TRANSLOCATION IN RAT LIVER MITOCHONDRIA

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1. Introduction

The translocation of adenine nucleotides across the inner mitochondrial membrane occurs via a specific 'permease' or 'translocase' located in the membrane. The action of this translocase is inhibited by atractyloside (for review see [1]). We have reported [2–5] that low concentrations of Ca^{2+} stimulate translocation of ATP while having little effect on that of ADP.

In efforts to further define the nature of the stimulation by Ca^{2+} we have carried out studies with the rare earth cation La^{3+}. This ion has proved to be a useful probe in studying interactions of Ca^{2+} with proteins, lipids and membranes in general [6–8], as well as with Ca^{2+}-induced energy-dependent processes in mitochondria [9–11].

2. Materials and methods

The preparation of rat liver mitochondria and assay of translocase activity using the back-exchange technique [12] were carried out exactly as described previously [3,4]. The materials used were as described [4]. $LaCl_3 \cdot 7H_2O$ was obtained from Ajax Chemicals Ltd., Sydney.

3. Results and discussion

The influence of La^{3+} on the translocation of ATP and ADP with and without added Ca^{2+} is shown in fig.1. The following effects can be seen: a) only that portion of ATP translocation which is stimulated by Ca^{2+} is inhibited by La^{3+} (K_i = 3.5 μM);

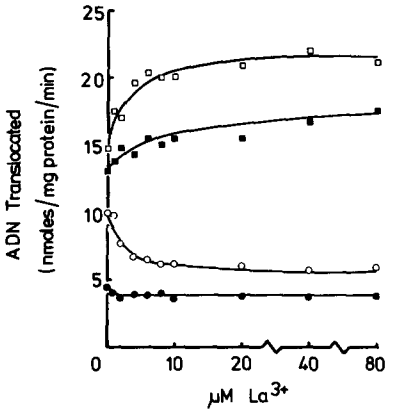

Fig. 1. Effect of La^{3+} on ATP and ADP translocation with and without added Ca^{2+}. Each reaction mixture contained 200 mM sucrose, 2 mM HEPES-KOH buffer (pH 7.4), 250–500 μg mitochondrial protein prelabelled with [^{3}H] ATP (see [4,12]). Ca^{2+} (200 μM) and varying concentrations of La^{3+} were present as indicated. The total volume was 250 μl. Incubations at 4° were started by the addition of 200 μM ADP or ATP, continued for 10 sec and stopped by the addition of 50 μM atractyloside. Translocase activity was measured as previously described [3,4]. Open symbols, Ca^{2+} present; closed symbols, Ca^{2+} absent; circles, ATP; squares, ADP.

b) ADP translocation in the absence of added Ca^{2+} is slightly stimulated by La^{3+} (K_m = 25 μM; maximal stimulation was 50%); c) ADP translocation in the presence of added Ca^{2+} is markedly stimulated by La^{3+} (K_m = 7 μM; maximal stimulation was 60%). These data show that La^{3+} has different effects on ADP and ATP translocation in the absence of Ca^{2+} and has completely opposite effects when Ca^{2+} is present. They also indicate that added Ca^{2+} enhances the stimulation of ADP translocation by La^{3+}.

Table 1
Effect of Ca^{2+} concentration on the inhibitor constant for La^{3+} of Ca^{2+}-stimulated translocation of ATP.

Ca^{2+} concentration (μM)	K_i for La^{3+}
100	2.4
200	4.0
400	12.5
1000	18.0

Incubations were carried out as described in the legend to fig. 1 except that the La^{3+} concentration was varied in the presence of the four indicated concentrations of Ca^{2+}.

In other experiments, the influence of Ru^{3+} and Nd^{3+} on ADP and ATP translocation was also examined. As with La^{3+}, only that portion of ATP translocation stimulated by Ca^{2+} was inhibited by Ru^{3+} (K_i = = 1.5 μM) and Nd^{3+} (K_i = 4.5 μM). Ru^{3+} and Nd^{3+}, like La^{3+}, also stimulated the translocation of ADP. The affinity for these rare earth cations was increased from about 30 μM to about 10 μM in the presence of Ca^{2+}. The rates of translocation were stimulated some 50% in all cases. Thus the effects on adenine nucleotide translocation seen with La^{3+} in the absence and presence of Ca^{2+} are seen also with other rare earth cations.

Data in table 1 show that the K_i for La^{3+} inhibition of Ca^{2+}-stimulated ATP translocation increases as the Ca^{2+} concentration increases. These findings further suggest that Ca^{2+} and La^{3+} compete for a common (binding) site in the vicinity of the translocase.

We have provided evidence [3–5] that the mechanism by which Ca^{2+} stimulated ATP translocation is different from that involving uncouplers of oxidative phosphorylation such as CCCP [12]. Data shown in fig. 2 strongly support our contention. Here it is seen that, in contrast to Ca^{2+}-stimulated ATP translocation (see fig. 1), uncoupler-stimulated ATP translocation is virtually unaffected by La^{3+} concentrations up to 80 μM.

Meisner [13] has shown and we have confirmed [4] that K^+ stimulates ATP translocation in rat liver mitochondria. Furthermore we have shown that K^+ and Ca^{2+} are additive in their effects on ATP translocation [4]. Fig. 3 shows results from an experiment in which the effect was studied of La^{3+} on ATP translocation with and without added K^+ and Ca^{2+}. La^{3+}

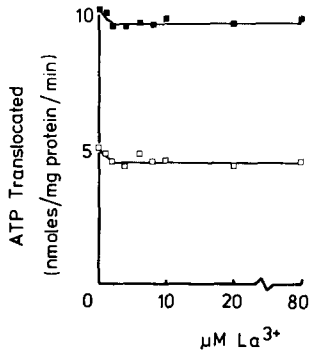

Fig. 2. Effect of La^{3+} on CCCP-stimulated translocation of ATP. Incubations were carried out as described in the legend to fig. 1. CCCP (2 μM) and La^{3+} were present as indicated. ■, CCCP present; □, CCCP absent.

clearly has no effect on the K^+-stimulated portion of ATP translocation but at the same time inhibits the Ca^{2+}-stimulated portion. Moreover the presence of K^+ does not alter the affinity of the inhibition for La^{3+} (K_i = 5 μM in the absence and presence of K^+).

4. Conclusions

This study illustrates once more the usefulness of the rare earth cations in elucidating the effects of Ca^{2+} on biological processes. Using this cation we have been able to show that the response of ADP translocation to La^{3+} is clearly different to that of ATP translocation. This was particularly evident when low concentrations of Ca^{2+} were simultaneously present in the incubation mixture.

Secondly, we have provided conclusive evidence that the stimulation of ATP translocation induced by Ca^{2+} is different from that induced by uncouplers and that induced by K^+. The observations that La^{3+} (i) inhibits only that portion of ATP translocation which is stimulated by Ca^{2+} and (ii) competes with added Ca^{2+}, suggests a mechanism of stimulation which involves a binding site(s) for Ca^{2+} in the vicinity of the translocase and not one involving, for example, chelation of metal ion with ATP. Finally, while these data are consistent with our proposal [3,4] that Ca^{2+} exerts its effects by interacting with phospholipids in the vicinity of the translocase, they do not exclude possible interactions of Ca^{2+} with the protein moiety of the translocase.

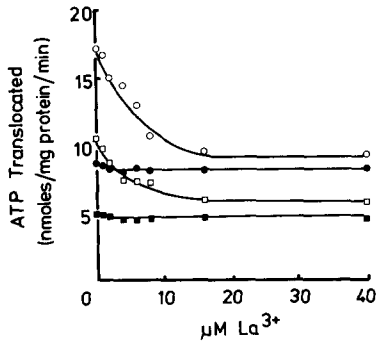

Fig. 3. Effect of La^{3+} on Ca^{2+}-stimulated and K^+-stimulated translocation of ATP. Incubations were carried out as described in the legend to fig.1. K^+ (20 mM), Ca^{2+} (200 μM) and La^{3+} were present as indicated. Open symbols, Ca^{2+} present; closed symbols, Ca^{2+} absent; circles, K^+ present; squares, K^+ absent.

References

[1] M. Klingenberg and E. Pfaff, in: Metabolic Roles of Citrate, ed. T.W. Goodwin (Academic Press, New York, 1968) p. 105.
[2] T. Spencer and F.L. Bygrave, Proc.Aust.Biochem.Soc. 4 (1971) 97.
[3] T. Spencer and F.L. Bygrave, Biochem.Biophys.Res. Commun. 43 (1971) 1290.
[4] T. Spencer and F.L. Bygrave, Biochem.J., in press.
[5] T. Spencer and F.L. Bygrave, J.Bioenergetics (1972) in press.
[6] J.Y. Lettvin, W.F. Pickard, W.S. McColluch and W. Pitts, Nature 202 (1964) 1338.
[7] D. van Breenen and C. van Breenen, Nature 223 (1969) 898.
[8] E.R. Birnbaum, J.E. Gomez and D.W. Darnall, J. Amer.Chem.Soc. 92 (1970) 5287.
[9] L. Mela, Arch.Biochem.Biophys. 123 (1968) 286.
[10] L. Mela, Biochemistry 8 (1969) 2481.
[11] A.L. Lehninger and E.Carafoli, Arch.Biochem. Biophys. 143 (1971) 506.
[12] E. Pfaff and M. Klingenberg, European J.Biochem. 6 (1968) 66.
[13] H. Meisner, Biochemistry 10 (1971) 3485.