# EFFECT OF METAL CATIONS ON THE INHIBITION OF ADENINE NUCLEOTIDE TRANSLOCATION BY ACYL-CoA

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## 1. Introduction

Thioesters of CoA with long and medium chain length fatty acids are strong inhibitors of the translocation of adenine nucleotides in mitochondria [1–3]. The inhibition of this translocation by lipophilic anions [4] and anionic detergents [5] suggests an ionic interaction between adenine nucleotides and the mitochondrial membrane or the translocase itself. Therefore, the ionic interaction between polar groups of acyl-CoA and specific translocase sites of the membrane can be postulated as well. If this is true, then the inhibition of the translocase produced by acyl-CoA should be modified by ionic strength of the medium.

Divalent cations, and $Mg^{2+}$ in particular, are well known complexing agents for phosphate groups [6]. They can thus form complexes with acyl-CoA as well as bind to phospholipid components of the mitochondrial membrane. The inhibition of adenine nucleotide translocase by tetraphenylboron is partly released by $Mg^{2+}$, probably due to a partial neutralization by this cation of the increased negative charge of the membrane [4]. It became therefore interesting to examine the effect of $Mg^{2+}$ on the inhibition produced by acyl-CoA.

The present investigation shows that KCl diminishes the inhibitory effect of palmitoyl-CoA on ADP translocation and decreases the affinity of the inhibitor to the translocase. It also shows that $Mg^{2+}$ can partly, and $La^{3+}$ completely, abolish the inhibition. Experiments with mitochondria depleted of magnesium by the use of the ionophore A23187 suggest that, in normal liver mitochondria, the translocase is not saturated from the inside, probably because intramitochondrial adenine nucleotides are mostly complexed with magnesium.

## 2. Material and methods

Mitochondria from livers of albino rats were isolated according to Hogeboom [7]. Translocation of adenine nucleotides was measured by the inhibitor-stop method using [$^{14}$C] ADP as substrate and carboxyatractyloside as inhibitor. The translocation in the 'forward' direction was followed as described previously [8] and in the 'back-exchange system' as described by Pfaff and Klingenberg [9].

Palmitoyl-CoA was synthesized by the procedure of Seubert [10]. [$^{14}$C] Palmitoyl-CoA was synthesized from [1-$^{14}$C] palmitic acid (Radiochemical Centre, Amersham, England) and CoA (Boehringer, Mannheim, F.R.G.) by the own modification (Duszyński and Wojtczak, unpublished) of the procedure of Seubert [10]. [8-$^{14}$C] ADP was obtained from the Radiochemical Centre (Amersham, England); carboxyatractyloside was a generous gift of Boehringer (Mannheim, F. R. G.) and the ionophore A23187 of Eli Lilly (Indianapolis, Indiana, USA).

## 3. Results

Replacement of sucrose by KCl in the incubation medium markedly decreased the inhibitory effect of palmitoyl-CoA on ADP translocation (fig.1). In both sucrose and KCl media the competitive character of this inhibition [3] was maintained (fig.2), but the $K_i$ value was four times higher in KCl than in sucrose whereas $K_m$ value for ADP remained practically unchanged.

The effect of $Mg^{2+}$ on the inhibition of the translocase by palmitoyl-CoA is illustrated in table 1. In the



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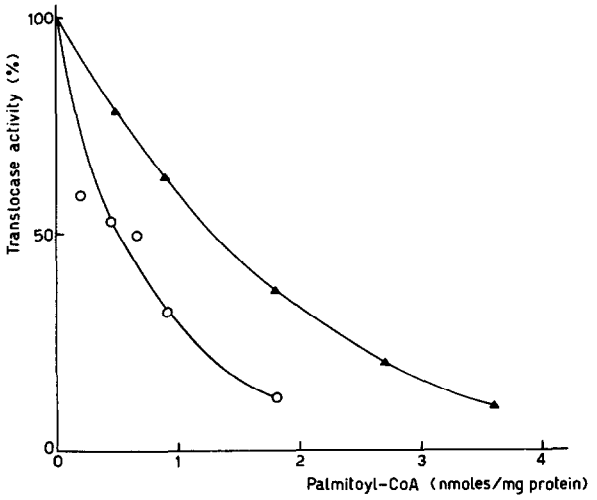


Fig.1. Effect of medium composition on the inhibitory action of palmitoyl-CoA on ADP translocation in rat liver mitochondria. The medium contained: (o—o—o) 250 mM sucrose, 1 mM EDTA and 10 mM TEA–HCl* (pH 7.4); or (▲—▲—▲) 110 mM KCl, 10 mM sucrose, 1 mM EDTA and 10 mM TEA–HCl (pH 7.4). The amount of mitochondria was 7 mg protein and the total volume 2.1 ml. Palmitoyl-CoA was added first, 2 min later followed by [$^{14}$C]ADP (final concentration 70 $\mu$M) and the translocation was stopped by carboxyatractyloside (final concentration 5 $\mu$M) after 20 sec. The temperature was 0°C.

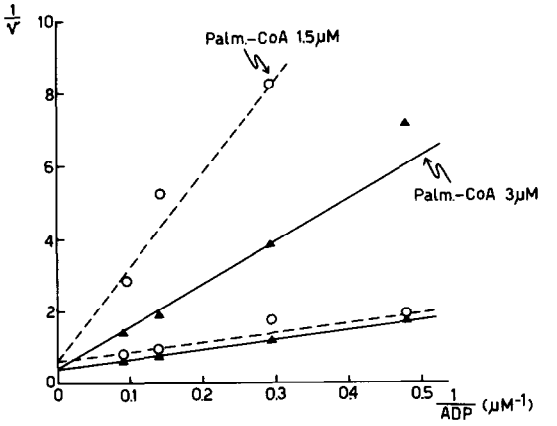


Fig.2. The Lineweaver-Burk plots of ADP translocation in sucrose and KCl media. Experimental conditions as in fig.1. (o—o—o) Sucrose medium; (▲—▲—▲) KCl medium. The rate of translocation is expressed in arbitrary units. $K_m$ values calculated from this experiment are 5.0 $\mu$M and 6.6 $\mu$M, $K_i$ values 0.2 $\mu$M and 0.9 $\mu$M in sucrose and KCl media respectively.

absence of the inhibitor the translocation of ADP is slightly decreased by magnesium ions, which is in agreement with earlier observations of Pfaff and Klingenberg [9] and could be interpreted as indicating that free nucleotides rather than their Mg-complexes are the substrate for the translocase. The inhibitory effect of palmitoyl-CoA was smaller when added in the presence than in the absence of $Mg^{2+}$ in the medium. However, magnesium ions did not abolish the inhibition when added *after* palmitoyl-CoA; in fact, the inhibition was usually somewhat greater in this case. Similar results were obtained when $Sr^{2+}$ was used instead of $Mg^{2+}$ (not shown).

A somewhat different picture was seen with the trivalent cation $La^{3+}$. In this case an abolishion of the

Table 1
Effect of palmitoyl-CoA and $Mg^{2+}$ on ADP translocation

| Additions | ADP translocation | | | |
|---|---|---|---|---|
| | without $MgCl_2$ | | with 10 mM $MgCl_2$ | |
| | (nmoles/mg prot./min) | (%) | (nmoles/mg prot./min) | (%) |
| None | 1.7 | 100 | 1.4 | 100 |
| Palmitoyl-CoA, | | | 1.2[1] | 86[1] |
| 1 nmole/mg protein | 1.1 | 65 | 0.7[2] | 50[2] |

The samples contained 2.1 ml of 120 mM KCl, 1 mM EDTA, 5 mM TEA–HCl* (pH 7.4) and 7 mg mitochondrial protein. The translocation was measured as described in the legend to fig.1.

[1] Palmitoyl-CoA was added after $MgCl_2$;

[2] Palmitoyl-CoA was added before $MgCl_2$;

* *Abbreviation:* TEA, triethanolamine.

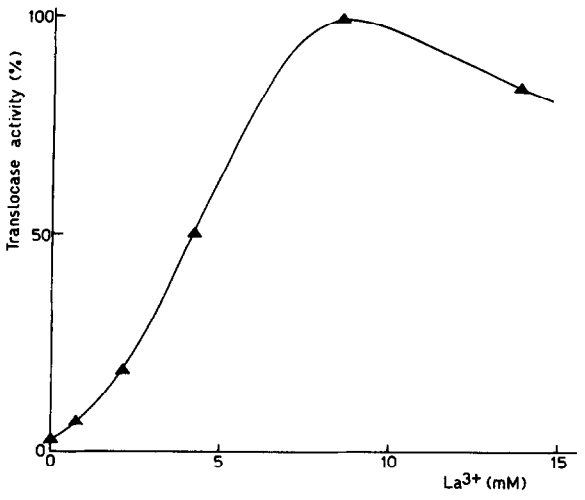


Fig.3. Effect of $La^{3+}$ on the inhibitory action of palmitoyl-CoA on ADP translocation. The incubation medium contained: 250 mM sucrose, 1 mM EDTA, 5 mM TEA-HCl (pH 7.2) and 4.6 mg mitochondrial protein in total volume of 2.1 ml. Palmitoyl-CoA (2 nmol/mg protein) was added first, followed by $La(NO_3)_3$ 1 min later. The translocation was measured as described in the legend to fig.1.

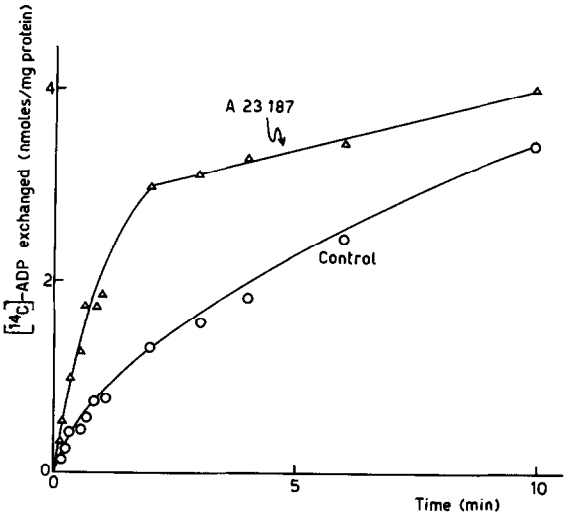


Fig.5. Effect of ionophore A23187 on ADP translocation. The incubation medium contained: 83 mM sucrose, 80 mM KCl, 1 mM EDTA, 10 mM TEA–HCl (pH 7.4) and 3.7 mg mitochondrial protein in total volume of 2.1 ml. Where indicated, the samples were preincubated for 1 min with 3 $\mu$g of the ionophore A23187 before the translocation was started. Other conditions as in fig.1.

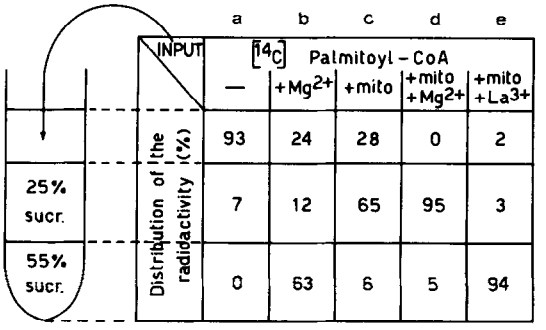


Fig.4. Binding of [$^{14}$C] palmitoyl-CoA to mitochondria in the absence and presence of metal cations. On top of the discontinuous density gradients composed of 4 ml 55% (w/v) sucrose and 4 ml 25% (w/v) sucrose (buffered with 5 mM TEA–HCl, pH 7.4) 1.5 ml samples were layered containing 250 mM sucrose, 1 mM EDTA, 10 mm TEA–HCl (pH 7.4), 5 nmoles [$^{14}$C] palmitoyl-CoA and the following additions: a) none, b) 9 mM $MgCl_2$, c) mitochondria corresponding to 3 mg protein, d) mitochondria followed by 9 mM $MgCl_2$ and e) mitochondria followed by 8 mM $La(NO_3)_3$. After centrifugation at 100 000 *g* during 3 hr the layers were collected and measured for radioactivity. In the centrifuge tubes c) and d) mitochondria accumulated at the 25%/55% interphase and were collected with the 25% layer; in the tube e) they sedimented at the bottom.

inhibition occurred when lanthanum ions were added before as well as after palmitoyl-CoA. A complete release of the inhibition produced by 2 nmol palmitoyl-CoA/mg mitochondrial protein was observed in the presence of 8 mM lanthanum nitrate (fig.3).

The following experiment (fig.4) illustrates the association of palmitoyl-CoA to mitochondria in the absence and presence of $Mg^{2+}$ and $La^{3+}$. Samples containing [$^{14}$C] palmitoyl-CoA without and with added mitochondria and $MgCl_2$ or $La(NO_3)_3$ were layered on top of a discontinuous sucrose gradient. After centrifugation the distribution of the radioactivity was analysed (fig.4). [$^{14}$C] Palmitoyl-CoA present alone in the medium did not move during centrifugation and was almost quantitatively recovered from the input. Magnesium ions added to the medium partly precipitated palmitoyl-CoA which was then collected at the bottom of the centrifuge tube. If mitochondria were present in the absence of divalent cations, about two third of the radioactivity was recovered from the interphase of 25%/55% sucrose where mitochondria were accumulated. This agrees fairly well with the finding [3] that the distribution between bound to mitochondria and free

palmitoyl-CoA is 2:1. However, when $Mg^{2+}$ was added after mitochondria, practically all palmitoyl-CoA accumulated at the 25%/55% interphase, indicating its stronger binding to mitochondria in this case. An increased binding of palmitoyl-CoA to mitochondria by increasing KCl concentration was observed by McMillin Wood [11]. When $La^{3+}$ ions were added, both mitochondria and all [$^{14}$C] palmitoyl-CoA were precipitated at the bottom of the centrifuge tube.

Since the discovery of the ionophore A23187 specific for divalent cations [12] it became possible to obtain mitochondria largely depleted of their endogenous magnesium and calcium. In connection with the present study it was intriguing to see how the translocation of adenine nucleotides proceeded in such particles. It was observed that in $Mg^{2+}$-depleted mitochondria the translocation of ADP was much faster and attained the equilibration level sooner than in untreated mitochondria (fig.5). Similar results were obtained in the back-exchange system.

## 4. Discussion

Metal cations have been already shown to influence the translocation process of adenine nucleotides in mitochondria. Monovalent cations increase the rate of ATP transport [13,14], especially in the presence of gramicidin or valinomycin. This effect has been explained by postulating charge equilibration, arising from the electrogenic exchange of external ATP against ADP [14]. The present investigation demonstrates that monovalent salt solutions substantially diminish the inhibition by acyl-CoA. The most likely explanation of this effect seems to be the assumption of an ionic interaction between polar groups of acyl-CoA and the translocase.

The effect of divalent and trivalent cations is more complex. They can form complexes with the polar moiety of acyl-CoA and can thus decrease its affinity to the receptor sites of the translocase. They can also partly neutralize the excessive negative charge of the membrane (cf. [4]) produced by adsorbed acyl-CoA. On the other hand, however, $Mg^{2+}$ increases the binding of acyl-CoA to mitochondrial membranes and can thus enhance the inhibition under certain conditions. Lanthanum ions are much stronger complexing agent for acyl-CoA than magnesium ions and can therefore completely reverse the inhibition produced by acyl-CoA.

Most of the studies on the inhibitory effect of acyl-CoA on adenine nucleotide translocase carried out so far have been done in the absence of divalent cations. The present investigation shows that under ionic composition more similar to that of the intercellular medium the inhibition is much smaller. This suggests that under in vivo conditions the inhibitory effect of long chain acyl-CoA may be much smaller and may be controlled in a rather complex way. In fact, it has been calculated [15] that rat liver contains 0.8 nmol long chain acyl-CoA per mg protein. Cellular acyl-CoA has been recently shown [16] to be mostly concentrated in mitochondria and hence mitochondria in situ may contain amounts of acyl-CoA which, in the isolated particles and in sucrose media without $Mg^{2+}$, are sufficient completely to block adenine nucleotide transport.

A stimulatory effect of the ionophore A23187 on adenine nucleotide translocation is an additional observation of the present study. Two explanations may be proposed for this effect. First, that the higher rate of the translocation is due to facilitated charge equilibration by mitochondrial divalent cations [13,14]. Second, that under normal conditions the translocase is not 'saturated' with the nucleotides from the inside, due to the fact that most of them are complexed by intramitochondrial magnesium and calcium. The first explanation does not hold, since the ionophore was also effective in the presence of EDTA, i.e. under conditions when the recycling of divalent cations was prevented, and also because the stimulation was observed in the case of ADP translocation which is not electrogenic. It should be therefore suggested that the second explanation may be valid. Although the amount of adenine nucleotides in mitochondria is rather high, corresponding to about 10 mM concentration in the matrix space, most of them must be present as magnesium, and perhaps also calcium, complexes. The concentration of free, not complexed, ADP + ATP is therefore very low. Though it has not been conclusively demonstrated, it might be assumed that not complexed molecules of ADP and ATP are better substrates for the translocase than are Mg-complexes of those nucleotides. Therefore, depletion of the matrix space of $Mg^{2+}$ and $Ca^{2+}$ increases the available concentration of nucleotides to be translocated.

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