EFFECT OF DETERGENTS ON ADP TRANSLOCATION IN MITOCHONDRIA

Jerzy DUSZYŃSKI and Lech WOJTCZAK
Department of Cellular Biochemistry, Nencki Institute of Experimental Biology, Pasteura 3, 02–093 Warszawa, Poland

Received 9 January 1974

1. Introduction

The inhibitory action of acyl-CoA thioesters on adenine nucleotide translocation has attracted attention of several laboratories because of its possible physiological importance [1–7]. Acyl-CoA contains polar and non-polar regions in its molecule and it might be therefore supposed that its inhibitory action on the translocase is due to the detergent effect on mitochondrial membrane. Vaartjes et al. [8] considered this possibility and compared the action of acyl-CoA with that of acyl-carnitine. They found that these two compounds strongly differed in their inhibitory effect, acyl-CoA being a strong inhibitor and acyl-carnitine having a low inhibitory effect, whereas they were equivalent as detergents.

The present investigation was undertaken with the aim further to compare the effect of acyl-CoA with that of a number of typical detergents on adenine nucleotide translocation in mitochondria. It is shown that although anionic and non-ionic detergents can inhibit the translocase, the action of acyl-CoA seems to be much more specific. It is also shown that the cationic detergent, cetyltrimethylammonium bromide, can abolish the inhibitory effect of palmitoyl-CoA. These results are discussed in terms of molecular interactions between acyl-CoA, detergents and the translocase.

2. Materials and methods

Mitochondria were isolated from livers of albino rats by the procedure described by Hogeboom [9]. Translocation of adenine nucleotides was measured by the inhibitor-stop method using [^{14}C] ADP. Translocation in the 'forward' direction (the uptake of [^{14}C] ADP by mitochondria from the medium) was followed as described previously [10]; the 'back-exchange' was measured according to Pfaff and Klingenberg [11]. Carboxyatractyloside (Gummiferin) was used as inhibitor of the translocation [12].

Palmitoyl-CoA was synthesized by the procedure of Seubert [13] or obtained from Sigma (St. Louis, Mo., USA). Its concentration was determined spectrophotometrically. ADP was obtained from Polskie Odczynniki Chemiczne (Gliwice, Poland) and [8-^{14}C] ADP from the Radiochemical Centre (Amersham, England). Lubrol WX was purchased by I.C.I. Organics (Providence, R.I., USA), sodium deoxycholate by Polfa (Jelenia Gora, Poland) and cetyltrimethylammonium bromide by Koch-Light (Colnbrook, England). Carboxyatractyloside (Gummiferin) was a generous gift of Boehringer (Mannheim, GFR).

3. Results

The study on detergents as inhibitors of adenine nucleotide transport in mitochondria is complicated by the fact that in the 'forward' exchange system the lytic action on mitochondrial membrane may be misinterpreted as inhibition of the transport. Therefore, to compare the effect of several commercial detergents with that of palmitoyl-CoA the 'back-exchange' system was used. In this system the inhibitory effect is manifested by a decrease of the efflux of the label from mitochondria to the medium whereas the lytic effect increases the efflux. It was found (table 1) that sodium dodecyl sulphate, deoxycholate and Lubrol WX had a much lower inhibitory effect on the translocation than palmitoyl-CoA, in

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Table 1
Effect of detergents on adenine nucleotide translocation in mitochondria from rat liver

Additions	Inhibition (%)
None	0
Palmitoyl-CoA, 3 nmoles/mg prot.	61
Sodium dodecylsulphate, 75 nmoles/mg prot.	37
Deoxycholate, 45 nmoles/mg prot.	23
Lubrol WX, 11 μg/mg prot.	34

Mitochondria were loaded with [^{14}C] ADP in the medium containing 250 mM sucrose, 10 mM Tris–HCl, 1 mM EDTA, pH 7.4, for 40 min at 0°C–4°C. After centrifugation portions of loaded mitochondria, containing 6.6 mg protein, were incubated in 2.2 ml of the following medium: 110 mM KCl. 10 mM sucrose, 9 mM Tris–HCl, 1 mM EGTA, 2 mM P_i and 5 mM 2-oxoglutarate, pH 7.4, for 10 min at 20°C and than cooled down to 0°C. The translocation was started by addition of unlabelled ADP to final concentration of 70 μM and stopped after 20 sec by addition of carboxyatractyloside to final concentration of 5 μM.

spite of the fact that they were used at higher concentrations. As tested separately, the concentrations of detergents and palmitoyl-CoA, as shown in table 1, had no or very little lytic action in the sense that they

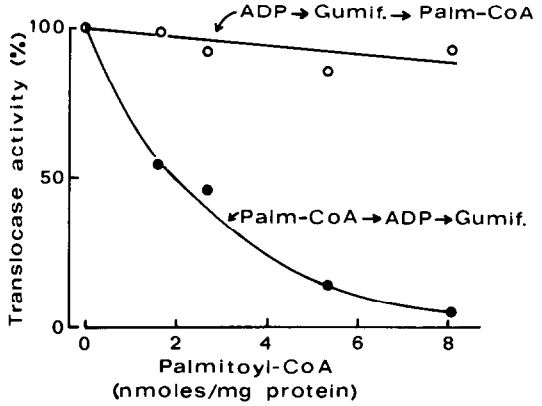

Fig. 1. Effect of palmitoyl-CoA on adenine nucleotide translocation in rat liver mitochondria. The incubation mixture contained 110 mM KCl, 10 mM sucrose, 1 mM EDTA, 9 mM Tris–HCl, pH 7.4 and 5.5 mg mitochondrial protein in total volume of 2.1 ml. (•–•–•) Palmitoyl-CoA was added first, 2 min later followed by [^{14}C] ADP (final concentration 70 μM), and the translocation was stopped by carboxyatractyloside (Gumif) at final concentration 5 μM added after 20 sec. The temperature was 0°C. (○–○–○) Palmitoyl-CoA was added immediately after corboxyatractyloside (Gumif.). (Gumif.).

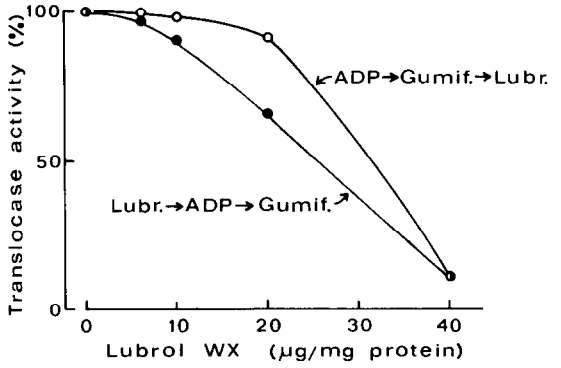

Fig. 2. Effect of Lubrol WX on adenine nucleotide translocation. Incubation medium and conditions as in fig. 1 except that the samples contained 5.1 mg mitochondrial protein. (•–•–•) Lubrol WX (Lubr.) was added first, followed by [^{14}C] ADP; carbocyatractyloside (Gumif.) was added after 20 sec; (○–○–○) Lubrol WX was added immediately after carboxyatractyloside.

did not affect the efflux of [^{14}C] nucleotides when added after carboxyatractyloside (see also figs. 1 and 2).

The effect of palmitoyl-CoA on the translocation of ADP, as measured in the 'forward' exchange system, is shown in fig. 1. Two parallel sets of experimental samples were run. To one of them palmitoyl-CoA was added first, followed by [^{14}C] ADP and the reaction was stopped by carboxyatractyloside 20 sec after addition of [^{14}C] ADP. To the other set of samples (control run) palmitoyl-CoA was added imediately *after* the exchange had been inhibited by carboxyatractyloside. The decrease in the radioactivity in these samples should therefore indicate the lytic action of palmitoyl-CoA. As can be seen in fig. 1, 50% inhibition was obtained with 2 nmoles palmitoyl-CoA/mg mitochondrial protein and an almost complete inhibition occurred at 8 nmoles/mg protein. Practically no lytic effect could be observed up to 8 nmoles palmitoyl-CoA/mg protein.

In contrast to this, Lubrol WX, sodium dodecyl sulphate and sodium deoxycholate were much less inhibitory in sublytic concentrations. This is exemplified for Lubrol WX by fig. 2. At higher concentrations an increase of the carboxyatractyloside-insensitive permeability to adenine nucleotides made the study of the inhibition impossible. Nevertheless, using low (sublytic) concentrations of sodium dodecyl sulphate it could be shown that the inhibition was

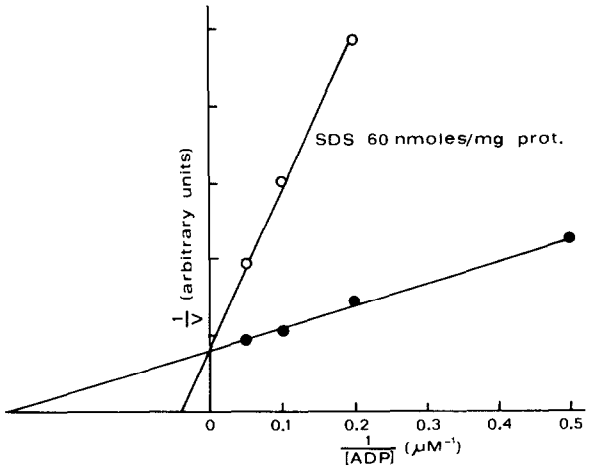

Fig. 3. The Lineweaver–Burk plot of ADP translocation and its inhibition by sodium dodecyl sulphate. The incubation medium contained 250 mM sucrose, 1 mM EDTA, 10 mM Tris–HCl, pH 7.4 and 4.3 mg mitochondrial protein in total volume of 3.0 ml. The temperature was 0°C. The translocation was started by addition of [^{14}C] ADP at different concentrations and stopped after 20 sec by addition of carboxyatractyloside to final concentration 5 μM. The samples with sodium dodecyl sulphate (SDS) were preincubated with the detergent for 2 min before addition of [^{14}C] ADP. The following constants are calculated from the plot: K_m for ADP, 3.7 μM; K_i for sodium dodecyl sulphate, 18.3 μM.

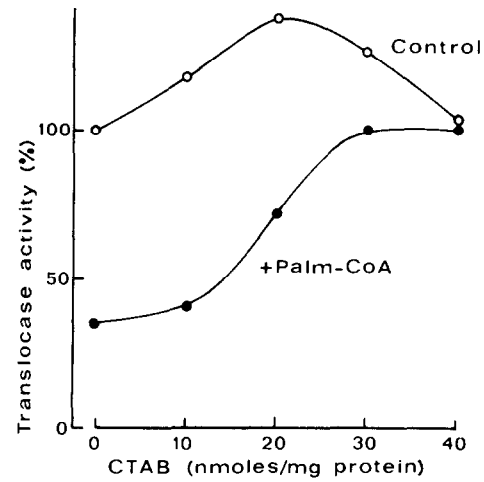

Fig. 5. Reversal by cetyltrimethylammonium bromide of the inhibitory effect of palmitoyl-CoA on adenine nucleotide translocation. The medium and conditions as in fig. 1.; 5.4 mg mitochondrial protein in the total volume of 2.1 ml. (o–o–o) Mitochondria were treated with cetyltrimethylammonium bromide (CTAB) followed by [^{14}C] ADP and carboxyatractyloside as in fig. 4.; (•–•–•) mitochondria were first preincubated for 2 min with palmitoyl-CoA, 2 nmoles/mg mitochondrial protein, before treatment with cetyltrimethylammonium bromide.

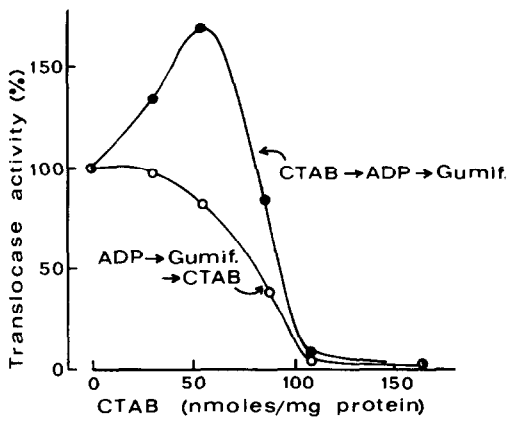

Fig. 4. Effect of cetyltrimethylammonium bromide on adenine nucleotide translocation. Incubation conditions as in fig. 1.; 4.7 mg mitochondrial protein in the total volume of 2.2 ml. (•–•–•) Cetyltrimethylammonium bromide (CTAB) was added first, followed by [^{14}C] ADP and carboxyatractyloside (Gumif.) was added after 20 sec; (o–o–o) cetyltrimethylammonium bromide was added immediately after carboxyatractyloside.

competitive with respect to ADP (fig. 3), similarly as is the inhibition produced by medium and long chain acyl-CoA [14]. The inhibition constant (K_i) for dodecyl sulphate was 18.4 μM.

The detergentexamined so far were anionic (dodecyl sulphate and deoxycholate) or non-ionic (Lubrol WX). A quite different effect was obtained with the cationic detergent cetyltrimethylammonium bromide. It was found (fig. 4) that sublytic lytic concentrations of this substance increased sublytic or slightly lytic concentrations of this substance increased sometime it amounted to more than 50% at optimum detergent concentration (e.g. in fig. 4), but occasionally it could not be observed at all. Because in some of our mitochondrial preparations the exchange was also stimulated by serum albumin, known to bind fatty acids and acyl-CoA, it could be supposed that the stimulatory effect of cetyltrimethylammonium ion was by protecting against an endogenous inhibitor, presumably medium and long chain acyl-CoA. This was verified by an experiment in

which cetyltrimethylammonium was added to mitochondria pretreated with palmitoyl-CoA. It was found (fig. 5) that the inhibitory effect of palmitoyl-CoA was fully overcome by the cationic detergent. The amount of cetyltrimethylammonium bromide needed to fully abolish the inhibition by palmitoyl-CoA was 15 times higher than that of the inhibitor.

4. Discussion

The inhibition by medium and long chain acyl-CoA of adenine nucleotide translocase is competitive with respect to ADP and depends on the chain length of the fatty acid moiety [14]. It can be speculated that the adenosine-phosphate end of acyl-CoA molecule, due to its structural similarity to ADP, binds to the adenine nucleotide-binding site of the translocase and that this binding is facilitated, and the local concentration of the inhibitor increased, by the binding of the hydrocarbon chain of the fatty acid moiety (non-polar end of acyl-CoA molecule) to mitochondrial membrane. A similar explanation may hold for the inhibitory action of certain anionic and non-ionic detergents, the non-polar part of the detergent attaching the molecule to the membrane and the polar part competing with adenine nucleotide for the binding site(s) of the translocase. However, due to a poor structural similarity to ADP of the polar groups of the detergents studied, the inhibition is much lower than by palmitoyl-CoA.

Recently, Meisner [15] has found that the lipophilic anion, tetraphenylboron, inhibits the translocation of ADP and ATP. He postulates that the binding of this anion to mitochondrial membrane increases the negative charge of this membrane and therefore decrease the association of the nucleotides and other anionic metabolites to be translocated. On the other hand, Mg^{2+} can neutralize inhibition produced by tetraphenylboron. A similar mechanism may be postulated for the protective effect of the cationic detergent, cetyltrimethylammonium, against the inhibition produced by palmitoyl-CoA as observed in the present investigation.

It can be thus concluded that anionic detergents inhibit the translocation of adenine nucleotides by increasing a negative surface potential of the inner mitochondrial membrane. Polar groups of certain anionic and non-ionic detergents may also compete for the adenine nucleotide-binding site of the translocase, thus increasing the potency of the inhibition. The inhibition produced by CoA thioesters of medium and long chain faty acids is therefore much stronger and more 'specific' than that of other detergents because of a great similarity of the polar part of acyl-CoA to ADP. It can also be speculated that the translocase is situated at the water–lipid interphase of the membrane in such a way that the distance between the ADP-binding site of the translocase and the lipophilic region of the membrane, or of the translocase itself, corresponds to the distance between polar and non-polar ends of medium and long chain acyl-CoA and of certain detergents.

The inhibition by certain detergents of adenine nucleotide translocation in mitochondrial membrane may explain, at least partly, the lowering of P:O ratio and inhibition of $^{32}P_i$–ATP exchange as observed by Pope et al. [16] and Kagawa [17].

Acknowledgement

Technical assistance of Mrs. Barbara Burcan is gratefully acknowledged.

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