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DIPHOSPHATIDYLGLYCEROL-INDUCED CHANGES IN THE ORGANIZATION OF MITOCHONDRIAL ATPase

A. BRUNI and E. BIGON

Institute of Pharmacology, University of Padova, Largo E. Meneghetti 2, 35100 Padova (Italy)

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SUMMARY

1. On submitochondrial particles from bovine heart, diphosphatidylglycerol produced a selective solubilization of ATPase. The solubilized enzyme was purified further by ammonium sulfate fractionation and shown to have the same reconstitutive activity as coupling factor F_1 (Pullman, M.E., Penefsky, H. S., Datta, A. and Racker, E. (1960) J. Biol. Chem. 235, 3322–3329).

2. Diphosphatidylglycerol-treated submitochondrial particles retained large amounts of the phospholipid and showed a decreased ATPase activity. Once the excess of phospholipid was removed, soluble ATPase could be again reincorporated in an oligomycin-sensitive complex.

3. On Mg–ATP particles the solubilization of ATPase induced by diphosphatidylglycerol was preceded by a stimulation of oligomycin-sensitive ATPase which indicated a dissociation of F_1 from the ATPase inhibitor (Pullman, M. E. and Monroy, G. C. (1963) J. Biol. Chem. 238, 3762–3769). Magnesium was required to obtain the oligomycin-sensitive stimulation whereas in the absence of magnesium the solubilization of ATPase was prevalent.

4. It is concluded that the decreased association of F_1 with the ATPase inhibitor produced by diphosphatidylglycerol, causes a labilization of ATPase–membrane interaction. Under particular conditions, e.g. a high amount of phospholipid and a low concentration of magnesium, this is followed by the detachment of ATPase.

INTRODUCTION

Diphosphatidylglycerol (cardiolipin) is a component of mitochondrial inner membrane comprising approximately 20 % of total phospholipids [1]. When added to phospholipid-depleted mitochondrial particulate ATPase, this phospholipid produced an oligomycin-sensitive stimulation [2, 3, 4]. More recently [5, 6], it was found that diphosphatidylglycerol produced a selective solubilization of mitochondrial ATPase and was very effective in preventing the inhibition of ATPase activity induced by the ATPase inhibitor [7]. These findings prompted an investigation to

study whether the solubilization of ATPase and the antagonism toward the ATPase inhibitor are related events.

MATERIALS AND METHODS

Bovine heart mitochondria [8], submitochondrial particles prepared in the presence of pyrophosphate [9], ammonia plus Sephadex filtration (AS particles) [10], and Mg–ATP particles [11] were obtained according to published procedures. The Mg–ATP particles were resuspended in a magnesium-free medium containing 0.25 M sucrose, 10 mM Tris–HCl, 1 mM EDTA (pH 7.4). Soluble mitochondrial ATPase (F_1) was obtained as described by Horstman and Racker [12] (spec. act. 112 μmoles ATP split/mg protein per min at 37 °C). A unit of ATPase activity was considered as the amount catalyzing the hydrolysis of 1 μmole of ATP per minute under the specified conditions.

Commercial samples of diphosphatidylglycerol from bovine heart prepared by the method of Pangborn [13] (General Biochemicals, lot: 42216, Sylvana Chemical or Sigma) were easily dispersed at neutral pH by ultrasonic oscillations and gave reproducible results. Diphosphatidylglycerol isolated by the method of Courtade and McKibbin [14] (General Biochemicals, lot: 45276), was dispersible only to a very limited extent and could not be used. Diphosphatidylglycerol gave only one spot on thin-layer chromatography [15] and was used after a 5–10-min sonication (Biosonik III apparatus) at 0 °C under a stream of pure nitrogen in 0.25 M sucrose, 10 mM Tris–HCl, 1 mM EDTA (pH 7.4) followed by centrifugation. The concentration was calculated on the basis of a phosphorus content of 4 % (cf. [14]).

Solubilization of mitochondrial ATPase and further purification

2.5 mg diphosphatidylglycerol were added to 4.0 ml of a mixture containing 0.25 M sucrose, 10 mM Tris–HCl, 1 mM EDTA, 4 mM ATP and 5 mg submitochondrial particles. The final pH was 7.4 at 37 °C. After 10 min at 37 °C, the sample was centrifuged at 20 °C for 30 min at 50 000 rev./min (Spinco No. 50 rotor) and the clear, colorless supernatant collected by inversion. As shown previously [5] this supernatant contained 8–13 % of the protein of submitochondrial particles and a high oligomycin-insensitive ATPase activity. The effectiveness of the phospholipid did not change when the concentration of protein was varied from 1.0–5.0 mg/ml. The visible spectra of a supernatant containing 0.3 mg protein/ml did not show bands of cytochromes. To 4.0 ml of supernatant 0.7 ml of saturated solution of ammonium sulfate were added dropwise under stirring at room temperature and the sample was centrifuged at 20 °C. The barely visible sediment was discarded and the supernatant was brought to 50 % saturation by the addition of 3.3 ml of a saturated solution of ammonium sulfate. After 10 min at 0 °C the sample was centrifuged in the cold and the sediment dissolved in 0.5 ml of 0.25 M sucrose, 1 mM EDTA, 10 mM Tris–HCl, containing 4 mM ATP (pH 7.4) warmed at room temperature [12]. Some insoluble material which aggregated at the high salt concentration appeared at this stage and was removed by centrifugation at 20 °C. With pyrophosphate–submitochondrial particles approximately 40 % of the units added with particles were recovered in the final solution. In terms of protein content the yield was 1.5–3.0 %. The specific activity was in most experiments between 40 and 60 μmoles ATP split/mg

protein per min at 37 °C. In a few cases, higher figures (75–85) were observed. This variability can be accounted for by the presence in the preparation of the ATPase inhibitor which was not removed by a heat-treatment step [16, 12].

Analytical procedure

Polyacrylamide-gel electrophoresis was performed with 10 % polyacrylamide [17]. Phosphate [18] and protein [19] were determined according to the standard procedures, and phospholipid phosphorus after dry-ashing [20] or wet-ashing [15]. The submitochondrial particles were extracted with 10 % trichloroacetic acid before the determination of phosphorus. Analysis of the individual phospholipids was performed as described [3]. ATPase activity was tested in 1.0 ml volume containing: 50 mM sucrose, 0.2 mM EDTA, 50 mM Tris–HCl, 2.5 mM $MgCl_2$, 3 mM ATP, 30 μg pyruvate kinase (320 units/mg), 2 mM phosphoenolpyruvate (final pH 7.4). 0.3 mg bovine serum albumin was added with soluble ATPase. The incubation time was 10 min at 37 °C. The reaction was initiated by the addition of ATP and $MgCl_2$ and stopped with 0.25 ml of 50 % trichloroacetic acid. Variations from this standard procedure are indicated in the legend to Tables II and IV.

RESULTS

Solubilization of ATPase

Fig. 1 shows the different effects of diphosphatidylglycerol and lysophosphatidylcholine, a monoacylphospholipid with marked detergent activity. After the

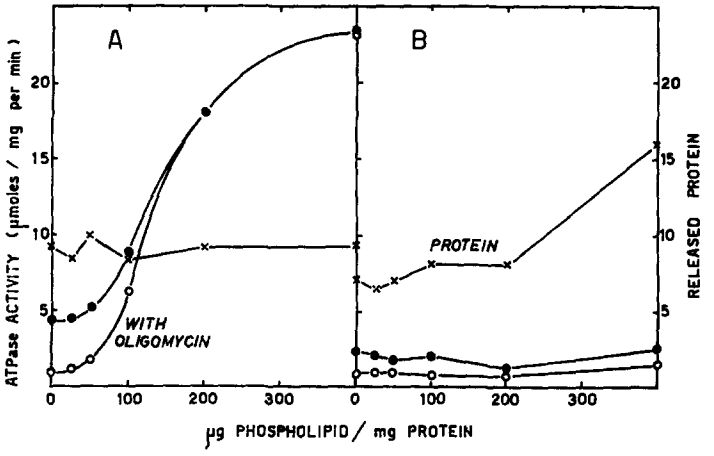

Fig. 1. Release of ATPase in the supernatant after treatment with diphosphatidylglycerol or lysophosphatidyl-choline. 0.6 mg pyrophosphate-submitochondrial particles were incubated for 10 min at 37 °C with the indicated amount of diphosphatidylglycerol or lysophosphatidylcholine in 0.5 ml of 0.25 M sucrose, 1 mM EDTA, 10 mM Tris–HCl, 4 mM ATP (pH 7.4). After a 30-min centrifugation at 50 000 rev./min (20 °C), the protein content and the ATPase activity were determined in the supernatant. The conditions for ATPase were as described in Materials and Methods with 6–7 μg protein of supernatant. When present, (○–○) oligomycin was added at 140 μg/mg protein (1 μg/tube). (×–×) values for released protein (percentage of submitochondrial particles). A, diphosphatidylglycerol. B, egg lysophosphatidylcholine.

incubation of the particles at 37 °C followed by centrifugation a small amount of membrane fragments with oligomycin-sensitive ATPase was recovered in the supernatant. When the incubation medium was supplemented with diphosphatidylglycerol (Fig. 1, A), the release of ATPase increased the activity found in the supernatant and lowered the oligomycin sensitivity. Only a slight increase in the amount of released protein accompanied the solubilization of ATPase. The complete effect was reached at 0.3–0.4 mg/mg protein. Lysophosphatidylcholine (Fig. 1, B) had no effect up to a concentration of 0.2 mg/mg protein. At 0.4 mg/mg protein the concentration of phospholipid in the medium reached 1 mM and was above the critical micellar concentration. "Solubilization" of the particles ensued with no great changes in the specific activity.

Although lysophosphatidylcholine produced a decrease in the oligomycin sensitivity, the released material was always partially inhibited. The effect of phospholipid in the two cases reflected different events: solubilization of ATPase with diphosphatidylglycerol and fragmentation of the particles with lysophosphatidylcholine.

Table I shows that the effect of diphosphatidylglycerol was present in submitochondrial particles prepared by different procedures and with different functional properties. However, differences in the specific activity and in the amount of released protein were observed. Mg–ATP particles which were obtained by the most conservative procedure and had a high content of ATPase inhibitor [11, 21], yielded a soluble ATPase with generally lower specific activity. This indicated either a lower proportion of ATPase to the total released protein or the detachment of an ATPase retaining some regulating association of bound enzyme or both. With these particles the ratio between the added and released units indicated a great effectiveness of diphosphatidylglycerol. Ammonia–Sephadex particles which on the contrary are highly resolved with respect to coupling factors and ATPase inhibitor [10, 22], gave the highest value of specific activity but a low recovery of released units. It is possible that two reasons contributed to the different effectiveness of diphosphatidylglycerol

TABLE I

EFFECT OF DIPHOSPHATIDYLGLYCEROL ON DIFFERENT SUBMITOCHONDRIAL PARTICLES FROM BOVINE HEART

0.5 mg submitochondrial particles were treated with 0.5 mg diphosphatidylglycerol/mg protein as described in Fig. 1. After centrifugation the protein content and ATPase activity were determined in the supernatant. ATPase activity is expressed as μmoles ATP split/mg protein per min. The specific activity of the particles was: 4.2 pyrophosphate particles, 1.6 Mg–ATP particles, 6.1 ammonia–Sephadex particles. The total units added were 2.1, 0.8, 3.0, respectively.

	Pyrophosphate particles	Mg–ATP particles	Ammonia–Sephadex particles
ATPase activity of solubilized protein	14.8	9.6	19.9
Protein released (μg)	60.0	47.5	35.0
Total units released	0.9	0.45	0.7
% of added units	42.8	56.2	23.4

in the two particles. In the case of Mg–ATP particles the solubilization of ATPase was probably accompanied by an increase in the specific activity due to a decreased influence of association with the ATPase inhibitor. This apparently resulted in a higher recovery of solubilized units. The consequences of solubilization are expected to be less relevant in ammonia–Sephadex particles considering the low content of ATPase inhibitor. In addition, the decreased availability of detachable ATPase in the ammonia–Sephadex particles which are known to be partially resolved with respect to F_1 [22], could be responsible for the reduced effectiveness of diphosphatidylglycerol in this case.

That a definite conformation or some associated component can be shown to influence the specific activity of freshly solubilized ATPase is illustrated by the experiment of Table II. Purified ATPase from Mg–ATP particles was incubated in a low chloride medium immediately after preparation and after 24 h storage at room temperature. A substantial increase in the specific activity was detected which was prevented by the addition of a low amount of magnesium. A higher concentration of cation inactivated the enzyme. Occasional activation during storage was also noticed with F_1 prepared by conventional procedures [23].

The ammonium sulfate fractionation of supernatant obtained in the presence of diphosphatidylglycerol yielded a cold-labile, soluble ATPase similar in all respects to coupling factor F_1. The protein profiles of crude supernatant and more purified fraction obtained with pyrophosphate particles are reported in Fig. 2.

It is noteworthy that the first supernatant already contained F_1 as the major component and the band corresponding to subunit 5 was clearly evident. This component is believed [24] to be identical with the ATPase inhibitor. The reconstitutive property of purified ATPase was tested on Sephadex–urea particles [10], Sephadex–urea particles extracted with ammonia [25] and ammonia particles [22] where it behaved like F_1 in restoring the oligomycin-sensitive ATPase and the reduction of NAD by succinate in the presence of ATP. The solubilization of ATPase by diphosphatidylglycerol constituted a simple way to obtain and purify reconstitutively active enzyme from a small amount of submitochondrial particles in a significant and reproducible yield.

TABLE II

EFFECT OF STORAGE ON THE ACTIVITY OF SOLUBILIZED ATPase

The ATPase was solubilized by diphosphatidylglycerol from 5 mg Mg–ATP particles and purified by $(NH_4)_2SO_4$ fractionation as described under Materials and Methods. EDTA was omitted in all solutions and Tris–2[*N*-morpholino]ethane sulfonic acid (MES) was used instead of Tris–HCl. After preparation the ATPase (0.2 mg/ml) was stored at 20 °C in 0.25 M sucrose, 10 mM Tris–MES, 4 mM ATP (pH 7.4) with and without $MgCl_2$. Conditions for ATPase activity, 170 mM sucrose, 7 mM Tris–MES (pH 7.4), 0.3 mg bovine serum albumin, 2.7 mM phosphoenolpyruvate, 10 μg pyruvate kinase, 4 mM ATP (pH 7.4), 4 mM $MgCl_2$, 1.1 μg solubilized ATPase. Final volume, 0.3 ml. Incubation, 10 min at 37 °C. The reaction was started with the enzyme and stopped with 0.1 ml 50 % cold trichloroacetic acid.

Determination	ATPase activity (μmoles/mg per min)	
	Without $MgCl_2$	With 0.2 mM $MgCl_2$
After preparation	17.2	12.9
After 24-h storage at 20 °C	40.1	15.8

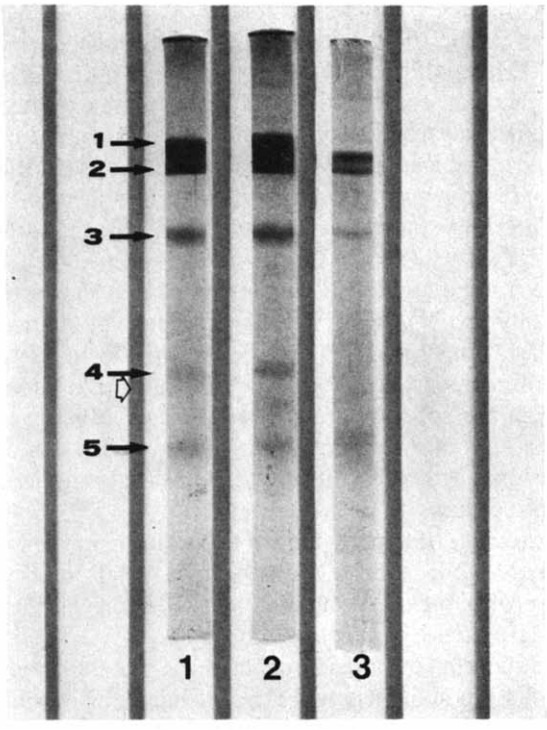

Fig. 2. Polyacrylamide gel electrophoresis of the ATPase solubilized by diphosphatidylglycerol. 1, 30 μg F_1 prepared according to Horstman and Racker [12]. 2, 28 μg F_1 extracted by diphosphatidylglycerol at the stage of ammonium sulfate fractionation. 3, 19 μg protein of supernatant after treatment of the particles with diphosphatidylglycerol. The electrophoresis was carried out at 22 °C in 10 % acrylamide gels, containing 0.1 % sodium dodecylsulfate [17]. The white arrow indicates the position of cytochrome *c*.

Diphosphatidylglycerol-treated particles

In Table III it can be seen that the incubation of pyrophosphate submitochondrial particles with diphosphatidylglycerol resulted in the extensive uptake of the phospholipid. In submitochondrial particles from rat liver, diphosphatidylglycerol was ineffective in releasing F_1 [5], yet the phospholipid was bound to the same extent as in the particles from bovine heart.

When the particles treated with diphosphatidylglycerol were washed at room temperature, a significant ATPase activity was retained (from 4.0 to about 2.0 μ-moles/mg protein per min after treatment with 0.25 mg phospholipid/mg protein). A large part of the activity was oligomycin-insensitive and cold-labile, indicating that some amount of ATPase, although dislocated, was not released in the medium. Washing the diphosphatidylglycerol-treated particles in the cold, low ATPase activity could be recovered (Table IV). After the addition of soluble ATPase only oligomycin-insensitive ATPase was restored. When the excess of phospholipid was partially removed with cholate, the oligomycin-sensitive ATPase activity of the particles increased, indicating that some bound enzyme was inhibited by the large amount of phospholipid present. In this case the addition of soluble enzyme was followed by

TABLE III

BINDING OF DIPHOSPHATIDYLGLYCEROL TO SUBMITOCHONDRIAL PARTICLES

0.5–1 mg pyrophosphate–submitochondrial particles were incubated with the indicated amount of diphosphatidylglycerol in the conditions used to solubilize F_1. After centrifugation and washing, the phospholipid content was estimated in the sediments as outlined under Materials and Methods. In the experiment No. 2 submitochondrial particles from rat liver were used instead of particles from bovine heart.

Expt No.	Diphosphatidylglycerol added (mg/mg protein)	Phospholipid content (mg/mg protein)
1	none	0.660
	0.125	0.745
	0.250	0.950
	0.500	1.260
2	none	0.680
	0.500	1.220

TABLE IV

RECONSTITUTION OF OLIGOMYCIN-SENSITIVE ATPase IN PARTICLES TREATED WITH DIPHOSPHATIDYLGLYCEROL

Pyrophosphate–submitochondrial particles were incubated (see Materials and Methods) with diphosphatidylglycerol and washed twice in the cold. Extraction with cholate (1 mg/mg protein) was performed with 10 mg particles in 2.0 ml of 1.5 mM dithiothreitol, 1 M KCl, 0.25 M sucrose, 10 mM Tris–HCl, 1 mM EDTA (final pH 8.0). After 1 h at 0 °C, the sample was centrifuged at 40 000 rev./min, 20 min at 0 °C and the sediment, washed twice, resuspended in 0.25 M sucrose, 10 mM Tris–HCl, 1 mM EDTA (pH 7.4). Reconstitution with the ATPase solubilized by diphosphatidylglycerol and purified by ammonium sulfate fractionation was performed with 0.6–0.9 mg particles in 0.3 ml of 0.25 M sucrose, 10 mM Tris–HCl, 1 mM EDTA medium containing 2.5 mM ATP. After 10 min at 20 °C the samples were centrifuged at 0 °C at 40 000 rev./min and the sediments resuspended in 0.3 ml of 0.25 M sucrose, 10 mM Tris–HCl, 1 mM EDTA pH 7.4. ATPase activity was measured as described under Materials and Methods with 70–100 μg protein. 10 μg oligomycin/mg protein. The phospholipid content of the original particles was 0.58 mg/mg protein. In parenthesis is the percentage inhibition induced by oligomycin.

	Addition of oligomycin	Particles treated with diphosphatidylglycerol (0.5 mg/mg protein)	Particles treated with diphosphatidylglycerol and extracted with cholate
Total phospholipid content (mg/mg protein)	–	1.15	0.75
Diphosphatidylglycerol content (mg/mg protein)	–	0.58	0.37
Residual ATPase activity (μmoles/mg per min)	–	0.26	0.68
	+	0.17 (35)	0.12 (82)
ATPase activity after reconstitution with 62 μg solubilized ATPase/mg protein	–	1.06	1.62
	+	0.89 (16)	0.65 (60)

the reconstitution of the oligomycin-sensitive ATPase, showing that the oligomycin-sensitivity conferring protein [25], although unoperative, was retained after treatment with diphosphatidylglycerol. It was of interest to note that the large amount of bound phospholipid protected the particles from the effect of cholate. Only 27% of protein were solubilized in comparison to 53% in untreated particles. A high concentration of salts or urea were ineffective in removing the bound diphosphatidylglycerol.

Effect on Mg–ATP particles

Previous observations [6] have shown that preincubation of the Mg–ATP particles with ATP and magnesium resulted in a decrease of the ATPase activity due to an induced association of ATPase with the endogenous ATPase inhibitor. Extending this observation it was found that a simpler and reproducible system to show this effect was the direct measurement of ATPase activity in a medium of small volume and low chloride anion content (Table V). The activity of Mg–ATP particles dropped from 1.5–2.0 μmoles ATP split/mg protein per min to 0.5–0.9 whereas that of the ammonia-sephadex particles remained unchanged, confirming the difference between these two preparations when the presence of endogenous inhibitor should become manifest. Under these conditions, the addition of diphosphatidylglycerol or phosphatidylserine stimulated the oligomycin-sensitive ATPase activity. As it was shown previously in the case of phosphatidylserine [6], this stimulation was likely due to the release of inhibition induced by the endogenous ATPase inhibitor. Chloride anions, added as sodium chloride, were less effective than phospholipids but their effect was improved when added as Tris–chloride. It was of interest to note that when diphosphatidylglycerol was added together with salts, the effect of phospholipid decreased or

TABLE V

STIMULATION OF ATPase ACTIVITY IN Mg–ATP PARTICLES

50 μg of Mg–ATP particles were incubated for 10 min at 37 °C in 0.3 ml of a medium containing 165 mM sucrose, 0.65 mM EDTA, 6.5 mM Tris–HCl (pH 7.4), 0.3 mg bovine serum albumin, 2.7 mM phosphoenolpyruvate, 10 μg pyruvate kinase, 4 mM $MgCl_2$, 4 mM ATP (pH 7.4) and the additions indicated. The reaction was initiated by the addition of Mg–ATP particles and terminated with 0.1 ml cold 50% trichloroacetic acid. Oligomycin, 1 μg/tube (25 μg/mg particles). Egg phosphatidylcholine, bovine brain phosphatidylserine.

Additions	ATPase activity (μmoles/mg per min)	
	Without oligomycin	With oligomycin
none	0.90	0.03
20 μg phosphatidylcholine	0.97	0.08
20 μg phosphatidylserine	1.39	0.06
20 μg diphosphatidylglycerol	1.77	0.19
none	0.89	0.04
20 μg diphosphatidylglycerol	1.85	0.44
50 mM NaCl	1.25	0.04
50 mM Tris–HCl (pH 7.4)	1.45	0.04
diphosphatidylglycerol plus NaCl	1.69	0.08
diphosphatidylglycerol plus Tris–HCl	1.41	0.08

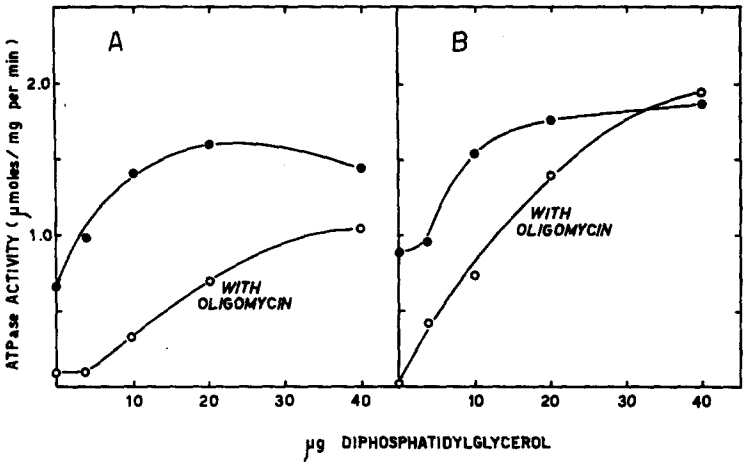

Fig. 3. Effect of diphosphatidylglycerol on Mg–ATP particles. 40 μg Mg–ATP submitochondrial particles were incubated as described in Table V, bovine serum albumin was omitted. A, the ATPase reaction was started by the addition of particles. B, the ATPase reaction was started by the addition of magnesium after a preincubation of 10 min at 37 °C. In order to verify that the loss of oligomycin sensitivity was due to the detachment of ATPase, samples containing 1.5 ml of the same medium, 200 μg of particles and corresponding amounts of diphosphatidylglycerol, were incubated at 37 °C for the same period of time and quickly centrifuged at 20 °C. The oligomycin-insensitive ATPase activity was measured in the supernatant.

disappeared probably through the neutralization of negative charges by the cation component. Since a high concentration of sodium chloride also inhibited the diphosphatidylglycerol-induced solubilization of ATPase [5], this was taken as an indication of a requirement for the negative charge in both effects. The inhibition of solubilization of ATPase by salts was manifest also in the experiment of Table V as a decrease of oligomycin-insensitive activity in the presence of phospholipid. The stimulation by anions of ATPase activity in Mg–ATP particles has been previously reported [26, 27].

In Fig. 3, A it can be seen that the stimulation of oligomycin-sensitive ATPase induced by diphosphatidylglycerol on Mg–ATP particles, clearly preceded the loss of oligomycin sensitivity due to the solubilization of ATPase. The determination of ATPase activity in the supernatant after centrifugation of the samples, confirmed that the detachment of ATPase paralleled the loss of the oligomycin sensitivity. However, when the particles were preincubated with diphosphatidylglycerol in the absence of magnesium, the stimulation of activity immediately corresponded to the solubilization of ATPase (Fig. 3, B) revealing that for the stimulation of oligomycin-sensitive ATPase magnesium was required, whereas in the absence of this cation the ATPase was easily solubilized. It should be added that the diphosphatidylglycerol-induced stimulation of ATPase activity was still evident when the concentration of magnesium was higher than that of ATP but declined at a large excess of cation (6.0 mM).

DISCUSSION

Two effects were evident upon the addition of diphosphatidylglycerol to submitochondrial particles: (a) release of inhibition induced by the ATPase inhibitor on membrane-bound ATPase and (b) solubilization of ATPase.

As suggested [6], the first effect might have physiological significance considering that diphosphatidylglycerol is a normal constituent of mitochondrial particulate ATPase. A close association of this phospholipid with mitochondrial ATPase was also recently proposed [28]. The solubilization of ATPase by diphosphatidylglycerol was remarkably selective as indicated by the absence of the fragmentation of particles and the results of polyacrylamide gel electrophoresis.

Mg–ATP particles [11, 21] prepared by a procedure which preserves some functional property of intact mitochondria (low ATPase activity, uncoupler-stimulated respiration) were more suitable for studying the effects of diphosphatidylglycerol. Only in this case it was possible to observe the effect of phospholipid on the association of F_1 with the ATPase inhibitor and to show that it preceded the solubilization of ATPase. This observation indicated that the initial specific effect of diphosphatidylglycerol required a membrane organization in which the normal relationship between the ATPase and the other components of the oligomycin-sensitive complex were preserved. On the contrary, the detachment of ATPase was produced in all particles. The change in the specific activity of solubilized enzyme observed upon storage and prevented by the addition of magnesium, suggested a slow rearrangement of subunit association in the ATPase complex after isolation. The rapid isolation procedure allowed by diphosphatidylglycerol may help to disclose undetected properties of the enzyme immediately after solubilization.

The influence of magnesium provided the basis for a possible explanation of the diphosphatidylglycerol effect. Magnesium is known to promote the binding of ATPase to membrane [29] and the association of F_1 with the ATPase inhibitor [12], whereas diphosphatidylglycerol showed the opposite effects. The interaction of diphosphatidylglycerol with magnesium at a level of particulate ATPase appears to be a likely event justifying the dissociation of the ATPase inhibitor and the destabilization of ATPase-membrane interaction. The detachment of ATPase would follow when more negatively charged liposomes are taken up by the particles. In this final effect the electrostatic repulsion on the negatively charged [25] enzyme may play a role. This is consistent with the observation that externally added cations inhibited the solubilization of ATPase [5]. The importance of magnesium in the association of several ATPase with membranes was assessed in a number of studies on mitochondrial, chloroplast and bacterial enzyme [29–34]. The ability of diphosphatidylglycerol to form a complex with magnesium is also well established [35].

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