SOLUBILIZATION OF MITOCHONDRIAL ATPase BY PHOSPHOLIPIDS

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Summary. Brief incubation of submitochondrial particles from bovine-heart with the acidic phospholipid, diphosphatidylglycerol (cardiolipin), resulted in the solubilization of mitochondrial ATPase (F_1). Little or no effect was evident with submitochondrial particles from rat-liver. The solubilized ATPase was oligomycin insensitive, cold labile and showed the same electrophoretic pattern as pure F_1. The specific activity and the polyacrylamide gel electrophoresis indicated that mitochondrial ATPase is extracted in a considerable degree of purity. Other phospholipids partially reproduced the effect of diphosphatidylglycerol whereas some anionic or non ionic detergents were ineffective under the same conditions.

It has been recently shown (1) that acidic phospholipids are more effective than neutral phospholipids in the reconstitution of oligomycin-sensitive ATPase from rat-liver mitochondria. The high reactivity of these phospholipids toward the protein components of the energy-transfer system is further indicated by the present experiments which show that acidic phospholipids, particularly diphosphatidylglycerol, are able to extract mitochondrial ATPase from bovine-heart submitochondrial particles. The observation provides a simple method to obtain purified F_1 from small amounts of particles and may be useful for studies on the structural organization of the ATP generating system.

Materials and Methods. Submitochondrial particles (SMP) from rat-liver or bovine-heart mitochondria were prepared as described by Kielley and Bronk (2) or Racker (3). Soluble mitochondrial ATPase (F_1) was extracted and purified according to Horstman and Racker (4). Bovine-brain or egg "ultrapure" phospholipids were purchased from General Biochemical. They were dispersed by ultrasonic oscillations in 0.25 M sucrose, 1 mM EDTA, 10 mM TRIS.HCl (pH 7.4) (1). Polyacrylamide gel electrophoresis was performed as described by Davis (5) at pH 8.3, omitting the sample

gel. Time of migration, one hour. The proteins were stained with 1 % (w/v) Amido-Schwartz in 7 % acetic acid.

Extraction of soluble ATPase by diphosphatidylglycerol (DPG) was routinely accomplished by incubating 10 min at 37° 500 µg submitochondrial particles and 250 µg diphosphatidylglycerol in 1.0 ml of 0.25 M sucrose, 10 mM TRIS-HCl, 1 mM EDTA, 4 mM ATP.TRIS (pH 7.4). The sample was centrifuged 30 min at 50 000 Rev./min (Spinco N° 50 rotor keeping the temperature around 20°) and the supernatant was assayed for ATPase activity as described in the legend to Tables. Proteins were estimated by the Lowry et al. (6) procedure.

Results and Discussion. In Table I it is seen that after incubation at 37° the oligomycin-sensitivity of SMP from bovine-heart was somewhat decreased. This effect which, however, was not always reproducible, confirms previous observations of Warshaw et al. (7). Under these conditions a certain amount of proteins (15-20 % of added SMP) were released in the supernatant and showed oligomycin-sensitive ATPase activity, indicating the presence of small membrane fragments. Addition of diphosphatidylglycerol to SMP was followed by the appearance of oligomycin-insensitive ATPase activity in the supernatant and a decrease of the oligomycin-sensitive ATPase in the residue. Release of oligomycin-insensitive ATPase was still clearly evident at 0.06 mg DPG/mg protein. On the basis of specific activity it can be estimated that mitochondrial ATPase (F_1) represents a large part (about 20 %) of released protein. DPG did not modify or only slightly increased the total amount of protein released in the supernatant.

The ATPase activity of supernatant after DPG treatment was cold labile and was not inhibited even after addition of a large excess of oligomycin (150 µg/mg protein). Control experiments showed that the small amount of DPG added to the incubation medium with the supernatant was not antagonist of this high concentration of antibiotic (cfr. also ref. 1). These observations strongly indicated that the soluble form of mitochondrial ATPase was present. When the structure

TABLE I

Release of oligomycin-insensitive ATPase by diphosphatidylglycerol.

260 μg submitochondrial particles (SMP), prepared according to Racker (3) from bovine-heart mitochondria were incubated with or without 150 μg diphosphatidylglycerol (DPG) as described under "Materials and Methods" in a total volume of 0.5 ml. The samples were centrifuged and the ATPase activity of sediments and supernatants was measured in the following incubation medium : 50 mM Tris-HCl (pH 7.4), 2.5 mM $MgCl_2$, 3.0 mM ATP (pH 7.4), 50 mM sucrose, 0.2 mM EDTA, 2 mM P-enolpyruvate (pH 7.4), 5 μg pyruvate kinase, 0.5 mg bovine serum albumine, 0.1 % (v/v) ethanol containing or not oligomycin. The amount of protein was 4-8 μg for F_1 and supernatants and 40-70 μg sediment or submitochondrial particles. Final volume, 1.0 ml. Incubation, 5 min at 37°. The reaction was terminated with trichloroacetic acid.

System	Released protein (% of added SMP)	ATPase activity (μmoles/min/mg)	
		Without oligomycin	With 1 μg oligomycin
Untreated SMP	-	3.2	0.2
Sediment after incubation of SMP at 37°	-	2.8	1.3
Sediment after incubation of SMP at 37° with DPG	-	1.0	0.9
Supernatant after incubation of SMP at 37°	12.3	8.5	0.0
Supernatant after incubation of SMP at 37° with DPG	14.8	15.1	15.1
Bovine-heart F_1	-	70.4	63.6

of DPG used as dispersion in aqueous solution almost free of inorganic cations was changed inducing depolarization of micelles by addition of 5 mM $MgCl_2$ or performing the incubation in 0.2 M NaCl, 25 mM TRIS-HCl, 4 mM ATP (pH 7.4) the release of F_1 was prevented.

In Table II it is seen that the effect of DPG was manifest using SMP from bovine-heart prepared by two different procedures. On the contrary, SMP from rat-liver were little or not depleted. This is in agreement with recent finding (8,9) on the isolation and purification of F_1 from rat-liver mitochondria showing that the liver-enzyme is more tenaciously bound to mitochondrial membrane and difficult to

TABLE II

Solubilization of ATPase in different submitochondrial particles.

Submitochondrial particles from rat-liver were prepared according to Kielley and Bronk (2) (rat-liver SMP-P), from bovine-heart according to Kielley and Bronk (bovine-heart SMP-P) or Racker (3) (SMP-PP). Pretreatment of 260 µg submitochondrial particles with 150 µg diphosphatidylglycerol (DPG) in a total volume of 0.5 ml, measurement of protein and ATPase activity found in the supernatants as described in Table I, without addition of P-enol-pyruvate and pyruvate kinase. Incubation time, 10 min at 37°.

Particles	DPG during pretreatment	Released protein (% of added SMP)	ATPase activity (µmoles/min/mg)	
			without oligomycin	with 1 µg oligomycin
Rat-liver SMP-P	-	18.3	1.9	0.0
"	+	19.2	1.2	1.0
Bovine-heart SMP-P	-	14.4	3.7	0.2
"	+	15.8	12.0	12.0
Bovine-heart SMP-PP	-	13.5	3.2	0.3
"	+	14.9	13.3	13.3

remove. Recently (1,10) it was shown that phospholipids can reverse the inhibition of particulate mitochondrial ATPase from rat-liver induced by lipofilic compounds (oligomycin, dicyclohexylcarbodiimide, tributyltin). The ineffectiveness of phospholipids in removing F_1 from rat-liver mitochondria exclude that the antagonism is due to solubilization of insensitive mitochondrial ATPase.

To test whether the effect of DPG was due to unspecific action such as detergent activity (cfr. ref. 11), other individual phospholipids and some detergent were examined. None of the compounds listed in Table IV had the same properties of DPG : the acidic phosphatidylinositol showed great effectiveness but largely increased the amount of released protein. The same effect was produced by lysophosphatidylcholine but not at the same extent. Phosphatidylserine reproduced the effect of DPG, but with lower activity. Phosphatidylethanolamine and phosphatidylcholine showed low, if any, effectiveness.

TABLE III

Effect of phospholipids and detergents

Experimental conditions as described in Table I. Individual phospholipids and detergents were used at the same concentration (0.57 mg/mg protein) with the exception of sodium dodecylsulfate which was 0.29 mg/mg protein. Protein and ATPase activity were determined in the supernatant. Except were indicated, phospholipids were from bovine-brain.

Phospholipid	Released protein (% of added SMP)	ATPase activity (μmoles/min/mg)	
		Without oligomycin	With 1 μg oligomycin
none	16.0	2.4	0.0
Diphosphatidylglycerol	15.0	16.1	15.1
Phosphatidylinositol	25.8	6.8	6.2
Phosphatidylserine	15.0	3.5	3.0
Phosphatidylethanolamine	16.4	1.8	0.9
Egg phosphatidylcholine	18.8	2.0	0.4
Egg lysophosphatidylcholine	25.0	1.8	1.2
none	17.4	3.0	0.3
Diphosphatidylglycerol	18.0	14.2	13.4
Cholate	18.3	4.0	0.3
Deoxycholate	18.3	2.8	0.3
Sodium dodecylsulfate	26.6	3.7	3.1
Lubrol 'W'	25.0	1.7	0.5

Among the detergents, the anionic cholate and deoxycholate, the non ionic lubrol were without effect at the same concentration of DPG. The anionic sodium dodecylsulfate promoted solubilization of proteins, but inactivation of ATPase activity was evident.

In Fig. 1 it is seen the electrophoretic pattern of protein released by DPG and phosphatidylinositol from SMP. It can be observed that part of the protein was formed by inactive components which failed to enter the gel. This was especially evident with phosphatidylinositol. These components could be removed by ammonium sulfate fractionation with increase in specific activity. The true solubilized components were represented by F_1 together with minimal contamination by other proteins. Adding corresponding amount of protein released in the absence of phospholipids the band corresponding to F_1 was not observed.

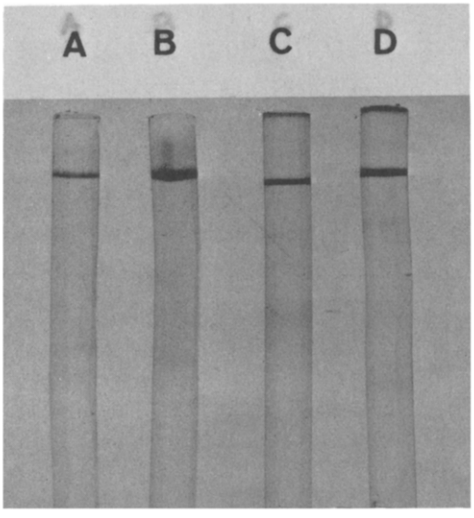

FIG. 1. Electrophoretic pattern of proteins released by diphosphatidylglycerol and phosphatidylinositol from bovine-heart submitochondrial particles (3). Polyacrylamide gel electrophoresis according to Davis (5). Submitochondrial particles were pretreated with phospholipids (0.5 mg/mg protein) as described in Table I. Aliquots of supernatant were subjected to electrophoresis without further purification.

A and B, 8 μg and 16 μg soluble mitochondrial ATPase (F_1)(4); C, 23 μg protein of diphosphatidylglycerol-treated sample ; D, 30 μg protein of phosphatidylinositol-treated sample.

Two possibilities are considered to explain the release of mitochondrial ATPase by acidic phospholipids : (a) a specific affinity for F_1 leading to formation of a complex which can not be longer bound by the mitochondrial membrane; (b) severe disorganization of mitochondrial inner membrane with release of proteins or other factors involved in the binding of F_1. Both effects could be produced simultaneously. The second possibility seems indicated by preliminary experiments which have shown that purified F_1 (4) could not be bound by the depleted particles. By contrast, DPG-extracted F_1 after further purification with ammonium sulfate fractionation, reconstituted oligomycin-sensitive ATPase in submitochondrial particles (12) depleted of F_1 by treatment with urea.

The high efficiency of external DPG in removing F_1 raises the

question of the partecipation of mitochondrial DPG in the organization of the ATP generating system. Guarnieri et al. (13) found little capacity by intact mitochondria and SMP to bind an antibody reactive with the polar head of DPG. Removal of F_1 by urea was not followed by increase in the binding of antibody.

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