ISOLATION OF AN ATP-Pi EXCHANGEASE FROM LYSOLECITHIN-TREATED ELECTRON TRANSPORT PARTICLES

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SUMMARY

A simple and rapid procedure has been developed for the isolation of an oligomycin-sensitive ATPase with a high level of ATP-Pi exchange activity using lysolecithin-treated ETP as the starting material. The ATP-Pi exchangease is deficient in electron transport components and is sensitive to uncouplers. Electron micrographs of negatively stained preparations show a variety of membrane fragments with attached headpieces. SDS gels show the presence of the same peptides found in an oligomycin-sensitive ATPase with no ATP-Pi exchange activity. The ATPase activity of the exchangease particles is stimulated by the uncoupler FCCP.

INTRODUCTION

No purified oligomycin-sensitive ATPase preparation has been shown to have the capacity for energy coupling as measured by ATP-Pi exchange activity. Of the oligomycin-sensitive ATPase preparations described in the literature (1-4), ATP-Pi exchange activity could be reconstituted in two of them (2,3) only after extensive dialysis and/or sonication in the presence of large amounts of lipid plus coupling factors (5,6). Even then, the ATP-Pi exchange activity was much lower than that of the original parent particle. Since the detergents used in the isolation procedures all inhibit or destroy ATP-Pi exchange activity, it is little wonder that a highly coupled oligomycin-sensitive ATPase has not yet been isolated.

ETP: electron transport particle

In recent work from our laboratory, lysolecithin has been used as a membrane-disruptive reagent which inhibits about 70% of the ATP-Pi exchange activity of the original ETP (7). Electron micrographs of negatively stained lysolecithin-treated ETP showed small membrane fragments with headpieces attached. This communication describes a rapid and simple procedure for the isolation of these fragments and contains data on their composition and enzymatic activities.

MATERIALS AND METHODS

ETP were prepared by the same method used by Linnane and Ziegler (8) for the preparation of ETP_H except for the omission of ATP and Mg^{+2} during sonication (7). ATPase activity was assayed at 30° for 3 minutes in a reaction mixture which was 50 mM in Tris-HCl, pH 8.5, 4 mM in $MgCl_2$ and 10 mM in ATP. Inorganic phosphate was separated from organic phosphate by the method of Lindberg and Ernster (9) and measured by the colorimetric method of Martin and Doty (10). ATP-Pi exchange was measured as described previously (7). NADH and succinic-ferricyanide reductase activities were determined at 22° in a medium 0.5 mM in NADH or 10 mM in succinate, 4 mM in ferricyanide, and 0.1 M in phosphate buffer, pH 7.4 (11,12). Cytochrome oxidase activity was determined polarographically (13). The concentrations of cytochromes were estimated according to Williams (14). Electron microscopy was performed as described previously (7). Protein was determined by the method of Lowry *et al.* (15). SDS gel electrophoresis was performed by the method of Fairbanks *et al.* (16), modified by using 0.1% SDS in the gels and incubating the samples in 1% SDS and 5 mm mercaptoethanol at 100° for 2 minutes. Lysolecithin prepared from egg lecithin (a product of Sigma) was used. FCCP was a generous gift of

FCCP: carbonylcyanide p-trifluoromethoxyphenyl hydrazone

Dr. Henry Lardy. Nigericin was a gift of Commercial Solvents.

RESULTS AND DISCUSSION

To prepare the ATP-Pi exchangease particles, lysolecithin was added to ETP to give a final concentration of 10 mg/ml protein and 6 mg/ml lysolecithin in a medium 10 mM in Tris-HCl, pH 7.8 and 250 mM in sucrose. The mixture was incubated for 30 minutes at 4°, diluted with an equal volume of 100 mM K^+ MES, pH 6.0, and incubated for an additional 20 minutes. The precipitate (105p15) was removed by centrifugation at 105,000 x g for 15 minutes. The supernatant was further centrifuged at 155,000 x g for 2 hours to obtain the ATP-Pi exchangease particles which were resuspended in a medium 50 mM in K^+ MOPS, pH 7.4 and 250 mM in sucrose.

Table 1 summarizes the ATPase and ATP-Pi exchange activities of the different fractions in a typical isolation run. The identical procedure has been repeated at least 15 times with relatively little variation in the activity data. There is a four-fold purification based on the specific activity of ATPase (for measurement of maximal ATPase activity, the assay was carried out in the presence of the uncoupler, FCCP). The specific activity of ATP-Pi exchange is increased six-fold when compared to the unfractionated lysolecithin-treated ETP. The yield of the exchangease varied from 3 to 6% of the original protein.

The electron transport activities and cytochrome content of the ATP-Pi exchangease are low when compared to either ETP or the 105p15 pellet (Table 2).

The ATPase activity of the exchangease particle was inhibited 88% by oligomycin (5 µg/mg protein). FCCP stimulated the ATPase activity, depending on the preparation, from a low of 10% to a

MOPS: morpholinopropane sulfonic acid
MES: 2(N-morpholino) ethane sulfonic acid

TABLE 1

Distribution of ATPase and ATP-Pi Exchange Activities

Fraction	ATPase (μmoles/min/mg protein)	ATP-Pi Exchange (nmoles/min/mg protein)
ETP	1.0	105
ETP + lysolecithin	1.2 (1.5)*	33
105p15	0.9	19
ATP-Pi Exchangease	3.5 (6.4)*	186
155s120	0	0

*plus FCCP (4 μM)

high of 270%. This variable sensitivity to FCCP may reflect a yet unknown structural variability in the particle.

The ATP-Pi exchange activity of the exchangease particle resembled that of the parent ETP in respect to its sensitivity to both oligomycin and the uncoupler, FCCP. Also, like ETP, the exchange activity of the particle was synergistically inhibited by the ionophoric combination of valinomycin and nigericin plus K^+. This sensitivity to the ionophores is an obvious difference between this particle and the S_1 fraction studied earlier which was insensitive to the synergistic inhibition of valinomycin and nigericin (7). This difference between the exchangease and the

NIG: nigericin
VAL: valinomycin

TABLE 2

Electron Transport Activities and Cytochrome Content of Fractions

	NADH Dehydrogenase (μmoles/min/mg protein)	Succinic dehydrogenase (μmoles/min/mg protein)	Cytochrome Oxidase (μmoles/min/mg protein)	Cytochrome b (nmoles/mg protein)	Cytochrome $c+c_1$ (nmoles/mg protein)
ETP	15.5	0.24	1.1	0.83	0.71
ETP-lysolecithin	13.0	0.18	2.9	----	----
105p15	7.9	0.05	3.0	0.91	0.85
Exchangease particles	1.0	0.01	0.3	0.12	0.07
155s120	0.9	0	0	----	----

S_1 fraction is probably a result of the lower concentration of lysolecithin present in the exchangease particles, since the addition of more lysolecithin (1.2 mg/mg of protein) to the exchangease resulted in almost complete loss of sensitivity to the synergistic inhibition of the ionophores (Table 3).

Electron micrographs (Figure 1A) of negatively stained exchangease preparations showed a variety of membrane fragments - some similar to the particles seen in S_1 (7) and others considerably larger. Addition of lysolecithin (1.2 mg/mg of protein - the same amount used in the experiment described in Table 3) to the exchangease caused a fragmentation of the larger structures (Figure 1B). This structural change on addition of lysolecithin is not dramatic like the structural change resulting from the

TABLE 3

Effect of Ionophores on ATP-Pi Exchange Activity

Addition	ATP-Pi Exchange Activity (nmoles/min/mg)	
	Control	+ Lysolecithin*
None	104	56
Val (2 μg)	103	57
Nig (2 μg)	74	27
Both	< 1	23

*1 mg of protein was preincubated 5 minutes with 1.2 mg of lysolecithin in the reaction mixture. ATP was then added to start the reaction.

addition of lysolecithin to a membrane like ETP, since the exchangease itself is already a fragmented membrane.

From this, we conclude that sensitivity to the synergistic action of valinomycin and nigericin is a property not only of membranes, but also of macromolecular structures of a critical complexity (the relevant property is still undefined). At the present time, we are aware of only one parameter by which we can distinguish between these two structural possibilities. High K^+ (o.1 M) has little or no effect on the synergistic action of the ionophore combination on exchangease activity in ETP, but it abolishes the synergistic action on exchangease activity of the isolated exchangease. This important secondary aspect of coupling

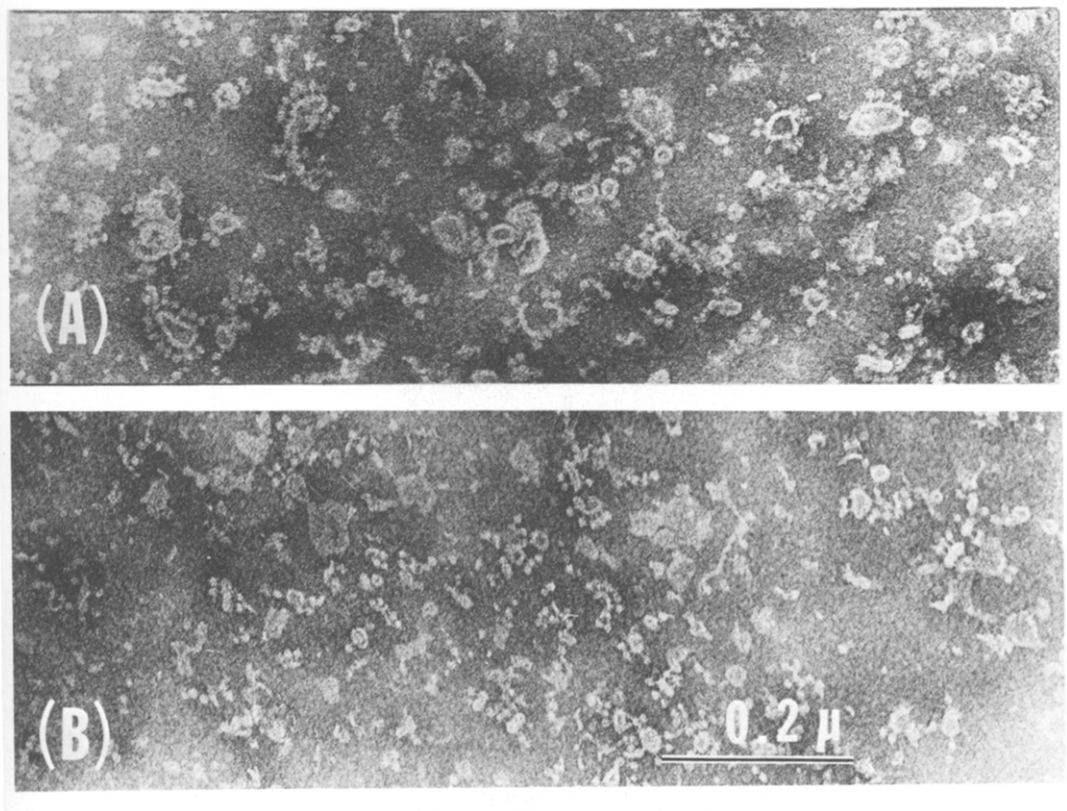

Figure 1: Electron micrographs of negatively stained exchangease particles (A), and exchangease particles plus lysolecithin (B) as described in the text.

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will be the subject of a later publication.

It is of considerable importance to compare the SDS gel patterns of our ATP-Pi exchangease with an oligomycin-sensitive ATPase which has no capabity for ATP-Pi exchange. Such a comparison could permit identification of peptides which would be indispensable for energy coupling, but unnecessary for ATP hydrolysis. SDS gels of the exchangease particles and of oligomycin-sensitive ATPase prepared by the procedure of Tzagoloff *et al.* (1) are shown in Figure 2. The gel patterns show that the peptide components of the two preparations are quite similar. Most importantly, all

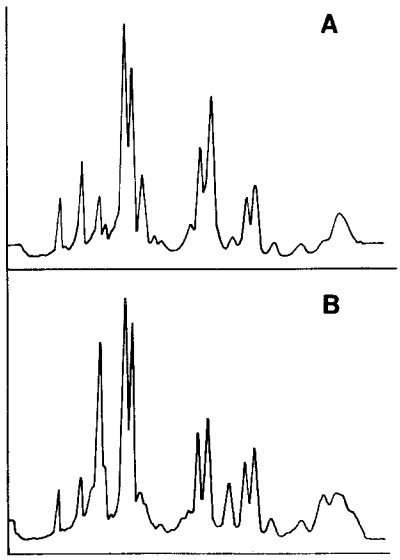

Figure 2: Densitometric traces of the exchangease (trace A) and of the oligomycin-sensitive ATPase (trace B). The gels were scanned with a Gilford linear gel scanner.

peptide bands present in the exchangease gel (A) are also present in the ATPase gel (B). However, some bands are more intense in the exchangease, in particular, one band of a 29,000 dalton peptide (17) which may be the peptide isolated by Capaldi *et al.* (18). Peptide bands not found in the exchangease, but present in the ATPase are contaminants known to be present in the preparation (1).

In summary, we have described a simple and rapid procedure for the isolation of an oligomycin-sensitive ATPase that has retained coupling capabilities, as demonstrated by the high level of ATP-Pi exchange activity. This ATP-Pi exchangease probably represents the minimum structure required for energy coupling. It contains both an exergonic complex - the ATP hydrolyzing unit, an endergonic complex - the ATP synthesizing unit, and the energy transducing unit. It is yet unknown whether the ATP hydrolase

and the ATP synthetase are one or two similar separate entities.

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