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Cross-reconstitution of isolated F_1-ATPase from potato tuber mitochondria with F_1-depleted beef heart and yeast submitochondrial particles

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Cross-reconstitution of isolated potato mitochondrial F_1-ATPase with F_1-depleted beef heart and yeast submitochondrial particles is reported. Potato F_1 binds to the heterologous membrane and confers oligomycin sensitivity on the ATPase activity of the reconstituted system. Binding of F_1 is promoted by the presence of Mg^{2+} with the maximal stimulatory effect at 20 mM. Mg^{2+} increase the sensitivity to oligomycin of the reconstituted system consisting of potato F_1 and yeast membranes, however, they do not influence oligomycin sensitivity of potato F_1 and beef heart membranes.

Cross-reconstitution; F_1-ATPase; H^+-ATPase; Oligomycin sensitivity; (Plant mitochondrion, Mammal, Yeast)

1. INTRODUCTION

H^+-translocating adenosine triphosphatases (H^+-ATPases) of mitochondria, chloroplasts and bacteria have basically similar structure and function. The enzymes are, however, not identical molecules and exhibit specificity of structural, catalytic and immunological properties. H^+-ATPases consist of a hydrophilic part, F_1, containing the catalytic site of the enzyme and a hydrophobic, membrane part, F_0, constituting the H^+-translocating moiety of the enzyme. In addition, the mitochondrial H^+-ATPases of mammals and yeast contain two more proteins which constitute a structural and functional link between F_1 and F_0, oligomycin sensitivity conferring protein (OSCP) and factor F_6 [1]. These proteins have not been found in chloroplasts and bacteria [2–4]. Sequence analysis shows, however, that there is homology between the mammalian OSCP and two subunits of *E. coli* F_1F_0 system, the δ-subunit of F_1 and the b-subunit of F_0, indicating an interesting structural relation of the subunits of H^+-ATPase in different organisms [5,6].

Limited information is available concerning plant mitochondrial H^+-ATPase. The F_1 part of the enzyme has recently been purified from a variety of sources [7–11]. No reports are, however, available concerning compositions and properties of F_0 in plant mitochondria.

Cross-reconstitution experiments between isolated F_1 and F_1-depleted submitochondrial particles derived from different sources are applied for studies of the structural relation between various H^+-ATPases. Several attempts to functionally bind mammalian F_1 as well as the chloroplast CF_1 to a heterologous membrane

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Abbreviations: Mops, 3-(*N*-morpholino)propanesulfonic acid; Tris, 1,3-bis(tris[hydroxymethyl]amino)propane

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system have failed. However, the purified yeast F_1 when added to the F_1-depleted bovine heart submitochondrial particles could substitute native F_1 in oligomycin-sensitive ATPase activity [12]. Chloroplast CF_1 could be substituted in reconstitution of photophosphorylation by both plant mitochondrial F_1 [13] and cyanobacterial F_1 [14].

In the present paper we demonstrate cross-reconstitution experiments between the plant mitochondrial F_1, purified from potato tubers mitochondria and the F_1-depleted beef heart and yeast submitochondrial particles. The binding of F_1, conferral of oligomycin sensitivity on the ATPase activity as well as specific cation requirement for the cross-reconstitution are discussed.

2. MATERIALS AND METHODS

2.1. *Preparation of mitochondria, submitochondrial particles and purification of F_1-ATPase from potato tubers*

Potato tubers mitochondria were isolated as described by Neuburger et al. [15]. Submitochondrial particles were prepared by sonication of the mitochondria 5 times for 20 s at 4°C with a Branson sonifier (model B-30, setting 5). Sonication was performed at a protein concentration of about 7 mg/ml and in a medium consisting of 0.25 M sucrose, 5 mM Mops (pH 7.5) and 20 mM $MgCl_2$. F_1-ATPase was isolated from ethylene glycol washed submitochondrial particles after treatment with 220 mM chloroform at 37°C, pH 7.5, followed by purification on a glycerol gradient (10–50%) as described by Glaser et al. [16] according to a method described by Fisher et al. [17] for purification of F_1-ATPase from rat liver mitochondria. The ATPase activity of the isolated potato F_1 was 21 μmol/min per mg protein.

2.2. *Preparation of F_1-depleted submitochondrial particles from beef heart mitochondria*

Submitochondrial particles were prepared from mixed beef heart mitochondria by sonication in the presence of EDTA as described in [18]. By passage through a Sephadex G-50 coarse column, the ATPase inhibitor protein was released from the particles [19]. F_1-depleted submitochondrial particles were prepared by extraction of the Sephadex particles with 8 M urea [19].

2.3. *Preparation of mitochondria, submitochondrial particles and F_1-depleted submitochondrial particles from baker's yeast*

Preparation of yeast mitochondria was done according to a modified method by Lang et al. [20]. All steps were performed in cold. Baker's yeast, 100 g, was washed twice in 1 l distilled water and collected by centrifugation at 3000 × *g* for 10 min. The washed yeast cells were suspended in 0.6 M sorbitol, 10 mM EDTA (pH 6.5), centrifuged and suspended again in 400 ml of the same medium. 100 ml of the yeast suspension was added to a bottle holding 1 l containing 350 g glass beads, 0.5 mm in diameter. The bottle was shaken vertically at a speed of twice a second and for 6 times at 20 s, each with cooling on ice in the intervals. The yeast suspension was poured off and the beads were rinsed with sorbitol-EDTA in order to get a high yield of yeast mitochondria. The yeast suspension was centrifuged at 4000 × *g* for 10 min, and the pellet was discharged. The supernatant was centrifuged at 10000 × *g* for 10 min. The final mitochondrial pellet was suspended in 15 ml of 0.25 M sucrose and 10 mM Tris-chloride (pH 7.5), giving a protein concentration of about 30 mg/ml.

Submitochondrial particles were prepared by sonication of the mitochondria 6 times for 20 s at 4°C. After centrifugation at 10000 × *g* for 10 min the pellet was discharged and the supernatant was recentrifuged at 105000 × *g* for 60 min. The pellet containing submitochondrial particles was resuspended in 0.25 M sucrose and 10 mM Tris-chloride (pH 7.5) and centrifuged once more. The submitochondrial particles were finally suspended in 1.5 ml of 0.25 M sucrose and 10 M Tris-chloride (pH 7.5). The ATPase activity of the particles was 3.9 μmol/min per mg protein.

F_1-depleted submitochondrial particles from yeast were prepared by treatment of the particles with 3.5 M NaBr, as described for beef heart particles [21].

2.4. *Cross-reconstitution of oligomycin-sensitive ATPase activity from purified potato mitochondrial F_1 and F_1-depleted beef heart or yeast submitochondrial particles*

Reconstitutions were done by incubating F_1-depleted beef heart submitochondrial particles (1.1 mg/ml) with various amounts of purified F_1 from mitochondria of potato tubers. The incuba-

tions were carried out for 30 min at room temperature in a medium containing 0.25 M sucrose. 10 mM Tris-SO_4 (pH 8.0), 0.15 mM EDTA and in the presence of MgAc at concentrations indicated in the figure legends. Reconstitutions with F_1-depleted yeast submitochondrial particles were performed in a similar manner except that the medium consisted of 50 mM Tris-Ac, pH 7.5. Not bound F_1 was separated from bound F_1 by centrifugation of the samples for 5 min in an Eppendorf centrifuge.

2.5. *Measurement of ATPase activity*

ATPase activity was measured by coupling the reaction to the pyruvate kinase and lactate dehydrogenase reactions and measuring NADH oxidation spectrophotometrically [22,23].

2.6. *Protein determination*

Protein was determined according to Peterson et al. [24].

3. RESULTS AND DISCUSSION

3.1. *Cross-reconstitution of oligomycin-sensitive ATPase activity by purified F_1 from potato tubers mitochondria and F_1-depleted beef heart submitochondrial particles*

Urea treatment of beef heart submitochondrial particles results in a virtually complete depletion of F_1, the residual ATPase activity of the particles being only 0.02 μmol/min per mg as compared to 5 μmol/min per mg for the original Sephadex G-50 treated particles. As shown in fig.1A purified potato F_1 can be bound to F_1-depleted beef heart submitochondrial particles and thereby conferring oligomycin-sensitive ATPase activity on these particles. Maximal levels of ATPase activity is reached when 16 μg potato F_1 is incubated per 100 μg particles. Only 15% of added potato F_1 is however bound under these conditions. The addition of 3 nmol oligomycin/mg particle protein to the assay system causes about 60% inhibition of the ATPase activity.

It can be seen in fig.1B that maximal binding of potato F_1 to F_1-depleted beef heart submitochondrial particles is dependent on the presence of Mg^{2+} in the reconstitution medium. When 16 μg potato F_1/100 μg particles is used in reconstitu-

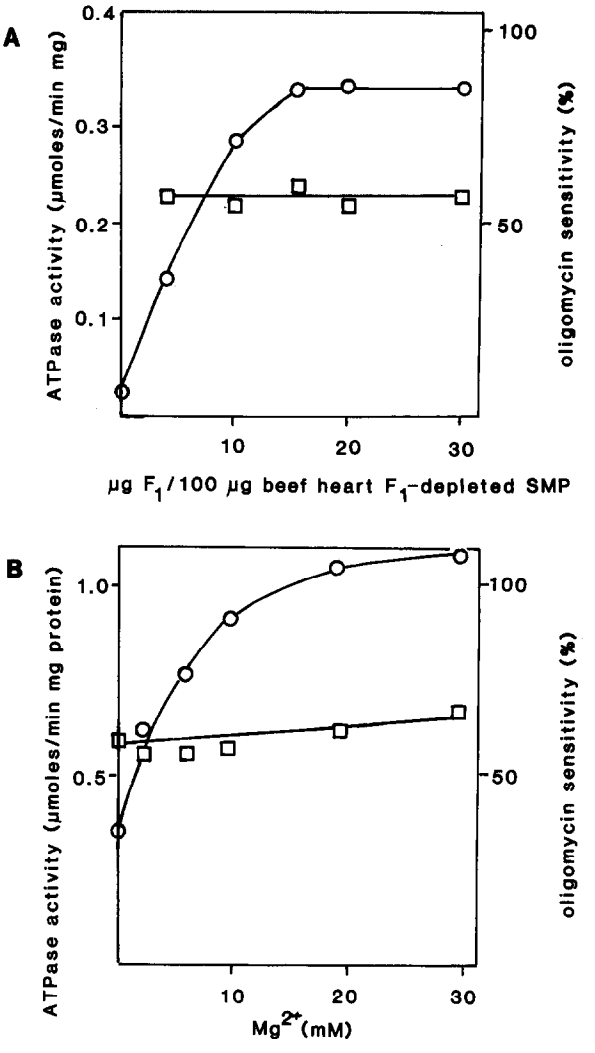

Fig.1. (A) Cross-reconstitution of oligomycin-sensitive ATPase in F_1-depleted beef heart submitochondrial particles by potato F_1. (○—○) ATPase activity; (□—□) oligomycin sensitivity of the ATPase activity. F_1-depleted beef heart submitochondrial particles were incubated with increasing amounts of potato F_1 in the conditions described in section 2; no MgAc is present. Oligomycin, 3 nmol/mg protein, is added directly to the cuvette during the ATPase assay. (B) The effect of Mg^{2+} on the binding of potato F_1 to F_1-depleted beef heart submitochondrial particles (○—○) and on the conferral of oligomycin sensitivity (□—□). Reconstitution was performed with 16 μg potato F_1/100 μg particles under conditions described in section 2. Conditions for incubation with oligomycin were as for A.

tion, the binding of potato F_1 to the particles increases more than 3-fold, in the presence of 30 mM MgAc as indicated by an enhancement of the ATPase activity of the particles from 0.3 μmol/min per mg protein to 1.1 μmol/min per mg protein. Oligomycin sensitivity seems to be independent of Mg^{2+} concentration being between 60 and 70% for all concentrations. When maximal activity of the particles is achieved at 30 mM MgAc, 50% of the added F_1 was bound to the particles i.e. 8 μg F_1/100 μg particles. In reconstitution experiments between the homologous beef heart F_1 and beef heart membranes [25] a ratio of 10 μg F_1/100 μg particles results in a maximal binding of 90% of added F_1, and an ATPase activity of 5 μmol/min per mg particle protein. The reconstitution medium contained no Mg^{2+} but 2 mM EDTA.

These results show that potato mitochondrial F_1 can substitute beef heart F_1 in binding to F_1-depleted beef heart submitochondrial particles. In the absence of Mg^{2+}, the binding efficiency of potato F_1 as well as the efficiency of conferral of oligomycin sensitivity are lower than in the case of the native enzyme. The binding of potato F_1 can be stimulated by Mg^{2+}. The amount of potato F_1 bound to the particles in the presence of Mg^{2+} approaches the amount of the native F_1 which can be maximally bound. However, the presence of Mg^{2+} does not improve sensitivity of the heterologous reconstituted system to oligomycin.

3.2. *Cross-reconstitution of oligomycin-sensitive ATPase activity by purified F_1 from potato tubers mitochondria and F_1-depleted yeast submitochondrial particles*

Fig.2A shows the binding of purified potato F_1 to F_1-depleted yeast submitochondrial particles in the presence of 30 mM MgAc. The F_1-depleted yeast submitochondrial particles have a low ATPase activity of 0.03 μmol/min per mg protein and by adding increasing amounts of potato F_1 the activity is increased to 1.1 μmol/min per mg particle protein. Maximal ATPase activity of the reconstituted system is achieved at 15 μg F_1/100 μg particles. Under these conditions 60% of added F_1 is bound. When 5 nmol oligomycin/mg particles is added in the assay system, ATPase activity is inhibited to about 55% at all ratios of F_1 to particles.

In fig.2B it can be seen that similar to the case

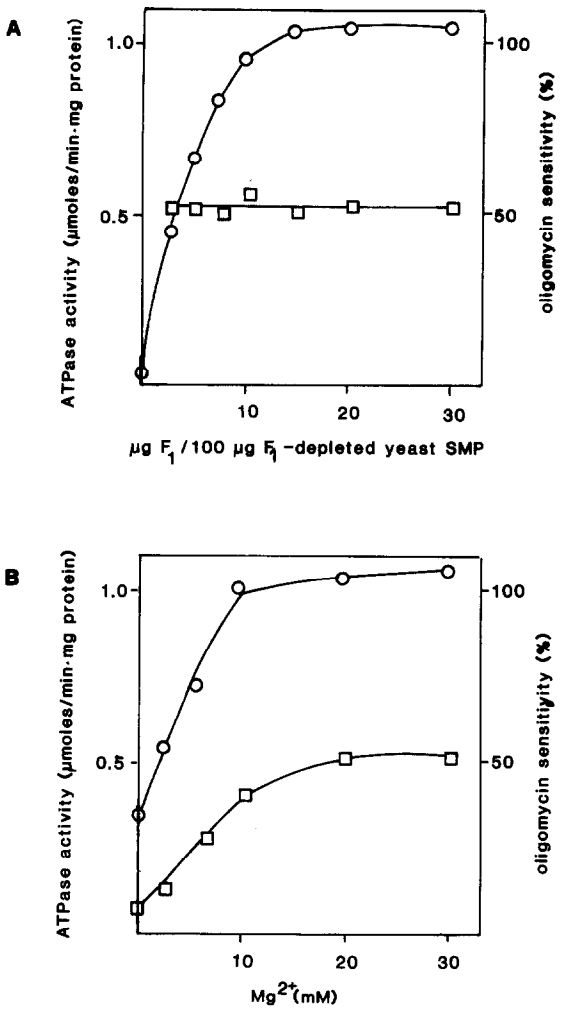

Fig.2. (A) Cross-reconstitution of oligomycin-sensitive ATPase in F_1-depleted yeast submitochondrial particles by potato F_1. (○—○) ATPase activity; (□—□) oligomycin sensitivity of the ATPase activity. F_1-depleted yeast submitochondrial particles were incubated with increasing amounts of potato F_1 under conditions described in section 2, 30 mM MgAc is present. Oligomycin, 5 nmol/mg protein, is added directly to the cuvette during the ATPase assay. (B) The effect of Mg^{2+} on the binding of potato F_1 to F_1-depleted yeast submitochondrial particles (○—○) and on the conferral of oligomycin sensitivity (□—□). Reconstitution was performed with 10 μg potato F_1/100 μg particles in the conditions described in section 2. Conditions for incubation with oligomycin were as for A.

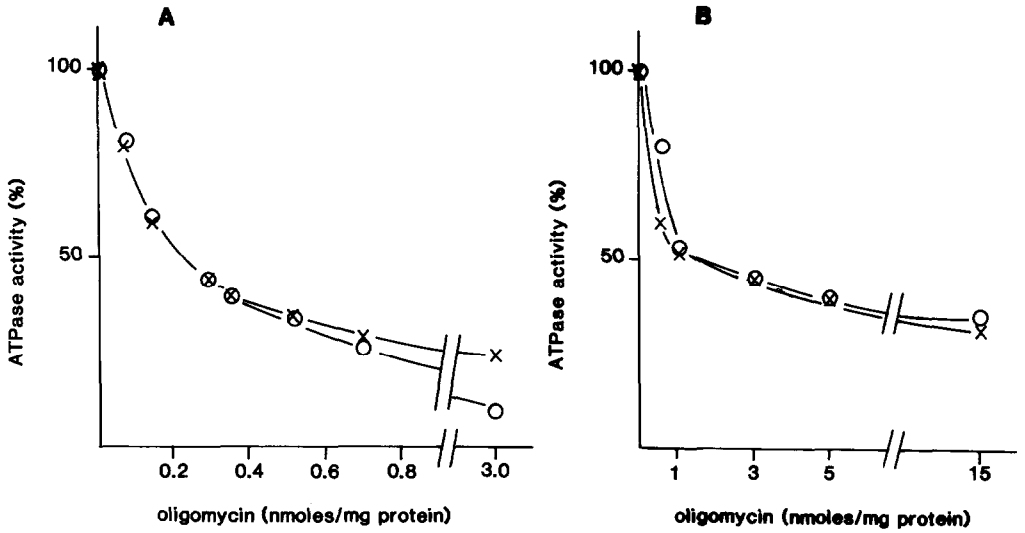

Fig.3. (A) Oligomycin titration of the ATPase activity of beef heart submitochondrial particles and of cross-reconstituted ATPase of F_1-depleted beef heart submitochondrial particles with potato F_1. Reconstitution was performed with 9 μg potato F_1/100 μg F_1-depleted particles in the presence of 30 mM MgAc and as described in section 2. The reconstituted ATPase (×—×) and the original beef heart submitochondrial particles (○—○) were incubated with the indicated amounts of oligomycin for 40 min at a protein concentration of 0.7 mg/ml before assay of ATPase activity. (B) Oligomycin titration of the ATPase activity of yeast submitochondrial particles and of cross-reconstituted ATPase of F_1-depleted yeast particles with potato F_1. Reconstitution and binding of oligomycin was performed as in A. Reconstituted system (×—×), yeast submitochondrial particles (○—○).

of F_1-depleted beef heart submitochondrial particles, binding of F_1 to yeast membranes is dependent on the presence of Mg^{2+} in the reconstitution medium. However, as seen in the same figure, not only binding of F_1 but also conferral of oligomycin sensitivity is dependent on Mg^{2+} in the case of yeast membranes, in contrast to the beef membranes (fig.1B). Both binding and conferral of oligomycin sensitivity seems to be optimal at about 20 mM MgAc.

These results show that potato mitochondrial F_1 can substitute yeast mitochondrial F_1 in both binding and conferral of oligomycin sensitivity on the ATPase activity of the reconstituted system. The presence of Mg^{2+} promotes both binding and sensitivity to oligomycin. The effect of Mg^{2+} has been reported on reconstitution of the homologous F_1 and F_1-depleted submitochondrial particles from beef heart [25,26].

3.3. *Oligomycin titers of the cross-reconstituted system*

Fig.3A shows the inhibition of oligomycin titration on ATPase activity of the cross-reconstituted ATPase between potato F_1 and F_1-depleted beef heart submitochondrial particles. The oligomycin titer coincides completely with the titer of the corresponding beef heart submitochondrial particles. The same is true for potato F_1 reconstituted with yeast membranes as compared to yeast submitochondrial particles (fig.3B). However, yeast submitochondrial particles and cross-reconstituted yeast membranes with potato F_1 are about 5 times less sensitive to oligomycin as beef heart submitochondrial particles and cross-reconstituted beef heart membranes with potato F_1.

These results show that the sensitivity to oligomycin is an intrinsic property of the membrane components of H^+-ATPase and independent of F_1.

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