

# 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  $\delta$ -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

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  $\mu$ mol/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  $3000 \times g$  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  $4000 \times g$  for 10 min, and the pellet was discharged. The supernatant was centrifuged at  $10000 \times g$  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  $10000 \times g$  for 10 min the pellet was discharged and the supernatant was recentrifuged at  $105000 \times g$  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  $\mu$ mol/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<sub>4</sub> (pH 8.0), 0.15 mM EDTA and in the presence of MgAc at concentrations indicated in the figure legends. Reconstitutions with F<sub>1</sub>-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<sub>1</sub> was separated from bound F<sub>1</sub> 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<sub>1</sub> from potato tubers mitochondria and F<sub>1</sub>-depleted beef heart submitochondrial particles

Urea treatment of beef heart submitochondrial particles results in a virtually complete depletion of F<sub>1</sub>, the residual ATPase activity of the particles being only 0.02  $\mu$ mol/min per mg as compared to 5  $\mu$ mol/min per mg for the original Sephadex G-50 treated particles. As shown in fig.1A purified potato F<sub>1</sub> can be bound to F<sub>1</sub>-depleted beef heart submitochondrial particles and thereby conferring oligomycin-sensitive ATPase activity on these particles. Maximal levels of ATPase activity is reached when 16  $\mu$ g potato F<sub>1</sub> is incubated per 100  $\mu$ g particles. Only 15% of added potato F<sub>1</sub> 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<sub>1</sub> to F<sub>1</sub>-depleted beef heart submitochondrial particles is dependent on the presence of Mg<sup>2+</sup> in the reconstitution medium. When 16  $\mu$ g potato F<sub>1</sub>/100  $\mu$ g particles is used in reconstitu-

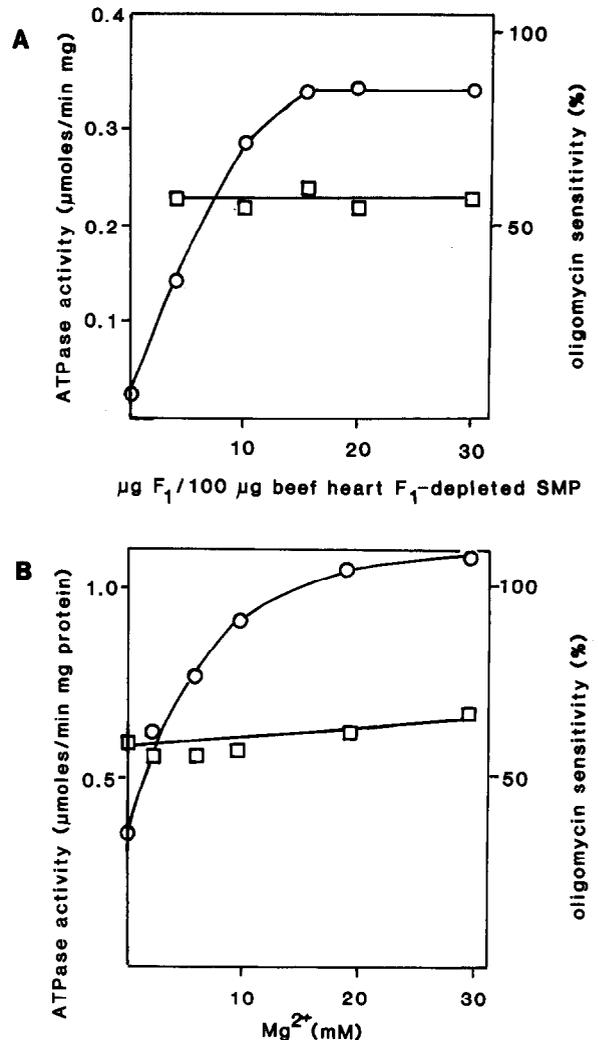


Fig.1. (A) Cross-reconstitution of oligomycin-sensitive ATPase in F<sub>1</sub>-depleted beef heart submitochondrial particles by potato F<sub>1</sub>. (○—○) ATPase activity; (□—□) oligomycin sensitivity of the ATPase activity. F<sub>1</sub>-depleted beef heart submitochondrial particles were incubated with increasing amounts of potato F<sub>1</sub> 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<sup>2+</sup> on the binding of potato F<sub>1</sub> to F<sub>1</sub>-depleted beef heart submitochondrial particles (○—○) and on the conferral of oligomycin sensitivity (□—□). Reconstitution was performed with 16  $\mu$ g potato F<sub>1</sub>/100  $\mu$ g 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  $\mu\text{mol}/\text{min}$  per mg protein to 1.1  $\mu\text{mol}/\text{min}$  per mg protein. Oligomycin sensitivity seems to be independent of  $\text{Mg}^{2+}$  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  $\mu\text{g}$   $F_1/100$   $\mu\text{g}$  particles. In reconstitution experiments between the homologous beef heart  $F_1$  and beef heart membranes [25] a ratio of 10  $\mu\text{g}$   $F_1/100$   $\mu\text{g}$  particles results in a maximal binding of 90% of added  $F_1$ , and an ATPase activity of 5  $\mu\text{mol}/\text{min}$  per mg particle protein. The reconstitution medium contained no  $\text{Mg}^{2+}$  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  $\text{Mg}^{2+}$ , 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  $\text{Mg}^{2+}$ . The amount of potato  $F_1$  bound to the particles in the presence of  $\text{Mg}^{2+}$  approaches the amount of the native  $F_1$  which can be maximally bound. However, the presence of  $\text{Mg}^{2+}$  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  $\mu\text{mol}/\text{min}$  per mg protein and by adding increasing amounts of potato  $F_1$  the activity is increased to 1.1  $\mu\text{mol}/\text{min}$  per mg particle protein. Maximal ATPase activity of the reconstituted system is achieved at 15  $\mu\text{g}$   $F_1/100$   $\mu\text{g}$  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

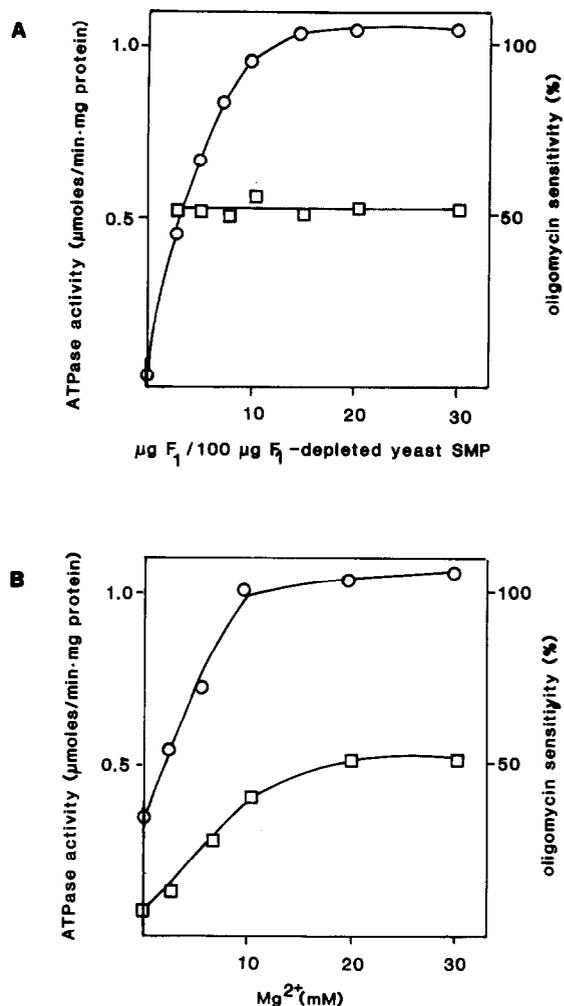


Fig.2. (A) Cross-reconstitution of oligomycin-sensitive ATPase in  $F_1$ -depleted yeast submitochondrial particles by potato  $F_1$ . ( $\circ$ — $\circ$ ) ATPase activity; ( $\square$ — $\square$ ) oligomycin sensitivity of the ATPase activity.  $F_1$ -depleted yeast submitochondrial particles were 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  $\text{Mg}^{2+}$  on the binding of potato  $F_1$  to  $F_1$ -depleted yeast submitochondrial particles ( $\circ$ — $\circ$ ) and on the conferral of oligomycin sensitivity ( $\square$ — $\square$ ). Reconstitution was performed with 10  $\mu\text{g}$  potato  $F_1/100$   $\mu\text{g}$  particles in the conditions described in section 2. Conditions for incubation with oligomycin were as for A.

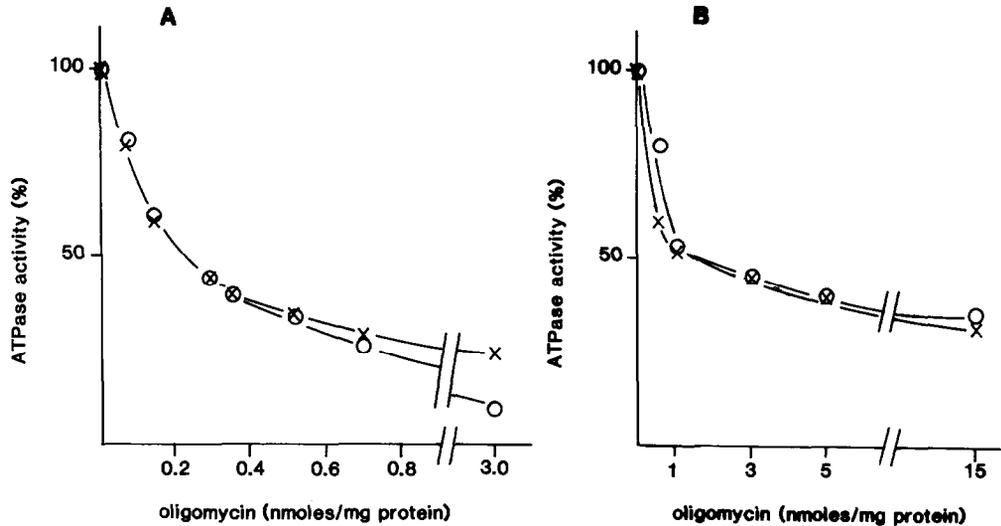


Fig.3. (A) Oligomycin titration of the ATPase activity of beef heart submitochondrial particles and of cross-reconstituted ATPase of  $F_1$ -depleted beef heart submitochondrial particles with potato  $F_1$ . Reconstitution was performed with 9  $\mu\text{g}$  potato  $F_1$ /100  $\mu\text{g}$   $F_1$ -depleted particles in the presence of 30 mM MgAc and as described in section 2. The reconstituted ATPase ( $\times$ — $\times$ ) and the original beef heart submitochondrial particles ( $\circ$ — $\circ$ ) 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 ( $\times$ — $\times$ ), yeast submitochondrial particles ( $\circ$ — $\circ$ ).

of  $F_1$ -depleted beef heart submitochondrial particles, binding of  $F_1$  to yeast membranes is dependent on the presence of  $\text{Mg}^{2+}$  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  $\text{Mg}^{2+}$  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  $\text{Mg}^{2+}$  promotes both binding and sensitivity to oligomycin. The effect of  $\text{Mg}^{2+}$  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 titra-

tion 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  $\text{H}^+$ -ATPase and independent of  $F_1$ .

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