Bioenergetics (1976) 8, 1-7

Functional Role of Soluble Mitochondrial ATPase Subunits

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Received 26 July 1975

Abstract

A preparation of soluble mitochondrial ATPase (coupling factor F_1) containing no γ and δ minor subunits has been isolated. The minor-subunits-deficient F_1 was found to be competent in ATP hydrolysis. However, it did not demonstrate a "coupling" effect in EDTA-submitochondrial particles. A portion of the ATPase activity of EDTA particles, stimulated by the minor-subunits-deficient F_1, was insensitive to oligomycin. ATPase activity of Na^+-particles was changed only slightly by this F_1. It is suggested that γ and δ subunits are necessary to form specific contacts between the F_1 molecule and components of the mitochrondrial membrane.

Introduction

Soluble mitochondrial ATPase plays an important role in the oxidative phosphorylation system. A preparation of the enzyme isolated by different authors [1, 2] is known to contain five subunits with molecular weights of 54,000 (α), 50,000 (β), 33,000 (γ), 16,000 (δ) and 11,000 (ϵ). According to Knowles and Penefsky, the latter subunit is a dimer of a protein inhibitor of mitochondrial ATPase [1]. In our previous paper [2] it was shown that α and β subunits play a major role in ATP

Abbreviations:
SMP—submitochondrial particles.
F_1—coupling factor (soluble mitochondrial ATPase).
PCB^-—phenyl dicarbaundecaborane anions.

hydrolysis and in the competitive ADP sorption. γ and δ subunits constitute about 15% of the weight of an F_1 molecule. Their functional role has not been elucidated. An analysis of the amino-acid composition of the subunits allowed us to make the suggestion that a molecule of the native enzyme consists of seven protein globules of more or less similar molecular weight [2]. The appearance of γ and δ subunits in a preparation of ATPase is in our opinion a consequence of degradation of the seventh globule in the course of isolation of the enzyme.

The results obtained in the present work show that the role of γ and δ subunits (the seventh protein globule) is that of providing specific contact between the F_1 molecule and the components of the mitochrondrial membrane.

Methods

Beef-heart mitochrondria were isolated as described by Crane et al. [3]. EDTA-SMP were prepared as described by Lee and Ernster [4]. Na^+-SMP were prepared according to MacLennan et al. [5]. Soluble mitochrondrial ATPase was isolated according to the method of Horstman and Racker [6]. To obtain an enzyme preparation containing no γ and δ subunits, this method was modified at the stage of sonication of the mitochondrial suspension (sonication was carried out at pH 5.0 instead of 7.4). The subunits-deficient F_1 preparations were found to have the same specific activity as F_1 containing a complete set of subunits, about 30 μmoles/mg protein/min at 25° C. Electrophoresis of the resulting ATPase preparations in polyacrylamide gel was performed as described by Weber and Osborn [7]. The initial rate of the ATPase reaction was measured according to Nishimura et al. [8]. The reaction mixture contained 3mM Tris-HCl buffer, pH 8.3; 4 mM ATP; 4 mM $MgSO_4$ and 1–2 mg SMP in a total volume of 8 ml. The reaction was initiated by adding SMP suspension to a sample. To study the inhibitory effect of oligomycin, the reaction mixture was supplemented with oligomycin (1.5 μg/ml) after SMP. SMP was regarded as having oligomycin-insensitive ATPase activity 3 min after the inhibitor had been added. The reconstitution of EDTA-SMP and Na^+-SMP with F_1 was carried out at 0°C in a total volume of 2 ml. The incubation mixture contained 50 mg of EDTA-SMP or Na^+-SMP and 800 μg F_1. Reconstitution was carried out for 1 h when the ATPase activity of the resulting reconstituted system and its sensitivity to oligomycin was studied. The values given in Table I are the average values of 4–10 measurements. Reconstitution lasted 15 min when the reconstituted SMP preparation was tested with respect to energy-dependent transport of PCB^- anions (the mixture was incubated for 5 min at room temperature and for 10 min in cold). The energy-dependent PCB^-

TABLE I. ATPase of reconstituted EDTA-SMP + F_1 (50 mg + 800 μg), and Na^+-SMP + F_1 (50 mg + 800 μg) systems and their sensitivity to oligomycin (1.5 μg/ml)

System	ATPase, nmoles/mg protein/min M ± m	Oligomycin-insensitive ATPase nmoles/mg protein/min
EDTA-SMP	1000 ± 30	<20
EDTA-SMP + F_1 (standard preparation)	1400 ± 50	<20
EDTA-SMP + F_1 (without γ and δ subunits)	1350 ± 50	160 ± 10
Na^+-SMP	110 ± 10	80 ± 5
Na^+-SMP + F_1 (standard preparation)	180 ± 15	72 ± 5
Na^+-SMP + F_1 (without γ and δ subunits)	120 ± 10	100 ± 10

transport inside SMP was to test the value of the membrane potential in the initial and reconstituted system. The change in the concentration of the PCB^- penetrant, by which the effectivity of the transport inside SMP was determined, was recorded by means of the artificial phospholipid membrane test [9].

Results and Discussion

For studying the functional role of the γ and δ subunits of F_1, it would be important to have two types of F_1 preparations: one, containing these minor components, and the other without them. Figure 1A shows an electrophoregram of a preparation of soluble ATPase isolated in conformity with the standard procedure of Horstman and Racker [6]. This method, if modified at the stage of sonication of mitochondrial suspension (pH 5.0, instead of 7.4) yields an F_1 preparation containing no γ and δ subunits (Fig. 1B)*. As was shown previously [2], the two ATPase preparations do not differ in the K_M and V_{max} values; they also

* It should be noted that the preparations of F_1 used in our experiments contained much larger amounts of ϵ subunits than might be expected if the ϵ subunit were a protein inhibitor of ATPase. It seems highly probable that ϵ subunits in preparations of F_1 isolated by different methods are of a different nature.

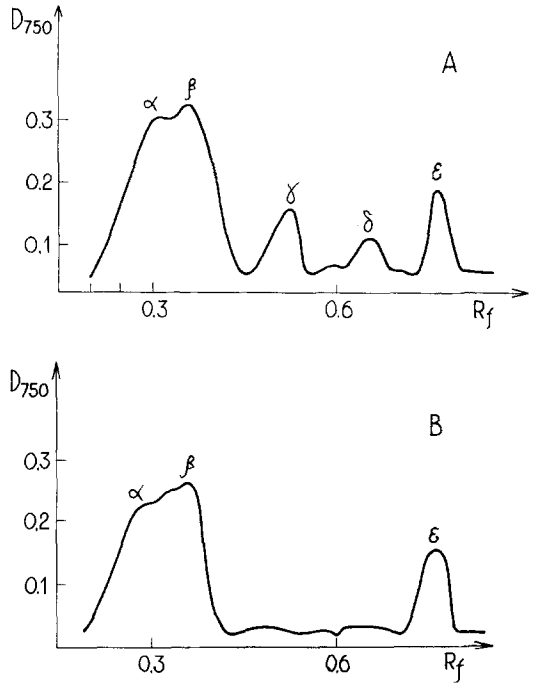

Figure 1. Electrophoresis of mitochondrial ATPase in polyacrylamide gel in the presence of sodium dodecylsulphate. (A) Standard F_1 preparation. (B) F_1 without γ and δ minor components; 50 μg of protein were layered onto the gel. The samples were stained with amino black. The electrophoretic mobility of some protein zones were measured relative to bromphenol blue which was assumed as being equal to unity.

possess a similar affinity to Mg-ADP, a competitive inhibitor of the ATPase reaction. In our previous paper [2] the suggestion was made that the functional role of the γ and δ subunits is to provide contacts between F_1 and the mitochondrial membrane. To verify this suggestion we have compared the ability of a standard F_1 preparation and one devoid of γ and δ subunits to reconstitute with F_1-deficient submitochondrial particles. Two kinds of particles of this type were used, namely Na^+-SMP and EDTA-SMP. whose ATPase activity was found to be 100 and 1000 nmol/mg protein/min, respectively.

Two effects of F_1 connected with F_1 reconstitution with EDTA-SMP were studied: (1) F_1-induced recoupling of EDTA-SMP estimated by means of measurement of respiration-driven movement of a penetrating ion, PCB^- [9], and (2) activation of the oligomycin-sensitive ATPase.

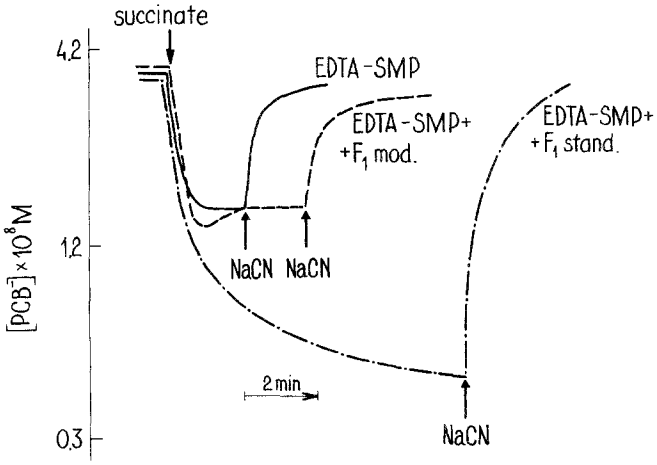

Figure 2. Succinate oxidation-coupled absorption of PCB^- anions by EDTA-SMP preparations. Incubation medium contained sucrose, 0.25 M; $MgSO_4$, 0.005 M; Tris-HCl, 0.05 M; pH 7.5, and various EDTA-SMP preparations (0.83 mg protein per ml of the medium). $F_{1\,stand}$ = factor F_1 obtained by the standard procedure; $F_{1\,mod}$ = factor F_1 obtained by modified procedure resulting in γ and δ subunits being lost. Additions: succinate-Tris, 6 mM; NaCN 2 mM.

Figures 2 and 3 show the results of the PCB^- experiments. One can see that energization of EDTA-SMP by succinate oxidation (Fig. 2) or ATP hydrolysis (Fig. 3) gives rise to a PCB^- uptake suggesting membrane-potential formation (plus inside EDTA-SMP). In both cases, addition of a standard preparation of factor F_1 significantly increases the energy-linked PCB^- response, whereas F_1 deprived of γ and δ subunits is without any measureable effect. In all systems, respiration-driven PCB^- uptake is completely inhibited by cyanide and ATP-driven uptake by oligomycin.

In the same experiments, ATPase activity of EDTA-SMP before and after reconstitution with two kinds of F_1 was estimated. The duration of the reconstitution procedure (1 h) was sufficient for the excess of F_1 molecules not involved in the reconstitution to be completely inactivated. The results are given in the Table I. It is seen that the ATPase activity in reconstituted EDTA-SMP increases by 30–40%. This increase is approximately the same in EDTA-SMP reconstituted with a standard F_1 preparation and with F_1 without the minor subunits. The difference between the two systems becomes evident when their sensitivity to oligomycin is compared. The ATPase activity of the initial EDTA-SMP and those reconstituted with a standard F_1 preparation is inhibited by oligomycin almost completely, whereas EDTA-SMP

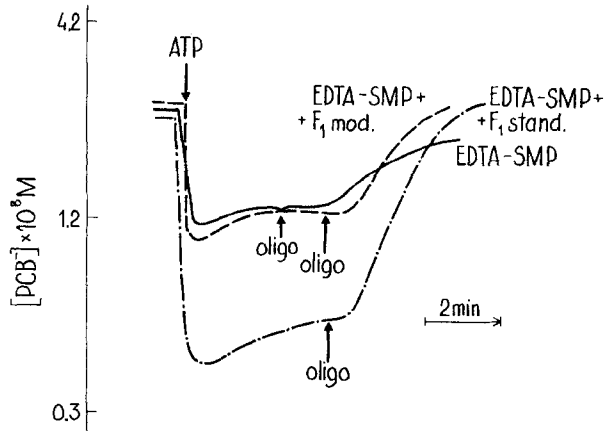

Figure 3. ATP hydrolysis-coupled absorption of PCB^- anions by EDTA-SMP preparations. For the composition of the incubation medium see Fig. 2. Additions: ATP, 3 mM; oligomycin, 2 μg per ml of the medium.

reconstituted with F_1 devoid of the γ and δ subunits retain about 10% of oligomycin-insensitive ATPase activity.

In contrast to EDTA-SMP, Na^+-SMP cannot be reconstructed with factor F_1 containing no γ and δ subunits, although a standard preparation of F_1 increases ATPase activity by about 60% (Table I).

The results obtained suggest that γ and δ subunits, which, in our opinion, originate from the seventh (decomposed) protein globule of F_1 [2], play a role in the formation of specific contacts between F_1 and the mitochondrial membrane. The role of the γ and δ subunits is particularly important when Na^+-SMP is used for reconstruction. The SMP obtained in milder conditions (EDTA-SMP) can be reconstructed with factor F_1 which has no γ and δ subunits. However, in this case some defects in the reconstruction process take place resulting in the loss of the recoupling F_1 activity and partial desensibilization of the ATPase system to oligomycin.

Salton and Shoor [10] and Bragg et al. [11] emphasized the role of the minor subunits in the processes of interaction of the bacterial coupling factors with the membrane. A comparison of their results with our data allowed more structural similarity between bacterial ATPases and the enzyme from beef-heart mitochrondria to be revealed.

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