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RECONSTITUTION OF ATPase FROM THE ISOLATED SUBUNITS OF COUPLING FACTOR F_1'S OF *ESCHERICHIA COLI* AND THERMOPHILIC BACTERIUM PS3

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SUMMARY: ATPase was reconstituted from mixtures of isolated subunits of coupling factor, F_1 ATPase of *E. coli* (EF_1) and thermophilic bacterium PS3 (TF_1); ability to hydrolyze ATP was attained from the combination of α and β subunits from EF_1 and γ subunit from TF_1, α and β from TF_1 and γ from EF_1, and α and γ from EF_1 and β from TF_1. The β subunit of TF_1 also could complement the EF_1 from an *E. coli* mutant defective in this subunit. This is the first demonstration of interspecies *in vitro* recombination of ATPase activity from isolated subunits.

The proton-translocating ATPases(F_1-F_o) in membranes of chloroplasts, mitochondria and bacteria synthesize ATP utilizing energy from the electron transport chains (1-4). The coupling factor F_1 is a peripheral membrane complex composed of 5 different subunits (α, β, γ, δ, and ε), and has ATPase activity. Recently the F_1's of the thermophilic bacterium PS3 (5,6) and *E. coli* (7,8), abbreviated as TF_1 and EF_1, respectively in this communication, were dissociated into individual subunits. None of the isolated subunits from TF_1 or EF_1 had ATPase activity when assayed individually (5-8). However, F_1 ATPase activity of TF_1 could be reconstituted from combinations of different subunits as long as the β subunit was present. On the other hand, only a mixture of α, β, and γ subunits of EF_1 reconstituted ATPase activity, and a mixture of any two of the subunits did not give activity (7). This three subunits complex was active as a functional F_1 after binding δ and ε subunits (8).

Abbreviations used are : F_1, that portion of the proton-translocating ATPase which is peripheral to the membrane; TF_1, the F_1 of a thermophilic bacterium PS3; EF_1, the F_1 of *E. coli*; $\alpha^T,\beta^T,\gamma^T$, the subunits of TF_1 in order of decreasing molecular weight; $\alpha^E,\beta^E,\gamma^E$, the subunits of EF_1; anti EF_1, antiserum against EF_1; anti β^T, antibody against $TF_1\beta$.

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To understand the assembly process of the F_1 complex, it became of interest to study reconstitution of ATPase from mixtures of subunits from TF_1 and EF_1. In the present communication we show that combinations of $\alpha^E + \beta^T + \gamma^E$(mixture of α and γ subunits from EF_1 and β subunit from TF_1), $\alpha^E + \beta^E + \gamma^T$, or $\alpha^T + \beta^T + \gamma^E$ gave ATPase activity after reconstitution. This observation suggests that subunits from different origins have similar roles in forming the catalytic portion of the ATPase.

MATERIALS AND METHODS

Preparations. Subunits of TF_1 were purified in the presence of 8 M urea by ion exchange chromatography (5,6). Subunits of EF_1 were purified by hydroxyapatite and EDAE-Sepharose chromatography from EF_1 dissociated after cold treatment and freeze-thawing (8). All subunits used in the present study were homogeneous by sodium dodecylsulfate polyacrylamide gel electrophoresis, as shown previously (5-8). Molecular weights used for the calculation of subunit ratios were values determined by Yoshida *et al.* (9). Antiserum against EF_1(anti EF_1) (10) and antibody against $TF_1\beta$(anti β^T) (9) were obtained as described previously. Mutant EF_1 was prepared from *E. coli* strain AN120 (*uncA401*) (11) or KF11 (*uncD11*) (12) by the published procedures. Other materials were as described previously (5-8).

Assays. ATPase activity was assayed as described previously (13) and one unit of the enzyme was defined as the amount hydrolyzing 1 μ mol of ATP per min at 37° under standard conditions (13). Protein was assayed by the method of Lowry *et al.* (14).

Reconstitution of ATPase from subunits. Subunits from EF_1 and TF_1 were mixed in 200 μl of 0.01 M succinate-Tris, pH 6.0, containing 0.2 M KCl, 10% glycerol and 0.3 mM β-mercaptoethanol. The mixture was dialyzed at 23° for about 8 hours against 400 ml of EF_1 reconstitution buffer (7) (0.05 M succinate-Tris, pH 6.0, containing 10% glycerol, 0.3 mM β-mercaptoethanol, 0.1 mM EDTA, 2 mM ATP and 2 mM $MgCl_2$).

RESULTS AND DISCUSSION

Reconstitution of ATPase from isolated subunits of EF_1 and TF_1. A combination of three subunits (α, β, and γ) from either EF_1 or TF_1 reconstituted ATPase activity, as shown previously (5-8). In this study one subunit was omitted from each combination, and the corresponding subunit from the different F_1 was added. As shown in Table 1, ATPase activity was reconstituted from the combination of $(\alpha^E + \beta^E + \gamma^T)$ or $(\alpha^T + \beta^T + \gamma^E)$, indicating that the γ subunit was mutually interchangeable between the two F_1 complexes during reconstitution. ATPase activity was also reconstituted from the combination of $(\alpha^E + \beta^T + \gamma^E)$, whereas

Table 1 Reconstitution of ATPase Activity from Combinations of Subunits of TF_1 and EF_1

Buffer		Subunit EF_1 α	β	γ	TF_1 α	β	γ	ATPase Activity Reconstituted (units/mg protein)
Exp.1 EF_1 reconstitution buffer								
	A. 1.	+	+	+				36.0
	2.	+	+					0.17[a]
	3.		+	+	+			0.00
	4.	+		+				0.00
	5.	+		+		+		2.25
	6.	+	+				+	2.10
	B. 1.				+	+	+	0.68[b]
	2.			+	+	+		1.48
	3.		+		+		+	0.00
	4.	+				+	+	0.00
Exp.2 EF_1 reconstitution buffer - ATP								
	1.	+	+				+	0.02
	2.	+		+		+		0.00
	3.			+	+	+		0.81

Subunits from EF_1 and TF_1 were mixed and dialyzed against EF_1 reconstitution buffer (Exp.1) or the same solution from which ATP was omitted (Exp.2). Amounts of subunits used were: α^E, 10.5 μg; β^E, 11.2 μg; γ^E, 1.8 μg; α^T, 10.0 μg; β^T, 11.0 μg; γ^T, 1.8 μg. After dialysis, ATPase and protein were assayed as described in the text. The following controls did not form ATPase activity: $(\alpha^E + \beta^E + \alpha^T(\text{or } \beta^T))$; $(\alpha^E + \gamma^E + \alpha^T(\text{or } \gamma^T))$; $(\beta^E + \gamma^E + \beta^T(\text{or } \gamma^T))$.

a) Although the mixture of α + β prepared from *E. coli* by hydrophobic column chromatography formed about 3-10% of the maximal activity (α + β + γ) (7), the mixture from the present preparation reconstituted only very low activity. The difference of the results between two preparations may be partly due to the amount of γ subunit contamination in the α and β subunit preparations used in the previous studies (7,8).

b) Slightly higher activity (0.85 unit/mg protein) was obtained when the three subunits were mixed in the same solution containing 4 M urea and dialyzed against reconstitution buffer. Other combinations did not reconstitute in this condition, except that $\alpha^T + \beta^T + \gamma^E$ gave 41% of the activity obtained without urea. The specific activity of TF_1 ATPase reconstituted $(\alpha^T + \beta^T + \gamma^T)$ was about half the value previously reported (5,6). This difference may be because EF_1 reconstitution buffer was used instead of TF_1 reconstitution conditions. It should be noted that TF_1 is usually assayed at 60° or 65° (6,20), although all enzyme activities were assayed at 37° in this experiment. Optimum temperature of TF_1 is reported to be 75°: Specific activities of TF_1 at 37°, 60°, and 75° were about 5,10 and 22 units/mg protein, respectively (20).

$(\alpha^T + \beta^E + \gamma^T)$ did not reconstitute. In contrast the α subunit of TF_1 or EF_1 did not reconstitute ATPase activity after mixing with β and γ subunits from different origin, suggesting that an active ATPase was not formed because the α subunits are species specific.

The specific activities of the ATPase reconstituted from subunits of mixed origins were lower than the activity reconstituted from EF_1 subunits alone (7,8).

However, it was about the same value as that of the ATPase reconstituted from TF_1 subunits.

As shown previously, ATP is required for reconstitution of EF_1 (7,8), but not for that of TF_1 (5,6). As shown in Table 1 (compare Exp. 1 and 2), ATP was essential to reconstitute ATPase from combinations including the α subunit from EF_1 ($\alpha^E + \beta^E + \gamma^T$ and $\alpha^E + \beta^T + \gamma^E$). On the other hand significant activity was reconstituted from the combination of ($\alpha^T + \beta^T + \gamma^E$) without ATP (Table 1, Exp. 2). These results suggest that interaction of the α subunit of EF_1 with nucleotide may be essential in the assembly with other subunits. In this regard a change in the conformation of this subunit after ATP binding has been reported (15). A combination of ($\alpha^T + \beta^E + \gamma^E$) did not reconstitute ATPase. This may be due to the difference in nucleotide binding properties of the $TF_1\alpha$ and $EF_1\alpha$ subunits(8,16).

Effect of the ratio of subunits from TF_1 and EF_1 on the reconstitution. In the experiment shown in Fig. 1a, the amounts of α and β subunits from EF_1 were held constant, while the amount of γ from TF_1 was varied. As the concentration of γ was increased (i.e. the α/γ molar ratio was lowered), the specific activity

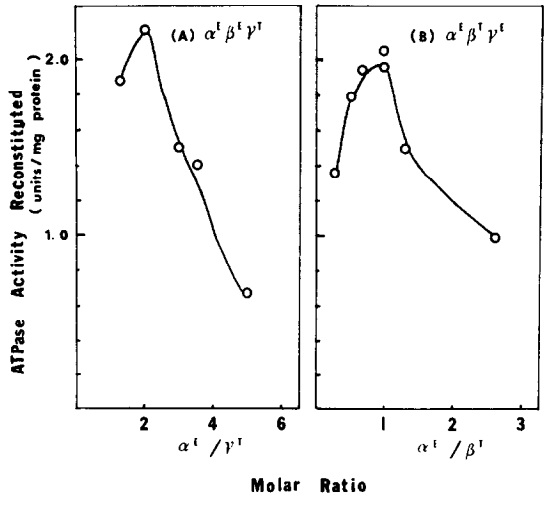

Fig. 1 Effect of the ratio of subunits from TF_1 and EF_1 on the reconstitution of ATPase activity
A) Various amounts of γ^T were mixed with α^E (10.3 µg) and β^E (11.0 µg).
B) Various amounts of β^T were mixed with α^E (10.3 µg) and γ^E (1.8 µg). Other procedures were as described in the text.

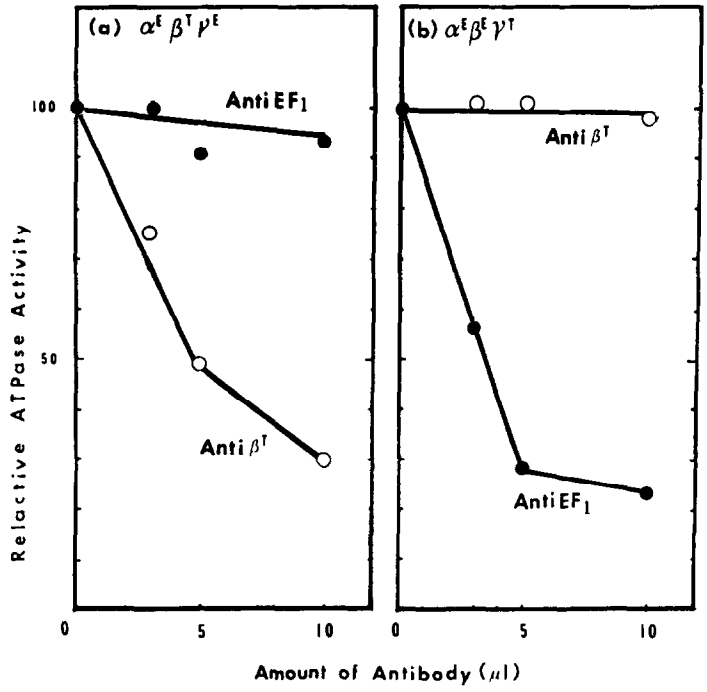

Fig. 2 Effects of antibodies on ATPase activity reconstituted from subunits of TF_1 and EF_1

ATPase activity of the reconstituted complex (0.5-2.0 μg protein) was measured in the presence of different amount of antibodies (anti EF_1, ●—● ; anti β^T, o—o), and expressed as relative activity. A) ATPase activity reconstituted from ($\alpha^E + \beta^E + \gamma^T$). B) ATPase activity reconstituted from ($\alpha^E + \beta^T + \gamma^E$). Amounts of subunits used for reconstitution were as described in the legend to Table 1. Other conditions were as described in the text.

of the reconstituted ATPase increased to a maximal level at an α/γ (α, EF_1; γ, TF_1) molar ratio of 2 and decreased significantly at a ratio lower than 2. In a similar experiment in which the amount of β was varied, maximal reconstitution was observed at an α/β (α, EF_1; β, TF_1) molar ratio of about 1 (Fig. 2b). These results suggest that a complex with stoichiometric amounts of polypeptides was formed after reconstitution. However, it is difficult to conclude the exact stoichiometry of subunits in the reconstituted complex, mainly because the reconstitution may not be complete.

Effect of antibodies on the reconstituted ATPase. The ATPase reconstituted from ($\alpha^E + \beta^T + \gamma^E$) was inhibited by anti β^T, but not by anti EF_1, confirming that $TF_1\beta$ is the catalytic subunit in the reconstituted ATPase (Fig. 2a). On immunodiffusion a precipitin line was observed between anti EF_1 and this ATPase

complex, suggesting that the antibodies reacted with the ATPase but did not inhibit. Anti EF_1 did not react with TF_1 or its subunits, and anti β^T did not react with other TF_1 subunits, EF_1, or EF_1 subunits. These control experiments indicate that the antibodies used in the present study were specific.

Essentially the same experiment was performed on the ATPase reconstituted from ($\alpha^E + \beta^E + \gamma^T$) (Fig. 2b). This ATPase was inhibited by anti EF_1 but not by anti β^T. Anti EF_1 reacted with isolated α or β subunits of EF_1 in immunodiffusion assay (data not shown). However, its reaction with the β subunit is essential to obtain inhibition of ATPase activity, because the antibody had no effect on the activity of the complex reconstituted from ($\alpha^E + \beta^T + \gamma^E$) (Fig. 2a).

Complementation of a mutant EF_1 (defective in β subunit) with $TF_1\beta$ subunit. The β subunit of TF_1 could complement a mutant EF_1 defective in the β subunit, confirming the above results showing that ($\alpha^E + \beta^T + \gamma^E$) reconstitute ATPase. The EF_1 from strain KF11 has low Mg^{2+}-ATPase activity but high Ca^{2+}-ATPase due to the mutation in β subunit: the ratio of Ca^{2+}-ATPase to Mg^{2+}-ATPase of the wild-type and the mutant F_1 were 0.8 and 3.5, respectively (12). In the experiment shown in Fig. 3, the amount of the dissociated mutant F_1 was kept constant, while the amount of β from TF_1 was varied. As the amount of β was increased, the Ca^{2+}-ATPase activity of the reconstituted complex decreased and the Mg^{2+}-ATPase activity increased to the approximate ratio of Ca^{2+}-ATPase to Mg^{2+}-ATPase of 0.9 (similar ratio to TF_1 or wild-type EF_1). The reconstituted ATPase was inhibited by antibodies against the β subunit from TF_1, whereas the defective EF_1 from the mutant KF11 was insensitive (data not shown). A similar experiment was performed using EF_1 from strain AN120 (17), which is defective in the α subunit (11,18, 19). Addition of the $TF_1\alpha$ subunit to the dissociated EF_1 from AN120 did not give ATPase activity (Fig. 3), confirming the above results that ($\alpha^T + \beta^E + \gamma^E$) did not reconstitute ATPase activity (Table 1).

The subunit nomenclature of F_1 is originally based on the mobility in polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate. The

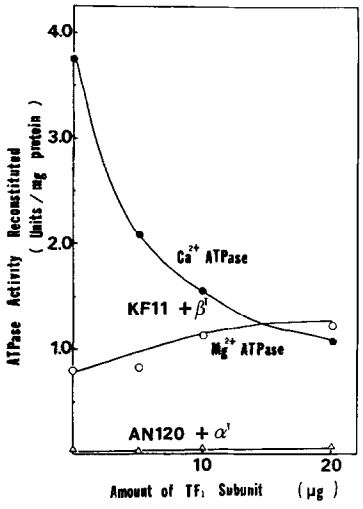

Fig. 3 *In vitro* complementation of mutated EF_1's with TF_1 subunits Defective EF_1 from AN120 (*uncA401*) (Δ—Δ) or KF11 (*uncD11*) (●—● and ○—○) was dissociated after cold treatment and freeze-thawing, and mixed with varying amounts of isolated subunits from TF_1. The mixture was dialyzed against reconstitution buffer, and ATPase (Mg^{2+}-dependent activity (13), Δ—Δ and ○—○; Ca^{2+}-dependent activity (12), ●—●) was assayed as described previously. Amounts of defective F_1 used were 43 μg protein for KF11 and 24 μg protein for AN120.

present results suggest that the β and γ subunits from EF_1 and TF_1 have similar functions because these subunits from EF_1 could be replaced by those from TF_1. It must be stressed that the energy transducing apparatus from entirely different bacteria have similarities. The thermophilic bacterium PS3 was isolated in a Japanese hot spring and is able to grow at 80° (maximum temperature) (20), whereas *E. coli* is of intestinal origin and is unable to grow at temperatures higher than 45°. Moreover, the former is a Gram-positive spore former, while the latter is Gram-negative.

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