

$\alpha_3\beta_3$ complex of thermophilic ATP synthase

Catalysis without the γ -subunit

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A complex of the α - and β -subunits of thermophilic ATP synthase showed about 25% of the ATPase activity of the $\alpha\beta\gamma$ complex. The $\alpha_3\beta_3$ hexamer structure was analyzed by sedimentation (11.2 S) and gel filtration (310 kDa). Dilution of the $\alpha\beta$ complex caused dissociation of the complex and rapid loss of ATPase activity which was restored by addition of the γ -subunit. A previous method using urea for isolating the subunits resulted in an $\alpha\beta$ complex with lower activity than that prepared by over-expression of the genes. The $\alpha\beta$ -ATP complex was formed from the $\alpha\beta$ complex, ADP and P_i in the presence of dimethyl sulfoxide.

F_1 -ATPase; Oligomer, $\alpha_3\beta_3$; ATPase; ATP synthesis; (Thermophilic bacteria)

1. INTRODUCTION

ATP synthase is a major enzyme for the energy supply of cells. Its catalytic portion is termed F_1 and consists of the α , β , γ , δ and ϵ subunits [1-3]. On reassembly of purified subunits, only the complexes containing $\alpha\beta\gamma$ of *E. coli* F_1 (EF_1) ATPase showed activity [3]. As truncation [3] and cross-linking [4] of γ resulted in loss of ATPase activity, the essential role of γ during ATP synthesis was proposed [4]. In contrast to these results obtained with EF_1 , a mixture of α and β of thermophilic F_1 (TF_1) showed weak ATPase activity [2,5], complex formation [5] and conformational $\alpha\beta$ interactions detected by H-D exchange reactions [6]. These

results on the $\alpha\beta$ complex have been neglected by others working on F_1 . Recently, the subunits of TF_1 were sequenced [7] and prepared by over-expression of their respective genes [7]. The over-expressed TF_1 subunits were more active than urea-treated subunits ($u\alpha$, $u\beta$) prepared according to classical methods [2,8]. The ATPase activity of the $\alpha\beta$ complex, prepared with our genes [7], showed asymmetric and allosteric properties [9]. As enzyme-bound ATP is synthesized by TF_1 from ADP and P_i [10], the $\alpha\beta$ complex is expected to display this activity.

This paper reports determination of the hexamer structure and activity of the $\alpha_3\beta_3$ complex which dissociates easily.

2. MATERIALS AND METHODS

TF_1 , $u\alpha$ and $u\beta$ were prepared by the urea method [8]. Thermophilic α , β and γ were obtained by over-expression of the respective genes [7], in *E. coli* strain DK8 lacking all EF_1 genes. Further purification of α and β to microcrystals, using a Green A column, was carried out as described (Shirakibara, Y., to be reported elsewhere). Gel filtration and sedimentation analysis of the $\alpha\beta$ mixture were performed as described in the legends to figs 1 and 2, respectively, and for ATPase activity, according to

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Abbreviations: F_1 , catalytic portion of ATP synthase; TF_1 , F_1 from thermophilic bacterium PS3; EF_1 , F_1 from *E. coli*; AMPPNP, adenylyl-5'-yl imidodiphosphate; DMSO, dimethyl sulfoxide; DTT, dithiothreitol; Mes, 4-morpholineethanesulfonic acid; α , β and γ , subunits of F_1 ; HPLC, high-performance liquid chromatography

the legends to figs 3 and 4. ATP synthesis was measured by adding the $\alpha\beta$ -ADP complex (final 2 mg/ml) to 20 mM $^{32}\text{P}_i$ (8200 cpm/nmol), 40% DMSO, 80 mM Mes-NaOH (pH 6.0) and 2 mM MgSO_4 as in [10].

3. RESULTS AND DISCUSSION

3.1. Association of the α - and β -subunits into the $\alpha_3\beta_3$ complex

The mixture of α and β eluted to give two distinct peaks corresponding to 310 and 53 kDa, respectively, on gel filtration (fig.1). The larger peak corresponded to $\alpha_3\beta_3$ (319 582 Da from the DNA sequence [7]), which eluted soon after that of TF₁ (380 kDa by HPLC [11]). The smaller peak contained both α and β . When analyzed separately, both α and β gave only a single peak at about 53 kDa. Similarly, two components were observed during sedimentation analysis (fig.2). The rapidly sedimenting component, 11.2 S, corresponded to $\alpha_3\beta_3$, which sediments more slowly than F₁ (11.9 S [12]). The *s* value of the smaller component, 4.1 S, corresponded to that of the mixture of free α and β . Polyacrylamide gel electrophoresis of the mixture of α and β [2] revealed no additional band apart from those of α and β due to dilution for 2 h. When the mixture of α and β was diluted from 5 to 1 mg/ml, the $\alpha\beta/(\alpha+\beta)$ ratio in the HPLC elution curve decreased (fig.1). A symmetrical arrangement of deuterated α - and β -subunits in the hexamer was shown by cold neutron scattering (with Ito, Y., Harada, M., Schefer, J. and Schoenborn, B.P., in preparation).

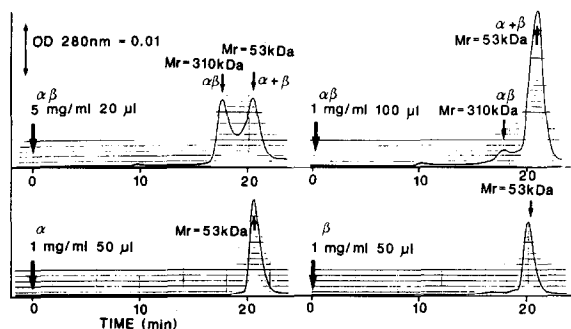


Fig.1. Gel filtration of the $\alpha\beta$ mixture. The indicated amounts of samples were analyzed in a Waters model 441 HPLC apparatus with a column of TSK gel G 400 SW (7.5×600 mm) at 22°C , and samples eluted with 0.1 M NaCl and 0.1 M Tris-Cl ($\text{pH } 7.8$) at a rate of 1 ml/min .

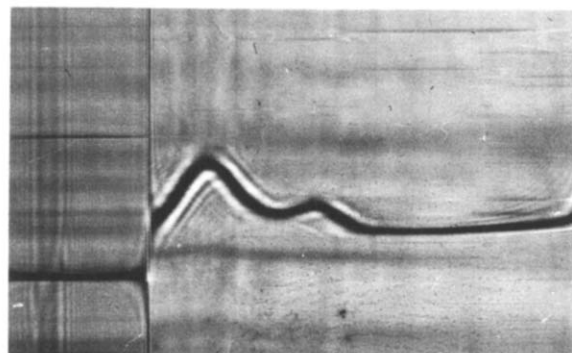


Fig.2. Sedimentation pattern of a mixture of α - and β -subunits at 40 min. The mixture of α and β [(1:1, 6.3 mg/ml, in 50 mM Tris-HCl (pH 7.75), 0.1 mM EDTA and 0.1 mM DTT)] was analyzed in a Beckman Spinco model E with rotor An-D, at 48 000 rpm and 20.0°C. $s_{20,w}$ values were 11.210 S (right) and 4.091 S (left).

3.2. Catalytic activities of the easily dissociating $\alpha_3\beta_3$ complex

The low ATPase activities were reconstituted when subunits were added directly to a cuvette (fig.3). The very weak ATPase activity of mesophilic β [13] was also detected even in β of crystalline purity, but was removed by site-directed

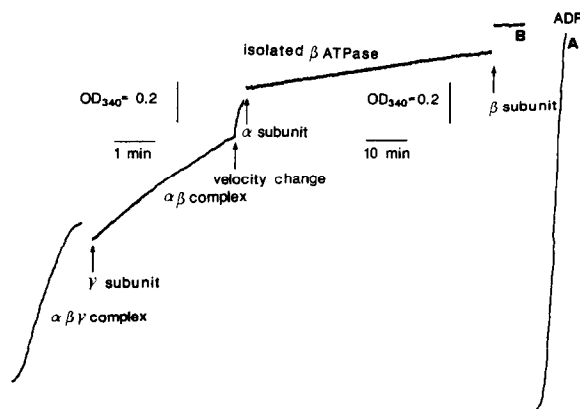


Fig. 3. ATPase activity of the β -subunit, and mixtures of the α - and β -, and α -, β - and γ -subunits. Activity was measured by coupling to the oxidation of NADH in a Beckman model 35 spectrophotometer (at 340 nm) in the presence of 4 mM ATP, 5 mM phosphoenolpyruvate, 0.3 mM NADH, 50 mM Tris- SO_4 (pH 7.9) and 4 $\mu\text{g}/\text{ml}$ each of lactate dehydrogenase and pyruvate kinase in a final volume of 0.25 ml at 22°C. (A) 5 μl of 0.2 M ADP. (B) At the times indicated by arrows, 80 μg subunit β (20 μl), 80 μg subunit α (20 μl) and 20 μg subunit γ (20 μl , turbid suspension) were added; ATPase activities were 1.6, 81 and 320 nmol/min per mg β , respectively.

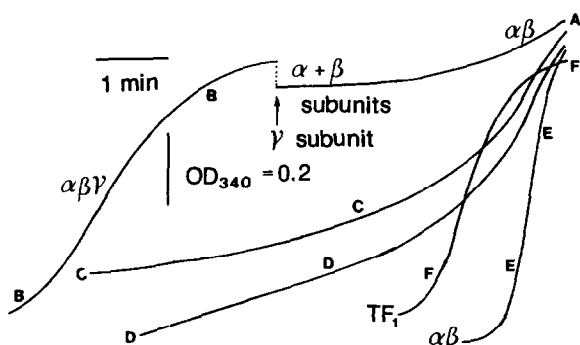


Fig.4. Inactivation of the $\alpha\beta$ ATPase by dilution, and its reactivation by the γ -subunit. Using a Beckman model DU70 spectrophotometer, the following amounts of the mixture of α and β (1:1, 4 mg/ml) or TF_1 were added to 50 μ l of the reaction medium described in the legend to fig.3. (A) 2 μ g mixture (0.5 μ l, final 39.6 μ g/ml), (B) 20 μ g subunit γ (1 μ l) added to (A), (C) 5.6 μ g mixture (1.4 μ l), (D) 8 μ g mixture (2 μ l), (E) 20 μ g mixture (5 μ l, final 363.6 μ g/ml), and (F) 2 μ g TF_1 (5 μ l, final 36.4 μ g/ml).

mutagenesis of the catalytic site of β [14]. After preincubation of α with β (4 mg/ml each, 22°C), the specific ATPase activity of the $\alpha\beta$ mixture was 556 nmol/min per mg ($K_m = 0.36$ mM) (fig.4E), while that of $\alpha\beta\gamma$ amounted to 1.9 μ mol/min per mg ($K_m = 0.28$ mM). Dilution of the mixture of α and β caused rapid inactivation, recovery being achieved by the addition of γ (fig.4A,B). Previous ATPase assays ($\alpha\beta < 40$ μ g/ml, 10 min incubation) [2,5] underestimated the activity of $\alpha\beta$. The $\alpha\beta$ was AMPPNP-sensitive and azide-insensitive. The mixture of $u\alpha$ and $u\beta$ showed low ATPase activity (11–34 nmol/min per mg β). Moreover, once $u\alpha$ had been mixed with β , the addition of α did not reconstitute the activity of the mixture of α and β . Similarly, restoration of the ATPase activity of the $u\beta + \alpha$ mixture by the addition of β was poor, indicating the formation of less active $u\alpha u\beta$, $u\alpha\beta$ and $u\beta\alpha$ complexes. Enzyme-bound [32 P]ATP [10] (0.202 mol ATP/mol $\alpha_3\beta_3$) was synthesized by $\alpha\beta$ in 40% DMSO. The extent of carbamoylation of the subunits with 8 M [14 C]urea (185 kBq/mmol) during the isolation procedure [8] was slight (1.02 mol/mol α and 0.54 mol/mol β).

Studies on EF_1 [3,4], in contrast to our findings with TF_1 - $\alpha\beta$, indicated that γ is essential for ATPase activity. These variations may be due to differences between unstable EF_1 and stable TF_1 [1]. Considering the highly conserved structures of both the α - and β -subunits in different species [1–3,7], the role of the γ -subunit [3,4] should be reassessed.

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