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Minireview

The $\alpha\beta$ Complexes of ATP Synthase: the $\alpha_3\beta_3$ Oligomer and $\alpha_1\beta_1$ Protomer

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The basic structures of the catalytic portion (F_1, $\alpha_3\beta_3\gamma\delta\varepsilon$) of ATP synthase are the $\alpha_3\beta_3$ hexamer (oligomer with cooperativity) and $\alpha_1\beta_1$ heterodimer (protomer). These were reconstituted from the α and β subunits of thermophilic F_1 (TF_1), and the $\alpha_3\beta_3$ hexamer was crystallized. On electrophoresis, both the dimer and hexamer showed bands with ATPase activity. Using the dimer and hexamer, we studied the nucleotide-dependent rapid molecular dynamics. The formation of the hexamer required neither nucleotide nor Mg. The hexamer was dissociated into the dimer in the presence of MgADP, while the dimer was associated into the hexamer in the presence of MgATP. The hexamer, like mitochondrial F_1 and TF_1, showed two kinds of ATPase activity: one was cooperative and was inhibited by only one BzADP per hexamer, and the other was inhibited by three BzADP per hexamer.

KEY WORDS: ATP synthase; F_1; oligomer; protomer; molecular dynamics.

INTRODUCTION

ATP synthase (F_0F_1) catalyzes oxidative and photosynthetic phosphorylation (Kagawa, 1972; Racker, 1976; Cross, 1981; Amzel and Pedersen, 1983; Kagawa, 1984; Senior, 1988; Futai *et al.*, 1989). The enzyme uses the energy of a protonmotive force generated by the redox chain to synthesize ATP (Mitchell, 1979). In fact, the proton current through F_0F_1 was demonstrated in both liposomes (Kagawa and Racker, 1971; Kagawa, 1972) and planar lipid bilayers (Muneyuki *et al.*, 1989). F_0F_1 is a multisubunit complex composed of two subcomplexes: a soluble ATPase, F_1, and a proton channel, F_0. The $\gamma\delta\varepsilon$ subunits were shown to form a connecting bridge between the $\alpha_3\beta_3$ hexamer and F_0 (Kagawa, 1978; Kagawa *et al.*, 1979). The asymmetric $\alpha_3\beta_3\gamma$ structure of F_1 was first suggested by Catterall and Pedersen (1971), and was reconstituted from subunits purified from both TF_1 (Yoshida *et al.*, 1977) and *E. coli* F_1 (EF_1) (Dunn and Futai, 1980). The direct interaction between the purified α and β subunits was implicated from the proton–deuteron exchange (Ohta *et al.*, 1980) and the $\alpha\beta$ reconstitution kinetics (Kagawa and Nukiwa, 1981). Thus, the existence of the $\alpha_3\beta_3$ hexamer was predicted (Kagawa, 1978). In fact, the $\alpha_3\beta_3$ hexamer of thermophilic F_1 (TF_1) was isolated by chromatography (Kagawa *et al.*, 1989; Miwa and Yoshida, 1989), ultracentrifugation (Kagawa *et al.*, 1989) and

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[6]Abbreviations: AMPPNP, adenylyl-imidodiphosphate; AMPPSP, adenosine-5′-O-(3-thiotriphosphate); 2-diazidoATP, 3′-arylazido-β-alanyl-2-azido ATP; 8-diazidoATP, 3′-arylazido-β-alanyl-8-azido ATP; BzADP, 3′-O-(4-benzoyl) benzoyl ADP; F_0, proton-channel portion of F_0F_1; F_0F_1, ATP synthase; F_1, catalytic portion of F_0F_1; HPLC, high-performance liquid chromatography; Rg, radius of gyration; PAGE, polyacrylamide gel electrophoresis; TF_1, thermophilic F_1.

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electrophoresis (Harada *et al.*, 1991b). The $\alpha_3\beta_3$ hexamer was crystallized (Shirakibara and Kagawa, to be published). The specific ATPase activity of the $\alpha_3\beta_3$ hexamer was about 15% (2.4 m mol/min/mg protein, at 30°C, pH 8.0) of that of TF_1 (Aloise *et al.*, 1991), which is why the $\alpha\beta$ complex had previously been overlooked. Small-angle X-ray scattering revealed MgADP-dependent dissociation into $\alpha_1\beta_1$ heterodimer (Harada *et al.*, 1991a), which was also purified by both HPLC and PAGE (Ohta *et al.*, 1990; Harada *et al.*, 1991b). The active $\alpha\beta$ complex has also been demonstrated in mesophilic F_1 (Avital and Gromet-Elhanan, 1991).

There have been many hypotheses on the nucleotide-induced conformational change of F_1 during energy transduction (Cross, 1981; Ysern *et al.*, 1988; Senior, 1988; Boyer, 1989). In the "rotational catalysis hypothesis," Gresser *et al.* (1982) speculated that each β subunit "tagged" by the $\gamma\delta\varepsilon$ subunits synthesizes or hydrolyzes ATP during the rotation of the $\alpha_3\beta_3$ hexamer around the $\gamma\delta\varepsilon$ axis.

Here we review the basic structures of F_1, which are the $\alpha_3\beta_3$ hexamer (oligomer) and $\alpha_1\beta_1$ heterodimer (protomer), and examine these hypotheses.

ISOLATION OF THE $\alpha_3\beta_3$ HEXAMER AND $\alpha_1\beta_1$ DIMER

The isolation of both the $\alpha_3\beta_3$ hexamer (M_r = 319,581) and $\alpha_1\beta_1$ dimer (M_r = 106,527) by HPLC and PAGE is the most convincing evidence for their existence. Since the HPLC and PAGE depend on the moving liquid phase, the equilibrium and dynamics of the hexamer–dimer interconversion were analyzed by the small-angle X-ray scattering of the $\alpha\beta$ mixtures. The reaction kinetics was analyzed by synchrotron radiation to the dimer and hexamer in a stopped-flow apparatus.

The $\alpha_3\beta_3$ hexamer was formed in a mixture ($\alpha:\beta$ = 1:1) of the α and β subunits (Kagawa *et al.*, 1989), which were obtained by overexpressing the genes for these subunits (Ohta *et al.*, 1988). The molecular weights of the α (54589.89) and β (51937.58) subunits were determined by sequencing the thermophilic F_0F_1 gene (Ohta *et al.*, 1988). In contrast to the reconstitution of the $\alpha_3\beta_3\gamma$ complex of mesophilic F_1, which strictly requires MgATP or MgADP (Dunn and Futai, 1980), the reconstitution of the $\alpha_3\beta_3$ hexamer does not require any nucleotide or Mg^{2+} (Kagawa *et al.*, 1989). The $\alpha_1\beta_1$ dimer was obtained by adding MgATP to the $\alpha_3\beta_3$ hexamer (Ohta *et al.*, 1990; Harada *et al.*, 1991a). The reconstitution stimulating thermophilic chaperonin 61 K is homologous to the α subunit (Tamada *et al.*, 1991) and was also shown to stimulate formation of the mesophilic $\alpha\beta$ complex (Avital and Gromet-Elhanan, 1991).

On gel permeation HPLC, the $\alpha_3\beta_3$ hexamer was eluted at 320 KDa in the absence of nucleotide and Mg^{2+}, and the $\alpha_1\beta_1$ dimer at 107 KDa in the presence of MgADP. The $\alpha_3\beta_3$ hexamer thus purified was crystallized by a similar method to TF_1 (Shirakibara *et al.*, 1991). When the peak fraction of 107 KDa was rechromatographed after removal of the MgADP using a centricolumn equilibrated with the control buffer, it reassembled and gave a peak at 320 KDa, with peaks of the partially dissociated α and β monomers, due to dilution. Thus, the nucleotide-dependent dimer–hexamer interconversion is reversible. On PAGE, both the dimer and hexamer gave bands of material with ATPase activity (relative mobilities; $TF_1:\alpha_3\beta_3:\alpha:\alpha_1\beta_1:\beta$ = 1:1.3:2.1:2.9:3.6, in 7.5% acrylamide separation gel at pH 8.8). The apparent ATPase activity of the $\alpha_1\beta_1$ dimer may be mainly due to reassociated $\alpha_3\beta_3$ produced in the conditions for ATPase assay. Both the dimer and hexamer were composed of equal amounts of the α and β subunits, as shown by a second PAGE of these $\alpha\beta$ complexes in the presence of sodium dodecyl sulfate (Ohta *et al.*, 1990).

MOLECULAR SHAPES OF THE $\alpha_3\beta_3$ HEXAMER AND $\alpha_1\beta_1$ DIMER

The molecular size and shape of the $\alpha_3\beta_3$ hexamer (R_g = 45.4 ± 0.2 Å) and $\alpha_1\beta_1$ dimer (R_g = 34.7 ± 0.2 Å) were deduced from small-angle X-ray scattering data (Fig. 1). The $\alpha_1\beta_1$ heterodimer, not the $\alpha\alpha$ and $\beta\beta$ dimers, was the possible molecular species, because X-ray scattering of solutions of pure α (R_g = 30.9 ± 0.5 Å) and pure β (R_g = 28.0 ± 0.4 Å) subunits each showed only one molecular species. TF_1 (R_g = 49.2 ± 0.1 Å) was larger than the $\alpha_3\beta_3$ hexamer. In the absence of ADP, the $\alpha_3\beta_3$ hexamer was not dissociated by dilution to 1.15 mg/ml, or by the addition of Mg, Pi, or Pi + Mg.

The $\alpha_3\beta_3$ hexamer was a hexagon with a central hole that was partially filled with the minor subunits in TF_1 (Yoshimura *et al.*, 1989). The hexagonal structure with tilted subunits (Fig. 1a) is similar to that deduced from X-ray crystallography of liver F_1

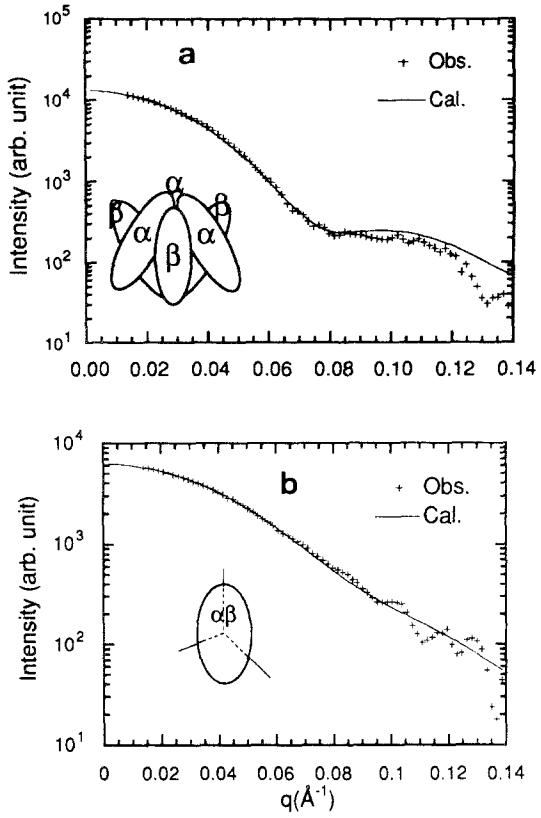

Fig. 1. Molecular shape of the $\alpha_3\beta_3$ hexamer (a) and the $\alpha_1\beta_1$ dimer (b) deduced from the small-angle X-ray scattering data. The scattering parameter q is defined as $q = 4\pi \sin\theta/\lambda$, where 2θ is the scattering angle and λ is the wavelength of the 1.541 Å (Cu$K\alpha$) X-ray. Neutron ellipsoidal values (Ito *et al.*, 1990) were used for the α subunit (40.8 × 40.8 × 106.0 Å) and the β subunit (40.0 × 40.0 × 112.0 Å). The best fit for the $\alpha_3\beta_3$ hexamer was obtained by using a tilt angle of 30° with respect to the hexamer's symmetrical axis. The best fit for the $\alpha_1\beta_1$ dimer gave a triaxial ellipsoid (a = 105.2 Å, b = 39.4 Å, and c = 108.2 Å) (Harada *et al.*, 1991a).

(Bianchet *et al.*, 1991) and neutron scattering analysis of the $\alpha_3\beta_3\gamma$ (Ito *et al.*, 1990).

PROTOMER AND OLIGOMER ATPASE ACTIVITY

The substrate specificity of the $\alpha_3\beta_3$ hexamer was similar to that of F_1: both hydrolyzed ATP, GTP, and ITP, but not CTP or UTP. The cooperative properties of the allosteric oligomer, such as F_1, were also demonstrated in the $\alpha_3\beta_3$ hexamer by biphasic kinetics and one-hit one-kill chemical modification (Aloise *et al.*, 1991; Yoshida and Allison, 1990). The Lineweaver–Burk plot of ATP hydrolysis catalyzed by the $\alpha_3\beta_3$ hexamer appeared to be biphasic and concave downward at high ATP concentrations. The apparent K_m values (150 and 490 μM) are similar to those of TF_1. Chemical modifications of MF_1, TF_1, and the $\alpha_3\beta_3$ hexamer with MgBzADP revealed two sequential covalent binding patterns for each enzyme. The oligomer activity was inhibited by one MgBzADP per one $\alpha_3\beta_3$ hexamer. After 66% of the oligomer activity had been inhibited, the remaining protomer activity was inhibited by low-affinity binding of one BzADP per $\alpha_1\beta_1$ dimer (Aloise *et al.*, 1991). Chemical modification of Tyr 307 of the β subunit in the $\alpha_3\beta_3$ hexamer by 7-chloro-4-nitrobenzofurazan also showed the one-hit one-kill phenomenon (Yoshida and Allison, 1990).

The term uni-site catalysis refers to ATP hydrolysis at a single catalytic site of multi-site ATPases (Penefsky and Cross, 1991). The ATPase activity of the isolated $\alpha_1\beta_1$ dimer was expected to represent the uni-site catalysis. However, in a micromolar range of ATP concentration, the $\alpha_1\beta_1$ dimers were reassociated into the $\alpha_3\beta_3$ hexamer. In the presence of 0.3 mM ADP, the [γ-^{32}P]ATPase activity of the $\alpha_1\beta_1$ dimer was 35.2 nmol/min/mg (Harada *et al.*, 1991b).

NUCLEOTIDE-DEPENDENT PROTOMER-OLIGOMER INTERCONVERSION

The purified α and β subunits both bind one AT(D)P/subunit and the site-directed mutagenesis identified these binding sites (Futai *et al.*, 1989; Senior, 1988; Yohda *et al.*, 1988). The cross-linking of both subunits with 2-diazidoATP (catalytic site specific) or 8-diazidoATP resulted in formation of the covalent $\alpha_1\beta_1$ dimer (Schaefer *et al.*, 1989). Thus, $\alpha_1\beta_1$ dimer binds the nucleotides at the $\alpha\beta$ interface. Titration of the dissociation of the $\alpha_3\beta_3$ hexamer was performed with various nucleotides in the presence of Mg (Fig. 2). For 50% dissociation of the $\alpha_3\beta_3$ hexamer, the concentration of ATP was 6 mM, and that of ADP was 30 mM. The K_d value for MgADP of the β subunit is 25 mM, while that of the α subunit is only 5.2 mM. ITP and IDP, which react only with the β subunit, also dissociated the hexamer. Therefore, the dissociation was due to interaction between the nucleotide and the β subunit. There are two kinds of $\alpha\beta$ interface in the F_1 molecule (Bianchet *et al.*, 1991), but the cross-linking of the $\alpha\beta$ with 2-diazidoATP (Schaefer *et al.*, 1989) suggested that the catalytic interface is present in the $\alpha_1\beta_1$ dimer.

The apparent dissociation of the $\alpha_3\beta_3$ hexamer by ATP or ITP is caused by the ADP or IDP produced.

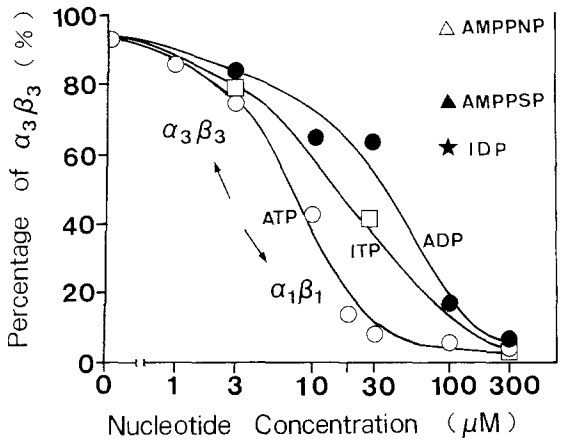

Fig. 2. Effects of various concentrations of nucleotides on the dissociation of the $\alpha_3\beta_3$ hexamer into the $\alpha_1\beta_1$ heterodimer. Gel permeation HPLC was performed as described by Harada *et al.* (1991b). Open circles, ATP; closed circles, ADP; open squares, ITP; other symbols as indicated. (ATP and ITP are hydrolyzed on the hexamer.)

AMPPNP stabilizes the hexamer. Synchrotron radiation combined with the stopped-flow method revealed a rapid ($t_{1/2}$ = 9 sec) MgATP-dependent association of the $\alpha_1\beta_1$ dimer into the $\alpha_3\beta_3$ hexamer, followed by its ADP-dependent dissociation (Sato, M., Kihara, H., Harada, M., Ito, Y., and Kagawa, Y., to be published). This corresponds to ATP-dependent shrinking of F_1 followed by its expansion (6%) (Neidhardt *et al.*, 1991). The one-hit one-kill phenomenon of the $\alpha_3\beta_3$ hexamer also suggests that the oligomeric structure is kept as long as one nucleotide binding site is occupied with ATP.

CONCLUSION

The importance of the asymmetry introduced by the small subunits ($\gamma\delta\varepsilon$) has been pointed out (Ysern *et al.*, 1988). The discovery of both $\alpha_1\beta_1$ dimer and $\alpha_3\beta_3$ hexamer disproved the role of the $\gamma\delta\varepsilon$ subunits in the "rotational catalysis" (Gresser *et al.*, 1982). In the absence of the $\gamma\delta\varepsilon$ subunits, one nucleotide or inhibitor bound to the symmetric $\alpha_3\beta_3$ hexamer induced the asymmetry and allosteric properties of the oligomer (Aloise *et al.*, 1991; Yoshida and Allison, 1990). The nucleotide specificity, two K_m values, presence of the protomer–oligomer activities, and one-hit one-kill phenomenon are common to both F_1 and the $\alpha_3\beta_3$ hexamer. A synchrotron experiment on the ATP hydrolysis cycle revealed the dynamic shrinkage and expansion of F_1 (Neidhardt *et al.*, 1991) that correspond, respectively, to the ATP-induced association and ADP-induced dissociation of the $\alpha_3\beta_3$ hexamer that lacks the binding γ subunit.

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