

A cryoelectron microscopy study of the interaction of the *Escherichia coli* F₁-ATPase with subunit *b* dimer

Stephan Wilkens^a, Stanley D. Dunn^b, Roderick A. Capaldi^{a,*}

^aInstitute of Molecular Biology, University of Oregon, Eugene, OR 97403, USA

^bDepartment of Biochemistry, University of Western Ontario, London, Ont. N6A 5C1, Canada

Received 31 August 1994

Abstract A complex between the *Escherichia coli* F₁-ATPase and a truncated form of the ECF₀-*b* subunit was formed and examined by cryoelectron microscopy in amorphous ice. Image analysis of single particles in the hexagonal projection revealed that the polar domain of the *b* subunit interacts with a β subunit different from the one which interacts with the ϵ subunit. The cavity in the enzyme, visible in the hexagonal projection, is not filled by the *b* polypeptide, therefore leaving enough room for extensive conformational changes of the γ and ϵ subunits within the native F₁F₀ complex.

Key words: Cryoelectron microscopy; F₁-ATPase; ATP synthase; *b* subunit

1. Introduction

An F₁F₀-type ATPase, found in the plasma membrane of bacteria, thylakoid membrane of chloroplasts and the inner membrane of mitochondria, functions to synthesize ATP in response to a light- or respiratory chain-driven proton gradient. This enzyme also works as an ATPase using the hydrolysis of ATP to establish a pH gradient for subsequent use in ion or substrate transport processes.

The simplest F₁F₀-type ATPases structurally are those from bacteria. The enzyme from *Escherichia coli* is made up of a total of eight different subunits; five in the F₁ part ($\alpha_3\beta_3\gamma\delta\epsilon$) and three in the F₀ part (ab_2c_{10-12}) [1–3]. The interaction between F₁ and F₀ is through a narrow stalk of around 45 Å in length [4] which contains subunits of the F₁ part [5], and, in addition, appears to involve the *b* subunits of the F₀. The amino acid sequence of the *b* subunit includes an N-terminal, hydrophobic and membrane-spanning segment, followed by a long hydrophilic, highly charged, C-terminal region. Recently, one of us has genetically produced a truncated form of the *b* subunit from residues 25 to 156, called *b*_{sol}, that represents the polar C-terminal domain of this subunit [6]. The *b*_{sol} forms a stable dimer in solution which interacts with ECF₁, as shown by size-exclusion chromatography and competition experiments, in which *b*_{sol} blocked rebinding of F₁ to intact F₀.

Cryoelectron microscopy studies of ECF₁ and ECF₁F₀ have provided important information about the overall shape, the location of subunits, and the energy-dependent conformational changes that occur in these complexes [4,7–9]. Here, we have used cryoelectron microscopy to study the ECF₁-*b*_{sol} complex. The results show that *b*_{sol} superimposes in the hexagonal projection on a β subunit which is different from the β subunit that interacts with the ϵ subunit.

2. Materials and methods

The truncated, soluble form of the ECF₀-*b* subunit (*b*_{sol}) was isolated and characterized as described by Dunn [6]. ECF₁ was purified from

an overproducing *E. coli* strain AN1460 according to Wise et al. [10] with the modifications described in Gogol et al. [11]. The Fab' fragments derived from a monoclonal antibody against the α subunit used in this study are described in Aggeler et al. [12]. For cryoelectron microscopy, ECF₁ at a concentration of 0.04 mg/ml in 20 mM Tris-HCl, pH 7.2, 100 mM NaCl, 1 mM ethylenediaminetetraacetic acid (EDTA) and 2 mM ATP was mixed with a 5-fold molar excess each of *b*_{sol} and anti- α Fab' fragments and incubated for 30 min at room temperature. The sample was then rapidly frozen for cryoelectron microscopy as described in Gogol et al. [7]. Cryoelectron microscopy and single particle image analysis was conducted as described in Wilkens and Capaldi [9]. For the image analysis, the SPIDER package of programs [13] was used.

3. Results

A complex between ECF₁ and *b*_{sol} was prepared according to Dunn [6], except that for cryoelectron microscopy a 5-fold excess of the *b*_{sol} dimer over F₁ was used to compensate for the moderate binding affinity of *b*_{sol} for F₁ (1–2 μ M) [6].

Fig. 1 shows an electronmicrograph of the ECF₁-*b*_{sol} complex, embedded in a thin layer of amorphous ice. The molecules are additionally labeled with Fab' fragments derived from a monoclonal antibody against the α subunit to ensure a uniform hexagonal orientation of the enzyme complexes in the thin ice layer. For single particle image analysis, a dataset of 603 molecular images was selected from two electron micrographs. The single images were first band pass filtered to remove high and low spatial frequencies, followed by an iterative alignment to the strong asymmetric features of the ECF₁(α I Fab')₃ complex (see below) and finally treated by correspondence analysis and classification methods.

Fig. 2A shows the total average of the dataset after three rounds of alignment; Fig. 2B shows an average of 366 ECF₁(α I Fab')₃ complexes, analysed the same way as the *b*_{sol}-labeled molecules. The major features of the averages in Fig. 2A and B are the three triangularly arranged α subunits, each labeled with an Fab' fragment, the three β subunits, alternating with the α subunits, a central mass located nearest to one of the β subunits and a central cavity located next to a different β subunit.

Wilkens and Capaldi [9] have shown that the ECF₁(α I Fab')₃ complex displays a strong asymmetry in the hexagonal projec-

*Corresponding author. Fax: (1) (503) 346-5891.



Fig. 1. Cryoelectron micrograph of the ECF_1 - b_{sol} complex, additionally labeled with anti- α Fab' fragments to ensure a uniform orientation of the molecules in the ice layer. Electron optical magnification $\times 60,000$. Bar = 50 nm.

tion which manifests itself in the projection intensities of the β subunits, the distances between the β subunits and the location of the central cavity and the central mass. Due to this asymmetry of the complex, the three different β subunits could be distinguished and were called $\beta 1$ (top), $\beta 2$ (bottom left) and $\beta 3$ (bottom right).

There is no obvious additional density visible in the molecule labeled with b_{sol} (Fig. 2A). However, careful examination of the

average indicated a greater density of the bottom left β ($\beta 2$) compared to the average of the unlabeled complex (Fig. 2B). This could be confirmed by subtracting the average shown in Fig. 2B from the average shown in Fig. 2A, creating the difference map shown in Fig. 2C. This difference map displays a strong positive density around the location of $\beta 2$, indicating additional density due to binding of b_{sol} to this particular β subunit.

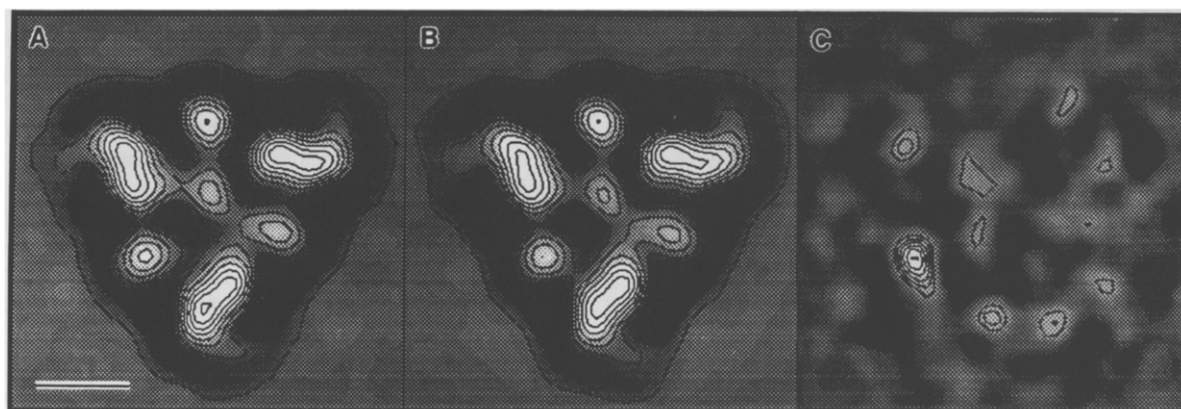


Fig. 2. (A) Total average of a dataset of 603 ECF_1 molecules, labeled with b_{sol} and anti- α Fab' fragments. (B) Average of 366 molecules, labeled with anti- α Fab's only. Due to the inherent asymmetry of the $\text{ECF}_1(\alpha\text{I Fab}')_3$ complex, the three β subunits are distinguishable, with the top β ($\beta 1$) being the strongest one, the bottom left β ($\beta 2$) located next to the cavity and intermediate in density, and the bottom right β ($\beta 3$) smeared out in density (see [9]). (C) Difference map generated by subtracting (B) from (A). Bar = 5 nm.

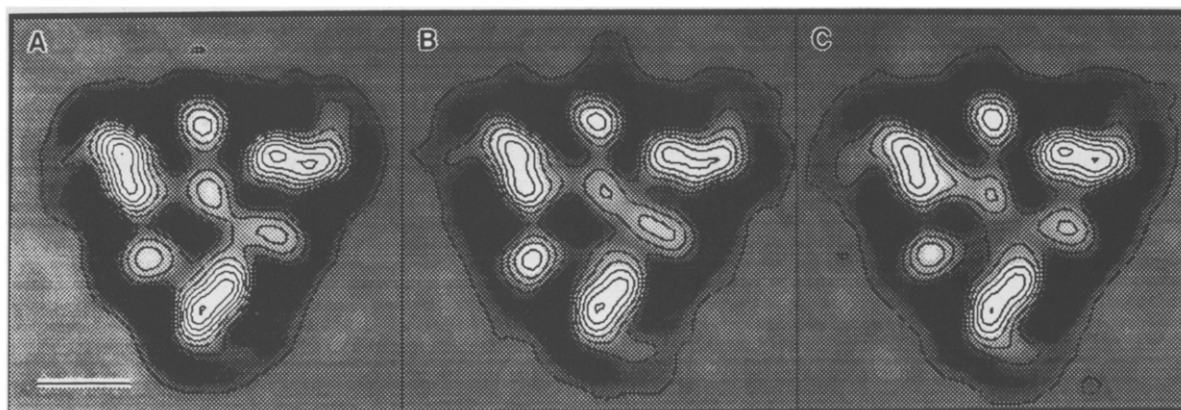
[illegible]

Fig. 4. Sequence homology between the *b* and ϵ subunits according to [16].

- [3] Fillingame, R. (1990) *The Bacteria: Bacterial Energetics*, vol. XII (Krulwich, T.A., ed.) pp. 345–391, Academic Press, New York.
- [4] Gogol, E.P., Lücken, U. and Capaldi, R.A. (1987) *FEBS Lett.* 219, 274–278.
- [5] Abrahams, J.P., Lutter, R., Todd, R.J., vanRaaij, M.J., Leslie, A.G.W. and Walker, J.E. (1993) *EMBO J.* 12, 1775–1780.
- [6] Dunn, S.D. (1992) *J. Biol. Chem.* 267, 7630–7636.
- [7] Gogol, E.P., Aggeler, R.J., Sagermann, M. and Capaldi, R.A. (1989) *Biochemistry* 28, 4717–4724.
- [8] Lücken, U., Gogol, E.P. and Capaldi, R.A. (1990) *Biochemistry* 29, 5339–5343.
- [9] Wilkens, S. and Capaldi, R.A. (1994) *Biol. Chem. Hoppe-Seyler* 375, 43–51.
- [10] Wise, J.G., Latchney, L.R. and Senior, A.E. (1981) *J. Biol. Chem.* 252, 3480–3485.
- [11] Gogol, E.P., Lücken, U., Bork, T. and Capaldi, R.A. (1989) *Biochemistry* 28, 4709–4716.
- [12] Aggeler, R.J., Mendel-Hartvig, J. and Capaldi, R.A. (1990) *Biochemistry* 29, 10387–10393.
- [13] Frank, J., Shimkin, B. and Dowse, H. (1981) *Ultramicroscopy* 6, 343–358.
- [14] Capaldi, R.A., Aggeler, R.J., Turina, P. and Wilkens, S. (1994) *Trends Biochem. Sci.* 19, 284–289.
- [15] Aris, J.P. and Simoni, R.D. (1983) *J. Biol. Chem.* 258, 14599–14609.
- [16] Walker, J.E., Gay, N.J., Saraste, M. and Eberle, A.N. (1984) *Biochem. J.* 224, 799–815.
- [17] Dallmann, H.G., Flynn, T.G. and Dunn, S.D. (1992) *J. Biol. Chem.* 267, 18953–18960.
- [18] Gogol, E.P., Johnston, E., Aggeler, R.J. and Capaldi, R.A. (1990) *Proc. Natl. Acad. Sci. USA* 87, 9585–9589.