FEBS Letters 351 (1994) 1–5

FEBS 14409

Hypothesis

Structural model of the ATP-binding domain of the F_1-β subunit based on analogy to the RecA protein

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Received 7 July 1994

Abstract

In contrast to the previous topological model of the ATP binding domain of the F_1-ATPase β subunit based on analogies to those of *ras* p21 and adenylate kinase, a more consistent model can be constructed with the known structure of the recA protein as a reference. The secondary structure of the F_1-ATPase β subunit predicted from the primary structure agrees well with that of the recA protein. The topology includes a repetitive $\beta\alpha_C\beta\alpha\beta\alpha\beta$ structure where all β strands are parallel and surround the central α_C helix above which bound ATP is located. Several residues thought to be located at catalytic site as reported in genetic and chemical labeling work can be consistently positioned in this model.

Key words: F_1-ATPase; ATP binding domain; ATP binding motif; RecA; Dicyclohexylcarbodiimide

1. Introduction

F_1-ATPase, a soluble part of H^+-ATP synthase, has a subunit structure $\alpha_3\beta_3\gamma\delta\varepsilon$ and catalytic sites are mostly on the β subunit [1–3]. The exact three-dimensional structure of F_1-ATPases has not yet been available although crystallographic analysis is making progress [4]. Several groups proposed essentially the same topological models for the ATP-binding domain of F_1-ATPase β subunit based on analogies to nucleotide-binding domains of the three-dimensional structures of *ras* p21, and/or adenylate kinase [5–8]. They tried to position the following residues[1] at reasonable location. They are Tyr^{307} which can be labeled by 7-chloro-4-nitrobenzofrazan (Nbf-Cl) [9], Tyr^{341} which can be labeled by 2-azido ATP [10–12] and *p*-fluorosulfonylbenzoyl inosine [13], and two nucleotide-binding sequence motifs which can be found in a wide range of nucleotide binding proteins, that is, motif A ($GXXXXGKT^{165}$; where X can be any residue) and motif B ($XXXXD^{252}$; where X is a hydrophobic residue) [14]. They also took into account the structural requirement that Tyr^{307} and Lys^{164} should be in proximity since, at alkaline pH, the Nbf group transferred from Tyr^{307} to Lys^{164} [9]. However, their models did not incorporate knowledge of the essential Glu^{190} of the β subunit which is labeled when the F_1-ATPase from a thermophilic *Bacillus* PS3 (TF_1) is inactivated by dicyclohexylcarbodiimide [15]. Inactivation by dicyclohexylcarbodiimide is observed only in the presence of ADP but not in the presence of ADP plus Mg^{2+} [16]. The essentiality of this Glu^{190} has been further proven by site-directed mutagenesis; replacement of this residue by other residues except for Asp caused almost complete loss of ATPase activity of the F_1-ATPase [17–20]. According to the adenylate kinase-based models, the position of this Glu is far apart from the ATP binding region of the β subunit molecule [5,7,8].

Story and Steitz determined the three-dimensional structure of the *Escherichia coli* recA protein [21,22]. This 352-residue protein catalyzes the ATP-driven homologous pairing and strand exchange of DNA [23]. In vitro the recA protein shows the activity of a DNA-dependent ATPase [24]. Although the recA protein, as well as *ras* p21 and adenylate kinase[2], contains consensus nucleotide-binding motifs A and B, its folding topology is quite different from those of *ras* p21, and adenylate kinase. It was also suggested that Glu^{96} of the recA protein was in a position to serve as a general base to activate a water molecule for an in-line attack of the γ-phosphate during ATP hydrolysis [22]. This kind of critical Glu (or Asp) does not exist in *ras* p21, and adenylate kinase. When the positions of Glu^{96} of the recA protein and Glu^{190} of F_1-ATPase β subunit in their primary structures were compared, we noticed that both of them

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Abbreviations: TF_1, F_1-ATPase from a thermophilic *Bacillus* strain PS3; Nbf-Cl, 7-chloro-4-nitrobenzofrazan.

[1] Numbering of the F_1-β subunit residues in this paper is according to that of TF_1.

[2] The nucleotide-binding motif A of adenylate kinase is GXXXXGKG instead of the typical GXXXXGKT/S.

SSDI 0014-5793(94)00796-9

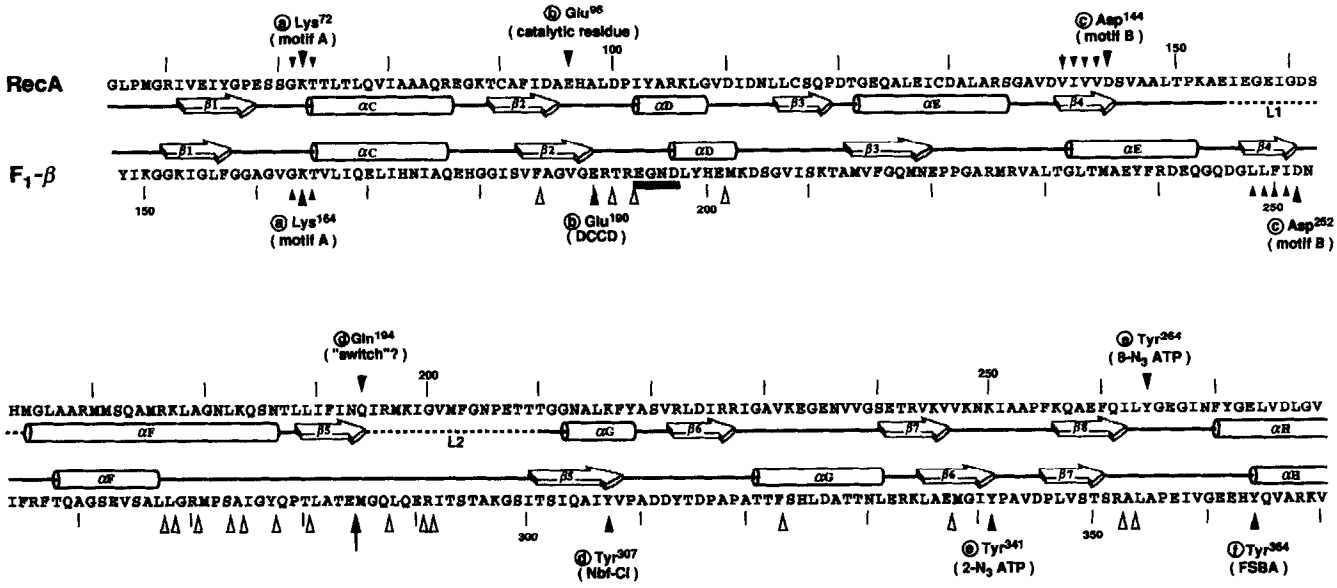

Fig. 1. Comparison of secondary structures of ATP binding domain of *E. coli* recA protein and F_1-β subunit. Structure of *E. coli* recA protein is according to the crystallographic results and that of F_1-β subunit is based on the prediction calculated by the joint prediction method [48] and 3D-1D compatibility method [49] using the amino acid sequence of F_1-β subunit from a thermophilic *Bacillus* PS3 [50]. α helices and β strands with too short length (<4 residues) were neglected. Some adjustments were made after comparison with predictions of F_1-β subunit by Walker [51], Duncan [5], and Garboczi [7], and with a map of protease cleaved sites [52]. Horizontal columns and arrows indicate α helices and β strands, respectively. Important residues are indicated by arrowheads (▲). Open triangles (△) show the cleaved sites by proteases under the condition of limited proteolysis [52]. Underlined residues, Glu^{194}–Asp^{197}, can be deleted from the primary structure leaving a four amino acid gap in the β subunit. This 'gapped' β subunit can be reconstituted into ATPase-active $\alpha_3\beta_3\gamma$ complex [31]. Also the β subunit with a peptide bond nick between Glu^{284} and Met^{285} shown by an arrow is reconstitutable into ATPase-active $\alpha_3\beta_3\gamma$ complex [31]. Abbreviations: DCCD, dicyclohexylcarbodiimide; Nbf-Cl, 4-chloro-7-nitrobenzofurazane; FSBA, *p*-fluorosulfonylbenzoyl adenosine.

occupy almost the same positions in relation to nucleotide-binding motifs A and B. This encouraged us to construct a topological model of the nucleotide-binding domain of F_1-ATPase β subunit with the three-dimensional structure of the recA protein as a reference. Almost all the requirements for the model to meet are satisfied by the model[3].

2. Comparison of the F_1-β subunit with the recA protein, ras p21, and adenylate kinase

When the positions of bound nucleotides in the known structures of the recA protein [21], *ras* p21 [25], and adenylate kinase [26] relative to one another and to the conserved peptide are compared, the positions of the α-and β-phosphates are very similar but the base and sugar positions of ADP in the recA protein are quite different from that of ADP bound to adenylate kinase [26] and that of GDP bound to *ras* p21 [25]. This implies that the structure of nucleotide binding domain of the recA protein might be organized in a different manner. In fact, the folding topology of the recA protein is significantly different from those of *ras p21* and adenylate kinase [27] (Scheme 1).

ras p21	$\beta_1\alpha_C\beta_2\beta_3\alpha_D\beta_4\alpha_E\beta_5\alpha_F\beta_6\alpha_G$
adenylate kinase	$\sim\beta_1\alpha_C\beta_2\alpha_D\alpha_E\alpha_F\beta_3\alpha_G\beta_4\alpha_H\alpha_1\beta_5\alpha_1$
recA protein	$\sim\beta_1\alpha_C\beta_2\alpha_D\beta_3\alpha_E\beta_4\alpha_F\beta_5\alpha_G\beta_6\beta_7\sim$
F_1-β subunit	$\sim\beta_1\alpha_C\beta_2\alpha_D\beta_3\alpha_E\beta_4\alpha_F\beta_5\alpha_G\beta_6\beta_7\sim$

Scheme 1

The numbering of the α helix and β strand is according to that of the recA protein so that the nucleotide-binding motif A is between β_1 and α_C. In the recA protein, $\beta\alpha$ repeats five times, whereas an antiparallel β strand (β_3) or three consecutive α helices ($\alpha_D\alpha_E\alpha_F$) follow the $\alpha_C\beta_2$ in *ras* p21 or in adenylate kinase, respectively. When the predicted secondary structure of F_1-β subunit from residue 150 to 370 (Fig. 1) is compared with those of the proteins with known structures, overall similarity to the recA is evident (Scheme 1). In addition, F_1-β subunit has an essential Glu at position 190 and this Glu^{190} is located at 26 residues downstream from Lys^{164} in the motif A. Also, an essential Glu^{96} of the recA protein which was proposed to be directly involved in catalysis is located at 24 residues downstream of Lys^{62} in the motif A (Fig. 1).

[3] The content of this report was presented orally at Meeting of Japanese Biochemical Society at Oct. 4th, 1993.

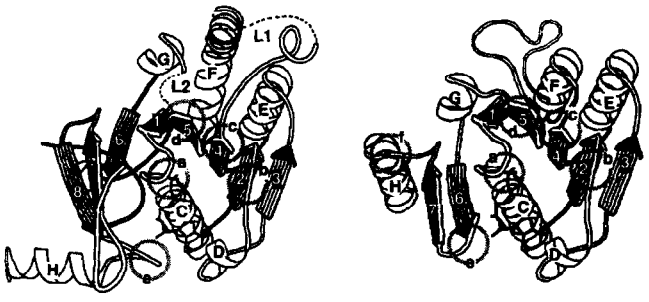

Fig. 2. (A) Structure of the nucleotide binding domain of the *E. coli* recA protein. (B) A model for the nucleotide binding domain of F_1-β subunit. Structure of the recA protein with a bound ADP is based on X-ray crystallography [22]. Circles from a to f show the positions of important residues indicated in Fig. 1.

No such critical Glu (or Asp) is found in the known structures of *ras* p21 and adenylate kinase. Therefore, construction of a topological model of ATP binding domain of F_1-ATPase β subunit based on analogy to the recA protein seems more promising than the previous models based on analogy to *ras* p21 or adenylate kinase.

3. Predicted structure of the F_1-ATPase β subunit

We have thus constructed a structural model of ATP binding domain of F_1-β subunit using the structure of the recA protein as a reference (Fig. 2). As is observed commonly in the proteins that have nucleotide-binding motif A, Lys^{164} of F_1-β subunit is at the amino end of α_C helix interacting with β- and γ-phosphates of the bound ATP (Fig. 2A,B, circle a; Fig. 3). The next residue, Thr^{165}, also interacts with β-phosphate and with Mg^{2+}. The essential roles of these two residues for the activity of F_1-ATPase has been established [28,29].

The α_C helix is an amphiphilic helix, that is, Ile^{168}, Leu^{171} and Ile^{172} are oriented to the same side, facing to the side of the β_4 strand. They possibly interact with Leu^{248} and Phe^{250} in the nucleotide-binding motif B in the β_4 strand through hydrophobic interaction. Glu^{190} can be positioned in a loop after the β_2 strand at a position almost identical to Glu^{96} of the recA protein (Fig. 2A,B, circle b). Similar to the recA protein, the carboxyl group of Glu^{190} may act as a general base to activate a water molecule which then attacks the γ-phosphorus of bound ATP (Fig. 3). If so, the deprotonated form of Glu^{190} is required for catalysis and the pK_a value of this residue determines the acidic-side slope in the pH-ATPase activity profile of F_1-ATPase [30].

The α_D helix may start from position 198 of F_1-β subunit. This assignment is justified by the observation that a fragmented β subunit made up from two peptides with a gap from Glu^{194} to Asp^{197} is reconstitutable into an ATPase-active $\alpha_3\beta_3\gamma$ complex [31]. Tyr^{199} in the α_D helix may stack the adenine base as is observed for Tyr^{103} of the recA protein. The fact that F_1-β subunits from animals and plants have an inserted amino acid sequence after Ile^{208} supports the assignment of the region around Ile^{208} as a loop since it has been recognized that deletions or insertions occurring in the multiple amino acid sequence alignment of homologous proteins provides accurate parsing points to distinguish loop regions from regular secondary structure [32].

The nucleotide-binding motif B is contained in the β_4 strand and Asp^{252} is positioned at the carboxyl end of the strand (Fig. 2A,B, circle c). From knowledge obtained from motif B-containing proteins with known structures, it is almost certain that the carboxyl group of Asp^{252} is involved in the binding of active-site Mg^{2+} through a water molecule (Fig. 3) [22,33]. The carboxyl group of Asp^{252} may be also hydrogen-bonded to Thr^{164} of the motif A sequence as reported for the *ras* p21 structure [33]. As stated above, side chains of Leu^{248} and Phe^{250} in the β_4 strand are protruding to the same side as Asp^{252} and they could interact with hydrophobic residues of the α_C helix.

In the recA protein structure, after the β_4 strand, there is a crystallographically 'invisible' disordered region (loop L1) and then the αF helix follows (Fig. 2A) [21]. However, we predict the reverse order of a helix and a loop, that is, the α_F helix and then a long stretch of loop (Figs. 1 and 2B). This prediction is based on two facts: the region from residues 267–292 is most easily cleaved when isolated the β subunit is treated with protease, and

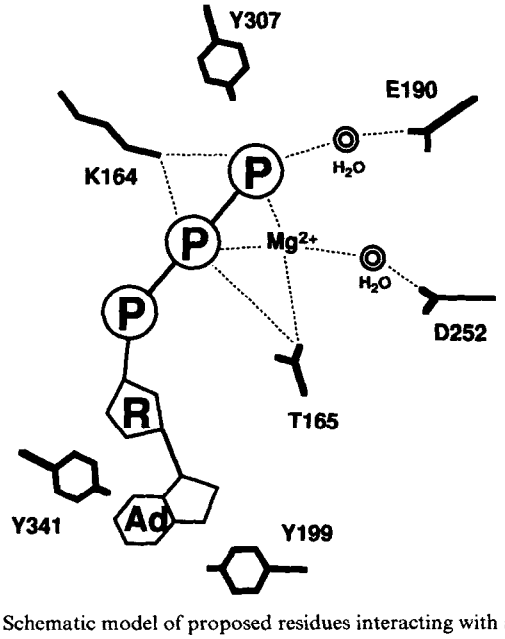

Fig. 3. Schematic model of proposed residues interacting with a bound ATP molecule on F_1-β subunit. The model is based on biochemical evidence of F_1-ATPase and on analogy to the recA protein [22].

there is an inserted sequence after I^{292} in an aligned sequence of homologous proteins, an indication of a loop region [32,34].

According to the model, Tyr^{307} of the F_1-β subunit is near the carboxyl end of the β_5 strand (Fig. 2A, circle d). The distance from Tyr^{307} to Lys^{164} in the tertiary structure of the model seems to be close enough to allow the direct transfer of Nbf moiety from the former residue to the latter [9]. The location of Tyr^{307} in the model structure of F_1-β subunit is equivalent to that of Gln^{194} in the recA protein structure (Fig. 2B, circle d). Gln^{194} in the recA protein is proposed to play a crucial role in the transmission of conformational change; Gln^{194}, when interacting with the γ-phosphate of ATP, stabilizes loop L2 ('invisible' region) and/or the α_G helix in a state that binds single-stranded DNA, and hydrolysis of ATP destroys this interaction resulting in a change in conformation of the DNA binding region(s) [22]. Similarly, we speculate that Tyr^{307} of the F_1-β subunit belongs to a 'switch' region responsible for transmission of conformational change from the β subunit to the adjacent α subunit which is then transmitted to the next β subunit. An unusual property of labeling of Tyr^{307} by Nbf-Cl [35], that is, labeling of a single β subunit out of three β subunits in a F_1-ATPase molecule results in complete loss of ATPase activity, can be relevant to this contention. The 'invisible' loop L2 region of the recA protein corresponds to the region from residue 309 to 320 of the F_1-β subunit where three Pro-Ala sequences are contained (Fig. 1).

Tyr^{254} of the recA protein is labeled by 8-azido ATP [36]. Tyr^{341} of F_1-β subunit is labeled by 2-azido ATP [10–12]. Although the Tyr^{341} itself is not involved in catalysis [37,38], it contributes greatly to binding affinity of ATP to the catalytic site [38–40]. It is reasonable to assume that, in the tertiary structure of the protein, position of Tyr^{341} of F_1-β subunit is similar to that of Tyr^{254} of the recA protein. The distance in the primary structure between the carboxyl end of the α_5 strand and the Tyr is 34 residues in F_1-β subunit and 70 residues in the recA protein. The presence of one α helix and two β strands is predicted in the F_1-β subunit but one additional β strand is present in the recA protein structure (Fig. 1). Thus, very good matching of the predicted secondary structure of the F_1-β subunit to the one observed for the recA protein discontinues at this region. Tyr^{341} of the F_1-β subunit is predicted to be located at the carboxyl end of the β_6 strand but this location is too far from the adenine base if the β_6 strand is parallel to the β_1 strand. Therefore, we assume that the β_6 strand is not in the same sheet which is formed by the five parallel ($\beta_1 \sim \beta_5$) strands. Fig. 2B is drawn on this assumption. However, if the α_G helix is shorter and the β_6 strand is present at several residues more to the amino terminal side than is predicted, the β_6 strand can be in the parallel position to the β_1 strand as is observed for the recA protein. In any case, the β_7 strand should be in an antiparallel arrangement with the β_6 strand.

4. Tyr^{364} of the β subunit might be interacting with an ATP binding site on the α subunit

We predict that Tyr^{364} is located far apart from the catalytic ATP binding site (Fig. 2B, circle f). The reason is as follows. The α and β subunits have homologous primary structure each other [41] and are arranged as alternative order in the hexagonal structure of F_1-ATPase [42]. The three dimensional structure at resolution of 6.5 Å revealed that the alignment of α and β subunits in the hexameric structure is parallel [4]; structural arrangement of one α/β contact region is essentially the same as that of the next β/α contact region. These facts imply that the AT(D)P binding sites of the β subunits are analogous to those of the α subunits and that they are arranged in six-fold symmetry.

Isolated α and β subunits can bind one AT(D)P individually [43] and the binding sites on the α and β subunits are catalytic sites and non-catalytic sites, respectively [40,44]. Apparently contradictory to the above statement, analyses of modification by 2-azido ATP and *p*-fluorosulfonylbenzoyl adenosine indicate that Tyr^{364} of the β subunit is supposed to be at a non-catalytic site of the F_1-ATPase. This contradiction is reconciled by the assumption that binding site on the α subunit is mainly made up from residues of the α subunit but Tyr^{364} of the β subunit also has some contribution to this site [45]. Indeed, bifunctional crosslinker, 8-azido-*p*-fluorosulfonylbenzoyl adenosine cross-linked Tyr^{364} and the α subunit [46]. If this assumption is right, Tyr^{364} of the β subunit might extend to the non-catalytic AT(D)P binding site of the α subunit. Bearing this in mind, we put Tyr^{364} at a position far apart from the catalytic ATP binding site (Fig. 2B, circle f). This agrees with the result from ESR spectroscopy that Tyr^{341} and Tyr^{364} are calculated to be approximately 15 Å apart [47]. Vice versa, Lys^{376} of the α subunit, which is an equivalent residue of Tyr^{364} of the β subunit, might be positioned closely at the catalytic site on the β subunit.

3. Experimental test for the feasibility of the model

Feasibility of the model can be tested by experiments. For example, introduction of Pro into the region predicted as the α_C helix and the β_2 strand in our model should result in serious impairment of stability and/or activity of F_1-ATPase. According to the model, Tyr^{199} might stack the adenine base and, if it really does so, replacement of this residue by other amino acid, Ala, for instance, will cause significant decrease of binding affinity of ATP to the β subunit and hence the K_m value of

F_1-ATPase will increase. Cross-linking the residues located in the adjacent β strands can also provide the support for the model. Finally, X-ray crystallography will justify the feasibility of the model.

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