Hypothesis

Structure of the nucleotide-binding domain in the β-subunit of *Escherichia coli* F_1-ATPase

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We propose a working model for the tertiary structure of the nucleotide-binding domain of the β-subunit of *E. coli* F_1-ATPase, derived from secondary structure prediction and from comparison of the amino acid sequence with the sequences of other nucleotide-binding proteins of known three-dimensional structure. The model is consistent with previously published results of specific chemical modification studies and of analyses of mutations in the β-subunit and its implications for subunit interactions and catalytic mechanism in F_1-ATPases are discussed.

Oxidative phosphorylation *F_1-ATPase* *β-Subunit* *Nucleotide-binding domain* *Secondary structure* *Tertiary structure*

1. INTRODUCTION

The catalytic sites of F_1-ATPases are believed to reside on the β-subunits [1–3]. There is very strong sequence homology between F_1 β-subunits across a broad spectrum of species [4]. From the results of all specific chemical modification studies and of mutant analyses that have been done on F_1-ATPases (irrespective of the source), eleven specific residues of β have been implicated as being essential for catalysis and/or located at the catalytic nucleotide-binding site. These residues are noted in fig.1 (see legend for references) in which the *E. coli* β-subunit sequence (459 amino acids) is shown. Ten of the eleven residues occur in the segment between residues 155 and 297. Four more, as yet undefined, point mutations which impair catalysis are also known to map within this segment [5]. Furthermore, there are two distinct regions of sequence in β between residues 148 and 242 which are homologous with specific sequences in several other nucleotide-binding proteins [6–9]; these specific sequences are located in a nucleotide-binding domain, the tertiary structure of which is already known in some of these nucleotide-binding proteins.

Therefore, the segment of residues 148–297 in β, comprising one-third of the protein, is strongly implicated as containing the catalytic nucleotide binding domain. From prediction of secondary structure and from the above-mentioned sequence characteristics and homologies, we propose here a model of the nucleotide-binding domain of *E. coli* F_1-β-subunit and discuss its implications for functional characteristics of F_1-ATPases.

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Abbreviations: AK, adenylate kinase; EF-Tu, *E. coli* protein synthesis elongation factor; PFK, phosphofructokinase; Nbf, 4-chloro-7-nitrobenzofurazan; DCCD, dicyclohexylcarbodiimide; FSBA, fluorosulfonylbenzoyl-5′-adenosine

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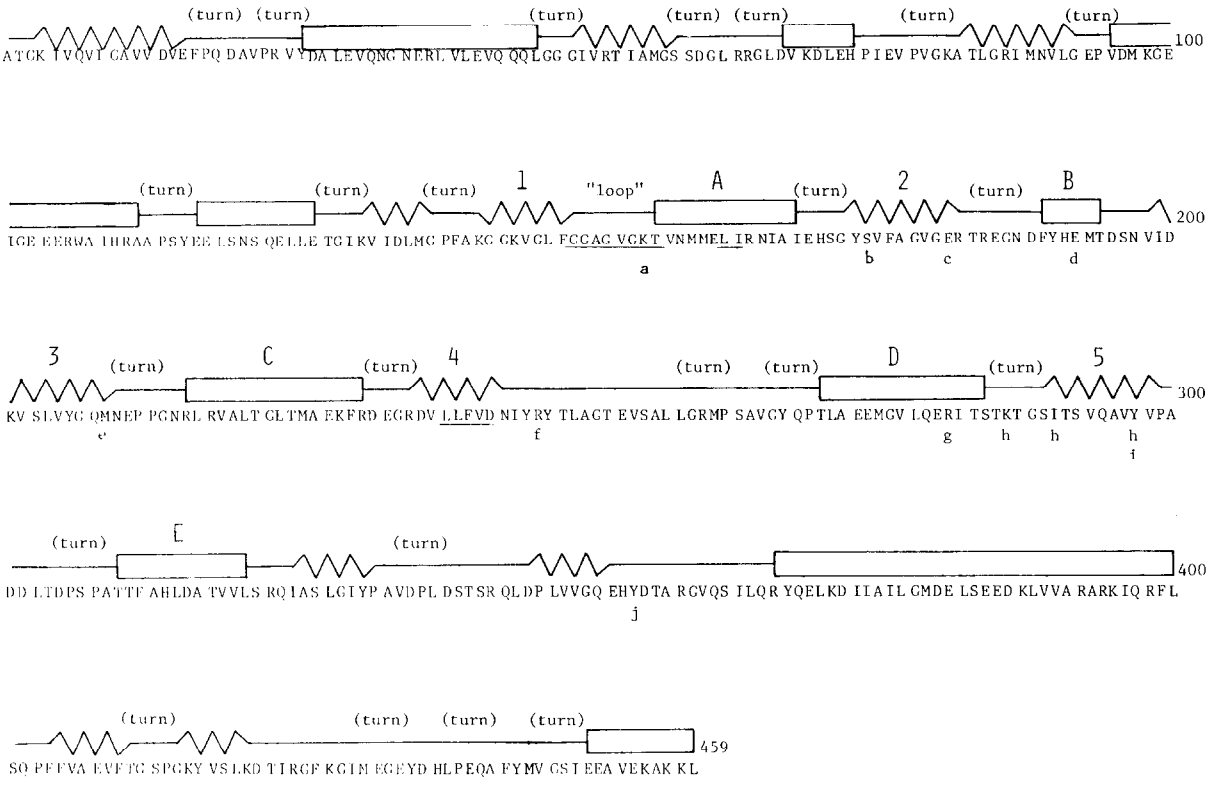

Fig.1. Predicted secondary structure of the *E. coli* F_1-β-subunit. The primary sequence is taken from Walker et al. [39]. The secondary structure is based on the published prediction of Walker et al. [39]. Some adjustments were made after comparison with predictions of Kanazawa and Futai [40], and of J.E. Bell (University of Rochester, Dept of Biochemistry, personal communication). β-turns were assigned after inspection of the aligned sequences of β-subunit from ten species, checking for conservation of the turns. (⊏⊐) α-helix, (vvvv) β-strand. The numbers and letters above the secondary structural elements are for reference to the tertiary folding model in fig.2. Underlining marks areas of sequence homology with other nucleotide binding proteins ([6] and see table 1). Lower-case letters below the sequence designate residues identified as essential for catalysis either by specific chemical modification or mutational analysis. (a) K-155, to which Nbf is transferred intramolecularly at high pH from the initially labeled Y-297 [19]; (b) S-174, mutated to F in *uncD11* [41]; (c) E-181 and (d) E-192, the two DCCD-reactive residues [32–34]; (e) M-209 mutated to I in *uncD484* [5]; (f) R-246 mutated to H in *uncD43* [42]; (g) R-281, labeled by phenylglyoxal [43]; (h) residues at positions 287, 290 and 297 labeled by 8-azido-ATP [18]; (i) Y-297, reacts with Nbf [20,21]; (j) Y-354, reacts with FSBA [37].

2. STRUCTURE PREDICTION

Many nucleotide-binding domains of known structure exhibit similar tertiary folding topology and are classified as doubly wound, parallel α/β domains [10]. All known doubly wound, parallel α/β domains have several features in common: the central β-sheet contains at least 4–5 predominantly parallel β-strands and has a right-handed twist; connections between strands of the sheet are usually α-helical, pack on both faces of the β-sheet, and are almost always right-handed [10]. These structures seem to be energetically [11] and geometrically [12] well-suited for nucleotide binding, and their similar topologies might arise from a common pathway of folding [10,13]. Thus, it should be feasible to predict the basic tertiary folding topology of the F_1 β-subunit nucleotide-binding domain by analogy with domains of known doubly

wound, parallel α/β structure with which it shares sequence homologies (see table 1).

The locations of sequence homologies A and B of table 1 in the tertiary structures of adenylate kinase (AK) [8,14], *E. coli* elongation factor Tu (EF-Tu) [15] and phosphofructokinase (PFK) [16] support a possible link between sequence conservation and structurally similar nucleotide-binding domains. In both AK and EF-Tu the sequence GXXXXGK (in homology A of table 1) forms a flexible loop structure connecting the N-terminal strand of the β-sheet with the first α-helical packing element. In AK, EF-Tu and PFK, the conserved sequence of hydrophobic residues (in homology B in table 1) forms part or all of the fourth strand (in sequence) of the β-sheet. Strands 1 and 4 of the β-sheet are adjacent to each other so that homologies A and B, although far apart in sequence, are quite close together in the tertiary structures of AK and EF-Tu. The locations of homologies A and B in the predicted secondary structure of the F_1 β-subunit (see fig.1) suggest a similar tertiary folding topology. If the β-strand before the sequence GGAGVGKT (homology A) is designated as β-strand 1 (N-terminus of the folding domain), then β-strand number 4 contains the conserved sequence of hydrophobic residues (homology B). AK and EF-Tu each contain the strand arrangement 2-3-1-4-5 in the β-sheet, but the predicted secondary structure of the F_1 β-subunit (fig.1) is most consistent with the overall topology of AK: both the β-subunit and AK exhibit α-helical connections between each of five successive β-strands, while EF-Tu has a hairpin connection between strands 2 and 3. Therefore, for the construction of the tertiary model, the predicted secondary structure of the *E. coli* F_1 β-subunit (fig.1), starting with the β-strand labeled 1 and continuing to the α-helix labeled E, was folded with the tertiary topology of AK, i.e. in a $-2X$, $+1X$, $+2X$, $+1X$ topology as defined by Richardson [10]. The characteristic twist of the β-sheet and spatial alignments of the α-helices and the conserved flexible loop were modelled after AK crystal form B [14]; lengths of α-helices and β-strands were adjusted according to predictions of fig.1 The resulting tertiary folding model is shown in fig.2. It is notable that, while the predicted connection between β-strands 2 and 3 of the β-subunit contains only one α-helix, it still accommodates the general folding pattern of AK, which has three α-helices in the analogous connection.

3. EVALUATION OF THE MODEL AND ITS FUNCTIONAL IMPLICATIONS

To aid in evaluation of the model in fig.2, a molecule of ATP has been positioned in the

Table 1

Sequence homologies between F_1-ATPase β-subunit and nucleotide-binding proteins of known tertiary structure

Protein	Residues	Sequences
Homology A		1 10 20 30
Bovine F_1-ATPase β-subunit	143–172	L A P Y A K G G K I G L F - G G A G V G K T V F I M E L I N
E. coli F_1-ATPase β-subunit	136–165	M C P F A K G G K V G L F - G G A G V G K T V N M M E L I R
Adenylate kinase	1–30	M E E K L K K S K I I F V V G G P G S G K G T Q C E K I V Q
EF-Tu	6–34	F G R T K P H V N V G T I - G H V D H G K T T L T A A I T T
Homology B		1 10 20 30
Bovine F_1-ATPase β-subunit	236–265	V A E Y F R D Q E G Q D V L L F I D N I F R F T Q A G S E V
E. coli F_1-ATPase β-subunit	226–254	M A E K F R D - E G R D V L L F V D N I Y R Y T L A G T E V
Adenylate kinase	102–130	G E E F E R K - I G Q P T L L L Y V D A G P E T M T K R L L
Phosphofructokinase	85–113	G I E Q L K K - H G I Q G L V V I G G D G S Y Q G A K K L T
EF-Tu	91–120	M I T G A A Q M D G A I L V V A A T D G P M P Q T R E H I L

All the alignments except for EF-Tu were taken directly from Walker et al. [6]. The EF-Tu alignment for homology A was taken directly from Halliday [7]; the EF-Tu alignment for homology B was made here by visual inspection of the EF-Tu sequence about 60 residues downstream from the end of its alignment in homology A (about the same distance between homologies A and B in the *E. coli* β-subunit sequence)

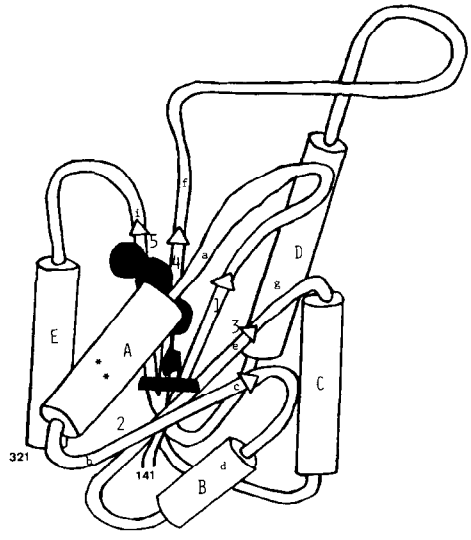

Fig.2. A model of the tertiary folding topology of the nucleotide-binding domain of the *E. coli* F_1-ATPase β-subunit. The secondary structural elements and specific residues are labeled as in fig.1. The asterisks in helix A are the conserved hydrophobic residues of homology A (table 1). The ATP molecule (solid black) is positioned in the putative binding cleft analogously to its location in the nucleotide-binding domain of adenylate kinase [8]. The purine ring plane is at right angles to the page.

putative binding cleft analogous to its position in AK as determined by NMR [8]. The conserved lysine in homology A of table 1 is near the α-phosphate of ATP bound to AK and may interact with it depending on the orientation of the lysine side chain [8]; in EF-Tu this conserved lysine also appears to be involved in electrostatic interaction with the α- or β-phosphate of bound GDP [15,17]. The conserved hydrophobic residues toward the end of homology A (table 1) are located in helix A and probably assist in forming a hydrophobic pocket for the adenine ring of bound ATP, as observed with AK [8]. The aspartate at the end of β-strand 4 (homology B of table 1) appears to interact with Mg^{2+} of bound MgATP on AK [8] and PFK [16]. The lack of a more highly conserved primary sequence is probably a result of the differing nucleotide-binding characteristics and functional activities of the proteins.

Results from chemical modification studies of F_1-ATPases are consistent with the model of the β-subunit nucleotide-binding domain in fig.2. First, three residues (designated h in fig.1) were identified by labeling mitochondrial F_1 with 8-azido-ATP [18]. Two residues, K-286 and I-290, are near the beginning of β-strand 5 and are close to the adenine ring of bound ATP in the model. The third residue, Y-297, is somewhat more distant from the bound adenine ring, but this may be due to the highly reactive nature of the azido group. Secondly, the model in fig.2 provides a very plausible explanation of the characteristic reactions of the β-subunit of F_1-ATPase with Nbf [19–21]. The proposed relative locations of bound ATP and the tyrosine that is initially labeled by Nbf (Y-297, marked i in fig.2) are consistent with inactivation studies which show that binding of P_i or Mg^{2+} plus P_i gives strong protection against inactivation by Nbf, whereas ATP gives weak protection and ADP has little or no protective effect [22,23]. At increased pH the Nbf group is transferred from Y-297 specifically to K-155 (marked a in fig.2) of the β-subunit [20,21]; these two residues, although well separated in the primary sequence of β are close in the model in fig.2. Furthermore, AK can undergo a pH-induced conformational change between crystalline form A and B [14] where the conserved flexible loop (in homology A, table 1) pivots towards strands of the β-sheet analogous to β-strands 4 and 5 in the β-subunit nucleotide-binding domain in fig.2. A similar conformational change in the conserved flexible loop of the proposed nucleotide-binding domain of the β-subunit (fig.2) could explain the facilitated transfer of Nbf from Y-297 to K-155 at increased pH.

In AK the conformational change mentioned above also can be induced by substrate binding [14,24]. The latter authors state that the flexible loop is "an epicenter of the structural changes" and is "directly connected mechanically to all other moving structural elements". In AK, when nucleotide binds, the contemporaneous movement of the loop and the α-helix above it (analogous to the segment between β-strand 4 and α-helix D of the β-subunit in fig.2) actually blocks the entrance to the binding cleft, making the bound nucleotide inaccessible from the aqueous environment [24]. This has obvious implications for the mechanism of the F_1-ATPase. For instance, such an induced conformational change could help to achieve the extremely high affinity of the 'first' catalytic site of F_1-ATPase for ATP. It could also participate in

several features of the highly cooperative binding change mechanism of F_1-ATPase [25] as pointed out recently by Fry et al. [9]. The binding change mechanism (see [26] for reviews) requires that conformational changes at alternate catalytic sites be tightly coupled to each other. The alternating arrangement of the α- and β-subunits in F_1 [27,28] and the results of the *uncA* (α-subunit) mutant studies of Wise et al. [29,30] indicate that this involves a pathway of conformational signal transmission from the nucleotide-binding domain of one β-subunit through a neighbouring α-subunit to an alternate β-subunit [1,31]. A possible clue to the pathway of the conformational signal transmission within the β-subunit comes from examination of the location in fig.2 of residues which, when altered, disrupt the conformational coupling (positive catalytic cooperativity) between F_1-ATPase catalytic sites. For instance, E-181 and E-192 (labeled c and d in fig.2), which are modified by DCCD [32–35], are near the edge of the β-sheet and may be involved in the conformational interactions with a neighbouring α-subunit. Alteration of residues E-181, M-209, R-246 and Y-297 (residues c, e, f and i respectively in figs 1,2) all affect catalytic cooperativity of F_1-ATPase and appear to alter residues near the C-termini of β-strands, suggesting the mechanism of conformational signal transmission through the β-subunit might involve deformations in the twist of the central β-sheet, congruent with the general mechanism suggested by Salemme [36].

Some discussion is warranted in regard to regions of the β-subunit structure which are not part of the model for the nucleotide-binding domain. The tyrosine (Y-354, marked j in fig.1) labeled by the ATP analog FSBA [37], is 33 residues beyond the last α-helix (E) shown in fig.2. With a 1X connection, the predicted β-strand just prior to Y-354 may form a sixth parallel strand of the β-sheet, placing Y-354 near the γ-phosphate of bound ATP in the model of fig.2. Even if the region including Y-354 were included in the nucleotide-binding domain, there remain two significant regions flanking the sequence shown in fig.2. Both the N-terminal segment of about 145 residues and the C-terminal segment of approx. 100 residues are sufficient in themselves to form distinct domains. These could be involved in structural/functional contacts with other subunits of F_1 and with subunits of F_0 in the intact F_1F_0-ATPase. The predicted long α-helix (35 residues) in the C-terminal sequence shown in fig.1 would extend for 5 nm, which is somewhat greater than the diameter of the large globular subunits of F_1 observed by electron microscopy [38]. This and the fact that it shows a high degree of sequence homology through 10 species [4] make it a primary candidate for participation in intersubunit interactions.

In summary, the model of the F_1 β-subunit nucleotide-binding domain presented here is speculative, but it can accommodate many functional characteristics of the enzyme and account for the behaviour of several residues identified by chemical labeling or mutation. Sufficient segments of the primary sequence exist outside this domain to produce separate domains possibly involved in intersubunit contacts. The model should provide inspiration for further analyses of structural and functional correlations in the β-subunit and in F_1-ATPase.

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