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ATP synthase: structure–function relationships

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Recent work has focused on obtaining a better understanding of the three-dimensional structural relationships between the α and β subunits of the F_1 moiety and the location of nucleotide binding domains within these subunits. Four types of approach are currently being pursued: X-ray crystallographic, chemical, molecular biological and biochemical. Here we briefly review some of the major conclusions of these studies, and point out some of the problems that must be resolved before an adequate model that relates structure to function in the ATP synthase molecule can be formulated.

Introduction

The mitochondrial ATP synthase consists of two major components, one called F_1 and the other F_0. F_1 is a soluble protein, which in mitochondria projects into the matrix space and in bacteria into the cytosol (for recent reviews see Refs. 1–4). It consists of five different subunit types in the stoichiometric ratio of $\alpha_3\beta_3\gamma\delta\epsilon$, a ratio that appears to hold for most F_1-like ATPase from bacteria to people. There is considerable homology among α and β subunits throughout the phylogenetic scale. The F_0 moiety of the ATP synthase complex forms the proton channel that directs protons to the F_1 moiety. F_0 consists of three subunit types in bacteria but many more in higher eukaryotes. Rat liver F_0 appears to contain at least eight distinct polypeptides [5].

F_1 has been studied extensively with respect to its nucleotide binding and kinetic properties. Although there is agreement that there is a minimum of six nucleotide binding sites on F_1, one each on α and β subunits, there is little agreement about their exact location or their function. Nor is there agreement about the exact number of nucleotide binding sites that function at any one time while the enzyme synthesizes ATP. Also, some workers have proposed that β subunits may contain more that one nucleotide binding site [6,7] raising the possibility that α and β subunits interact within regions of their respective nucleotide domains, or that F_1 contains more than six nucleotide binding sites. Finally, it should be pointed out that despite the fact that there is evidence from amino acid sequence data that a minimum of six nucleotide binding sites exist on F_1, few workers have been able to directly demonstrate this unless photoaffinity or hydrophobic nucleotide analogues are used [8,9]. When more natural substrates or analogues are used only the tightest sites are detected and the stoichiometry falls below 6 mol nucleotide per mol F_1 [10]

There is also little agreement as to how the subunits of the F_1 moiety work mechanistically during ATP synthesis. Thus, some workers believe that the key to understanding enzyme mechanism involves understanding the dynamics of the small subunits [10–12]. Do they move from one $\alpha\beta$ pair to another specifying each one in turn for an ATP synthetic role? Or do the small subunits restrict their roles to binding F_1 to F_0, and perhaps to regulating proton flow to F_1?

In the brief review of our work presented below it will be seen that we have not resolved these difficult questions. However, we do believe that this work together with work from other laboratories has taken us one step closer to better understanding structural–functional relationships within the F_1 moiety of ATP synthase complexes.

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Materials and Methods

Materials and Methods have been documented in detail in previous publications. These include reagents used, assays employed, the preparation of F_1 [13] and F_1 crystals [14], molecular biological techniques [15–17], peptide synthesis [18] and X-ray crystallography [19]. All studies below refer to the F_1 moiety of the rat liver ATP synthase.

Results and conclusions: a brief overview

Biophysical studies

The model presented in Fig. 1 summarizes our current view of the quaternary structure of the F_1 moiety of the rat liver ATP synthase molecule derived from X-ray studies. The model which is described in detail elsewhere [19] is based on 3.6 Å resolution data. In Fig. 1A, a view is shown looking down on the F_1 molecule from the top. In Fig. 1B, an illustration is shown of how the F_1 molecule may look from the side. In the model, β subunits are shown at the top and α subunits at the bottom closer to the proton channel, as there is biochemical data indicating that α subunits lie near the membrane.

There are five structural features of the model that are of interest: (1) a trimeric set of three β subunits sits on a trimeric set of three α subunits, and are offset so that the αs are seen between the βs; (2) β subunits do not interact physically; (3) α subunits do interact but only near the 3-fold axis; (4) α and β subunits do interact in significant areas of their structures; and (5) at the bottom, α and β subunits have empty density areas between them, forming pockets.

A sixth feature of the model that may have functional implications is the location of the heavy atom sites for the mercurial agent mersalyl (Fig. 1A). It will be noted that all three heavy atom sites lie near α/β interfaces. The β subunit of the rat liver enzyme has no cysteine residues whereas the α subunit has two, both of which are located between the Walker A and B consensus motifs thought to be involved in nucleotide binding. These results would suggest that each nucleotide binding site on an α subunit lies near an interface with a β subunit.

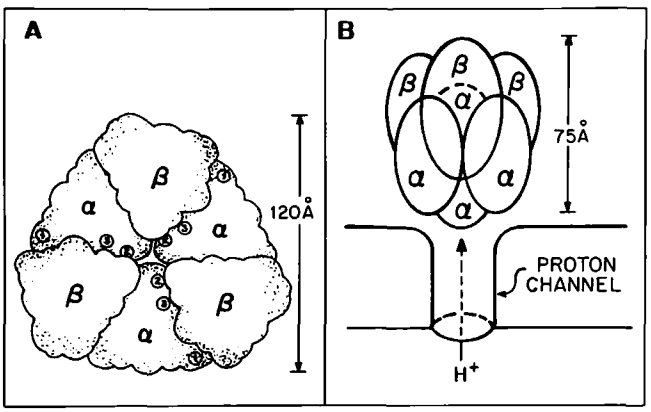

Fig. 1. (A) Schematic representation of the F_1 moiety of the rat liver ATP synthase molecule looking down on the top of the molecule. Heavy atom positions are indicated by numbers. (B) Schematic representation of the complete F_1 ATPase molecule showing relative positions of α and β subunits.

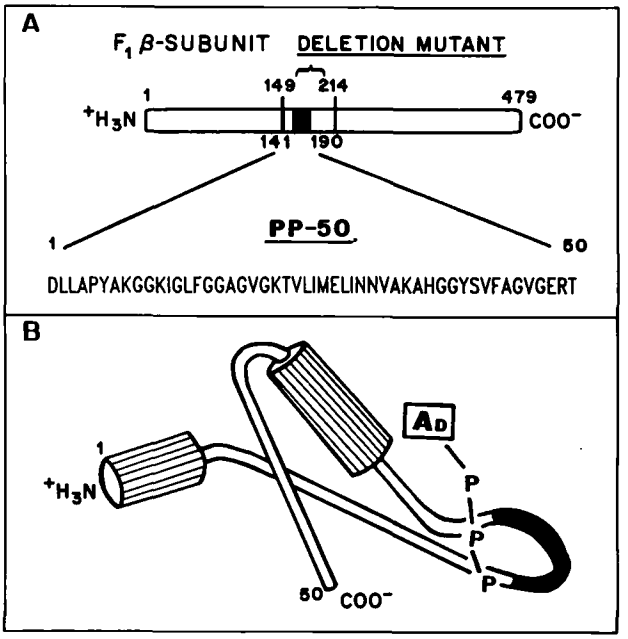

Fig. 2. (A) Regions are shown in the F_1-β subunit that correspond to the deletion mutant (Gly-149 through Lys-214) and the 50 amino-acid peptide called PP-50 (Asp-141 through Thr-190) referred to in this report. (B) Scheme emphasizing that the pyrophosphate group of ATP binds to the β subunit in the region near the glycine flexible loop.

Current work is focused on defining the location of the nucleotide binding domains by infusing heavy atom nucleotide analogues into single crystals, and by determining whether the crystallographic coordinates of one or more other ATP binding proteins fit within those of the F_1 moiety.

Chemical and molecular biological studies

Both types of studies focussed initially on determining whether the Walker A consensus motif in the β subunit is involved in nucleotide binding, and, if so, the nature of this interaction.

In chemical studies [18] a 50 amino-acid peptide (Asp-141 through Thr-190) was synthesized which spanned that region in the β subunit containing the Walker A consensus motif GX_4GKT and the entire 'predicted' glycine flexible loop region corresponding to that found in adenylate kinase [20]. This peptide called 'PP-50' (Fig. 2A) was purified to homogeneity, shown to exhibit significant secondary structure by circular dichroism, and to interact quite dramatically

with ATP. These results, which are summarized in detail elsewhere [18], showed that in 10 mM Tris-HCl buffer (pH 7.4) PP-50 does interact with ATP and is precipitated from solution. Significantly, GTP and ITP, which are substrates for F_1, also induced this precipitation as did pyrophosphate. AMP which fails to bind well to F_1 produced no precipitation response, nor did ions like phosphate or $MgCl_2$. These results suggested that the major interaction between PP-50 and the ligands that gave a precipitation response involved pyrophosphate. By using trinitrophenyl nucleotide analogues, interactions between PP-50 and the analogue could be followed fluorometrically in solution without precipitating PP-50. These studies demonstrated quite clearly that TNP-ATP and TNP-ADP interact strongly with PP-50 whereas TNP-AMP has little interactive capacity. Here, the conclusion remained the same, namely that the pyrophosphate group of ATP is contributing to the interaction with PP-50.

In molecular biological studies [15–17] a much longer β subunit fragment (Glu-122 through Ser-479) was overexpressed in *Escherichia coli* and shown also to bind TNP-ATP and TNP-ADP [16]. These studies were extended to mutational analysis within a region containing the Walker A consensus motif [17]. Significantly, upon deletion of a section of the β subunit corresponding to Gly-149 though Lys-214, which contains both the A consensus motif and the glycine flexible loop (Fig. 2A), the resultant protein displays a marked reduction in affinity for TNP-ATP, increasing the K_d from 5 to 60 μM.

Taken together, these results emphasize that the region of the β subunit containing the Walker A consensus region and the glycine flexible loop may be directly involved in binding either ATP or ADP, and that this binding reaction involves a pyrophosphate linkage. Thus, this region of the β subunit, may be a 'pyrophosphate gripper'. These results show also, that this region of the β subunit is not the only region involved in ATP/ADP binding, as its deletion does not completely prevent the binding of nucleotide. Rather, it lowers the affinity of binding.

Current work on the nature of ATP/ADP binding to F_1 continues to be pursued using both chemical and molecular biological approaches. Thus, the structure of PP-50 is being investigated by Drs. A. Mildvan and W. Chuang at this university using NMR, and the contributions of other regions of the β subunit to ATP/ADP binding are being investigated by molecular biological approaches.

Biochemical studies

Nucleotide binding studies have been carried out on rat liver F_1 in some detail. In these studies we have set three strict criteria so that the results obtained would be meaningful, and not subject to the criticism of many other studies. First, all F_1 preparations used were shown in advance to restore ATP synthesis to F_1-depleted inner membrane vesicles. Thus, we know that our enzyme is competent in restoring the normal physiological function [21]. Secondly, our F_1 preparations have been shown by immunological procedures to be free of the ATPase inhibitor protein, which is now known to increase nucleotide binding to F_1 [22]. Third, we have avoided the use of highly hydrophobic nucleotide analogues, or photoaffinity nucleotide analogues, which may bind to low affinity or non-specific sites. We have used only ADP, the natural substrate for ATP synthesis, and AMP-PNP a non-hydrolyzable analogue of ATP.

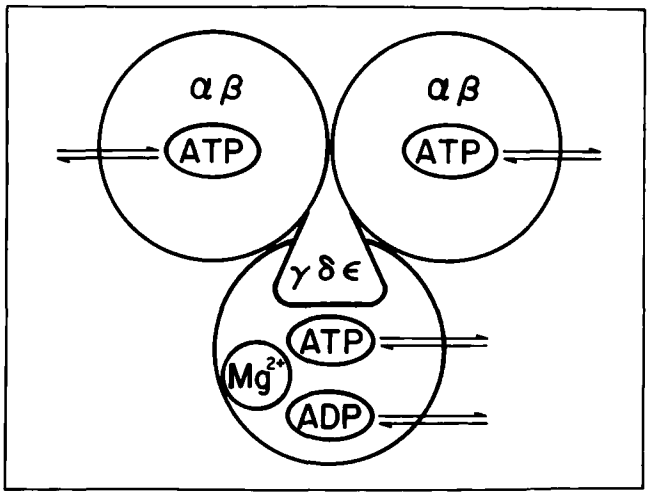

Fig. 3. Scheme emphasizing the asymmetrical nature of nucleotide and Mg^{2+} binding to rat liver F_1, and the importance of the small subunits in 'inducing' one $\alpha\beta$ pair to bind ligands more tightly than the other two $\alpha\beta$ pairs.

Taking the above precautions, we find that 1 mol of intact rat liver F_1 binds reversibly 4 mol of nucleotide [10]. There is only a single detectable ADP site ($K_d \sim 1$ μM), a single AMP-PNP site with a similar K_d, and two AMPPNP sites with a lower K_d (20–30 μM). Significantly, AMP-PNP has no effect on ADP binding, and ADP has no effect on AMP-PNP binding indicating that these sites are separate and distinct on the surface of F_1. The enzyme also contains one site which binds Mg^{2+} nonexchangeably [10]. These experiments clearly demonstrate that F_1 distributes nucleotides and Mg^{2+} in an asymmetrical manner. The simplest interpretation (Fig. 3) is that the asymmetric $\alpha\beta$ pair, i.e., the one in contact with the small subunits, binds ADP, AMPPNP, and Mg^{2+} much tighter than the other two $\alpha\beta$ pairs, each of which binds detectably only one AMPPNP.

Although the tighter nucleotide binding sites on F_1 are frequently called 'noncatalytic', this usually means in most investigator's reports that nucleotides bound at these sites do not exchange during ATP hydrolysis.

However, the real physiological reaction of interest is ATP synthesis, and it is assumed from current models that the role of the proton gradient is to release tightly bound nucleotide [23]. If this is so, it seems likely that this nucleotide release takes place from the asymmetric $\alpha\beta$, with the small subunits then specifying in sequence the remaining two $\alpha\beta$ pairs for tight nucleotide binding followed by an energy-induced release.

Experiments in progress are focused on determining the exact subunit location of the four reversible nucleotides that bind to rat liver F_1, and the effect of the electrochemical proton gradient on the binding of these nucleotides, as well as its effect on endogenously bound nucleotides not detected by the studies reviewed here.

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