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Minireview

Quaternary Structure of ATP Synthases: Symmetry and Asymmetry in the F_1 Moiety

L. Mario Amzel,[1] Mario A. Bianchet,[1] and Peter L. Pedersen[2]

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It has been proposed that during ATP synthesis/hydrolysis F_1 ATPases experience a complex pattern of nucleotide binding and release during the catalytic cycle (binding change mechanism). This type of mechanism has implications that can be correlated with the structure of the enzyme. F_1-ATPases (stoichiometry $\alpha_3\beta_3\gamma\delta\varepsilon$) are essentially a symmetrical trimer of pairs of the major subunits (α and β); the minor subunits (γ, δ and ε) are in single copies and interact with the trimer in an asymmetrical fashion. The asymmetry introduced by the minor subunits has important structural and functional consequences: (1) it introduces differences between the potentially equivalent binding and catalytic sites in the major subunits, (2) it restricts the ways in which a binding change mechanism can occur, and (3) it governs the way in which the F_1 interacts with the (asymmetrical) F_0 sector.

INTRODUCTION

The structure and function of F-type ATPases have been extensively reviewed in the past (Amzel and Pedersen, 1983; Pedersen and Carafoli, 1987; Ysern *et al.*, 1988; Walker *et al.*, 1990). One of the most interesting aspects of ATP synthesis is the ability of F_1 to synthesize enzyme-bound ATP from ADP and Pi (the equilibrium constant for the enzyme-bound reaction (ADP + Pi = ATP + H_2O) is approximately 1.0. This observation has led to proposals in which H^+ ions from the H^+ gradient bring about the release of enzyme-bound ATP; both direct (Mitchell, 1985) and indirect (Boyer, 1983) effects of the H^+ ions have been proposed. Most of the proposals invoke a complex pattern of nucleotide binding and release involving conformational changes in the enzyme: the binding change mechanism (Grubmeyer *et al.*, 1982; Cross *et al.*, 1982, 1984; Penefsky and Grubmeyer, 1984). According to this mechanism, the catalytic sites on each of the three β subunits exhibit different affinities for nucleotide depending on whether only one or more of the sites is occupied. When F_1 binds ATP(Mg) in such a way that only one catalytic site is occupied per mole of enzyme (unisite conditions), the bound ATP(Mg) has a very tight affinity ($K_d \simeq 10^{-12}$ M). This bound ATP undergoes a reversible hydrolysis at that site with a $K_{eq} \simeq 1$, but products are not readily released. To exhibit high steady-state ATPase activity ($K_{cat} \simeq 600\,\text{sec}^{-1}$), additional ATP (Mg) must promote product release by filling at least one other catalytic site (multi-site conditions) (Grubmeyer and Penefsky, 1981; Choate *et al.*, 1979; Cross and Nalin, 1982). The complex kinetics of cooperativety between the catalytic sites of F_1 suggests that the enzyme has at least two equivalent catalytic (exchangeable) sites that are assumed to function alternately (Boyer, 1987; Cunningham and Cross, 1988; Al-Shawi *et al.*, 1990). Whether two or three catalytic sites on the β subunits actually operate in an alternating sequence during steady-state ATP hydrolysis and synthesis (Boyer, 1983) is a subject of some controversy. However, changes in the affinity for nucleotides at these β sites, depending on whether one or more than one is filled, seems to be a very consistent observation. These

[1] Department of Biophysics and Biophysical Chemistry, Johns Hopkins School of Medicine, Baltimore, Maryland 21205.
[2] Department of Biological Chemistry, Johns Hopkins School of Medicine, Baltimore, Maryland 21205.

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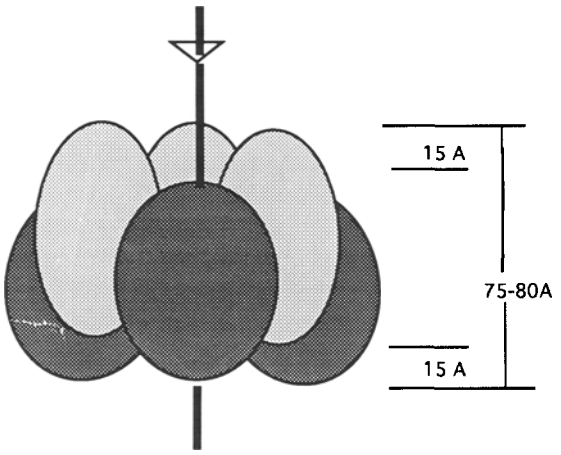

Fig. 1. Diagram of the arrangement of the major subunits in the F_1-ATPase. The α subunits (dark shade) occur at the bottom of the diagram and the β (light shade) at the top. The minor subunits (not shown in the diagram) are thought to be located at the bottom. The three-fold axis of symmetry relating the three copies of the major subunits is represented by a thick vertical line.

mechanisms have very explicit structural implications that need to be evaluated as soon as atomic level structural information becomes available.

STRUCTURE AND SYMMETRY OF F_1ATPases

The highest-resolution structure available to date is provided by an x-ray diffraction study to 3.6 Å resolution that describes the quaternary structure of the rat-liver F_1 (Bianchet *et al.*, 1991). Although this study does not provide atomic resolution, it contains information relevant to the proposed enzymatic mechanisms. In the crystal the enzyme sits on a crystallographic three-fold axis (parallel to the *c* axis of the hexagonal cell) that relates the three copies of each major subunit. Both the α and β subunits have similar ellipsoidal shapes and sizes—α of dimensions 48 × 48 × 50 Å and β of 40 × 48 × 50 Å (Fig. 1). The major axis of the β subunits is almost parallel to the three-fold axis, while that of the α is tilted by about 30°. Starting at the plane defined by the three two-fold axes ($z = 0$, "bottom"), one finds density corresponding to the three α subunits; they are elongated in the plane in such a way that they interact with each other at the three-fold axis. They continue in a very similar arrangement for about 50 Å measured along the z direction. The β subunits start about 10–15 Å from the $z = 0$ plane with their major axes running parallel to the z direction and the center of the subunits approximately 30 Å from the three-fold axis. In this arrangement the β subunits interact strongly with the α subunits, but little or not at all with each other (see cover figure). Since the α subunits are tilted, the relative arrangement of the α's and the β's varies from plane to plane, but on average can be described as having the centre of the α subunits at 40–50° from the center of the β's. At the "top" of the molecule—about 75 Å from the "bottom"—only the β subunits are present, leaving the region surrounding the three-fold axis devoid of density.

Since in the crystals the enzyme lies on a crystallographic three-fold axis of symmetry, the minor subunits—being in single copies in the complex—cannot conform to the crystallographic symmetry. This poses two questions: (1) How does the crystal packing accommodate molecules in different orientations at equivalent lattice points, and (2) What structural and functional information can be inferred from this observation. The unit cell of the crystals of the rat liver F_1 is rhombohedral, but the cell dimensions are such that the arrangement of molecules can be described approximately by a face-centered cubic (fcc) packing. In the approximate fcc cell, the packing unit is an approximate sphere formed by a pair of F_1 molecules interacting bottom-to-bottom. If this arrangement of the pair of molecules places the minor subunits in contact with the major subunits in the inside of the sphere, no additional contacts involving the minor subunits need exist in the crystal. That is, the sphere-to-sphere contacts (i.e., those required to conform to the translational symmetry of the crystal) involve only the major subunits. Since the major subunits are present in three copies each in the molecule, they can conform to the crystal symmetry. (Since the orientation of the minor subunits is different at each lattice point, in the x-ray image these subunits are seen as a disordered average of three different orientations; see Fig. 2).

The location of the minor subunits in the F_1 sector has been the subject of extensive studies using electron microscopy and immunoelectron microscopy (Lündsdorf *et al.*, 1984; Tiedge *et al.*, 1985; Gogol *et al.*, 1989). Micrographs of the complete F_1 as well as those of the $\alpha_3\beta_3\gamma$ complex show the presence of a stain excluding mass at the center of the molecule, while complexes lacking the γ subunit have no mass in that position (Yoshimura *et al.*, 1989; Gogol *et al.*, 1990). These observations place the γ subunit at the center of the F_1 molecule. In a recent paper using

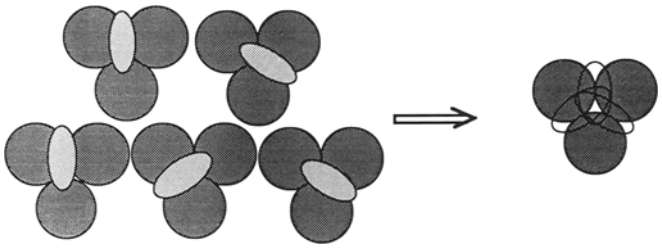

Fig. 2. Diagrammatic representation of the behavior of the minor subunits in the rat liver F_1 crystals. Pairs of major subunits (α/β) are represented by the shaded circles and the minor subunits by the ellipses. One F_1 molecule comprises three circles (α/β pairs) and one ellipse (γ, δ, and ε). At each of the lattice points (represented on the left) the orientation of the minor subunits is random with respect to a rotation of 120°. The image obtained as a result of the x-ray diffraction analysis is an average of all lattice points and therefore the density of the minor subunits appears in the map as an average of images rotated by 120° (image at the right of the diagram). Since the total density of these subunits is distributed over the three images, their density appears on the average at one-third of the density of the major subunits.

cryoelectron microscopy, Capaldi and coworkers (Gogol *et al.*, 1990) obtained images of *Escherichia coli* F_1 decorated with monoclonal Fab' fragments against the α, γ, δ and ε subunits. This study confirmed the central location of the γ subunit (although some γ epitopes were found on the periphery) but showed asymmetric location of γ epitopes. Subunits δ and ε were found at the periphery of the F_1 molecule.

FUNCTIONAL IMPLICATION OF THE SYMMETRY/ASYMMETRY OF THE F_1-ATPase

The fact that the major subunits do conform to the crystallographic three-fold symmetry of the crystal has structural as well as functional implications (Ysern *et al.*, 1988; Pedersen and Amzel, 1985). Since the minor subunits are in single copies in the complex, they must have different interactions with each of the copies of the α and the β subunits. One can expect that, as a consequence of these different interactions, the three copies of the major subunits might show some conformational differences that would extend to regions of the molecule far from the actual places of interaction (an allosteric effect of the interaction with the minor subunits). This is clearly not the case in the crystal: the presence of the minor subunits appears to break the symmetry of the complex only locally. Even under these conditions this asymmetry can have a profound effect on the way the F_1 interacts with the F_0. The F_0 moiety has a number of different subunits —most of them in single or double copies. Interaction of the F_1 with the F_0—especially with the **a** and **b** subunits of F_0—must involve interactions that are different with each α/β pair. Most likely, the asymmetry in the interactions of the F_1 with the F_0 is not independent of that introduced by the minor F_1 subunits. Probably the **a** and the **b** F_0 subunits recognize particular α/β pairs tagged by specific interactions with the minor F_1 subunits.

In principle, each of the minor subunits can interact with one, two, or three of the α/β pairs. Interaction with only one of the pairs would be very difficult to reconcile with a binding change mechanism unless there are massive movements (rotation) of the minor subunits during the catalytic cycle of the enzyme. More likely, the minor subunits (and in particular γ) interact asymmetrically with more than one α/β pair (Ysern *et al.*, 1988). That is, each β (and each α) in the trimer interacts with different portions of the minor subunits. During a catalytic cycle (if binding change actually plays a role in the mechanism of the enzyme) changes occur in the contacts between the minor subunits and each α/β pair which affect the binding and catalytic properties of the three different catalytic and/or regulatory sites. In the F_0F_1 complex these changes must be coordinated with the flow of protons through the F_0 sector. The symmetry of the F_0 sector places restrictions on how this coordination can be accomplished. There are enough **c** subunits (10–12) (Fillingame, 1981) to provide one proton channel for each α/β pair. However, since the **a** subunit—which exists as a single copy in the complex—is required for the formation of the channel, it has been suggested that the complete complex only contains a single proton channel (Friedl *et al.*, 1983; Lightowlers *et al.*, 1987; Vik *et al.*, 1988; Schneider and Altendorf, 1987). Alternatively, three channels can be formed by assembling **c** subunits around a single, required **a** subunit. In either case, it appears most likely that the extramembrane domain of the **a** subunit interacts with the central portion of the F_1, close to the position of the three-fold axis in a specific orientation with respect to the position of the single-copy F_1 subunits. In such an arrangement the role of the minor F_1 subunits in the F_0–F_1 interactions could be, at least in part, to provide

a non-three-fold symmetrical environment for the attachment of the **a** subunit (Englebrecht and Junge, 1988; Englebrecht *et al* ., 1986; Dupuis and Vignals, 1987; Jounouchi *et al.*, 1992).

BINDING AND LABELING STUDIES

Binding and labeling studies carried out with the F_1-ATPase provide additional information about the symmetry characteristics of the enzyme. Although it appears clear that there are six nucleotide binding sites in the complete F_1 (Garret and Penefsky, 1975; Cross and Nalin, 1982; Xue *et al.*, 1987; Wise *et al.*, 1983; Girault *et al.*, 1988) (three in α subunits and three in β subunits) (Schuster *et al.*, 1975; Harris *et al.*, 1978), the sites in the same subunit types are not equivalent at all times. (A good discussion of the symmetry/asymmetry properties of the catalytic and regulatory site on F_1 can be found in Pedersen and Amzel, 1985). For example, derivatization of the mitochondrial F_1 by 5′-p-fluorosulfonylbenzoylinosine (FSBI) (Bullough and Allison, 1986a), or 2-azido-ATP (Garin *et al.*, 1986; Cross *et al.*, 1987; Xue *et al.*, 1987; Wise *et al.*, 1987) at a single β subunit per F_1 results in the complete inactivation of the complex, while inactivation by fluorosulfonylbenzoyladenosine (FSBA)—which binds at a noncatalytic site—requires three sites per mole of enzyme for complete inactivation (Esch and Allison, 1978; Bullough and Allison, 1986a,b). Also, F_1 inactivated with 1 mole of 7-chloro-4-nitrobenzofurazan (Nbf-Cl) (Andrews *et al.*, 1984) per mole of enzyme retains the capacity to bind 3 moles of AMP-PNP per mole of enzyme. Inactivation of the enzyme can also be carried out with 5′-*p*-fluorosulfonylbenzoylethenoadenosine (FSBεA) (Verbug and Allison, 1990); this reagent labels the α subunit, probably at a noncatalytic site. Labeling by FSBεA is prevented if the enzyme has been previously inactivated by FSBA but not if the inactivation was carried out with FSBI. These complex patterns of labeling and inactivation are consistent with the symmetry/asymmetry suggested by the x-ray model in which all three α/β pairs are identical and in identical environments except for the small portions of the molecules that are in contact with the minor subunits. Understanding in detail the way in which binding of one label affects the binding of a different label will require atomic resolution studies of covalently labeled F_1 molecules.

CONCLUSIONS

From all the considerations presented in this discussion, a picture emerges of the F_1ATPase that has very interesting symmetry properties. The molecule is essentially a symmetrical trimer of pairs of the major subunits but the minor subunits, which occur at one end of the molecule, interact asymmetrically with the trimer (i.e., differently with each α/β pair). These asymmetric interactions have mainly local effects on the structure. However, the asymmetry introduced by the minor subunits has extremely important functional consequences: it creates structural and functional differences between potentially equivalent binding sites (catalytic, regulatory), it restricts the number of ways in which binding change mechanisms can ocur, and it governs the symmetry (or lack of it) in the interactions between the F_0 and the F_1.

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