Structural Analysis of the Yeast F_1-ATPase at a Resolution of 2.8 Å

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Introduction

Mitochondrial ATP synthase is the enzyme responsible for the synthesis of 90% of cellular ATP under aerobic conditions. The enzyme is composed of a water-soluble portion, F_1-ATPase (molecular weight [MW] = 360 kDa), and a membrane portion, F_0(MW = 190 kDa). F_1-ATPase is composed of five different subunits with the stoichiometry $\alpha_3\beta_3\gamma\delta\varepsilon$ [1]. The active sites are located in the β-subunits but have some contributions from the α-subunits [2]. As a result, there are three active sites within the enzyme composed of chemically identical α/β pairs. The subunit stoichiometry of F_0 is not as certain, but for the yeast enzyme, $a_1b_1c_{10}$ [3-5] appears to form a minimal structure on which a functional F_0 is made.

The high-resolution crystal structure of F_1-ATPase was first determined for the bovine enzyme at a resolution of 2.8 Å [2]. This study provided critical evidence in support of the binding-change mechanism for ATP synthesis [6], including the clear asymmetry of the enzyme. Although the three active sites were composed of identical subunits, the conformations of the active sites were not identical. One site was filled with AMP-PNP (the β_{TP} subunit); the second is filled with ADP (the β_{DP} subunit); and the third site was empty (the β_E subunit). The structure also provided a clear insight into the role of the γ-subunit, which was within the α/β core of F_1, and it provided the first details on the asymmetric associations of the γ-subunit with the active sites of the enzyme. This gave insight into a mechanism by which the rotation of the γ-subunit within the core of F_1 resulted in sequential conformational changes at the active sites. Indeed, ATP-dependent rotation of the γ-subunit was later demonstrated by single molecule fluorescent studies [7, 8].

Since the seminal publication on the structure of the bovine F_1-ATPase [2], there have been 10 additional high-resolution structures of the bovine enzyme in the presence of a variety of natural and artificial inhibitors from the same laboratory (for instance, see Ref. 9), and there have been a few lower-resolution structures of the enzyme, or subcomplexes of the enzyme, from rat liver [10], spinach chloroplast [11], thermophilic *Bacillus* PS3 [12], and *E. coli* [13]. With the exception of the *E. coli* structure (which is only available at low resolution), none of the structures clearly displayed the asymmetric features of the bovine F_1-ATPase because these were averaged by the crystallographic symmetry. Because it has not been proven possible to reconstitute the bovine enzyme from recombinantly expressed subunits, no model system was available that would allow the analysis of genetically modified forms of F_1-ATPase by way of x-ray crystallographic analysis. For this reason, we undertook an effort to crystallize the yeast F_1-ATPase with the future goal of analyzing mutant enzyme structures. We have determined the crystal structure of yeast F_1-ATPase at a resolution of 2.8 Å, and the structure supports all of the major conclusions from the structure of the bovine F_1-ATPase.

Methods and Materials

The β-subunit of yeast F_1-ATPase was modified by adding 6-His codons to the amino end just adjacent to the sequence encoding the leader peptide for mitochondrial import. The modified gene was integrated into the yeast genome in a strain of yeast that had a deletion mutation in the gene encoding the β-subunit, ATP2. The yeast was grown in a 50-L carboy (1% yeast extract, 2% peptone, 3% glycerol medium, 30 L) that was fitted with four sterile air in-ports and stirred with four propellers fitted on a drive shaft. Air was added at a rate of 40 L/min with rapid mixing. The yeast was harvested after 48 hours, the mitochondria were isolated, and the enzyme purified by a modified method [14]. Details on the genetic constructs and the purification method will be given elsewhere.

The enzyme was crystallized at room temperature by using polyethyelene glycol as the precipitant in the presence of AMP-PNP and ADP. The crystals were frozen by being plunged into liquid nitrogen. Diffraction data were collected at –173°C at DND-CAT beamline ID-5 at the APS with the MAR Research 225 mosaic charge-coupled device (CCD) detector. More than 600 images were recorded ($\Delta\Phi$ = 0.3°, 10-second exposures) and processed with MOSFLM [15] and SCALA [16]. The crystals belong to the $P2_1$ space group with unit cell dimensions of a = 111.8 Å, b = 294.4 Å, c = 190.8 Å, and β =101.7°. The structure was solved by molecular replacement using AMoRE [16] by using the coordinates of the bovine enzyme structure. After

the model was built by using O [17] and XtalView [18], it was refined by using Refmac [16, 19] and CNS [20]. Details on the crystallization and processing of the data will be given elsewhere.

Results and Discussion

The asymmetric unit contains three molecules of yeast F_1-ATPase (>10,500 residues) providing contents consisting of 55% solvent. The overall structures of the three molecules are similar but not identical. The density is defined much better for one of the three molecules than for the other two, with clear density present for nearly all of the residues in the nine chains. The asymmetry of the enzyme is clear, but, in contrast to the bovine enzyme, the yeast enzyme contains AMP-PNP in two of the active sites rather than in one. The third site is devoid of nucleotides, as in the bovine enzyme. The β_{DP} subunit, which contains AMP-PNP in the yeast structure, adopts a more open conformation and may represent a different point along the biochemical pathway toward the synthesis of ATP.

It had been suggested that the structure of the bovine enzyme represented that of the ADP-inhibited form of the enzyme, largely because ADP was present in one site and a second site was empty [21]. The yeast enzyme was also crystallized in the presence of ADP, and the overall structure is very similar to that of the bovine enzyme. The absence of ADP in any one of the active sites is definitive proof that the structure of the yeast enzyme is not that of the ADP-inhibited form. The close similarity of the structure of the yeast enzyme and the structure of the bovine enzyme indicates that while the bovine structure may be of the ADP-inhibited form, the structure is not that different from (if not nearly identical to) that of the active enzyme form.

The γ-subunit shows the same asymmetric contacts with the α- and β-subunits of the ATPase as those seen in the bovine structure. However, the position of the central coiled-coil of the γ-subunit within the $(\alpha\beta)_3$ subassembly is not identical in the three molecules of the yeast enzyme and is slightly different than those of the bovine enzyme structures. It is not clear at this stage of the analysis if these differences represent conformationally important forms of the enzyme or if they are caused by different crystal packing forces.

The structures of the δ- and ε-subunits are well defined in only one of the three molecules. The subunits' overall structures and their positions relative to the core of the enzyme are similar to those seen in the bovine enzyme. Thus, despite the low sequence similarity between the yeast and bovine subunits, the structures and also probably the functions of these minor subunits are conserved.

The close similarity in the biochemistries of the yeast and bovine enzymes indicates that the information derived from the analysis of the yeast enzyme is directly applicable to understanding the mammalian enzyme. Thus, crystallization and structural analysis of the yeast enzyme has opened the door to understanding the molecular details of the bovine enzyme on the basis of the genetic and structural analysis of the yeast enzyme.

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