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# The three-dimensional structure of rat liver mitochodria $F_1$-ATPase: X-ray diffraction studies

Mario Bianchet [a], Djamel Medjahed [a], Joanne Hulihen [a], Peter L. Pedersen [b], L. Mario Amzel [a,*]

[a] *Department of Biophysics and Biophysical Chemistry,*
[b] *Department of Biological Chemistry, The Johns Hopkins University, School of Medicine, Baltimore, MD 21205, USA*

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The ATP-synthase of bacteria, chloroplast and mitochondria is a complex, membrane-bound enzyme that catalyzes the phosphorylation of ADP by inorganic phosphate utilizing the electrochemical gradient of protons established by the electron transfer processes. Although membrane-bound, the enzyme contains a large extramembrane portion – the $F_1$-ATPase – that can be separated from the membrane and purified as a soluble protein. The $F_1$-ATPase is itself very large ($M_r$ 370000) and contains all the catalytic and nucleotide binding sites of the synthase. It contains five different subunits: $\alpha$ (55 kDa), $\beta$ (50 kDa), $\gamma$ (35 kDa), $\delta$ (25 kDa), and $\epsilon$ (12 kDa) in the stoichiometry $\alpha_3\beta_3\gamma\delta\epsilon$ [1,2]. The $F_1$-ATPase from rat liver mitochondria has been crystallized in our laboratory and subjected x-ray diffraction analysis.

The crystals of the rat liver $F_1$-ATPase belong to space group R32 with hexagonal cell dimensions of $a = b = 146$ Å and $c = 368$ Å (Table 1). The crystals, obtained by ammonium sulfate precipitation in the presence of phosphate and ATP, contain one-third of the $F_1$-ATPase in the asymmetric unit [3]. Data collected to 3.2 Å resolution phased using multiple isomorphous replacement were used to compute an electron density map that was further improved by cycles of solvent flattening and Fourier inversion.

The electron density map shows that two $F_1$-ATPase molecules are located at the intersection of the crystallographic three-fold and two-fold axes of the crystal. Each $F_1$-ATPase has therefore crystallographic three-fold symmetry relating one $\alpha\beta$ pair to the other two. The small subunits – $\gamma$, $\delta$, and $\epsilon$ – cannot conform to the crystalographic symmetry and have probably different orientations in different molecules in the crystal [4].

The electron density map was analyzed using the graphics program 'O' [5]. Elements of secondary structure – $\alpha$-helices and $\beta$-sheets – were easily identified and built using main chain atoms plus $\beta$-carbons (polyalanine chain). Although density for side chain atoms was clearly present in many of these regions no attempt was made at this time to fit the sequence to the electron density. After main chain atoms of over 400 residues were built, two regions were recognized as having an arrangement of helices and $\beta$-sheets similar to that found in the nucleotide binding portion of adenylate kinase (ADK). Regions of sequence corresponding to the consensus sequences found in nucleotide binding domains of several ATP-utilizing proteins have been identified in the alpha and the beta subuinits of $F_1$-ATPase (Table 2) [6]. It was then clear that these sequences had to correspound to the por-

Table 1
Crystallographic characterization

| Space group: | R32 |
|---|---|
| Cell parameters (Hexagonal setting) | |
| *a&b*: | 146 Å |
| *c*: | 368 Å |
| Crystallization condition: | 1.1 M $(NH_4)_2SO_4$, 0.2 M $KP_i$, 5 mM, ATP |
| Crystal morphology: | cubic |
| Crystal size: | 0.4–1.0 mm |
| Resolution | 4.0–2.7 Å |

* Corresponding author. Fax: +1 (410) 9550637.


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Table 2
Homology regions between $F_1$-ATPase subunits and related proteins

| | Consensus region A | |
|---|---|---|
| α $F_1$-ATPase | LVPIGRGQRELII_GDRQTGKTSIAIDTII | (155–184) |
| β $F_1$-ATPase | LAPYAKGGKIGLF_GGAGVGKTVLIMELIN | (143–171) |
| recA | GAGGLPMGRIVEIYGPESSGKTTLTLQVIA | (52– 84) |
| Myosin | MLTDRENQSILIT_GESGAGKKVNTKRVIQ | (165–193) |
| EFTu | FGRTKPHVNVGTI_GHVDHGKTTLTAAITT | (6– 34) |
| Adenylate kinase | MEEKLKKSKIIFVVGGPGSGKGTQCEKIVQ | (1– 30) |
| | Consensus region B | |
| α $F_1$-ATPase | MGEYFRDNGKRALIIYDDLSKQ | (253–273) |
| β $F_1$-ATPase | VAEYFRDQEGQDVLLFIDNIFR | (239–258) |
| recA | ALKFYASVRLDIRRIGAVKEGE | (214–235) |
| EFTu | MITGAAQMDGAILVVAATDGPMPQTR | (91–116) |
| Adenylate kinase | GEEFERK_IGQPTLLLYVDAGPETMT | (102–126) |

tions of the map having the ADK-type fold. The alpha and the beta subunits were differentiated by the existence of a cysteine residue in position 201 of the alpha subunit – right in the center of the nucleotide binding domain. This cystein residue was the site of binding of one of the heavy atoms in the mercurial derivatives used for the determination of the phases. Once the cysteine was identified other indications of the sequence of the alpha subunit were found in the electron density map. Using similar methods the nucleotide binding domains of the alpha (residues 165 to 316) and the beta (residues 144 to 327) subunits were built in the electron density.

Table 3
Data collection statistics

| Resolution (Å) | Shell (%) | *R*-merge | $\langle I/\sigma \rangle$ |
|---|---|---|---|
| 30.00 | 93.2 (93.2) | 5.6 | 14.19 |
| 20.00 | 96.2 (97.7) | 4.9 | 16.94 |
| 10.00 | 98.2 (98.6) | 5.2 | 15.27 |
| 7.50 | 98.6 (99.0) | 5.6 | 12.81 |
| 5.00 | 99.0 (99.2) | 7.1 | 7.53 |
| 4.00 | 99.2 (99.3) | 8.5 | 6.03 |
| 3.50 | 99.3 (99.5) | 9.7 | 3.51 |
| 3.30 | 99.3 (99.4) | 10.3 | 2.42 |
| 3.00 | 99.3 (99.5) | 11.2 | 1.87 |
| 2.80 | 97.1 (87.3) | 11.6 | 1.66 |

The number of independent reflections is 35348 out of a total of 530498 accepted measurements. The *R* value for symmetry related reflections is defined as $R_{sym} = \Sigma_{ij} | I_{ij} - \langle I_j \rangle | / \Sigma_i | I_{ij} |$, where $I_{ij}$ is the intensity of an individual measurement and $\langle I_i \rangle$ is the mean value for all measurements for each independent reflection.

Since the completion of this stage of the studies at 3.2 Å resolution, we have been able to improve the quality of the diffraction by a combination of an increase in the crystal size and the utilization of flash-freezing during the diffraction data collection. The resolution of the present data set extends to 2.8 Å with a degree of completness that exceeds 85% (Table 3). This new data set is not only a higher resolution set but was, in addition, obtained from a single crystal with very high redundancy. This high redundancy allowed a very detailed analysis of the symmmetry of the diffraction pattern that was confirmed to correspond to that of the space group R32. The new data set represents an increase in the number of reflexions from 25812 (in the 3.2 Å data set) to 35348. Structural analysis using this data set is underway.

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