Mitochondrial ATP Synthase

CRYSTAL STRUCTURE OF THE CATALYTIC F1 UNIT IN A VANADATE-INDUCED TRANSITION-LIKE STATE AND IMPLICATIONS FOR MECHANISM*

Chen Chen1, Ajay K. Saxena1,2, William N. Simcoke1,2, David N. Garboczi3, Peter L. Pedersen4,5, and Young H. Ko3

From the 1Department of Biological Chemistry, Johns Hopkins University School of Medicine, Baltimore, Maryland 21205-2185 and 2Structural Biology Section, Laboratory of Immunogenetics, NIAID, National Institutes of Health, Rockville, Maryland 20852

ATP synthesis from ADP, P, and Mg takes place in mitochondria on the catalytic F1 unit (αβγδε) of the ATP synthase complex (F0F1), a remarkable nanomachine that interconverts electrochemical and mechanical energy, producing the high energy terminal complex (Fig. 1). In currently available structural models of F1, the P-loop (amino acid residues —GAGVGTK—) contributes to substrate binding at the β subunit catalytic sites. Here, we report the first transition state-like structure of F1 (ADP•V/MgF) from rat liver that was crystallized with the phosphate (P) analog vanadate (VO4− or V). Compared with earlier “ground state” structures, this new F1 structure reveals that the active site region has undergone significant remodelling. P-loop residue alanine 158 is located much closer to V than is to P, in a previous structural model. No significant movements of P-loop residues of the α subunit were observed at its analogous but non-catalytic sites. Under physiological conditions, such active site remodelling involving the small hydrophobic alanine residue may promote ATP synthesis by lowering the local dielectric constant, thus facilitating the dehydration of ADP and P. This new crystallographic study provides strong support for the catalytic mechanism of ATP synthesis deduced from earlier biochemical studies of liver F1 conducted in the presence of V (Ko, Y. H., Bianchet, M., Amzel, L. M., and Pedersen, P. L. (1997) J. Biol. Chem. 272, 18875–18881; Ko, Y. H., Hong, S., and Pedersen, P. L. (1999) J. Biol. Chem. 274, 28853–28856).

The mammalian mitochondrial ATP synthase (F0F1) is a large protein complex (Fig. 1A) located in the inner membrane, where it catalyzes ATP synthesis from ADP, P, and Mg2+ at the expense of an electrochemical gradient of protons generated by the electron transport chain (reviewed in Refs. 1 and 2). Excluding regulators, the mammalian ATP synthase consists of 15 subunit types (3, 4), of which five in the stoichiometric ratio αβγδε

5 The abbreviations used are: V, orthovanadate; MOPS, 3-(N-morpholino)propanesulfonic acid; r.m.s., root mean square; AMP-PNP, 5′-adenylyl-β,γ-mididophosphate; OSCP, oligomycin sensitivity-conferring protein.
these same investigators used phosphate analogs other than Vi (e.g., AlF₄⁻/H₂O) to give an ADP-/H₂O⁻AlF₄⁻/H₂O⁻Mg⁺⁻myosin complex, the crystal structure of which was solved also at atomic resolution (19), they concluded that the ADP-/H₂O⁻Vi⁻Mg⁺⁻myosin structure resembled most closely that expected for a true transition state (18).

With the above in mind, our earlier biochemical studies (15, 16) with the catalytic F₁ moiety of the ATP synthase demonstrated first that in the presence of ADP, Mg²⁺, and Vi, the ATPase activity of the F₁ catalytic unit is markedly inhibited as expected if an ADP-/H₂O⁻Vi⁻Mg⁺⁻F₁ transition-like state is formed (15) and, second, that upon exposure to UV light, cleavage of the P-loop (GGAGVGKT) occurs also at the third position (Ala¹⁵⁸) of the P-loop (GGAGVGK). In control studies, prior incubation of F₁ with Vi alone, ADP + Vi, and ADP + Mg²⁺ had little or no effect on ATPase activity. Taken together, these findings suggested, consistent with experiments that had been observed earlier for myosin (17, 18), that an ADP-/H₂O⁻Vi⁻Mg⁺⁻F₁ transition-like state had formed in which the γ-phosphate of ATP and the third residue of the P-loop, in this case conserved Ala¹⁵⁸, reside close to one another (Fig. 1B).

Here, we report the first crystal structure of an ATP synthase F₁ moiety (catalytic unit) crystallized in the presence of ATP, Mg²⁺, and Vi and discuss its apparent validation of the conclusions made from our earlier biochemical studies (15, 16). In addition, we discuss the possible direct relevance of this new structure to the reaction pathway for ATP synthesis in mitochondria and compare it with crystal structures (20, 21) obtained for bovine heart F₁ in complex formation with aluminum fluoride, a potential transition state analog (22).

**EXPERIMENTAL PROCEDURES**

**Materials**

Rats (Harlan Sprague-Dawley CD, white males, retired breeders) were obtained from Charles River Breeding Laboratories and cared for and used...
experimentally according to guidelines approved by the Animal Care and Use Committee of the Johns Hopkins University School of Medicine. ATP, ADP, MgCl₂, ammonium sulfate, and sodium orthovanadate were obtained from Sigma. Crystallization plates were from Hampton Research. All other reagents were of the highest purity commercially available.

**Methods**

**Preparation and Crystallization of F₁**—Rat liver F₁ was purified by the method of Catterall and Pedersen (23) with the modification described by Pedersen et al. (24). It was then lyophilized to dryness in P₁ and stored at −20 °C until use. For crystallization, the lyophilized enzyme was dissolved at 25 °C in 100 μl of water and precipitated twice in 3 M ammonium sulfate, 5 mM EDTA. It was then dissolved in 5 mM ATP, 5 mM MgCl₂, 5 mM V₁, 25 mM sodium azide, and 50 mM MOPS, pH 8.8, at 23 °C and crystallized by the sitting drop vapor diffusion method. The V₁ stock solution was carefully prepared as described previously (15), a very important series of steps, and the concentration was determined by measuring the optical density at 265 nm using the extinction coefficient reported by Amzel and Pedersen (29) for rat liver F₁ crystallized in the ADP, ATP, ADP, MgCl₂, ammonium sulfate, and sodium orthovanadate were omitted in the phase calculation. The trigonal-bipyramidal model of V₁ was obtained from the ADP·V₁·Mg²⁺·myosin structure (18) and manually docked into the 3α density proximal to ADP. Electron density maps were calculated using the program CNS (30).

**TABLE 1**

| Parameters | Values |
|------------|--------|
| Space group and unit cell (Å) | R32, a = b = 144.6, c = 363.0 |
| Wave length (Å)/X-ray source | 0.99092/ANL 191D |
| Resolution (Å)* | 73.5–3.0 (3.16–3.0) |
| Observed/Unique reflections | 257,373/29,647 |
| Redundancy | 8.7 (5.9) |
| Completeness (%) | 100.0 (100.0) |
| Rwork (%) | 14.3 (75) |
| Rfree (%) | 12.1 (3.2) |

*Values in parentheses are for the highest resolution shell.

**Rationale for Crystallization Conditions**—P₂ added to the medium to obtain the earlier ADP·P₁·F₁ crystals (1MAB) of rat liver (10) was omitted from the crystallization medium designed to obtain ADP·V₁·MgF₁ crystals in order to minimize competition between P₁ and V₁ for the active site of F₁. A final crystallization mixture consisting of ATP, MgCl₂, V₁, and azide was selected (see “Experimental Procedures”). Azide, an inhibitor of ATP hydrolysis catalyzed by F₁, was included, since it is known that this inhibitor does not immediately prevent the cleavage of the γ-phosphate of ATP (34). Rather, azide inhibits after some ATP hydrolysis has occurred, implicating its stabilization of intermediate states (34). Therefore, it was rationalized that subsequent to F₁-catalyzed hydrolysis of ATP in the presence of V₁, the transition-like state may be stabilized. In fact, ADP·V₁·MgF₁-containing crystals were obtained.

**Description of ADP·V₁·MgF₁ Crystals**—Table 1 provides a summary of the x-ray data collected and refinement statistics. Like the earlier ADP·P₁·F₁ crystals (10), the ADP·V₁·MgF₁ crystals (Fig. 2A, inset) obtained in the presence of ATP, MgCl₂, and V₁ belong to space group R32, indicating that the three αβ subunit pairs of the functional unit have 3-fold crystallographic symmetry and that the single γ subunit in the crystal lattice resides in one of three possible orientations. Therefore, the occupancy of the γ subunit is 0.33. Space groups other than R32 were considered and excluded. These included three other possible C2 space groups, which are subgroups of R32, and space group P1. There is no difference in the Rsym among these space groups even to the highest resolution shell. Finally, twinning was excluded based on the ratio (Iₚ/I₀) calculated by the program CNS (30). The Rfree was carefully checked throughout the refinement, which included many trials of simulated annealing and energy minimization that were performed until no further improvement could be achieved.
Mechanism of ATP Synthase in the Transition State

Description of the Overall ADP-Vi•MgF1 Structure Relative to the ADP-Pi•F1 Structure (1MAB)—The ADP-Vi•MgF1 structure (Fig. 2) was solved by molecular replacement with the program MOLREP (28) with the ADP-Pi•F1 structure (1MAB) as the search model (see “Experimental Procedures”). The coordinates of the ADP-Vi•MgF1 structure described here (red) were aligned with those (blue) of the original ADP-Pi•F1 structure (10). Fig. 2A depicts the alignment of the αβγ parts of each structure, and Fig. 2B shows the alignment of the αβγ catalytic unit. The alignment was based on amino acid residues 24–91 of the α subunit that have been reported to undergo little conformational change during catalysis (21). Overall, the two structures almost overlap as the r.m.s. difference of all peptide atoms is only 0.52 Å. Thus, the presence of V3 Mg at the active site of rat liver F1 induces little global conformational movement. (Although present, the small δ and ε subunits are not resolved in either the 2.8 Å ADP-Pi•F1 structure (10) or in the ADP-Vi•MgF1 structure reported here but have been visualized in one F1 structure (35) in 1:1 complex formation associated with the bottom of the γ subunit.)

The αβγ part of the ADP-Vi•MgF1 structure (Fig. 2A) contains one α subunit (residues 23–502), one β subunit (residues 1–45, 74–87, and 206–270, occupancy 0.33), one γ subunit (residues 1–45, 74–87, and 206–270, occupancy 0.33), one ATP, one ADP, two Mg2+, and one V3. The ATP is complexed with Mg2+ to give ATPMg (yellow) in the α subunit site, and the ADP is complexed with both Mg2+ and V3 to give ADP-V3•Mg (green) in the β subunit “catalytic” site (Fig. 2, A and B). Within the β subunit, the V3•Mg part of the ADP-V3•Mg was located as described under “Experimental Procedures” and shown to interact with the P-loop (Fig. 2C).

The Active Site—Fig. 3A shows the atoms around the active site of the ADP-Vi•MgF1 structure relative to the P-loop, 156GGAGVGKT163. The Mg2+ (dark blue) is coordinated by P-loop Thr163 that plays the same role in myosin (18), and the vanadate (red) lies in a charged pocket surrounded by Lys159, Glu188, the catalytic base, and also Arg199 and Arg260 that are not shown. The stick model depicted without nucleotide
Mechanism of ATP Synthase in the Transition State


table 2. Distances (Å) between atoms of γ-phosphate or analogues to Ala158 and Lys162 in the ADP, P−F1, (Protein Data Bank code 1MAB) structure and the ADP, V−Mg,F1 structure.

| Atom          | Lys162 e-N | Ala158 Cα | Ala158 Cβ |
|---------------|------------|-----------|-----------|
| ADP-Pi-bound  | P          | 3.5       | 5.6       | 6.2       |
|               | O          | 3.0       | 4.5       | 5.2       |
| ADP-Vi,Mg-bound | V         | 3.9       | 3.8       | 4.7       |
|               | O          | 2.7       | 3.2       | 3.8       |

*Calculated from coordinates of Protein Data Bank code 1MAB; P is the phosphate atom of phosphate, and the closest oxygen atom of phosphate was selected.

Calculated from coordinates (Protein Data Bank code 2F43) of the ADP-Vi,Mg,F1 structure in this study; V is the vanadium atom of vanadate, and the closest oxygen atom of vanadate was selected.

of the two structures were aligned, the average distance between all amino acid atoms within residues 1–399 and 406–477 in each structure was determined, and the differences were calculated. A plot was then made of the differences versus residue number. The results presented in Fig. 4A show clearly that a distinct difference exists between the location of the β subunits in the two structures within that part of the active site that includes the P-loop. Thus, the overall average difference in distance among all β subunits is only 0.36 Å, whereas the average difference among atoms in the P-loop region is 1.0 Å. Some positional differences also occur between residues 406 and 440 near the C terminus that are unlikely to be involved with function. These differences result from our use of different side chain rotamers from those used in 1MAB to fit the electron density encountered in this region.

Consistent with a change in the P-loop regions induced by Vi−Mg as inferred by our earlier biochemical studies (15, 16), Fig. 4B shows an overlay of the P-loop region of the β subunit of the ADP-Vi−Mg,F1 structure (black) with that of the earlier ADP-Pi−F1 structure (gray). From this overlay, it is clear that a significant difference exists between the position of Ala158 in the P-loop in the two structures and also a significant difference in the position of Vi and Pi. Thus, the α-carbon of Ala158 in the ADP-Vi−Mg,F1 structure has moved 1.1 Å in the N-terminal direction of the main chain, away from the α-carbon of Ala158 in the ADP-Pi−F1 structure (gray). In addition, the Vi (red) is much closer to the P-loop in the ADP-Vi−Mg,F1 structure than is Pi (green) in the ADP-Pi−F1 structure.

in Fig. 3B shows that the P-loop of the ADP-Vi−Mg,F1 structure is stabilized also by hydrogen bonds between Arg137 and the carbonyl oxygens of Gly157 and Ala158 and by a hydrogen bond between the carbonyl oxygen of Gly158 and the imino nitrogen of Val157. Finally, both A and B of Fig. 3 show that within the active site of the ADP-Vi−Mg,F1 structure, the distance between the β carbon of P−loop Ala158 and the nearest oxygen atom of vanadate (red) is 3.8 Å, a value significantly less than the distance of 5.2 Å between the β carbon of Ala158 in the ADP-Pi−F1 structure and the nearest oxygen atom of phosphate. This finding indicated that, whereas V−Mg had induced little global change in the F1 structure as noted above, significant changes within the active site had occurred.

Structural Differences between the Active Sites of ADP-Vi−Mg,F1 and ADP-Pi−F1. —As indicated above, a distinct difference in the active sites of the ADP-Vi−Mg,F1 structure and the ADP-Pi−F1 structure (1MAB) was observed as it relates to the distance from the β carbon of Ala158 to the Vi and Pi oxygen atoms, respectively. To determine whether structural differences in β subunits were confined to the active site, the β subunits...
Mechanism of ATP Synthase in the Transition State

Comparison of Changes Observed in the P-loop Active Site Region When ADP, Vi, and Mg2⁺ Are Present Relative to When ADP, AlF4⁻/AlF₃⁻, and Mg2⁺ Are Present—Aluminum fluoride, a phosphate analog and potential transition state analog (10), has been co-crystallized recently with bovine heart F₁ (20, 21), resulting in two different high resolution structures, one designated by the authors as (ADP-AlF₄⁻)F₁, structure (1101GDRTQOIK1770), and the other as (ADP-AlF₃⁻)F₁, structure (1E1R). Specifically, the overall average distance difference between corresponding amino acid atoms in the two ADP-AlF₄⁻ subunits is 0.32 Å, a value essentially the same as the difference of 0.33 Å found between corresponding amino acid atoms in the P-loop region. This is shown more clearly in Fig. 5A, which depicts an overlay of the P-loop regions in the two cases.

In the P-loop region. This is shown more clearly in Fig. 5B, which depicts an overlay of the P-loop regions in the two cases.

Comparison of Changes Observed in the P-loop Active Site Region When ADP, Vi, and Mg²⁺ Are Present Relative to When ADP, AlF₄⁻/AlF₃⁻, and Mg²⁺ Are Present—Aluminum fluoride, a phosphate analog and potential transition state analog (10), has been co-crystallized recently with bovine heart F₁ (20, 21), resulting in two different high resolution structures, one designated by the authors as (ADP-AlF₄⁻)F₁ (Protein Data Bank code 1E1R) with two subunit catalytic sites filled with ADP, AlF₄⁻, and Mg²⁺, and a second designated (ADP-AlF₃⁻)₂F₁ (Protein Data Bank code 1H8E) with two subunit catalytic sites filled with ADP, AlF₃⁻, and Mg²⁺. Therefore, it was important to inquire to what extent ADP, AlF₄⁻/AlF₃⁻, and Mg²⁺ induce changes in the P-loop/active site region within these bovine F₁ structures relative to that induced by ADP, Vi, and Mg²⁺ in the rat liver F₁ structure. With regard to the first reported structure, (ADP-AlF₄⁻)F₁, that contained ADP, AlF₄⁻, and Mg²⁺ in a single β-subunit (20), the authors state that there is little change in the structure of this subunit relative to that of the same β-subunit (β₁₋₁₅₈) in the original bovine F₁ structure that contained ADP·Mg (9) (Protein Data Bank code 1BMF). With regard to the second reported bovine F₁ structure, (ADP-AlF₃⁻)₂F₁, in which two β-subunits are filled with ADP, AlF₃⁻, and Mg²⁺, no obvious change is observed (Fig. 6A) in the P-loops relative to the β-subunit (β₁₋₁₅₈) of the original bovine F₁ structure that contained only ADP·Mg (9). Therefore, both aluminum fluoride-containing bovine F₁ structures appear to be very near “ground state,” a conclusion reached earlier by Allison et al. (37) for the (ADP-AlF₄⁻)F₁ structure. This is in sharp contrast to that of the rat liver ADP·V₅·MgF₁ structure (Fig. 6B) reported here in which the P-loop region has undergone a significant conformational change, moving the β-carbon atom of Ala¹⁵₈ and the vanadyl atom nearly 1.5 Å closer than the β-carbon atom of Ala¹⁵₈ to phosphate atom distance in the original ADP·P₅·F₁ structure (10).

Conclusions—For the past 3 decades, a major objective related to work on the mitochondrial ATP synthase (F₁F₀) has been to understand the mechanism by which ATP·Mg is made from ADP, P₅, and Mg²⁺, a process that takes place primarily on β subunits. The study reported here, in which the F₁ catalytic moiety has been crystallized for the first time in the presence of Vi, a known transition state analog, has resulted in a structure (ADP·V₅·MgF₁) that meets two expectations of a transition state-like structure. One expectation is that one or more residues at...
whereas the methyl group of Ala$^{158}$ that has moved into the active site helps lower the dielectric constant and facilitate release of water (Fig. 7, center) and ATP formation (Fig. 7, bottom). These suggestions are consistent with our earlier biochemical studies with vanadate (15, 16) and with a study (38) demonstrating that low dielectric media (i.e. organic solvents) facilitate F$_1$-catalyzed ATP synthesis.

Acknowledgements—We thank Dr. L. Mario Amzel and Dr. Mario Bianchet (Department of Biophysics and Biophysical Chemistry, Johns Hopkins University School of Medicine) for many vigorous and helpful discussions about x-ray crystallography. We also thank Dr. Daniel Leahy of the same department for more recent insightful discussions. We are grateful also for the help and advice provided by members of the Structural Biology Center at the Argonne National Laboratory, especially Dr. Frank Rotella, in correcting reflection images. Joanne Huihui is acknowledged for technical assistance and David Bram for daily discussions.

REFERENCES

1. Pedersen, P. L., Ko, Y. H., and Hong, S. (2000) J. Bioenerg. Biomembr. 32, 325–332
2. Pedersen, P. L., Ko, Y. H., and Hong, S. (2000) J. Bioenerg. Biomembr. 32, 423–432
3. Buchanan, S. K., and Walker, J. E. (1996) Biochem. J. 318, 343–349
4. Ko, Y. H., Huihui, J., Hong, S., and Pedersen, P. L. (2000) J. Biol. Chem. 275, 32931–32939
5. Catterall, W. A., Coty, W. A., and Pedersen, P. L. (1973) J. Biol. Chem. 248, 7472–7431
6. Collinson, I. R., van Raaaij, M. J., Ruswick, M. J., Fearnley, I. M., Skehel, J. M., Oriss, G. L., Miroux, B., and Walker, J. E. (1994) J. Mol. Biol. 242, 408–421
7. Golden, T. R., and Pedersen, P. L. (1998) Biochemistry 37, 13871–13881
8. Karrash, S., and Walker, J. E. (1999) J. Biol. Chem. 274, 379–384
9. Johnson, J. S., Pedersen, P. L., and Amzel, L. M. (1994) Structure 2, 15–23
10. Ko, Y. H., Huihui, J., Pedersen, P. L., and Amzel, L. M. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 11065–11070
11. Noji, H., Yasuda, R., Yoshida, M., and Kinoshita, K. (1997) J. Biol. Chem. 272, 299–302
12. Sambongi, Y., Ko, Y., Tanabe, M., Oosane, H., Iwamoto-Kihara, A., Ueda, Y., Yanagida, T., Wada, Y., and Futaki, M. (1999) Science 286, 1722–1724
13. Ko, Y. H., Delannoy, M., Huihui, J., Chiu, W., and Pedersen, P. L. (2003) J. Biol. Chem. 278, 12305–12309
14. Chen, C., Ko, Y., Delannoy, M., Ludkte, S. J., Chiu, W., and Pedersen, P. L. (2004) J. Biol. Chem. 279, 31761–31768
15. Ko, Y. H., Bianchet, M. A., Amzel, L. M., and Pedersen, P. L. (1997) J. Biol. Chem. 272, 18875–18881
16. Ko, Y. H., Hong, S., and Pedersen, P. L. (1999) J. Biol. Chem. 274, 28853–28856
17. Cremo, C. R., Grammer, J. C., and Yoont, R. G. (1989) J. Biol. Chem. 264, 6608–6611
18. Smith, C. A., and Rayment, I. (1995) J. Mol. Biol. 260, 1718–1725
19. Braig, K., Menz, R. I., Montgomery, M. G., Leslie, A. G., and Walker, J. E. (2000) Acta Crystallogr. Sect. F, 567–573
20. Menz, R. I., Walker, J. E., and Leslie, A. G. (2001) Cell 106, 331–341
21. Kleuss, C. R., Raw, A. S., Lee, E., Sprang, S. R., and Gilman, A. G. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 9828–9831
22. Catterall, W. A., and Pedersen, P. L. (1971) J. Biol. Chem. 246, 4987–4994
23. Pedersen, P. L., Huihui, J., and Weible, I. P. (1981) J. Biol. Chem. 256, 1362–1369
24. Pughraith, J. W. (1999) Acta Crystallogr. Sect. D 55, 1718–1725
25. Leslie, A. G. W. (1992) J. Mol. Biol. 222, 505–524
26. Menz, R. I., Walker, J. E., and Leslie, A. G. (2000) J. Mol. Biol. 298, 157–162
27. Otwinowski, Z., and Minor, W. (1997) Methods Enzymol. 276, 307–326
28. Collaborative Computational Project 4 (1994) Acta Crystallogr. Sect. D 50, 5967–5970
29. Amzel, L. M., and Pedersen, P. L. (1978) J. Biol. Chem. 253, 2067–2069
30. Menz, R. I., Montgomery, M. G., Leslie, A. G., and Walker, J. E. (2000) Struct. Fold Des. 15, 567–573
31. Menz, R. I., Walker, J. E., and Leslie, A. G. (2001) Cell 106, 331–341
32. Menz, R. I., Walker, J. E., and Leslie, A. G. (2000) Proc. Natl. Acad. Sci. U. S. A. 91, 9828–9831
33. Amzel, L. M., and Pedersen, P. L. (1978) J. Biol. Chem. 253, 2067–2069
34. Menz, R. I., Montgomery, M. G., Leslie, A. G., and Walker, J. E. (2000) Acta Crystallogr. Sect. D 55, 1718–1725
35. Leslie, A. G. W. (1992) J. Mol. Biol. 222, 505–524
36. Merritt, E. A., and Bacon, D. J. (1997) J. Mol. Biol. 278, 505–524
37. Allison, W. S., Ren, H., and Dou, C. (2000) J. Biol. Chem. 275, 32531–32538
38. Gomez Puyou, A., Gomez Puyou, M. T., and de Meis, L. (1986) Eur. J. Biochem. 159, 133–140