The F₁c₁₀ subcomplex of the yeast F₁F₀-ATP synthase includes the membrane rotor part c₁₀-ring linked to a catalytic head, (αβ)₃γ, by a central stalk, γ6ε. The Saccharomyces cerevisiae yF₁c₁₀-ADP subcomplex was crystallized in the presence of Mg-ADP, dicyclohexylcarbodiimide (DCCD), and azide. The structure was solved by molecular replacement using a high-resolution model of the yeast F₁ and a bacterial c-ring model with 10 copies of the c-subunit. The structure refined to 3.43-Å resolution displays new features compared with the original yF₁c₁₀ and with the yF₁ inhibited by adenylylimidodiphosphate (AMP-PNP) (yF₁(1–III)). An ADP molecule was bound in both β₅DP and β₅TP-catalytic sites. The α₅DP-β₅TP pair is slightly open and resembles the novel conformation identified in yF₁, whereas the α₅TP-β₅TP pair is very close and resembles more a DP pair. yF₁c₁₀ provides a model of a new Mg-ADP-inhibited state of the yeast F₁. As for the original yF₁ and yF₁c₁₀ structures, the foot of the central stalk is rotated by ~40° with respect to high-resolution structures. The assembly of the F₁ central stalk with the F₀ c-ring is mainly provided by electrostatic interactions. On the rotor ring, the essential cGlu⁹⁹ carboxylate group is surrounded by hydrophobic residues and is not involved in hydrogen bonding.

The F₁F₀-ATP synthase is an essential membrane rotary motor that uses transmembrane electrochemical ion gradients to synthesize ATP. To date, only cryo-electron microscopy has provided a complete view of the yeast ATP synthase (1). The structure of the Saccharomyces cerevisiae F₁c₁₀-ATP synthase subcomplex provided the first model at 3.9-Å resolution of the molecular assembly between the membrane rotor ring and the central stalk of a F₁F₀-ATP synthase (2). Both adenylylimidodiphosphate (AMP-PNP) and ADP were added to the crystallization medium, a structure that will be referred to as yF₁c₁₀. It was solved by molecular replacement using the Cα coordinates of bovine and E. coli homologous proteins, the structure was fitted successfully in the envelope of the electron microscopy map of F₁F₀-ATP synthase to obtain the envelope of the peripheral stalk (1, 6).

The first F₁-ATPase x-ray structure (3) supports Boyer’s binding change mechanism for catalysis (7). It was proposed that (i) the empty and open catalytic site β₅ was the open site with low affinity for nucleotides, (ii) the β₅DP site filled by ATP (or AMP-PNP) was the loose conformation, and (iii) the β₅TP site filled by ADP was the tight conformation where synthesis occurs (8). The opening of catalytic sites can be estimated by means of the buried area at the α-β interface. The loose and tight conformations were further identified in the structures of the ground state (9) and of an intermediate state analog (10), but they remained debatable because the β₅TP site has also been proposed as the high affinity catalytic site (11).

The inconsistent presence of ADP in the β₅TP site of the bovine reference structure (3) has been explained by the Mg-ADP-inhibited state of the enzyme. Dicyclohexylcarbodiimide (DCCD) inhibits both the H⁺ transport and the ATPase activity (12). It has been used to block the enzyme for crystallization (13). It reacts preferentially with a single c-subunit of the F₀ rather than a single β-subunit of the F₁. During the past decade, the detailed structures of the bovine F₁-sector inhibited by Mg-ADP (bF₁;ADP, Protein Data Bank (PDB) code 1w0k) (10) or covalently inhibited on β₅Glu¹⁹⁹ by DCCD (bF₁;DCCD) (13) and of the yeast enzyme in the absence of AMP-PNP and ADP at 2.8-Å resolution (yF₁) (14) have been refined. There are three independent copies of the enzyme.

The atomic coordinates and structure factors (code 2WPD) have been deposited in the Protein Data Bank, Research Collaboratory for Structural Bioinformatics, Rutgers University, New Brunswick, NJ (http://www.rcsb.org/).

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The on-line version of this article (available at http://www.jbc.org) contains supplemental Tables S1–S3 and Figs. S1–S6.

The abbreviations used are: AMP-PNP, adenylylimidodiphosphate; r.m.s.d., root mean square deviation; PDB, Protein Data Bank; DCCD, dicyclohexylcarbodiimide; DCU, dicyclohexyl-N-acylurea.
in the yF₁ asymmetric unit, yF₁(I) and yF₁(III) were similar, and yF₁(II) was different. In the nucleotide-free yeast F₁ structure (yF₁,apo(I–III)), the DP and TP pairs adopt "closed" conformations despite the absence of bound nucleotides (15).

Concerning the membrane rotors, the structure solved at 2.4-Å resolution of the Na⁺-F₁-ATPase c-ring from *Hylobacter tartaricus* contains 11 monomers (16). The K-subunit (NtpK) of the Na⁺-V-ATPase of *Enterococcus hirae* with four transmembrane helices forms decameric rings (17). In the *E. hirae* DCCD-inhibited K₁₀-ring structure (PDB code 2DB4), cyclohexyl-N-acetylene (DCU) was found to be linked to Glu¹₃₉⁹ equivalent to the yeast cGlu⁵⁹⁹, with DCU groups protruding outside the ring.

Here, we report the x-ray structure of the F₁c₁₀ subcomplex from *S. cerevisiae* inhibited by Mg ADP and DCCD (yF₁c₁₀,ADP) refined to a resolution of 3.43 Å. However, DCC modifications on both sectors could not be ascertained on the electron density maps. The β₂₅₆ and β₅₆₇ catalytic sites adopt closed conformations and are both occupied by ADP. This structure represents the first view of the Mg-ADP-inhibited state of the yeast enzyme. The structure of the F₁,sector was compared with the structures of the bovine enzyme and with all independent copies found in the yeast F₁ with bound AMP-PNP and without bound nucleotides. Compared with the bovine enzyme, the yeast central stalk is twisted. In the F₁ rotor ring, the essential cGlu⁵⁹⁹ carbohydrate group is only surrounded by apolar residues. Its closest hydrogen bond acceptor, the cLeu⁵⁷ carbonyl oxygen of the adjacent c-subunit, is too far away to make a direct hydrogen bond. The proton binding has specific features compared with the bacterial Na⁺ transporting (16) or the cyanobacterial (19) and chloroplast (20) H⁺-transporting F-type ATP synthase rotor structures.

**EXPERIMENTAL PROCEDURES**

*yF₁,F₀, Enzyme Purification and yF₁c₁₀ Crystallization—**All purification steps were performed as described previously (21) except that the nickel-nitriolactate acid elution fraction was concentrated and subjected to a purification step on a Superdex 200 gel filtration column (HR 10/30). The gel filtration buffer contained 0.64 mM dodecyl β-d-maltoside, 100 mM NaCl, 2 mM MgCl₂, 25 mM trehalose, 0.5 mM EDTA, 3 mM sodium azide, 20 mM Tris-HCl, pH 8.0. Fractions corresponding to the major peak were analyzed by SDS-PAGE electrophoresis. These fractions contained all the known subunits of the monomeric F₁,F₀ (subunits α, β, γ, 4, 6, OSCP, d, δ, h, f, e, i, 8, and 9) (supplemental Fig. S1). Assembly states were asserted by non-denaturing Blue native PAGE. The purified F₁,F₀,ATP synthase is active, migrated as a fully assembled monomeric form (21). Neither ADP nor ATP was added during the purification. The protein solution was concentrated with a Centricron concentrator unit (YM-100, Millipore). The final enzyme concentration (9 mg/ml) was assessed by the Lowry method. The enzyme was incubated for 1 h at 20 °C with 660 μM ADP and then incubated for 1 h with 100 μM DCCD in the presence of 2.5 mM DTT and 0.5 mM PMSF.

Crystallizations were carried out at 20 °C, by the sitting drop vapor diffusion method, after mixing equal volumes of protein and reservoir solutions (100 mM NaCl, 12% PEG MME 5000, 3 mM sodium azide, 100 mM Hepes, pH 7.5). Crystals grew in a few days as flattened rods of typically 150 to 400 μm in length and 20 μm in thickness. Crystals were cryo-protected with mother liquor containing 15% (v/v) glycerol and flash-frozen in liquid nitrogen.

**Structure Determination—**X-ray diffraction data were collected on beamline ID29 (European Synchrotron Radiation Facility). Many crystals mounted from the same drop diffracted at an ~4-Å resolution, with the best one diffracting at 3.2-Å resolution. Severe anisotropic diffraction and radiation damage limited the quality of the data sets. The best data set (77% complete at 3.43 Å) was merged with one of lower resolution at 3.67 Å. All data were processed with IMOSFLM (22) and the CCP4 suite (23). The cell parameters of yF₁c₁₀,ADP are close to those of the original yF₁c₁₀ structure (2). The current structure was solved by molecular replacement with the PHASER program (24). The three independent conformations yF₁(I–III) in which nucleotides were removed (PDB code 2HLD) were used successively as search model (14). The solutions obtained with yF₁(I) and yF₁(III) were better (Z-score, 50; R_{work}/R_{free} 0.44) than with yF₁(II) (Z-score, 30; R_{work}/R_{free} 0.47). Because the central stalk of yF₁(I) was more complete than that of yF₁(III), the yF₁(I) solution was retained. The asymmetric unit contains one molecule resulting in a solvent content of 67% (V_{m} = 3.7 Å³/Da). Rigid body refinement of individual subunits resulted in R_{work} and R_{free} equal to 0.38 using 3.7 Å data. In the F₁,sector, the electron density maps are mainly continuous along the backbones and are good enough to rule out the possibility of statistical disorder.

A c-monomer template was built using the coordinates of the *I. tartaricus* rotor c₁₁-ring (PDB code 1YCE) (16) by removing six and seven residues at the N and C termini, respectively. To ascertain the number of c-subunits in the crystal, ring models containing from 8 to 12 copies (N) of the c-subunit were constructed as described below. The main axis of the *I. tartaricus* ring was oriented along the Z-axis and then rotated to place the center of the template on the X-axis. Rings were finally generated by successive (N–1) rotations of (2π/N) degrees of the template about the Z-axis. Optimization of the ring radii was based on an analysis of intersubunit contacts. With the fixed current F₁ model as input, molecular replacement searches were run using each ring model with PHASER (24). The best solution was clearly obtained for the decameric ring, i.e., a Z-score of 24, instead of a Z-score of 15 with the other ring models. Rigid body and overall temperature factor refinement of individual subunits resulted in R_{work} and R_{free} equal to 0.33. In the c-ring, the side chains were added to the model with the help of the *I. tartaricus* model (32% of identity, 59% of similarity with *S. cerevisiae*). The backbone electron density was better defined in a third and in the lower half of the ring where the sequence could be assigned with certainty. When no side chain information was present in the template or no density visible in the map, the most favored rotamer with acceptable van der Waals exclusion test was added to the model. Strong noncrystalllographic symmetry restraints were applied separately on helices H1 and H2 but not on the loop. The chains of the c-ring were labeled from J to S in the direction of the hydrolysis (25), starting with the chain having the lowest overall temperature factor. The assignment was checked on 2Fo – Fc unaveraged...
and Non-crystallographic Symmetry-averaged (calculated using the DM program) electron density maps on chains with the lowest temperature factors (Fig. 1) using COOT (26).

RESULTS

Overall Description of the Structure—The structure was solved by molecular replacement using the coordinates of the yeast yF₁ (14) and of the l. tartaricus rotor ring (16). These high resolution structures provided accurate models to phase and then refine the yeast F₁c₁₀ subcomplex structure. According to the functional α- and β-subunits reference scheme, as in Ref. 3, chains A, B, and C were named α-C, α-TP, α-DP-subunits, and chains D, E, and F were named β-DP, β-C, and β-TP-subunits, respectively (supplemental Fig. S2). The final model contains all subunits of both the F₁-sector (α₁₂–510, α₉₇–259, α₆₆–509, α₃₉–475, β₆–478, β₃₉–138, and e₁–59) and the F₉₅ membrane rotor composed of a ring of 10 c-subunits (c₁–76) (Fig. 2). No densities that could be attributed to any peripheral stalk subunits were visible. The overall structure of the F₁-sector of yF₁c₁₀ (3.43 Å) was more similar to the three yeast yF₁ (I–III) structures (root mean square deviation (r.m.s.d.) < 1.7 Å) than to those of bovine bF₁ structures (r.m.s.d. > 4.5 Å) (Table 2).

Table 1: Data processing and refinement statistics

| Dataset     | Crystal 1 | Crystal 2 | Merged data |
|-------------|-----------|-----------|-------------|
| Data collection |           |           |             |
| Space group  | a, b, c (Å) |           |             |
| Resolution range (Å) | 40–3.43 | 40–3.67 | 40–3.43 |
| Highest resolution (Å) | 3.43 | 3.67 | 3.43 |
| No. of unique reflections | 64,957 (8463) | 26,999 (4220) | 74,433 (7927) |
| Multiplicity (i) | 4.2 (4.4) | 1.5 (1.4) | 2.8 (2.4) |
| Completeness (%) | 77.2 (69.0) | 39.9 (42.2) | 88.1 (64.4) |
| Rmerge (i) | 0.19 (0.72) | 0.15 (0.73) | 0.19 (0.72) |
| Rsmooth (i) | 0.19 (0.72) | 0.15 (0.73) | 0.19 (0.72) |
| β-factor Wilson (Å²) | 4.2 (1.3) | 3.0 (1.3) | 4.3 (1.3) |
| Refinement statistics |           |           |             |
| No. of protein atoms | 30,671 |           |             |
| Protein B-factors (Å²) | 106 |           |             |
| r.m.s.d. bond lengths (Å) | 0.005 |           |             |
| Ramachandran plot (%) | 77.2 (69.0) | 39.9 (42.2) | 88.1 (64.4) |

*Statistics for the highest resolution bin are shown in parentheses.

The structure was solved by molecular replacement using the coordinates of the yeast yF₁ (14) and of the l. tartaricus rotor ring (16). These high resolution structures provided accurate models to phase and then refine the yeast F₁c₁₀ subcomplex structure. According to the functional α- and β-subunits reference scheme, as in Ref. 3, chains A, B, and C were named α-C, α-TP, α-DP-subunits, and chains D, E, and F were named β-DP, β-C, and β-TP-subunits, respectively (supplemental Fig. S2). The final model contains all subunits of both the F₁-sector (α₁₂–510, α₉₇–259, α₆₆–509, α₃₉–475, β₆–478, β₃₉–138, and e₁–59) and the F₉₅ membrane rotor composed of a ring of 10 c-subunits (c₁–76) (Fig. 2). No densities that could be attributed to any peripheral stalk subunits were visible. The overall structure of the F₁-sector of yF₁c₁₀ (3.43 Å) was more similar to the three yeast yF₁ (I–III) structures (root mean square deviation (r.m.s.d.) < 1.7 Å) than to those of bovine bF₁ structures (r.m.s.d. > 4.5 Å) (Table 2). yF₁(l), the most complete model, and yF₁(lII) were found to be more similar (r.m.s.d. < 1.47 Å). Thus, yF₁(l) will be considered as the yeast reference structure.

Structure of the F₉₅ Rotor Ring—The c-subunit folds as a helical hairpin with the N-terminal helix c2–38 (helix H1) connected by a polar loop c39–45 containing an R(N/Q)P motif to the C-terminal helix c46–74 (helix H2) (Fig. 3A). A decameric c-ring displays a hourglass shape with a central pore. The ring is 60 Å in height and has a diameter of 57 Å at the top, 51 Å at the bottom, and 43 Å in the middle. The above-mentioned distances are between local helical axes on the cGly21-Cα atoms plane. As shown in Fig. 3B, the neighboring helices H1 are tightly packed with 7.5 Å between their local axes. One outer helix H2N interacts with two inner helices, H1N at 7.4 Å and
S. cerevisiae F$_1$c$_{10}$-ATP Synthase Structure

H$_{1N+1}$ at 9.0 Å to form a tightly packed three-helix bundle (open angle ~50°) turned inwards. Furthermore, one inner helix H$_{1N}$ interacts with two outer helices, H$_{2N}$ and H$_{2N-1}$, to form a more open three-helix bundle (open angle ~80°) turned outwards that harbors the proton binding site. The neighboring helices H$_2$ are further away at 11.5 Å (Fig. 3B). In helix H$_1$, a 14° kink in the cLeu$^{26}$ region follows a two-turn long 3$_1$0 helix (c16–20) (Fig. 3A). A conserved GxGxGx(G/A) motif in the middle of the helix H$_1$ (c21–27) (supplemental Fig. S3) participates in this close packing (50). In helix H$_2$, a more pronounced 29° kink is located below cGlu$^{59}$ in the region opposite the glycine-rich motif (Fig. 3A). The central constriction is caused by the bend of both inner and outer helices. The pore has a diameter of 14–16 Å in the middle and 23 Å at the bottom and is lined by Ile$^{10}$, Ile$^{14}$, Ile$^{17}$, Ile$^{24}$, Ile$^{28}$, and Leu$^{20}$ residues (Fig. 3A). There are some narrow clefts between the outer helices, and one of these clefts (Fig. 3B) is lined by Leu$^{19}$, Gly$^{21}$, Ala$^{22}$, Gly$^{23}$, and Ile$^{26}$ from H$_{1N}$, Gly$^{59}$, and Gly$^{62}$ from H$_{2N}$ and Ser$^{58}$, Ala$^{60}$, Thr$^{61}$, Phe$^{64}$, from H$_{2N-1}$. There is no intrinsic channel lined by polar groups in the upper and lower parts of the c$_{10}$-ring. The cGlu$^{59}$ side chains with the lowest temperature factors (J–M) display well defined electron densities (Fig. 1). Nearby, small residual densities are not sufficiently high to model likely DCU modifications. One of oxygen atoms of the cGlu$^{59}$ side chain is accessible from the outer surface, whereas the second atom is turned toward the center of the outer three-helix bundle and is not hydrogen bonded (Fig. 3, B and C). Large rotations of the cGlu$^{59}$ side chains are sterically hindered by cLeu$^{26}$, cPhe$^{55}$, cAla$^{56}$, and cLeu$^{63}$ of the same c-subunit.

In yF$_1$c$_{10}$-ADP, TP Pair Resembles the DP Pair of Yeast and Bovine F$_{1}$—Despite the limited data resolution, residual electron densities appeared clearly in five nucleotide binding sites. These densities were sufficiently defined to be able to distinguish between di- and triphosphate nucleotides (supplemental Fig. S4). Mg-ATP molecules were modeled in the three noncatalytic sites, whereas Mg-ADP molecules were modeled in both the $\beta_{DP}$ and $\beta_{TP}$ catalytic sites. The refined temperature factors of nucleotides were compatible with fully occupied binding sites. No densities were observed in the $\beta_{E}$ catalytic site. The mean overall temperature factor of the $\alpha_{c}$-subunits was 1.18 Å. When $\alpha_{c}$- and $\beta_{c}$-subunits were individually compared with those of the yF$_1$(l) model (14) (Table 2), there were no significant differences in any subunits (r.m.s.d. values 0.42–0.54 Å), with the exception of the $\alpha_{TP}$-subunit (r.m.s.d. 1.18 Å). When the $\alpha_{c}$-subunit was split into subdomains I (a$_{TP}$25–147 and a$_{TP}$162–351) and II (a$_{TP}$148–186 and a$_{TP}$352–509), the deviations were decreased to 0.58 Å and 0.71 Å, respectively (supplemental Fig. S5). The change can be described as a rigid body rotation of 7.7° of subdomain II. When E, D, and TP pairs are compared with yF$_1$(l) pairs (Table 2), no significant differences are observed for the E and TP pairs (r.m.s.d. values 0.54 and 0.67 Å, respectively), whereas the TP pair appears quite different (r.m.s.d. 1.64 Å). The E pair is largely open (buried area of 1820 Å$^2$, supplemental Table S1) and similar to the yF$_1$(l) E pair (r.m.s.d. 0.54 Å), but slightly less open than the E pair in the bF$_1$-DCCD structure (1780 Å$^2$, r.m.s.d. 1.17 Å). The

| TABLE 2 |

r.m.s.d. values (Å) in C$\alpha$ positions between $\alpha$- and $\beta$-subunits, E, TP, and DP pairs, and $\gamma$, $\delta$, and $\epsilon$-subunits of yF$_1$c$_{10}$-ADP with yF$_1$(l) (PDB code 2HLD, chains A–I), yF$_1$(l) (PDB code 2HLD, chains J–R) (13), and bF$_1$ (bF$_1$-DCCD, PDB code 1E79) (12) | yF$_1$(l) | yF$_1$(l) | bF$_1$

| $\alpha_{c}$/$\alpha_{c}$ | 0.42 | 1.21 |
| $\alpha_{TP}$/$\alpha_{TP}$ | 0.50 | 1.12 |
| $\alpha_{TP}$/$\alpha_{TP}$ | 1.18 | 1.88 |
| $\gamma_{TP}$/$\gamma_{TP}$ | 1.29 | 1.27 |
| $\gamma_{TP}$/$\gamma_{TP}$ | 1.47 | 3.05 |
| $\beta_{c}$/$\beta_{c}$ | 1.05 | 2.16 |
| $\beta_{TP}$/$\beta_{TP}$ | 0.50 | 0.87 |
| $\beta_{TP}$/$\beta_{TP}$ | 0.54 | 0.87 |
| $\beta_{TP}$/$\beta_{TP}$ | 0.53 | 0.89 |
| $\beta_{TP}$/$\beta_{TP}$ | 0.71 | 0.73 |
| $\gamma_{TP}$/$\gamma_{TP}$ | 0.91 | 2.60 |
| $e/e$ | 0.70 | 2.64 |
| E/E | 0.54 | 0.89 | 1.17 |
| DP/DP | 0.67 | 1.43 | 1.76 |
| TP/TP | 1.64 | 1.43 | 2.46 |
| TP/TP | 1.70 | 1.02 | 1.14 |
| F$_1$F$_1$ | 1.47 | 1.68 | 4.53 |
| ($\alpha_{TP}$)$_{TP}$ | 1.22 | 1.43 | 2.13 |
S. cerevisiae F₅c₁₀-ATP Synthase Structure

DP interface is moderately open (2180 Å²) and is similar to the TP interface of yF₁(I) (2010 Å², r.m.s.d. 0.67 Å) (Fig. 4). The α- and β-subunits of yF₁c₁₀-ADP are superimposed. The αTP- and βTP-subunits of yF₁c₁₀-ADP are shown in red and blue, respectively. The yF₁(II) and yF₁(III) subunits are shown in green. The αArg₁⁶⁷ side chains and nucleotides are drawn as ball and sticks.

The TP interface is highly buried (2960 Å²) similar to the TP interface in yF₁apo(III) (2840 Å², r.m.s.d. 0.90 Å) (15). Interestingly, the TP pair resembles the DP pairs of yF₁(II) (2740 Å², r.m.s.d. 1.45 Å) and of bF₁-DCCD (2940 Å², r.m.s.d. 1.76 Å) are 6.3° more closed.

The TP closure cannot be attributed to direct lattice contacts of the α-subunit.

Faint residual densities were observed in the hydrophobic binding pocket around the βDP- and not βTP-Glu₁⁸⁵ side chain without structural changes in the neighborhood when the βDP-subunit was compared with yF₁(I) and βTP-subunit to yF₁apo(III). Attempts to model DCU modification were unsuccessful. Indeed, no structural changes were observed previously between bF₁-DCCD (13) and bF₁-ADP (11) structures. Finally, the yF₁c₁₀-ADP structure represents an Mg-ADP-inhibited state of the yeast F₁ enzyme, which is relatively different from those observed in bovine structures.

Central Stalk—A whole γε central stalk was observed in yeast F₁ structures (2, 14, 15) and in bF₁-DCCD (13). The γ, δ- and ε-subunits of yF₁c₁₀-ADP are individually similar to those of yF₁(I) (r.m.s.d. < 1.05 Å, Table 2). Overall, the γε central stalks of the yeast F₁ structures (yF₁c₁₀-ADP, yF₁(I), yF₁(II), yF₁c₁₀) are also similar (r.m.s.d. < 1.7 Å), whereas they differ significantly from the bovine one (r.m.s.d. = 3.05 Å, Table 2). The overall temperature factors of γ-, δ- and ε-subunits are 120 Å², 147 Å², and 149 Å², respectively. In F₁ complexes, the orientation of the γ-subunit relative to the (αβ)₃ component...
S. cerevisiae \( F_{1}c_{10} \)-ATP Synthase Structure
determines the catalytic state of the enzyme and, according to
the binding change mechanism, a 120° rotation of the \( \gamma \)-subunit
during the ATP hydrolysis cycle results in \( E \rightarrow TP, TP \rightarrow DP, \) and \( DP \rightarrow E \) interconversions. The three \( \alpha \)-subunits were
used to superimpose the central pseudo 3-fold axes. The upper
part (\( \gamma 256–278 \)) of the C-terminal helix of the \( \gamma \)-subunit are
well superimposed (Fig. 5A). This helical segment is
constrained to lie inside the narrowest part of the \( \alpha_{3}\beta_{4} \) inner channel lined by the proline-rich collar (\( \alpha_{\text{Pro}}^{290(288)}, \alpha_{\text{Pro}}^{291(289)}, \alpha_{\text{Gly}}^{292} \) and \( \beta_{\text{Pro}}^{276(276)} \)) (3). A conserved \( \gamma_{\text{Gly}}^{273(268)} \) allows
this helix to nestle against the \( \alpha_{\text{DP}} \) and \( \beta_{\text{TP}} \) subunit interface. Below, the N- and C-terminal helices interact to form a coiled
coil (Fig. 5B). From a tight turn of the yeast backbone residue
\( \gamma_{\text{Tyrr}}^{255(250)} \), the stalk deviates increasingly as it becomes closer
to the c-ring rotor, with ~18° shifts relative to \( \text{bF}_{1} \)-DCCD at
the periphery of the foot (Fig. 5D). Accordingly, the long C-terminal
helix is more curved in yeast (75 Å of curvature radius) than in
bovine structures (100 Å of curvature radius). The \( \gamma \)-subunit
twist is definitely an intrinsic property of the yeast enzyme. In
\( yF_{1c_{10}} \)-ADP, the region \( 235–242 \) of the C-terminal helix interacts with
the helical domains of \( \alpha_{\text{TP}} \) and \( \beta_{\text{TP}} \)-subunits (Fig. 5C), and the TP
closure results in a significant rotation/translation of this helix.
In yeast, the region \( 18–25 \) of the N-terminal helix interacts with
the C-terminal helical domains of \( \alpha_{e} \) and \( \beta_{TP} \)-subunits, and the partial
opening of the DP interface in the yeast structures could result from
these interactions. The \( \alpha_{DP} \)-subunit interacts with the globular domain
of the \( \gamma \)-subunit, whereas the helical domain of \( \beta_{e} \)-subunit does not
interact directly with the \( \gamma \)-subunit (Fig. 5C). The closure of the
TP pair and the subtle differences in the relative orientation and
position of the \( \gamma \)-subunit result from the MgADP inhibition. At
the \( F_{1}F_{0} \) rotor interface, the foot of the yeast central stalk is rotated
by ~40° (36° for \( yF_{1}c_{10} \), 41° for \( yF_{1}c_{10} \)-ADP) relative to the \( \text{bF}_{1} \)-DCCD
in the direction of the hydrolysis (Fig. 5D and
supplemental Fig. S6D). As a consequence, the presence of the
\( c_{10} \)-ring and the crystal packing have no significant influence on
the \( \gamma \)-subunit twist. The \( \delta \)-subunit interacts extensively with the \( \gamma \)-
and \( e \)-subunits along with the c-ring (supplemental Tables S2
and S3). In the \( \delta \)-subunit, the sec-
dermines the catalytic state of the enzyme and, according to
the binding change mechanism, a 120° rotation of the \( \gamma \)-subunit
during the ATP hydrolysis cycle results in \( E \rightarrow TP, TP \rightarrow DP, \) and \( DP \rightarrow E \) interconversions. The three \( \alpha \)-subunits were
used to superimpose the central pseudo 3-fold axes. The upper
part (\( \gamma 256–278 \)) of the C-terminal helix of the \( \gamma \)-subunit are
well superimposed (Fig. 5A). This helical segment is
constrained to lie inside the narrowest part of the \( \alpha_{3}\beta_{4} \) inner channel lined by the proline-rich collar (\( \alpha_{\text{Pro}}^{290(288)}, \alpha_{\text{Pro}}^{291(289)}, \alpha_{\text{Gly}}^{292} \) and \( \beta_{\text{Pro}}^{276(276)} \)) (3). A conserved \( \gamma_{\text{Gly}}^{273(268)} \) allows
this helix to nestle against the \( \alpha_{\text{DP}} \) and \( \beta_{\text{TP}} \) subunit interface. Below, the N- and C-terminal helices interact to form a coiled
coil (Fig. 5B). From a tight turn of the yeast backbone residue
\( \gamma_{\text{Tyrr}}^{255(250)} \), the stalk deviates increasingly as it becomes closer
to the c-ring rotor, with ~18° shifts relative to \( \text{bF}_{1} \)-DCCD at
the periphery of the foot (Fig. 5D). Accordingly, the long C-terminal
helix is more curved in yeast (75 Å of curvature radius) than in
bovine structures (100 Å of curvature radius). The \( \gamma \)-subunit
twist is definitely an intrinsic property of the yeast enzyme. In
\( yF_{1c_{10}} \)-ADP, the region \( 235–242 \) of the C-terminal helix interacts with
the helical domains of \( \alpha_{\text{TP}} \) and \( \beta_{\text{TP}} \)-subunits (Fig. 5C), and the TP
closure results in a significant rotation/translation of this helix.
In yeast, the region \( 18–25 \) of the N-terminal helix interacts with
the C-terminal helical domains of \( \alpha_{e} \) and \( \beta_{TP} \)-subunits, and the partial
opening of the DP interface in the yeast structures could result from
these interactions. The \( \alpha_{DP} \)-subunit interacts with the globular domain
of the \( \gamma \)-subunit, whereas the helical domain of \( \beta_{e} \)-subunit does not
interact directly with the \( \gamma \)-subunit (Fig. 5C). The closure of the
TP pair and the subtle differences in the relative orientation and
position of the \( \gamma \)-subunit result from the MgADP inhibition. At
the \( F_{1}F_{0} \) rotor interface, the foot of the yeast central stalk is rotated
by ~40° (36° for \( yF_{1}c_{10} \), 41° for \( yF_{1}c_{10} \)-ADP) relative to the \( \text{bF}_{1} \)-DCCD
in the direction of the hydrolysis (Fig. 5D and
supplemental Fig. S6D). As a consequence, the presence of the
\( c_{10} \)-ring and the crystal packing have no significant influence on
the \( \gamma \)-subunit twist. The \( \delta \)-subunit interacts extensively with the \( \gamma \)-
and \( e \)-subunits along with the c-ring (supplemental Tables S2
and S3). In the \( \delta \)-subunit, the sec-

FIGURE 5. Four serial cross-sections perpendicular to the pseudo 3-fold axes (A) are viewed from the top
determined.
S. cerevisiae F₁c₁₀-ATP Synthase Structure

DISCUSSION

The S. cerevisiae c-subunit resembles the c-subunit from I. tartaricus (r.m.s.d. 1.2 Å for a monomer, 1.4 Å for a dimer) and Spirulina platensis (r.m.s.d. 1.4 Å for a monomer, 1.6 Å for a dimer) (19) and differs greatly from the solution structure of the E. coli c-monomer (r.m.s.d. ~4.5 Å) (5). Accordingly, the c-subunit sequence of the mitochondrial H⁺-F-ATPase (S. cerevisiae) is closer to bacterial Na⁺-F-ATPase (32% identity with I. tartaricus) than to cyanobacterial (25% with S. platensis), chloroplast (21% with Spinacea oleracea) and bacterial (18% with E. coli) H⁺-F-ATPase (supplemental Fig. S3). In addition, the essential residue cGlu⁵⁹ conserved in all V- and F-ATPase is replaced by cAsp⁶¹ in E. coli (supplemental Fig. S3).

In the I. tartaricus Na⁺-F-ATPase, the Na⁺ is bound by Gln⁶⁵, two polar side chains (Gln⁶⁶ and Ser⁶⁷), one backbone carbonyl (Val⁶⁸), and a water molecule (35). In chloroplast and cyanobacterial H⁺-F-ATPase, the carboxyl oxygen atoms of the essential Gln⁶¹/⁶² are bound by Gln⁷⁸/⁷⁹, Tyr⁶⁶/⁶⁷, and the backbone carbonyl Phe⁵⁹/⁶⁰. In S. cerevisiae, the polar residues are replaced by hydrophobic ones (Ile²⁶, Ala⁶⁰, and Phe⁶⁴) (supplemental Fig. S3 and Fig. 7) and the kink in outer helix H₂ disrupts the intrahelical hydrogen bond networks, so cAla⁵⁶ and cLeu⁵⁷ backbone carbonyl groups are not hydrogen-bonded. The cLeu⁵⁷ carbonyl is 5.1 Å apart from the cGlu⁵⁹ carboxyl oxygen atoms (Fig. 3C); therefore, a nonbonded interaction would require a molecular bridge. As a result, a water molecule could be inserted at a position close to that of the Na⁺ in I. tartaricus c₁₁-ring and could establish hydrogen bonds with cGlu⁵⁹ carbonyl and cLeu⁵⁷ carbonyl groups. The equivalent distance is 4.5 Å in the Na⁺ binding site (16) and 2.7 Å in the proton binding site with a direct hydrogen bond (16, 19). Hence, the current structure does not rule out the involvement of a hydronium ion in the proton translocation mechanism in yeast. Two types of proton binding sites could exist, with or without hydronium ion requirement. Recently, the observed bell-shaped pH profile for DCCD labeling of the acidic c-ring residues of H⁺-transporting F-ATP synthase (36) were taken to be the involvement of a hydronium ion in the binding site, as proposed earlier by Boyer (37). A putative hydronium binding scheme for yeast H⁺-F-ATP synthase could be built on that of the sodium binding of Na⁺-F-ATPases (38), with a working model similar to the "push and pull" functional model (39).

The outer surface of the c-ring is essentially hydrophobic with the exception of the essential cGlu⁵⁹ embedded at 24 Å from the two sides of the membrane, if cLys⁴⁴/cAsp⁴⁵ and cLys⁸/carboxyl-terminal groups, at a distance of ~43 Å, mark the inner and outer surfaces of the mitochondrial membrane, respectively (Fig. 3A). On the matrix side, the pore is closed partly by the cArg⁵⁹ side chains that could act as a socket to receive the foot of the central stalk. An enlargement to 25 Å near a collar of cAsn⁵⁸ could indicate the top of a lipid plug. At the pore openings, a few residual density peaks cannot be attributed with certainty to ordered lipids or detergent molecules. However, there is enough space to accommodate, in vivo, two opposite plugs of a few lipids. It has been shown in E. coli that the pore is occupied by phospholipids (40).

The βᵥ and βᵥTP binding sites contain an Mg·ADP molecule with the "arginine finger" αArg⁷⁷⁵(37₃) pointing in toward the site (supplemental Fig. S4). The structure reported here is probably equivalent to the ADP-inhibited state of the enzyme because our crystallization conditions leading to bound
Mg\textsuperscript{2+}/H\textsubscript{18528} ADP and no free phosphate (41). This form is probably not an intermediate in either ATP hydrolysis or ATP synthesis but rather a pause state from which reactivation occurs upon the presence of protonmotive force in mitochondrial ATP synthase (7, 42). The \(yF_1c_{10}\) crystals were grown in the presence of ADP and azide without ATP analogs like various bovine F\textsubscript{1}-ATPase crystals. Azide is known to enhance Mg\textsuperscript{2+}/H\textsubscript{18528} ADP inhibition (43, 44). The \(bF_1\) ADP\textsubscript{N\textsubscript{3}} structure at 1.95-Å resolution revealed how azide enhances the binding of the ADP molecule in the \(\beta\)\textsubscript{DP} subunits (45). Afterward, the hypothetical presence of an azide molecule was proposed in bovine structures in the \(\alpha\beta\)\textsubscript{3} catalytic sites (3, 10, 13). Similarly, it cannot be ruled out that an azide molecule is bound to the \(\beta\)\textsubscript{DP} and \(\beta\)\textsubscript{TP} sites of \(yF_1c_{10}\) ADP.

Whatever the crystallization method, the detergent concentration or the inhibitor used to crystallize the yeast F\textsubscript{1}F\textsubscript{0}-ATP synthase, all subunits of the peripheral stalk were lost as indicated by SDS-PAGE analysis of crystals. SDS-PAGE analysis of three-dimensional crystals of the bacterial F\textsubscript{1}F\textsubscript{0}-ATP synthase from \textit{Chloroflexus aurantiacus} obtained by slow detergent removal also indicates the loss of the peripheral stalk during crystallization (46). The crystallization conditions may induce the destabilization of the a-subunit/c-ring and the peripheral stalk/F\textsubscript{1} interfaces, leading to the F\textsubscript{1}-c-ring subcomplex.

In \textit{E. coli} (47) and \textit{I. tartaricus} (48), three acidic residues have been identified in the \(\gamma\)-subunit as good candidates for interaction with the c-ring. However, we cannot exclude that the positive charges of conserved cArg\textsuperscript{39} could be partially neutralized by the polar heads of a phospholipid plug. The loss of the peripheral stalk and the large crystal lattice interface (980 Å\textsuperscript{2}) between the c-ring and the \(\beta\)-barrels domains of the (\(\alpha\beta\))\textsubscript{3} could disturb the interface of a similar area (890 Å\textsuperscript{2}) between the central stalk and the c-ring (supplemental Table S3), so the densities of cArg\textsuperscript{39} and \(\gamma\)Glu\textsuperscript{198} side chain residues are very poorly defined.

The winding of the central stalk observed in different conformational states of yeast F\textsubscript{1} complexes, with or without the c-ring, appears specific to the yeast F\textsubscript{1}. The structure of a complex between bovine F\textsubscript{1} and a truncated stator containing OSCP-, b-, d-, and F\textsubscript{6}-subunits reveals the position of the peripheral stalk along the cleft of a non-catalytic interface (49) and suggests a low degree of freedom for the localization of the membrane domain of the stator relative to the membrane rotor. The peripheral stalk prevents rotation of the (\(\alpha\beta\))\textsubscript{3} head but does not prevent its essential conformational changes. The predictions of the transmembrane regions and the secondary structures of the yeast subunit b (subunit 4) suggest that the peripheral stalks are highly similar. In \(yF_1c_{10}\) ADP, the stochiometry of 10 monomers per c-ring and the rotation of \(\sim 40^\circ\) of the central stalk foot with respect to bovine are compatible with a step-by-step rotation of \(36^\circ\) of the rotor, without involving any shift of the membrane stator and hence modification of the overall conformation of the peripheral stalk. Finally, when comparing the \(yF_1(I)\) and \(yF_1c_{10}\) ADP structures, it appears that the presence of the c-ring does not modify the position of the \(\gamma\)-subunit and of the foot sole of the central stalk (Fig. 5).

Refinement of the crystal structure of the yeast F\textsubscript{1}c\textsubscript{10}-ATP synthase inhibited by AMP-PNP should unveil some unknown features of this complex. High resolution structures of rotor rings of bacterial or mitochondrial H\textsuperscript{+}-transporting F-ATP synthase are now required to clarify the nature of the proton binding site in mitochondrial ATP synthases.

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**FIGURE 7. Comparison of sodium and proton binding sites.** A, proton binding site in \textit{S. cerevisiae} \(yF_1c_{10}\)ADP; B, Na\textsuperscript{+} binding site in \textit{I. tartaricus} c11-ring (PDB code 2WGM); C, proton binding site in the c\textsubscript{15} ring from \textit{S. platensis} cyanobacterium (PDB code 2WIE); and D, proton binding site in the c\textsubscript{14} ring from \textit{S. oleracea} chloroplast (PDB code 2W5J) with Glu\textsuperscript{28} built in the best rotamer conformation.
S. cerevisiae F\textsubscript{1},\textsubscript{10}-ATP Synthase Structure

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