V-Type H\(^+\)-ATPase/Synthase from a Thermophilic Eubacterium, *Thermus Thermophilus*

SUBUNIT STRUCTURE AND OPERON

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V-type ATPase (V\(_o\)V\(_1\)) capable of ATP-driven H\(^+\) pumping and of H\(^+\) gradient driven ATP synthesis was isolated from a thermophilic eubacterium, *Thermus thermophilus*. When the enzyme was analyzed by gel electrophoresis in the presence of sodium dodecyl sulfate, it showed eight polypeptide bands of which four were subunits of V\(_1\). We also isolated the V\(_o\)V\(_1\) operon, containing nine genes in the order of atpG-I-L-E-X-F-A-B-D, which encoded proteins with molecular sizes of 13, 43, 10, 20, 35, 11, 64, 53, and 25 kDa, respectively. The last four genes were identified as those for V\(_1\) subunits; atp\(_A\), B, D, and F encoded the A, B, \(\gamma\), and \(\delta\) subunits, respectively. The first five genes, atpG-atpX, were identified as genes for the V\(_o\) subunits. The product of atpL, the proteolipid subunit, lacked a 19-amino acid presequence and, unlike V-type ATPases, contained two membrane-spanning domains rather than four. The hydrophobic 43-kDa product of atpI is the smallest member so far found of the eukaryotic 100-kDa subunit family. Its electrophoretic band overlapped with the band of the A subunit. Therefore, all the gene products were found in our purified V\(_o\)V\(_1\). We isolated the A\(_{12}\)B\(_3\) subcomplex reconstituted from the isolated subunits and the A\(_{12}\)B\(_3\)γ subcomplex from subunit-expressing *Escherichia coli*. Electron microscopic observation of these subcomplexes revealed that the \(\gamma\) subunit of V\(_1\) filled the central cavity of A\(_{12}\)B\(_3\) and might be central subunit, similar to the \(\gamma\) subunit of F\(_1\)-ATPase.

F-type H\(^+\)-ATPase (F\(_o\)F\(_1\)) and vacuolar type H\(^+\)-ATPase (V\(_o\)V\(_1\)) are two classes of a superfamily of H\(^+\)-translocating ATPases (1, 2). F\(_o\)F\(_1\) is responsible for ATP synthesis and is present in inner membranes of mitochondria, thylakoid membranes of chloroplasts, and plasma membranes of bacteria (2, 3). These enzymes are composed of a hydrophobic membrane part (F\(_o\)) and a hydrophilic catalytic part (F\(_1\)). F\(_o\) forms a proton channel across the membranes and is composed of at least three kinds of transmembrane subunits, one of which is the most evolutionarily conserved 8-kDa proteolipid subunit (2). F\(_1\), which can easily be separated from the membranes and shows ATPase activity, is composed of two kinds of nucleotide binding subunits, \(\alpha\) and \(\beta\), and three minor subunits, \(\gamma\), \(\delta\), and \(\epsilon\), with a stoichiometry \(\alpha_2\beta_2\gamma\delta\epsilon\) (2, 3). The \(\gamma\) subunit penetrates the central cavity of the hexagonal \(\alpha_2\beta_3\) like a shaft (4) and rotates when F\(_1\) hydrolyzes ATP (5).

On the other hand, V\(_o\)V\(_1\) is present in the membranes of yeast vacuoles (6), clathrin-coated vesicles (7), chromaffin granules (8), lysosomes (9), and plant vacuoles (10) in eukaryotic cells, and it pumps H\(^+\) into vesicles. Like F\(_o\)F\(_1\), the V\(_o\)V\(_1\) is composed of a water-soluble set of subunits (V\(_1\)) and a membrane-integral set of subunits (V\(_o\)). The two major A and B subunits and other minor subunits comprise the V\(_1\) domain (1). The A and B subunits are homologous to the \(\beta\) and \(\alpha\) subunits of F\(_o\)F\(_1\). By analogy to F\(_1\), V\(_1\) has also been proposed to have a central shaft subunit that rotates in the A\(_{12}\)B\(_3\) hexagon. However, an equivalent subunit with indisputable sequence homology to the F\(_1\)γ subunit has not been identified in V\(_o\)V\(_1\). The hydrophobic V\(_o\) is thought to be composed of at least five different subunits (1, 11). The 16-kDa proteolipid subunit containing four transmembrane domains is the major subunit of V\(_o\) (1). Unlike F\(_o\)F\(_1\), V\(_o\) dissociates into individual subunits with concomitant loss of ATPase activity, and V\(_o\) does not show activity as a H\(^+\) channel by itself once V\(_o\) is detached from V\(_1\) (12).

We have previously identified V\(_o\)V\(_1\) in prokaryotic cells at first in archea and then in a thermophilic eubacterium, *Thermus thermophilus* (13–16). Because T. thermophilus, a strict aerobe, does not have F\(_o\)F\(_1\) but carries out oxidative phosphorylation, V\(_o\)V\(_1\) is responsible for ATP synthesis in this eubacterium, and indeed, the V\(_o\)V\(_1\) purified from this bacterium can catalyze ATP synthesis driven by H\(^+\) flow (17). Based on SDS-polyacrylamide gel electrophoresis (PAGE), the isolated T. thermophilus V\(_o\)V\(_1\) has been thought to be composed of eight kinds of polypeptides with the following apparent molecular sizes: 100, 66, 56, 38, 30, 24, 13, and 12 kDa (18). Four of them, A (66 kDa), B (56 kDa), γ (30 kDa), and δ (12 kDa), are found in the purified V\(_o\), whereas the 100-, 38-, 24-, and 13-kDa polypeptides are thought to be V\(_1\) subunits. In this study, to determine the subunit composition, we compared the nine genes in the T. thermophilus V\(_o\)V\(_1\) operon and partial peptide sequences of polypeptides in the isolated V\(_o\)V\(_1\). Furthermore, we reported the electron microscopic observation of the subcomplexes.

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The nucleotide sequence(s) reported in this paper has been submitted to the GenBank\(\ddagger\) (accession number(s) D63799.)

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\(\ddagger\) The abbreviations used are: PAGE, polyacrylamide gel electrophoresis; bR, bacteriorhodopsin; HPLC, high pressure liquid chromatography.
Purification of VV1 from T. thermophilus Plasma Membrane—Cultivation and disruption of T. thermophilus cells were carried out as described (16). Membranes were precipitated by centrifugation at 100,000 × g for 15 min and washed with 50 mM Tris-SO4 (pH 8.0) containing 5 mM MgSO4 five times to avoid contamination with soluble proteins. The washed membranes (30 g) were suspended in 100 ml of 50 mM Tris-SO4 (pH 8.0), 5 mM MgSO4, and 0.1% Triton X-100. The suspension was sonicated for 2 min. Debris and insoluble materials were removed by centrifugation at 100,000 × g for 15 min, and the supernatant was applied to a DEAE-Sephacel column (3 × 10 cm) equilibrated with 50 mM Tris-SO4 (pH 8.0), 5 mM MgSO4, and 0.2% Triton X-100. The column was washed with 200 ml of the same buffer. Proteins were eluted with a linear NaCl gradient (0–0.5 M). The purity of recombinant VVoV1 was confirmed using an ABI373A sequencer. The DNA fragment obtained was ligated into the EcoRI/SalI site of pT7e 99A (Amansham Pharmacia Biotech). After transformation of E. coli strain JM103, the expression vector was incubated at 0.4 mM (final concentration) isopropyl-β-D-galactopyranoside. Purification of the recombinant Aβγ complex expressed in E. coli harboring the expression vector were grown in 2 liters of 2×YT containing 0.2 M ampicillin at 37 °C. About 20 g of cells were obtained from 1 liter of overnight culture medium. Cells were suspended in 50 mM Tris-CI (pH 8.0), 50 mM NaCl, and 0.1 mM EDTA and disrupted by sonication. The debris was removed by brief centrifugation. The cell lysate was incubated at 65 °C for 30 min, and insoluble denatured proteins were removed by centrifugation for 30 min at 60,000 × g. The supernatant was applied to a DEAE-Sephacel column (2 × 20 cm) equilibrated with 50 mM Tris-CI (pH 8.0), 0.1 mM EDTA (buffer A). After the column was washed with buffer A, proteins were eluted with a linear Na2SO4 gradient, and Aβγ complex was eluted with Aβγ complex was collected and concentrated to 1 ml by ultrafiltration using a Mini-Model, module NM-3 (Funakoshi). The concentrated solution was applied to a Sephacryl S-300 column (1.8 × 90 cm) equilibrated with 50 mM Tris-SO4 (pH 8.0), 5 mM MgSO4, 50 mM Na2SO4, and 0.1% Triton X-100. Proteins were eluted with the same buffer, and the fractions were analyzed by polyacrylamide gel electrophoresis in the presence of the alkyl ether sulfate and SDS. The fractions containing VV1 were combined and dialyzed against 50 mM Tris-SO4 (pH 8.0), 5 mM MgSO4 for 5 h. The dialyzed solution was applied to a Porosh IQ/M (4.6 × 10 cm, PerSeptive Biosystems Corp.) equilibrated with 50 mM Tris-SO4 (pH 8.0), 5 mM MgSO4, and 0.1% Triton X-100. Proteins were eluted with a linear NaCl gradient (0–0.5 M). The purity of each subunit was analyzed by SDS-PAGE. The fractions containing VVoV1 were combined, and the VV1 was stored at 4 °C until use. ATP driven H+ Translocation and Light-induced ATP Synthesis by the VV1—Protoproteoliposomes containing VV1 and bacteriorhodopsin (bR) were reconstituted at 25 °C in the reaction buffer (25 mM potassium phosphate buffer (pH 7.3), 50 mM KCl, and 50 mM Na2SO4) according to the procedure described by Richardson and co-workers (15–21) with minor modification. Unilamellar liposomes were prepared by phase evaporation using phosphatidylcholine and resuspended at an equal molar ratio. Then, 49 ml of 14.8% of 0.1 M ATP-Mg and incubated at room temperature for 3 h. The incubated mixture was subjected to gel permeation high performance liquid chromatography (G-3000SXL, Tosoh) and eluted with 50 mM Tris-CI (pH 7.5) and 0.1 mM Na2SO4. The fractions containing Aβγ complex were collected and analyzed immediately.

Electron Microscopy—The negatively stained samples were diluted with 50 mM Tris-HCl (pH 8.0) to final protein concentrations of 10–100 µg/ml, and the specimens on carbon support films were embedded in negative stain (2% uranyl acetate) using the drop method (24). Specimens were examined with a Hitachi HF-2000 electron microscopy equipped with a Gatan camera system and analyzed with a software de-vice. Grids were scanned at low magnification under dark illumination to reduce radiation damage. After adjusting the astigmatism and focus at adjacent areas, the photographs of the target specimen area were recorded on Kodak SO163 film using an electron dose of about 30 eÅ2. The accelerating voltage was 200 kV, and the direct magnification was 50,000. Micrographs were digitized with a scanner (SCAI, Zeiss) at a pixel size of 0.14 nm for single particle analysis. About 40 particles were selected. The images were rotated to maximize correlation with each other and averaged after rejecting the particles that correlated poorly. All images were processed using the software we developed: the extensible object-oriented system (25).

RESULTS

Properties of Isolated VV1—Previously, we reported isolation of VvV1 from T. thermophilus membranes using two column chromatography steps, i.e. DEAE cellulose and Sepharose
proteoliposomes reconstituted by dialysis or the freeze-thaw method (26) failed to show H+ pumping activity (data not shown). The V1-V1 preparation was also capable of ATP synthesis; vesicles containing br and V1-V1 synthesized ATP by illumination (Fig. 1d). Neither bafilomycin A1 nor azide inhibited the ATP synthesis of V1-V1 (1, 27) and F1-ATPase (28), respectively, affected the illumination-driven ATP synthesis of T. thermophilus V1-V1 (Fig. 1d).

**atp Operon Contains Nine Genes**—We cloned the entire operon of the V1-V1, which contains nine genes in the order of atpG-I-L-E-X-F-A-B-D (Fig. 2). The sizes of the G–D genes were 360, 1188, 297, 561, 969, 324, 1749, 1434, and 681 base pairs, and the approximate molecular sizes of the encoded proteins are 13, 43, 10, 20, 35, 12, 64, 53, and 25 kDa, respectively. All of the nine genes have Shine-Dalgarno sequences in their upstream regions (29). At 17–50 bases downstream from the last codon of atpD, there is transcriptional termination signal (5’-GCCCGGGGCTTCCAGGCCCCGGCCTTCTTCTTTTT-3’), suggesting that atpD is the last gene in the operon. In 20–32 bases upstream from the initiation codon of atpG, there is a consensus sequence for a promoter of the T. thermophilus atp operon (5’-TTGACCTGCACTTCCGCCGCTAATATACATT-AGGCCGGG-3’, consensus sequences and a probable transcriptional start point being underlined). Therefore, the operon contains no gene(s) other than the nine genes.

**atpL Encodes a Hydrophobic 43-kDa Subunit**—The atpL gene is predicted to encode a 43-kDa polypeptide, but we could not find a protein band at the corresponding position in 18% SDS-PAGE (Fig. 1b). Therefore, we prepared an anti-atpL polypeptide antibody raised against the deduced peptide sequence as a 359GHMG-LQPILLLWEEETFKEF776 as an antigen and analyzed V1-V1 with Western blotting. A 60-kDa polypeptide was stained with the antibody (data not shown). The position of the stained band overlapped with that of the A subunit, but the atpL product is not a component of V1 because there was no positive band in the V1 preparation. In fact, a new band appeared just below the band of the A subunit when V1-V1 was analyzed with SDS-PAGE at a lower (12%) gel concentration (Fig. 3a, arrow). The N-terminal amino acid sequence of this polypeptide was VIA-MEKLVLAGPKG, which agreed with the predicted N-terminal sequence of the atpL product. Thus, the atpL product was contained in the purified V1-V1 preparation, and we call it the 43-kDa subunit hereafter. The C-terminal amino acid residue of the 43-kDa subunit has sequence similarity to those of rat VPP1 (96 kDa; Ref. 30), Saccharomyces cerevisiae Stv1 (95 kDa; Ref. 31), Vph1 (95 kDa; Ref. 32), and the atpL product of Enterococcus hirae (75 kDa; Ref. 33) (Fig. 3b). The hydrophobic plot of the 43-kDa subunit indicated that this stretch contained a large hydrophobic region that is commonly observed for the homologues mentioned above (Fig. 3c).

**atpL Encodes the 8-kDa Proteolipid Subunit**—atpL encodes a highly hydrophobic 10-kDa polypeptide with three apparent transmembrane domains (Fig. 4b), and its sequence shows significant sequence similarity to the sequences of the proteolipid subunits of F1-F1 and V1-V1 (Fig. 4a). The glutamic acid residue that plays an essential role in H+ conduction (2) is conserved at position 57 in the predicted transmembrane domain. Previously, no protein band of the corresponding molecular size was found in SDS-PAGE of V1-V1 (Fig. 4c, lane 2) (18). However, a 5-min boiling prior to electrophoresis in 2% SDS resulted in appearance of a new 9-kDa polypeptide band with concomitant disappearance of the 100-kDa band, which was previously interpreted as a candidate for a subunit of V1-V1 (Fig. 4c, shown by an asterisk). Analysis of the N-terminal sequence of the 9-kDa band (and also the 100-kDa band) revealed that the protein in the band was a product of atpL but...
lacked the N-terminal 19 amino acid residues that constituted the putative first transmembrane domain (Fig. 4b). Consequently, the proteolipid subunit of *T. thermophilus* *VoV*1 contains two rather than three transmembrane helices. The real molecular size of the subunit is approximately 8 kDa, and we therefore call it the 8-kDa subunit hereafter.

The Operon Contains All Genes for Nine Subunits of *VoV*1—It was already known that atpA and atpB encode the A and B subunits of V1 part (22). To identify the remaining five gene products, we compared the partial peptide sequences of the putative subunit bands in SDS-PAGE and deduced amino acid sequences of the genes (data not shown). The results showed that the products of atpF and atpD are d and g subunits of V1 part, respectively, and that the products of atpG, atpE, and atpX are 13-, 24-, and 38-kDa subunits of Vo part, respectively. The names 24- and 38-kDa subunits are changed to 20- and 35-kDa subunit hereafter. These Vo subunits are hydrophilic proteins rather than transmembrane protein; hence these subunits may be associated directly or indirectly with the hydrophobic subunits of V1. Thus, the products of all of nine genes in the operon are found in the V1/Vo preparation, and the V1/Vo is composed of nine kinds of subunits, four in V1 and five in Vo.

Correspondence to V1/Vo from Other Sources—We searched amino acid sequence similarity of 13-, 20-, and 35-kDa subunits of Vo and d and g subunits of V1 from *T. thermophilus* to the subunits of V1/Vo from other organisms. The amino acid residues of the 20-kDa subunit are 22.4, 18.7, and 19.4% identical with those of *E. hirae* ntpE (33), yeast VATE (34), and bovine VATE (35), respectively. Between the 35-kDa subunit and *E. hirae* ntpC and yeast VATX (36), 19.7 and 17.8% amino acid residues are found to be identical, respectively. The 13-kDa subunit has 26.0% amino acid identity to *E. hirae* ntpF gene product, 21.8% to yeast VATG (37), and 33% to the N-terminal 50 amino acid residues of bovine VATG (38). The sequence of the g subunit is 25.0, 31.5, 27.7, and 29.5% identical with those of *Sulfolobus acidocaldarius* g subunit (39), *E. hirae* 29-kDa subunit, yeast VATD, and bovine VATD (40), respectively. The d subunit shows overall 20% identity with the product of *E. hirae* ntpG gene. Correspondence of subunits of *T. thermophilus* *VoV*1 to those of other organisms is summarized in Table I.

Electron Microscopic Observation of A3B3, A3B3g, and A3B3gδ Complexes—The rotor subunit, g, of F1-ATPase occupies the central cavity of the hexagonal cylinder made up from the α and β subunits (24). To see which subunit fills the center of V1, we isolated the A3B3 and A3B3g subcomplexes and observed them using electron microscopy. The A3B3 complex...
is due to the central pore of A3B3 when A3B3 is compared with V1. The central cavity of A3B3 can be identified more easily after averaging the particles. Averaged top views of the particles are shown in Fig. 6 (d–f). A small mass is lost in an A3B3 complex with the confidence level of 99.7% when it is compared with V1 and/or A3B3 subunit.

**Correspondence among V0V1 subunits in T. thermophilus, E. hirae, S. acidocaldarius, S. cerevisiae, and bovine**

All sequence data of subunits were cited from data base (SWISS-PROT). The molecular sizes deduced from nucleotide sequence are indicated. Values in parentheses are in kDa.

| T. thermophilus | E. hirae | S. acidocaldarius | S. cerevisiae | Bovine H+ pump |
|----------------|----------|------------------|--------------|----------------|
| atpA (64)      | ntpA(66) | ATPA(66)         | VATA(69)     | VATA(68)       |
| atpB (53)      | ntpB(51) | ATPB(51)         | VATB(58)     | VATB(57)       |
| atpD (25)      | ntpD(27) | ATPD(24)         | VATD(29)     | VATD(28)       |
| atpE (20)      | ntpE(23) | VATE(27)         | VATE(27)     | VATE(26)       |
| atpF (11")    | ntpF(11) | MTPF(13)         | VATF(13)     | VATE(27)       |
| atpG (13)      | ntpF(14) | VATG(13)         | VATG(14)     | VATE(12)       |
| atpI (43)      | ntpF(76) | VHP1(95)         | VATP1(96)    | VPP1(96)       |
| atpL (8)       | ntpK(16) | ATPL(8)          | VATL(16)     | VATL(16)       |
| atpX (35")    | ntpC(33)| VATX(40)         | VATX(31)     | VATX(31)       |

A meaningful similarity was not observed between atpF and MTPF (and VATF) and atpX and bovine VATX.

**DISCUSSION**

The Purified V0V1 Is an ATP Synthase / H+ Pump—We modified the method of purification of V0V1 from *T. thermophilus* and consequently obtained highly purified V0V1. In this study,
in d were mixed with 5 mM ATP, and the mixture was fractionated by gel permeation HPLC. The isolated A and B subunits were reconstituted into liposome by 15% SDS-PAGE. Each subcomplex was purified by gel permeation HPLC before electrophoresis. Other experimental details are under “Experimental Procedures.”

![Fig. 5. Elution profiles of A3B3, A3B3γ, and A3B3γδ (V1) complexes from gel permeation HPLC](image)

The isolated A and B subunits were mixed with 5 mM ATP, and the mixture was fractionated by gel permeation HPLC (a), and the purity was then checked by rechromatography (b). c, analysis of A3B3, A3B3γ, and A3B3γδ (V1) by 15% SDS-PAGE. Each subcomplex was purified by gel permeation HPLC before electrophoresis. Other experimental details are under “Experimental Procedures.”

![Fig. 6. Electron micrographs of the A3B3, A3B3γ, and A3B3γδ (V1).](image)

d–f, overview of negatively stained A3B3, A3B3γ, and A3B3γδ, respectively, on the carbon support films. Proteins are shown in white. Many particles are observed in an end-on orientation indicated by arrowheads. The scale bar represents 50 nm. d–f, the averaged top views of A3B3 (n = 24), A3B3γ (n = 23), and A3B3γδ (n = 22) are shown in d, e, and f, respectively. Proteins are shown in white. The scale bar for d–f represents 5 nm. g, difference of density among A3B3, A3B3γ, and V1 by analysis of variance (25). Significant difference of density among them is shown in white. h, difference of density between A3B3 and A3B3γ by Student’s t test. Significantly lower density regions are shown in white.

the reconstitution of V1 into liposome was carried out by reverse phase method (19–21). As shown in Fig. 1c, the ATP-driven H+ pumping by V1 was clearly observed. In a previous study, we demonstrated that the ATPase activity of V1 was rapidly decreased during turnover because of ADP inhibition (17). The lower rate of H+ pumping by V1 than by F1, F2 may be a reflection of ADP inhibition of V1. On the other hand, the bR-V1 co-reconstituted liposomes synthesized ATP driven by proton motive force (Fig. 1d), and the rate of ATP synthesis was higher than that of F1, F2. Thus, it is concluded that the purified V1 is an ATP synthase/H+ pump. This is the first report showing that V1 is capable of both ATP synthesis driven by proton motive force and proton translocation across membranes driven by ATP hydrolysis.

Bafilomycin A1, a specific inhibitor of eukaryotic V1, that does not affect the activity of prokaryotic V1 (1, 13–16), did not show any inhibition of the ATP synthesis reaction catalyzed by V1. Azide, which inhibits the ATPase activity of F1, F2 because of the entrapment of the inhibitory ADP in the catalytic site, does not inhibit the ATP synthesis reaction of either F1, F2 (28) or V1 (Fig. 1d). In this respect, the V1 ATP synthase/H+ pump of T. thermophilus is more similar to an F1, F2 ATP synthase than to eukaryotic H+ pump V1.

**ATP Operon Encodes All Subunits of the V1—**Comparison of peptide sequence and deduced amino acid sequence of the genes in the atp operon enabled us to conclude that T. thermophilus V1 is composed of nine kinds of subunits. The putative 100-kDa subunit turned out to be an aggregated form of the 8-kDa proteolipid subunit, and a previously undetected hydrophobic 43-kDa subunit was identified in the protein band migrating with the A subunit in SDS-PAGE. The V1 operon of T. thermophilus shares the commonly observed feature of V1 with F1 and F2 operons in various prokaryotes that genes of the membrane part (V or F) precede those of the soluble part (V1 or F1).

**V1 Contains Two Transmembrane Subunits—**T. thermophilus V1 contains two kinds of transmembrane subunits, a 43-kDa subunit and an 8-kDa subunit. This is in contrast to F1, F2, which contains three kinds of transmembrane subunits. The 43-kDa subunit is so far the smallest member of the 100-kDa subunit family of V1, and its C-terminal hydrophobic stretch probably represents an indispensable part for all V1. The 8-kDa proteolipid subunit of T. thermophilus V1 is also unique. Although the proteolipid subunits of F1, F2 have molecular sizes of 6–9 kDa and two transmembrane helices (2, 3), the corresponding subunits of V1 are double sized, ~17 kDa, and have four transmembrane helices (1). Sequence similarity between the N- and C-terminal halves suggests that they have arisen from gene duplication of the two-helix proteolipid (41). An exception is the V1 from S. acidocaldarius, whose proteolipid subunit has two transmembrane helices after the loss of a hydrophobic presquence (39, 42). Similarly, although T. thermophilus atpL encodes a 10-kDa protein with three predicted transmembrane helices, the mature subunit did not have the presquence corresponding to the N-terminal transmembrane helix. Therefore, the proteolipid subunit of T. thermophilus belongs to the two-helix class.

**The γ Subunit May Be the Central Subunit—**Because A3B3 in T. thermophilus V1 should form a stator barrel and the subunit composition of V1 is A3B3γδ, the candidates for the central rotor shaft subunit should be either the γ or δ subunit. Our electron microscope study indicated that the central mass in V1 is mainly composed of the γ subunit. The predicted secondary structure of γ subunit reveals the existence of several long α helices, which are prerequisite for the shaft structure (data not shown). It is also worth mentioning that the features of A3B3 and A3B3γ are very similar to those of the αβ2γ αβ2γ complex of F1-ATPase from a thermophilic Bacillus PS3. The αβ2γ complex is unstable and tends to dissociate when exposed to nuclease. The αβ2γ complex is as stable as F1-ATPase and...
can be expressed in *E. coli*. Electron microscopic images of the αβγ subunit of V₁V₀ have a central cavity, but those of the αβγ subunit of F₁-ATPase do not (24). Taken together, the findings appear to indicate that the γ subunit of V₁V₀ is a counterpart of the γ subunit of F₁-ATPase. It may be possible to extend the conclusion to the γ subunit homologues of other organisms.

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