Structure of an ATPase Operon of an Acidothermophilic Archaeabacterium, Sulfolobus acidocaldarius*

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The nucleotide sequence of the operon of the ATPase complex of an acidothermophilic archaeabacterium, Sulfolobus acidocaldarius, has been determined. In addition to the three previously reported genes for the α, β, and c (proteolipid) subunits of the ATPase complex (Denda, K., Konishi, J., Oshima, T., Date, T., and Yoshida, M. (1989) J. Biol. Chem. 264, 7119–7121), the operon contains three other genes encoding hydrophilic proteins with molecular masses 25, 13, and 7 kDa. The 25-kDa protein is the largest subunit (γ), the 13-kDa protein is most likely the fourth subunit (δ), and the 7-kDa protein may correspond to an unknown subunit of the ATPase, tentatively named as ε subunit. They do not have significant sequence similarity to subunits in F0F1-ATPases and eukaryotic V-type ATPases, whereas the other three subunits, α, β, and c, have homologous counterparts in F0F1- and V-type ATPases. The order of the genes in the operon was αβγδε. The S. acidocaldarius ATPase operon differed from the eucarcbacterial F0F1-ATPase operon in that the former contains only one gene for a hydrophobic subunit at the most downstream part of the operon whereas the latter has three hydrophobic F0 genes preceding five hydrophilic F1 genes.

F0F1-ATPase in membranes of mitochondria, chloroplasts, and bacteria, synthesizes ATP coupled with an electrochemical gradient of protons generated by the electron transfer chain (1–3). It consists of two sectors: the water-soluble F1, which retains ATP hydrolytic activity and the membranous F0, which mediates proton transport across membranes. F1 and F0 are comprised of five (α, β, γ, δ, and ε) and at least three (a, b, and c) kinds of subunits, respectively. These subunits are encoded in the same operon in genomes of Escherichia coli (4, 5) and the thermophilic bacterium PS3 (6). The order of the genes in their operon is αaβbcδεγγδε where F1 is a 14-kDa polypeptide whose exact role is still unknown. It had been long believed that F0F1-ATPase is the only operational proton flow-driven ATP synthase, H+-ATP synthase, in all kinds of cells. However, novel ATPases, most likely a water-soluble part of membranous ATPase complexes which act as H+-ATP synthases in the cells, have been recently purified from the membranes of archaeabacteria, such as Sulfolobus acidocaldarius (7, 8), Methanosarcina barkeri (9), Halobacterium holohiobium (10), and Halobacterium saccharovorum (11, 12), and their enzymatic properties are significantly different from those of F1. Genes of their major subunits, the α and β subunits, have been cloned and sequenced. Comparison of their amino acid sequences with those of other ATPases revealed that archaeabacterial ATPases are related more closely to V-type ATPases than F0F1-ATPases (13–22). V-type ATPase is a class of H+-ATPase found in eukaryotic endomembrane systems, such as chromaffin granules, Golgi apparatus, lysosomes, and plant vacuoles (23–36). However, it was recently found that the c (proteolipid)2 subunit of membranous sector of the S. acidocaldarius ATPase complex is more similar to that of F0F1-ATPase than that of V-type ATPase (37, 38). The implication of these relationships in the evolution of H+-ATPase/synthase, as well as in the origin of eukaryotic cells, has been lately discussed (37, 39, 40).

Here, we report the structure and sequence of the S. acidocaldarius ATPase operon and compare them with those of other ATPases. The arrangement of genes and deduced amino acid sequences of the newly found genes in the S. acidocaldarius ATPase operon are totally different from those of F0F1-ATPase.

MATERIALS AND METHODS

Sequencing and Sequence Analysis of the ATPase Operon—The DNA fragment which we cloned for the first time contained the full-length operon (13). We have determined the nucleotide sequence of the atp operon by both strands. The method of sequencing and computer analysis of the gene have been described in our previous papers (13, 14, 37, 41).

Peptide Sequencing.—The soluble S. acidocaldarius ATPase was purified as described in our previous paper (7). Purified soluble S. acidocaldarius ATPase (250 μg) was dissolved in a solution containing 4% sodium dodecyl sulfate, 12% glycerol (w/v), 50 mM Tris-HCl, pH 6.8, 2% mercaptoethanol (v/v), 0.01% bromphenol blue, heated at 100 °C for 3 min, and subjected to 13% polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate. Subunit bands of the S. acidocaldarius ATPase in an electrophoresed gel were electroblotted using a transfer apparatus (ATTO, Tokyo). The transfer buffer contained 40 mM Tris, 240 mM glycine, 0.03% sodium dodecyl sulfate, and 20% (v/v) ethanol. Electrophoblotting onto polyvinylidene difluoride membrane (Immoblin, Millipore Inc., MA) was performed at 25 °C for 60 min. Blotted membrane was washed with distilled

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The nucleotide sequence(s) reported in this paper has been submitted to the GenBank™/EMBL Data Bank with accession number(s) J05671.

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The abbreviations used are: F1, a water-soluble sector of F0F1-ATPase; TF, F1, from a thermophilic bacterium PS3.
Fig. 1. Arrangements of genes in the atp operon encoding ATPase subunits in *S. acidocaldarius*. The letters α, β, γ, and ε indicate subunits of ATPase encoded in the genes A, B, G, and P, and the letters δ and ϵ show putative ATPase subunits encoded in the genes atpD and E.

**RESULTS AND DISCUSSION**

*Structure and Sequence of the Operon.*—The operon contains six genes, designated as *atpD, A, B, G, E, and F*, as shown in Fig. 1. There is no potential open reading frame which is immediately upstream of the 5′ end of *atpD* or downstream of the 3′ end of *atpP*. Consistently, preliminary results of Northern blot hybridization showed the presence of a 5-kilobase transcript which can cover the whole operon.4

Fig. 2 shows nucleotide sequence of the operon and the deduced amino acid sequences. The molecular weights and amino acid compositions of the six encoded polypeptides are shown in Table I. Gene products of *atpA, atpB, and atpP* have already identified as the cy, p, and c subunits of *S. acidocaldarius* ATPase complex, respectively (13, 14, 37), and their sequences are omitted from the figure. Gene products of *atpD, atpG, and atpE*, as well as those of *atpA* and *atpB*, are hydrophilic polypeptides as shown in Table I. Gene products of *atpG* and *atpE* are basic proteins and a product of *atpG* is a neutral one.

Soluble ATPase of *S. acidocaldarius* was at first reported to have three kinds of subunits, α, β, and γ (7), but later the fourth subunit was found by Lübben et al. (8). We also found the fourth subunit, the δ subunit, in our preparation.4 The estimated molecular weights of the γ and δ subunits were 28,000 and 12,000, respectively, from the mobility in sodium dodecyl sulfate-polyacrylamide gel electrophoresis (7, 8). These molecular weights are in good agreement with those estimated from the nucleotide sequences of genes *atpG* and *atpD*, respectively. The NH2-terminal amino acids of the γ and δ subunits are probably blocked since no significant amount of PTH amino acid was detected when they were analyzed with Edman degradation in a gas-phase sequencer. CNBr cleavage of the blotted membranes was effective to obtain an internal peptide of the γ subunit of which sequence was found in the deduced amino acid sequence of the *atpG* gene (Fig. 2, underlined sequence). *atpG* is thus identified as a gene for the γ subunit. However, the same procedure was not effective for the δ subunit and no internal sequence has been obtained. Attempts to isolate the δ subunit using a reverse-phase high performance liquid chromatography were unsuccessful. Therefore, we tentatively assume that *atpD* is the gene encoding the δ subunit, but further study is necessary to prove that its product is the δ subunit. Any candidate of the product of *atpE* has not been found in our preparation of the purified soluble ATPase. It is possible that this small
TABLE I

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<th>Amino acid compositions of proteins encoded by the atp operon</th>
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<td>Protein</td>
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<tr>
<td>No. of residues</td>
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(a) S. acidocaldarius : 
(b) E. coli :
(c) Synechococcus :
(d) Chloroplasts :
(e) Rsp. rubrum :

Fig. 3. Arrangements of genes encoding ATP synthase/ATPase subunits in S. acidocaldarius (a), E. coli (b), Synechococcus 6301 (c), spinach chloroplast (d), and Rhodospirillum rubrum (e). The letters a, b, c, α, β, γ, δ, and ε represent subunits encoded in the gene. The genes encoding hydrophobic subunits are shaded. Chloroplast b contains an intron (57). The dashed line signifies that the genes are at least 15 kilobases apart and are separately transcribed.

A polypeptide is a subunit of the soluble ATPase, tentatively named as the ε subunit, which is easily dissociated from the soluble ATPase during purification. Similar subunit loss during purification has been observed for some of F1 (43-45). If the product of atpE is, in fact, a subunit of the soluble ATPase, then, the intact soluble ATPase of S. acidocaldarius is comprised of five kinds of subunits, the α, β, γ, δ, and ε subunits, similar to F1.

It was already shown that the major two subunits, the α and β subunits, of the S. acidocaldarius ATPase are homologues of the 70- and 60-kDa subunits of the V-type ATPase, respectively, and that the ε subunit of the S. acidocaldarius ATPase complex is a homologue of the ε subunit of F1ATPase and a 10-kDa hydrophobic subunit of the V-type ATPase (37, 38). However, the amino acid sequences of the γ subunit and two other putative subunits of S. acidocaldarius ATPase deduced from the nucleotide sequence of atpG, D, and E genes do not have significant sequence similarity to any of subunits of V-type ATPases and F1ATPases (46, 47). Their hydrophathy profiles do not show apparent similar-
ity to those of any subunits of V-type ATPases and F_{0}F_{1}-ATPases, either data not shown). Furthermore, a search through the NBRF database did not detect any significantly similar sequences.

**Putative Initiation, Termination, and Ribosome Binding Sequences—AT-rich consensus base sequences of promoter regions have been speculated for archaebacterial genes (49–50). However, since sequence conservation at speculated promoter regions is not always strict and only a few protein-coding *S. acidocaldarius* genes have been identified, it seems premature to speculate about the location of the promoter region of this operon (51–55). Complementary sequences (GAGGTGAT) to the 3′ termini of 16 S rRNA from the same genus *S. solfataricus* or its similar sequences are found at several nucleotides upstream of initiation ATG of each gene of the operon as indicated by the *over- and underlined sequences* in Fig. 2. They might be the ribosome-binding sites. The intercistronic non-coding regions in *S. acidocaldarius* atp operon are extremely short and even gene overlappings are observed between genes β/γ and γ/ε. The intergenic region between atpE and atpP (17 base pairs) is the longest. A comparison of transcription termination sites so far determined for archaebacterial operons reveals a common, readily distinguishable archaebacterial signal, which is a T-rich sequence (56). A similar sequence, hence a candidate for transcription termination site of atp operon, was found at the 14 base pair downstream of the stop codon of the c subunit gene. In preliminary experiments, a new 2-kilobase transcript was detected when a DNA fragment corresponding to the downstream region of the c subunit gene was used as a probe for Northern blot hybridization. This indicates that atp operon ends after the c subunit gene and then another operon starts.

**Comparison of the Structure of the Operon with F_{0}F_{1}-ATPase Operons—**Since the *S. acidocaldarius* ATPase operon is the first example of an archaebacterial ATPase in which the whole sequence has been determined, it is interesting to compare the arrangement of the genes in the operon with that of F_{0}F_{1}-ATPase operon. One of the characteristics of the arrangement of genes in the F_{0}F_{1}-ATPase operon is that genes for hydrophobic F_{0} subunits precede genes for hydrophilic F_{1} subunits (Fig. 3). This feature of gene arrangement in the F_{0}F_{1}-ATPase operon has been typically observed in F_{0}F_{1}-ATPase operons of *E. coli* and a thermophilic bacterium PS3. F_{0}F_{1}-ATPase operons of two cyanobacteria, *Synechococcus* 6301 and *Synechococcus* 6716, and spinach chloroplast also retain this feature even though some of subunits are encoded in a second operon (57, 58). On the other hand, five genes, *atpD, A, B, G, and E*, encoding hydrophilic polypeptides in the ATPase operon of *S. acidocaldarius* precede a gene, atpP, encoding a very hydrophobic c subunit. Thus, the order of genes encoding hydrophobic and hydrophilic polypeptides in the operon of *S. acidocaldarius* ATPase is the reverse of that in the F_{0}F_{1}-ATPase operon.

Another feature of the operon of *S. acidocaldarius* ATPase is that there is only one gene which encodes a hydrophobic polypeptide, the c subunit. Here, two possibilities may be pointed out. The first possibility is that the operon contains all of the genes of the whole ATPase complex and the c subunit alone constitutes a H^{+} channeling membrane sector. The second possibility is that the genes related to a H^{+} channeling membrane sector are split into two (or more) independent operons and some hydrophobic subunits other than c are encoded in other operon(s) as observed for a purple non-sulfur photobacterium *Rhodospirirus rubrum* in which the F_{0} and F_{1} subunits are encoded by a two separately transcribed gene clusters (59, 60). Since attempts to isolate whole ATPase complex consisting of a membrane sector and a soluble ATPase from *S. acidocaldarius* membranes with good yield have been unsuccessful and the precise subunit organization of the ATPase has not been known, judgment of the above two possibilities should await further study.

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