Nucleotide sequence, organization and characterization of the *atp* genes and the encoded subunits of *Mycoplasma gallisepticum* ATPase

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The nucleotide sequence of a 7.8 kbp DNA fragment from the genome of *Mycoplasma gallisepticum* has been determined. The fragment contains a cluster of nine tightly linked genes coding for the subunits of the *M. gallisepticum* ATPase. The gene order is *I* (I-subunit), *B* (a-subunit), *E* (c-subunit), *F* (b-subunit), *H* (δ -subunit), *A* (α -subunit), *G* (γ -subunit), *D* (β -subunit) and *C* (ϵ -subunit). Two open reading frames were identified in the flanking regions; one (ORFU), preceding the *I* gene, encodes at least 110 amino acids and the other (ORFS), following the *C* gene, encodes at least 90 amino acids. The deduced amino acid sequences of the various subunits are presented and discussed with regard to the structure, function and differing sensitivity of the *M. gallisepticum* enzyme to dicyclohexylcarbodi-imide and aurovertin. The α - and β -subunits of the F_1 portion are well conserved (51 % and 65 % identity with those of *Escherichia coli*), whereas the γ -, δ - and ϵ -subunits, as well as the F_0 -subunits, show a low percentage identity. Nonetheless, the secondary structure of the F_0 -subunits show a high degree of similarity to the corresponding subunits of *E. coli*. Two very strong potential amphipathic α -helices are predicted in the δ -subunit and the *N*-terminus of the b-subunit contains two hydrophobic helical stretches. The possible roles of these structural properties in the close association of the F_1 and F_0 multisubunit complexes among mycoplasmas are discussed.

INTRODUCTION

All mycoplasmas tested so far possess a membrane-bound ATPase (Pollack *et al.*, 1965; Rottem & Razin, 1966; Razin, 1978) suggesting an important role for this enzyme in cell function. Monospecific antibodies generated against the β -subunit of *E. coli* F-ATPase were found to cross-react with a protein present in the cell membrane of all tested *Mycoplasma*, *Acholeplasma* and *Spiroplasma* species (Zilberstein *et al.*, 1986; Rottem *et al.*, 1987). This led to the suggestion that mycoplasmas possess an F-type ATPase, similar to the enzyme known in other bacteria.

The F-type ATPase is an enzyme complex which has been isolated from mitochondria, chloroplasts and bacteria and it has been shown to have a common function and closely related structures (Hoppe & Sebald, 1984; Walker *et al.*, 1984; Futai *et al.*, 1989; Nelson & Taiz, 1989; Senior, 1990). The complex is composed of two portions designated F_1 and F_0 which function in catalysis and proton translocation respectively. In most organisms the enzyme complex catalyses the synthesis of ATP from ADP and P_i by utilizing the energy stored in the electrochemical membrane potential gradient for protons (Hoppe & Sebald, 1984). In anaerobic bacteria and micro-organisms lacking an electron-translocating chain (e.g. the mycoplasmas), the enzyme complex is utilized to maintain a proton gradient by the hydrolysis of ATP coupled to the pumping of protons out of the cells (Futai *et al.*, 1989). The ATPase activity in mycoplasmas, however, contains several general characteristics that are not consistent with the features of commonly known F-ATPases. Unlike many bacterial F-ATPases, the mycoplasmal ATPase

activity could not be detached by varying the osmolarity of the medium (Rottem & Razin, 1966; Linker & Wilson, 1985) and was found to depend on the physical state of membrane lipids (Razin, 1978; Rottem, 1980). Furthermore, the ATPase activity of *M. gallisepticum* has been found to have special characteristics such as not being inhibited by oligomycin or azide (Linker & Wilson, 1985; Shirvan *et al.*, 1989) and, in the more alkaline pH range, being stimulated by Na^+ (Shirvan *et al.*, 1989). These observations suggest that either two distinct membrane ATPases are present in mycoplasmas or the F-ATPase complex of mycoplasmas is dissimilar to the known F-type ATPase (Shirvan *et al.*, 1989).

In this study a DNA probe from the *atp* operon of *Mycoplasma* species strain PG50 was used to identify and isolate two clones harbouring the *atp* operon of *M. gallisepticum*. The *atp* operon contains a cluster of nine genes that, as in *E. coli* (Walker *et al.*, 1984; Nelson & Taiz, 1989), are tightly linked. The complete sequence of the *M. gallisepticum atp* gene cluster, the deduced amino acid sequence and the secondary structures of the proteins are presented and discussed with respect to their relationship to structural and functional aspects of the ATPase complex. A preliminary report of part of this study has been presented (Rasmussen *et al.*, 1990).

MATERIALS AND METHODS

Strains, media and growth conditions

M. gallisepticum (A5969) was grown in a modified Edward medium containing 4 % horse serum (Razin & Rottem, 1976). The cells were grown at 37 °C for 20–22 h, harvested by

Abbreviations used: 1 $\times$ SSC, 0.15 M-NaCl/0.015 M-sodium citrate; DCCD, dicyclohexylcarbodi-imide; ORF, open reading frame.

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These sequence data have been submitted to the EMBL, GenBank and DDBJ Nucleotide Sequence Databases under the accession number X64256.

centrifugation at 12000 *g* for 20 min and washed twice with a 0.25 M-NaCl solution containing 20 mM-Hepes buffer, pH 7.5.

Southern blotting and hybridization procedures

Chromosomal DNA was prepared by the method of Marmur & Doty (1962). The DNA was cleaved with *Bgl*II, *Eco*RI, *Hind*III, *Hpa*I, *Pst*I or *Xba*I as recommended by the suppliers (New England Biolabs, BRL, Gaithersburg, MD, U.S.A.). The digested DNA fragments were separated by electrophoresis on 1% agarose gels and transferred to a nitrocellulose filter (Schleicher and Schull) as described by Southern (1975). The filters were hybridized at 55 °C in 6 × SSC/0.5% SDS/0.1% Ficoll/0.1% BSA/0.1% polyvinylpyrrolidone/10 mM-EDTA (where 1 × SSC = 0.15 M-NaCl/0.015 M-sodium citrate). After hybridization, the filters were washed 2 × 15 min in 2 × SSC/0.5% SDS at room temperature and 1 × 2 h plus 1 × 30 min in 0.2 × SSC/0.5% SDS at 40 °C. These low-stringency conditions allow hybridization with 40% mismatch. As a DNA probe, nick-translated pMYC405 was used. This clone harbours the central part of the ATPase operon of *Mycoplasma* subsp. strain PG50 (Rasmussen & Christiansen, 1987).

Cloning procedures

Partial DNA libraries were constructed by cloning *Bgl*II fragments of 6.6–7.0 kb and *Hind*III fragments of 4.2–4.6 kb into *E. coli* strain DH5 α . pGEM blue (Promega Corp., Madison, WI, U.S.A.) was used as vector. Screening of the *Bgl*II library with pMYC405 led to the identification of pOR201 and this clone was used to identify pOR203 in the *Hind*III library.

DNA sequencing and data analysis

Sequencing was done with Sequenase version I or II (US Biochemicals Corp., Cleveland, OH, U.S.A.). The sequence was fully determined on both strands and across all restriction sites of the 7.8 kbp fragment of *M. gallisepticum* genomic DNA which encodes for the ATPase subunits. Sequence analyses were carried out on a modified Staden (1985) program.

To predict the structure of the subunits, several tools were utilized, mainly the secondary-structure prediction of Chou & Fasman (1978) and prediction of the hydropathic character of the subunits according to the Kyte & Doolittle (1982) hydrophobicity scale.

Analysis of amphipathic α -helices

Amphipathic α -helices were analysed by converting the amino acid sequence into a sequence of hydrophobicity values [by the Fauchere-Pliska scale (Fauchere & Pliska, 1983)], dividing the sequence into overlapping blocks of length 11 and searching for runs of consecutive blocks with periodicity in hydrophobicity that fits a helical periodicity (Cornette *et al.*, 1987; Margalit *et al.*, 1987). A power spectrum procedure [at least-squares fit to a sinusoid which for short segments performs better than a discrete Fourier transform (Cornette *et al.*, 1987)] was used to identify the amphipathic blocks. The intensities of the power spectrum in the range of 0–180° were calculated. A block with maximum intensity at 100 ± 20° is considered a potential amphipathic α -helix. An additional criterion, the amphipathic index, was used to evaluate the relative magnitude of the intensity around 100° compared with the whole range of angles used in the analysis (0–180°) (Cornette *et al.*, 1987; Margalit *et al.*, 1987).

RESULTS AND DISCUSSION

Cloning and sequencing of the *atp* operon

The pMYC405 probe which harbours the central part of the *atp* operon of *Mycoplasma* subsp. strain PG50 (Rasmussen &

Christiansen, 1987) hybridized under low-stringency conditions to specific *M. gallisepticum* DNA fragments (not shown). Two of these fragments, a 6.8 kb *Bgl*II fragment and a 4.4 kb *Hind*III fragment, were ligated into pGEM-blue to yield pOR201 and pOR203 respectively. The fragments overlap by 1.1 kb (Fig. 1). A 7.8 kb region of pOR201 and pOR203 was sequenced and is presented in Fig. 2.

Identification of genes

Comparison of the protein sequences of the *E. coli* ATP synthase subunits with the amino acid sequences deduced from the 7.8 kb region enabled the identification of the *atp* genes in *M. gallisepticum* (Table 1). The gene order is *atpB*, *atpE*, *atpF*, *atpH*, *atpA*, *atpG*, *atpD*, *atpC* (Fig. 1) as found in *E. coli* (Walker *et al.*, 1984) and gene clusters encoding the subunits of F-ATPases of other micro-organisms (Walker *et al.*, 1984; Futai *et al.*, 1989; Senior, 1990).

Upstream of the *atpB* gene, an open reading frame (ORF) of 169 amino acids is located. The deduced protein is highly hydrophobic indicating that this putative gene is analogous to the I-subunit gene of *E. coli* (Walker *et al.*, 1984). Similar genes have been identified in several other micro-organisms including *Rhodospirillum rubrum* (Falk & Walker, 1988) and the thermophilic bacterium PS3 (Ohta *et al.*, 1988).

Alignments of the sequences of *M. gallisepticum atp* gene products with the corresponding F-ATPase subunits of *E. coli* (Table 1) revealed that the α - and β -subunits are rather well conserved (51% and 65% identity, respectively), whereas those of γ , δ and ϵ as well as a , b , c and I , are poorly conserved (16–28% identity). Most remarkable is the conservation of the β -subunit (472 amino acid residues) which was found to have 309, 313, 316, 311 and 315 residues identical with those of *E. coli* (Kamazawa *et al.*, 1982), *Bacillus megaterium* (Hawthorne & Brusilow, 1988), *R. rubrum* (Falk *et al.*, 1985), maize chloroplast (Krebbes *et al.*, 1982) and bovine mitochondria (Runswick & Walker, 1983), respectively.

Flanking regions and sequence features

The intergenic region between *atpB* and *atpE* is fairly long, 100 bp. Otherwise the intergenic regions are short, less than 8 bp, suggesting that the *atp* genes form an operon. The I-subunit gene and *atpB* overlap by 39 bp. A less extensive overlap of 8 bp is found for *atpD* and *atpC*. Several lines of evidence indicate that *Mycoplasma* is related to Gram-positive bacteria (Woese, 1987). In *Bacillus*, overlaps are common, and overlaps in the size of 40 bp have been found (Hansson *et al.*, 1991).

An ORF (ORFU) of at least 110 amino acids terminates 175 bp upstream of the start codon for the I-subunit gene. A search in the protein database PROT did not reveal homology with any protein. ORFU is succeeded by a sequence which has the potential to form a stem-loop structure followed by a run of thymidines. This structure resembles the ρ -independent transcription termination signal of *E. coli*. It appears to be widely conserved in eubacteria and has previously been found at the 3' end of *Mycoplasma* genes (Su *et al.*, 1987).

In the 100 bp succeeding the stop codon of *atpC*, no sequences with resemblance to the ρ -independent transcription termination signal are found. However, overlapping the *atpC* stop codon, a start codon of an ORF (ORFS) with at least 90 amino acids is located. A search of the PROT database did not show any similarity to any known protein sequences. Whether ORFS is indeed a gene and, if so, whether the gene product is part of the *M. gallisepticum* F-ATPase must await further studies.

ORFs presented start with an ATG initiation codon and end with a TAG or TAA stop codon. The 15 bp upstream of the start codons are shown in Table 2. All genes and ORFs except *atpC*

Fig. 1. Physical map of the region in the *M. gallisepticum* genome encoding the *atp* operon

The region between the bold vertical lines has been sequenced. The *atp* genes are denoted by capital letters. U and S are two open reading frames flanking the *atp* operon. Stippled boxes show the reading frames of the nine *atp* genes (lightly stippled) as well as U and S (heavily stippled). The following restriction sites are shown: A, *Ava*I; B, *Bam*HI; Bg, *Bgl*II; E, *Eco*RI; H, *Hind*III; X, *Xba*I. Arrow shows the direction of translation.

have potential ribosome-binding sites similar to the Shine-Dalgarno (1974) sequence. Some are fairly weak and some, particularly the one for *atpD*, are located at a relatively long distance from the start codon. The significance of this is as yet unknown.

F₁-subunits

The F₁ portion of the F-ATPase of all bacteria, chloroplasts and mitochondria investigated is characterized by a typical stoichiometry of the subunits. In *E. coli* the stoichiometry is $\alpha_3\beta_3\delta_1\gamma_1\epsilon_1$ (Futai *et al.*, 1989). As all these subunits are encoded by genes present in a single operon, several explanations for how different amounts of various proteins are obtained from a polycistronic message can be conceived. Among these is the hypothesis emphasizing the importance of codon/anticodon recognition for the efficiency of translation (Walker *et al.*, 1984). According to this hypothesis, bacterial genes that produce large amounts of protein use more abundant codons, whereas genes that produce low amounts of protein use rare codons (Gouy & Grantham, 1980). We therefore analysed the codon usage of the various genes of the *atp* operon of *M. gallisepticum*. We found, among all the genes, a strong bias against codons ending with G or C (results not shown). This is to be expected as the G+C content of *M. gallisepticum* is as low as 31.8–35.7% (Dupiellet *et al.*, 1990). Comparing the codon usage for α and β with those of γ , δ and ϵ revealed that subunits α and β (the multicopy subunits) have a high preference for codons ending with T or A in the variable third position (74.7 and 75% respectively) compared with subunits γ , δ and ϵ (71.7%, 64% and 67.7% respectively). As was pointed out in other systems (Gouy & Grantham, 1980), the most abundant codons often have T or A in the third position which result in 'intermediate' binding energies and rapid turnover.

It has previously been pointed out that in *E. coli* the α - and β -subunits are somewhat similar to each other in sequence and are likely to have similar secondary structures and tertiary folding (Walker *et al.*, 1984; Senior, 1990). Alignment of the *M. gallisepticum* α -subunit with the β -subunit revealed a 23.5% identity in 349 residues, suggesting their evolutionary relatedness. Both the α - and β -subunits have the conserved sequence GXX-XXGKT shown to be present in a variety of nucleotide-binding proteins (Walker *et al.*, 1982; Hsu *et al.*, 1987) and thought to interact with the phosphate groups of the bound nucleotide. In the α -subunit the conserved sequence is between residues 170 and

177 and in the β -subunit between residues 156 and 163. Nonetheless, whereas in the β -subunit of *M. gallisepticum*, 24 residues downstream from the conserved sequence, the sequence G-E-R is found, this sequence is missing from the α -subunit of *M. gallisepticum*. It has been suggested that the G-E-R sequence is also important for nucleotide binding (Bowman *et al.*, 1988).

The α -subunit is the largest subunit in the *M. gallisepticum* ATPase (57 kDa, see Table 1). In *E. coli* two functional regions have been located in addition to the nucleotide-binding domain described above: at the N-terminus, a membrane-binding region and, between residues 345 and 375, an α/β signal transmission region (Maggio *et al.*, 1987; Senior, 1990). As the amino acid sequences of the α -subunit from *M. gallisepticum* and *E. coli* show a high degree of similarity, it is most probable that these subunits have essentially the same three-dimensional structure and similar activities. The β -subunit contains the catalytic ATPase site predicted in *E. coli* to be formed by residues 140–335 (Duncan *et al.*, 1986; Senior, 1990). According to computer alignment (not shown), the equivalent region in *M. gallisepticum* is between residues 140 and 339. The corresponding regions show 90% identity (175 out of 195 residues).

The ATPase activity of *M. gallisepticum* is markedly inhibited by dicyclohexylcarbodi-imide (DCCD) although the concentrations required were higher than those required to inhibit other F-type ATPases (Rottem *et al.*, 1981; Linker & Wilson, 1985). The two glutamic acid residues at positions 181 and 192 of the β -subunit of *E. coli* were considered to be the DCCD-reactive residues (Yoshida *et al.*, 1981). These residues are present in *M. gallisepticum* as well. Nonetheless, in most F-type ATPases, a membrane-buried carboxy group of a conserved aspartic acid residue at position 61 of the c-subunit was shown to be the binding site of DCCD (Hoppe & Sebald, 1984). This residue is missing from *M. gallisepticum*. The equivalent residue to D-61 of the *E. coli* c-subunit is A-77 in *M. gallisepticum*. However, a glutamic acid residue occupies position 78 of *M. gallisepticum* c-subunit. This residue is at a distance of 18 residues (upstream) from a highly conserved AR×P sequence, present in *E. coli* at the same distance from D-61. By analogy with the role of the D-61 residue in *E. coli*, it is possible to assume a similar role for E-78 in *M. gallisepticum*. However, further support for this will have to await site-directed mutagenesis studies to attempt to confer DCCD resistance on *M. gallisepticum*.

The antibiotic aurovertin inhibits the activity of F-type ATPases of different species. A mutation conferring aurovertin resistance on the *E. coli* F-ATPase was identified as R-398→H in the β -subunit (Lee *et al.*, 1989). The equivalent residue in the β -subunit of all studied aurovertin-sensitive species is usually arginine, whereas in the aurovertin-insensitive ATPase of the thermophilic bacterium PS3 this residue is phenylalanine (Kagawa *et al.*, 1986). Notably in *M. gallisepticum* the equivalent residue to *E. coli* R-398 is N-406, and, as expected, the ATPase activity of *M. gallisepticum* was found to be almost completely insensitive to concentrations of aurovertin as high as 20 mM (< 20% inhibition at pH 7.5). Under identical assay conditions, 1 mM-aurovertin inhibited bovine heart mitochondrial F-ATPase activity by 80% (J. M. Jault, W. S. Allison & S. Rottem, unpublished work).

The δ - and ϵ -subunits are considered to participate in the binding of F₁ to F₀ (Bragg *et al.*, 1973; Senior, 1990), and the δ -subunit is believed to form the stalk linking F₁ to F₀. As in *E. coli* (Walker *et al.*, 1984; Futai *et al.*, 1989), this subunit is predicted to be highly helical. The regions of *M. gallisepticum* δ -subunit predicted to form α -helices have been analysed for their potential to form amphipathic helices in which one face is hydrophobic and the opposite face hydrophilic. In an amphipathic α -helix the hydrophobicity varies sinusoidally with a period of 3.6 residues

[illegible]

Fig. 2. DNA sequences of the *atp* operon and flanking regions of *M. gallisepticum*. Above is shown the deduced amino acid sequence of the *atp* genes. Translation initiation codons are underlined. The stop codon of ORFU and the start codon of ORFS are indicated and the inverted repeat regions succeeding ORFS are boxed.

Table 1. Characterization of the *M. gallisepticum* ATPase subunits encoded by genes of the *atp* operon

<i>atp</i> genes		ATPase subunits			
Genes	G+C (%)	Subunit	No. of residues	Molecular mass (kDa)	Percentage identity with <i>E. coli</i>
<i>atpI</i>	32.6	I	169	18.7	16.1 (19/120)*
<i>atpB</i>	37.8	a	297	33.3	22.6 (61/269)
<i>atpE</i>	38.3	c	96	10.0	25.6 (20/78)
<i>atpF</i>	32.4	b	198	22.7	21.3 (33/155)
<i>atpH</i>	32.0	δ	181	21.6	25.3 (44/174)
<i>atpA</i>	37.2	α	518	57.8	51.2 (256/500)
<i>atpG</i>	33.0	γ	289	33.1	27.9 (78/279)
<i>atpD</i>	38.0	β	470	51.5	65.2 (307/471)
<i>atpC</i>	35.8	ϵ	123	14.9	19.2 (25/130)

* In parentheses is given the number of identical amino acids/number of amino acids compared.

Table 2. Potential ribosome-binding site

Sequences 5' to the ATG initiation codons (bold) were analysed. Potential Shine-Dalgarno (1974) sequences are underlined.

Subunit	Sequence	Distance (bp)
I	TATATTTAAGATCAGATG	4
a	AAGGAATTAATAACAATG	11
c	TAAGCAAATAAAAACCATG	9
b	CGTAGCACGATAGTTATG	2-10
δ	ATTTAGACTAACTTATG	8
α	AAGGAAAACTAGACTATG	11
γ	TGGAGTAATTTTCTATG	10
β	AGGAGGCTGCTAAATAGTTTATG	(3), 15
ϵ	AAACATTAACACGTAATG	-
ORFS	AAAAGAAGCTAAATAATG	-

per turn of the helix or a frequency of 100° per residue (Fauchere & Pliska, 1983; Cornette *et al.*, 1987). Two very strong potential amphipathic α -helices are predicted in the δ -subunit (Fig. 3): one is located at residues 85-104 with maximum block intensity and amphipathic index of 17.09 and 3.86 respectively (at the block 91-101). The second segment is 121-141, and its best scoring block is 123-133 with intensity and amphipathic index of 12.98 and 4.11 respectively. For comparison, most of the blocks in other segments with maximum intensity around 100° have average intensity values of 7-8 and average amphipathic indices of 2-2.5.

Alignment of the identified amphipathic helices with other known sequences of the δ -subunit (Kimura *et al.*, 1990) indicates a low degree of similarity (results not shown). There are only a few common residues at the same position in all the sequences. However, in certain positions, there is a conservation of the chemical properties. An amphipathic analysis of the corresponding sequences shows a conservation of the two strong amphipathic segments. All the sequences have a very strong potential amphipathic segment in the region aligned with residues 85-104 with amphipathic indices above 3.5. Also, in all the sequences this region is predicted as an α -helix by the Chou-Fasman and Garnier-Osguthorpe-Robson algorithms. The second region, 121-141, which shows a high potential to fold as an amphipathic α -helix in the *M. gallisepticum* sequence, is predicted in five out of the other six sequences to have a helical

Fig. 3. Amphipathic characteristics of the *M. gallisepticum* δ -subunit

(a) Profile of the amphipathic index for the δ -subunit sequence. The amphipathicity index, a measure of the intensity of amphipathicity, is defined as the fractional area under the power spectrum curve in the range 85-100° divided by the fractional width of the interval. (b) Helical wheel presentation of the two predicted strong amphipathic α -helices in regions 85-104 and 121-141. Hydrophobic residues are shaded.

amphipathicity, but the values of the intensity and amphipathic index are lower in most of them.

The F₀-subunits

The F₀ portion of the F-type ATPase complex is integral to the membrane and is composed of three subunits a, b and c, which have an important role both as structural organizers as well as participating in proton conduction (Futai *et al.*, 1989; Senior, 1990). The deduced amino acid sequences of the *M. gallisepticum* a-, b- and c-subunits show a rather low percentage of identity with the corresponding *E. coli* subunits (Table 1). However, secondary-structure predictions show a high degree of similarity between corresponding subunits. The subunits are predominantly hydrophobic and contain a relatively high number of α -helices located at equivalent positions and of equal lengths (Fig. 4).

The a-subunit of *M. gallisepticum* contains several hydrophobic α -helices in agreement with previous models predicting five, six or seven transmembrane helices in other organisms (Hermolin *et al.*, 1983; Cain & Simoni, 1989; Senior, 1990). Structural analysis of the b-subunit of *M. gallisepticum* revealed that it consists of two hydrophobic segments (residues 8-29 and 45-68) at the N-terminus followed by a polar 130-residue segment (Fig. 4). In *E. coli* the b-subunit contains a single hydrophobic N-terminal segment of 30 residues (Kumamoto & Simoni, 1986). In this organism the b-subunit is not directly involved in H⁺ translocation but has a structural role (Senior, 1990). It was suggested that the single hydrophobic segment of *E. coli* is intercalated into the membrane whereas the bulk of the molecule projecting from the membrane on the cytoplasmic side interacts

Fig. 4. Predicted α -helicity (1) and hydrophobicity (2) of the F_0 -subunits of *M. gallisepticum* ATPase

The scales on the abscissae are in amino acid residues.

with the F_1 -subunits (Fig. 5a). The two hydrophobic helical stretches at the *N*-terminus of *M. gallisepticum* ATPase may suggest two alternative types of structure: (a) a hairpin-like structure with the two hydrophobic domains transversing the membrane and the few residues at the *N*-terminus and the polar segment at the *C*-terminus exposed on the cytoplasmic surface (Fig. 5b) or (b) a rod-like structure with the second hydrophobic stretch being an extramembranous domain (Fig. 5c) which may participate in the interaction with the hydrophobic parts of the amphipathic helices of the d-subunit. The latter possibility is consistent with the inability to detach *M. gallisepticum* ATPase activity from the membrane by varying the osmolarity of the medium or by treating the membrane with EDTA (Linker & Wilson, 1985; Shirvan *et al.*, 1989).

Structural analysis of c-subunit shows that it is composed of two transmembrane helices separated by a polar segment. This is in accordance with a hairpin-like structure with two hydrophobic

Fig. 5. Proposed models for the *M. gallisepticum* b-subunit in the cell membrane

The two models (b and c) proposed for the b-subunit of *M. gallisepticum* are compared with the accepted model for the b-subunit of *E. coli* (a) (from Kumamoto & Simoni, 1986).

segments separated by a polar loop oriented towards the cytoplasm that was predicted for c-subunits from different species (Senior, 1990).

Our findings have shown that *M. gallisepticum* possesses an F-type ATPase which is similar to the known bacterial ATPases. Knowledge of the complete nucleotide sequences and the deduced amino acid sequences of the various subunits has allowed us to provide possible explanations for some of the differences between the established bacterial F-ATPases and that of *M. gallisepticum*. These differences include a low sensitivity of the mycoplasmal enzyme to aurovertin, and a more firm attachment of F_1 to F_0 . Although our findings cannot rule out the presence of two distinct ATPases in *M. gallisepticum* (Southern, 1975), the cloned *atp* operon of *M. gallisepticum* might prove to be a valuable tool in resolving this question.

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REFERENCES

- Bowman, E. J., Tenney, K. & Bowman, B. J. (1988) *J. Biol. Chem.* **263**, 13994-14001
- Bragg, P. D., Davies, P. L. & Hou, C. (1973) *Arch. Biochem. Biophys.* **159**, 664-670
- Cain, B. D. & Simoni, R. D. (1989) *J. Biol. Chem.* **264**, 3292-3300
- Chou, P. Y. & Fasman, G. D. (1978) *Adv. Enzymol. Relat. Areas Mol. Biol.* **45**, 45-148
- Cornette, J. L., Cease, K. B., Margalit, H., Spouge, J. L., Berzofsky, J. A. & DeLisi, C. (1987) *J. Mol. Biol.* **195**, 659-685
- Duncan, T. M., Parsonage, D. & Senior, A. E. (1986) *FEBS Lett.* **208**, 1-6
- Dupiellet, J. P., Vuillaume, A., Rousselot, D., Bove, J. M. & Bradbury, J. M. (1990) *Zbl. Bakt. Suppl.* **20**, 859-864
- Falk, G. & Walker, J. E. (1988) *Biochem. J.* **254**, 109-122
- Falk, G., Hampe, A. & Walker, J. E. (1985) *Biochem. J.* **228**, 391-407
- Fauchere, J. & Pliska, V. (1983) *Eur. J. Med. Chem.* **18**, 369-375
- Futai, M., Noumi, T. & Maeda, M. (1989) *Annu. Rev. Biochem.* **58**, 111-136
- Gouy, M. & Grantham, R. (1980) *FEBS Lett.* **115**, 151-155
- Hansson, M., Rutberg, L., Schroder, I. & Hederstedt, L. (1991) *J. Bacteriol.* **173**, 2590-2599
- Hawthorne, C. A. & Brusilow, W. S. A. (1988) *Biochem. Biophys. Res. Commun.* **151**, 926-931

- Hermolin, J., Gallant, J. & Fillingame, R. H. (1983) *J. Biol. Chem.* **258**, 14550–14562
- Hoppe, J. & Sebald, W. (1984) *Biochim. Biophys. Acta* **768**, 1–27
- Hsu, S. Y., Noumi, T., Takeyama, M., Maeda, M., Ishibashi, S. & Futai, M. (1987) *FEBS Lett.* **218**, 222–226
- Kagawa, Y., Ishizuka, M., Saishu, T. & Nakao, S. (1986) *J. Biochem. (Tokyo)* **100**, 923–934
- Kamazawa, H., Kayano, T., Kiyasa, T. & Futai, M. (1982) *Biochem. Biophys. Res. Commun.* **105**, 1257–1264
- Kimura, T., Takeda, S., Ashai, T. & Nakamura, K. (1990) *J. Biol. Chem.* **265**, 6079–6085
- Krebbers, E. T., Larrinna, I. M., McIntosh, L. & Bogorad, L. (1982) *Nucleic Acids Res.* **10**, 4985–5002
- Kumamoto, C. A. & Simoni, R. D. (1986) *J. Biol. Chem.* **261**, 10037–10042
- Kyte, J. & Doolittle, R. F. (1982) *J. Mol. Biol.* **157**, 105–132
- Lee, R. S. F., Pagan, J., Stare, M., Vignais, P. V. & Senior, A. E. (1989) *FEBS Lett.* **253**, 269–272
- Linker, C. & Wilson, T. H. (1985) *J. Bacteriol.* **163**, 1258–1262
- Maggio, M. B., Pagan, J., Parsonage, D., Hatch, L. & Senior, A. E. (1987) *J. Biol. Chem.* **262**, 8981–8984
- Margalit, H., Spouge, J. L., Cornette, J. L., Cease, K. B., DeLisi, C. & Berzofsky, J. A. (1987) *J. Immunol.* **138**, 2213–2229
- Marmur, J. & Doty, P. (1962) *J. Mol. Biol.* **5**, 109–117
- Nelson, N. & Taiz, L. (1989) *Trends Biochem. Sci.* **14**, 113–117
- Ohta, S., Yohda, M., Ishizuka, M., Hirata, H., Hamamoto, T., Otawara-Hamamoto, Y., Matsuda, K. & Kagawa, Y. (1988) *Biochim. Biophys. Acta* **993**, 141–155
- Pollack, J. D., Razin, S. & Cleverdon, R. C. (1965) *J. Bacteriol.* **90**, 617–622
- Rasmussen, O. F. & Christiansen, C. (1987) *Isr. J. Med. Sci.* **23**, 393–397
- Rasmussen, O. F., Shirvan, M. H., Rottem, S. & Christiansen, C. (1990) *Zbl. Bakt. Suppl.* **20**, 619–621
- Razin, S. (1978) *Microbiol. Rev.* **42**, 414–470
- Razin, S. & Rottem, S. (1976) in *Biochemical Analysis of Membranes* (Maddy, A. H., ed.), pp. 3–26, Chapman and Hall, London
- Rottem, S. (1980) *Biochim. Biophys. Acta* **604**, 65–90
- Rottem, S. & Razin, S. (1966) *J. Bacteriol.* **92**, 714–722
- Rottem, S., Linker, C. & Wilson, T. H. (1981) *J. Bacteriol.* **145**, 1299–1304
- Rottem, S., Shirvan, M. H., Barile, M. F. & Zilberstein, D. (1987) *Isr. J. Med. Sci.* **23**, 389–392
- Runswick, M. J. & Walker, J. E. (1983) *J. Biol. Chem.* **258**, 3081–3089
- Senior, A. E. (1990) *Annu. Rev. Biophys. Biophys. Chem.* **19**, 7–41
- Shine, J. & Dalgarno, L. (1974) *Proc. Natl. Acad. Sci. U.S.A.* **71**, 1342–1346
- Shirvan, M. H., Schuldiner, S. & Rottem, S. (1989) *J. Bacteriol.* **171**, 4417–4424
- Southern, E. M. (1975) *J. Mol. Biol.* **98**, 503–517
- Staden, R. (1985) in *Genetic Engineering; Principles and Methods* (Setlow, J. K. & Hollander, A., eds.), vol. 7, pp. 67–114, Plenum Publishing Co., New York and London
- Su, C. J., Tryon, V. Y. & Baseman, J. B. (1987) *Infect. Immun.* **15**, 3023–3029
- Walker, J. E., Saraste, M., Runswick, M. J. & Gay, N. J. (1982) *EMBO J.* **8**, 945–951
- Walker, J. E., Saraste, M. & Gay, N. J. (1984) *Biochim. Biophys. Acta* **768**, 164–200
- Woese, C. R. (1987) *Microbiol. Rev.* **51**, 221–271
- Yoshida, M., Poser, J. W., Allison, W. S. & Esch, F. S. (1981) *J. Biol. Chem.* **256**, 148–153
- Zilberstein, D., Shirvan, M. H., Barile, M. F. & Rottem, S. (1986) *J. Biol. Chem.* **161**, 7109–7111

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