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Short Sequence-Paper

The Na^+-translocating ATPase of *Acetobacterium woodii* is a F_1F_0-type enzyme as deduced from the primary structure of its β, γ and ϵ subunits ☆

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Abstract

A 4.5 kbp *Eco*RI fragment hybridizing to a fragment of *uncD* (coding for subunit β of F_1F_0-ATPases) was cloned from chromosomal DNA of *Acetobacterium woodii*. The nucleotide sequence was determined and revealed five open reading frames (ORF), four of which were identified to code for subunits of the Na^+-ATPase. The deduced amino acid sequences of these ORF's are homologous to subunit α (partial coding sequence, C-terminal end), γ, β and ϵ of F_1F_0-ATPases from various organisms; furthermore, the organization of the genes in the order *uncA* (α), *uncG* (γ), *uncD* (β), *uncC* (ϵ) is identical to the structure of *unc* operon as present in most bacteria. Downstream of *uncC* is an ORF whose deduced amino acid sequence has 53% sequence homology to AlgD from *Pseudomonas aeruginosa*. The structure and organization of the *unc* genes are the final proof that the Na^+-ATPase from *A. woodii* is a member of the family of F_1F_0-ATPases.

Keywords: ATPase, Na^+-; DNA sequence; Acetogenic bacterium; (*A. woodii*)

The homoacetogenic bacterium *Acetobacterium woodii* is a strictly anaerobic bacterium whose bioenergetics are based on a sodium ion cycle across the membrane [1]. The Na^+-translocating ATPase has been purified and was, by biochemical and immunological criteria, identified as a F_1F_0-ATPase [2]. However, despite these similarities the ATPase from *A. woodii* differs from other F_1F_0-ATPases, including the Na^+-translocating enzyme from *Propionigenium modestum* [3], in three regards: as judged from an SDS-PAGE of the purified complex it contains just 6 subunits, the F_1–F_0 interaction is different and it is inhibited by nitrate [2]. Therefore, in order to clearly assign the Na^+-ATPase from *A. woodii* as a F_1F_0-ATPase it was necessary to determine the DNA sequence of its major subunits and compare the deduced amino acid sequence with those of different ATPases.

In order to clone the genes coding for the ATPase from *A. woodii* we took advantage of the fact that subunit β of F_1F_0-ATPases is very well conserved among various organisms [4]. Therefore, a homologous subunit β fragment was amplified by means of the polymerase chain reaction (PCR) using degenerated oligonucleotides corresponding to amino acids 54–58 and 208–214 of subunit β of *Escherichia coli*. The identity of this fragment was verified by sequencing and it was further used as a homologous probe. All techniques used were standard methods [5]. Southern blot analysis of genomic DNA restricted with different enzymes revealed a 4.5 kbp *Eco*RI fragment that

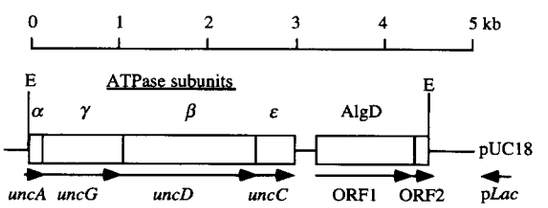

Fig. 1. Pysical map of plasmid pAF1 and organization of genes in the *unc* operon of *A. woodii*. The *Eco*RI fragment was cloned into the *Eco*RI site of pUC18. Genes are indicated by boxes and the direction of transcription is indicated by the arrows. E, *Eco*RI.

☆ Dedicated to Professor Gerhard Gottschalk on the occasion of his 60th birthday.

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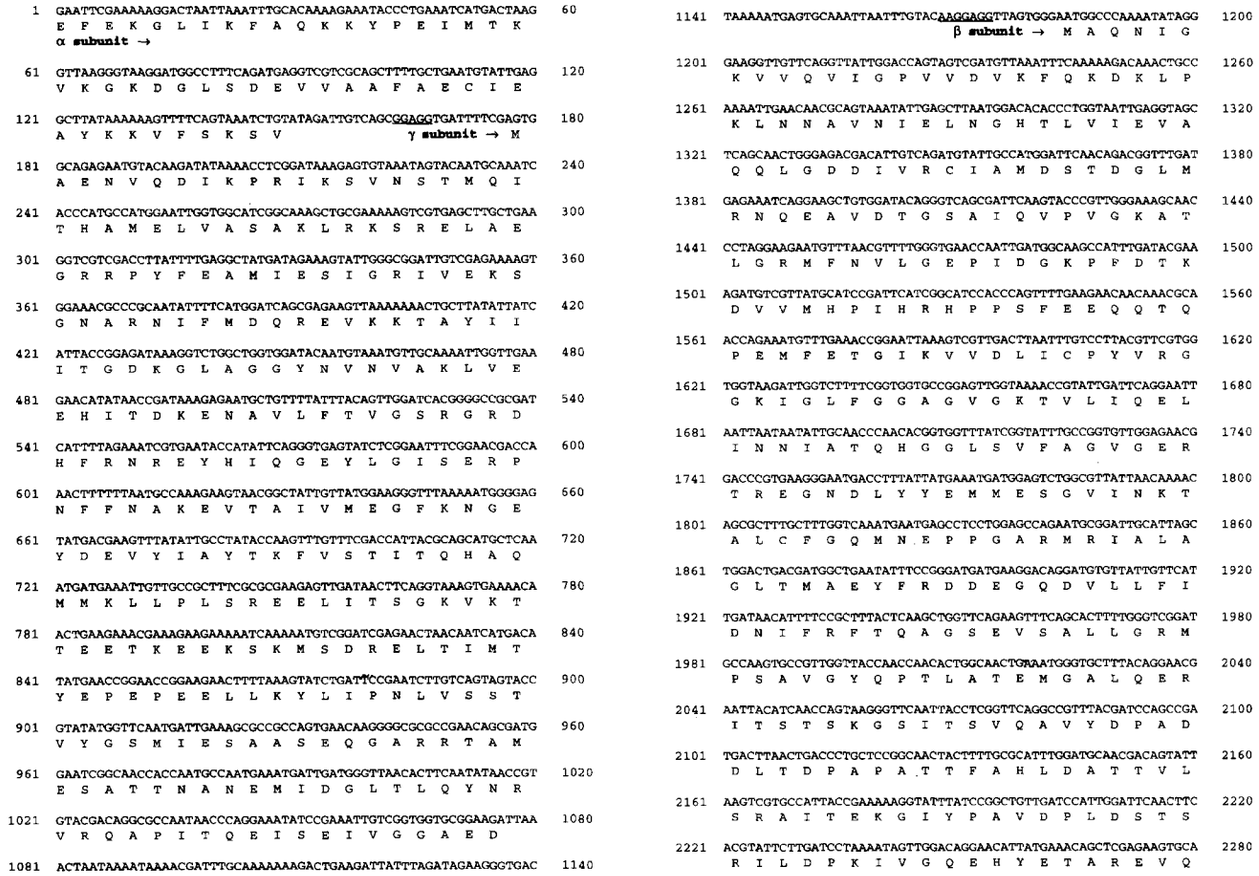
α subunit →
γ subunit → M
β subunit → M A Q N I G
ε subunit → M A E T F R L K I I A P T G
algD → M

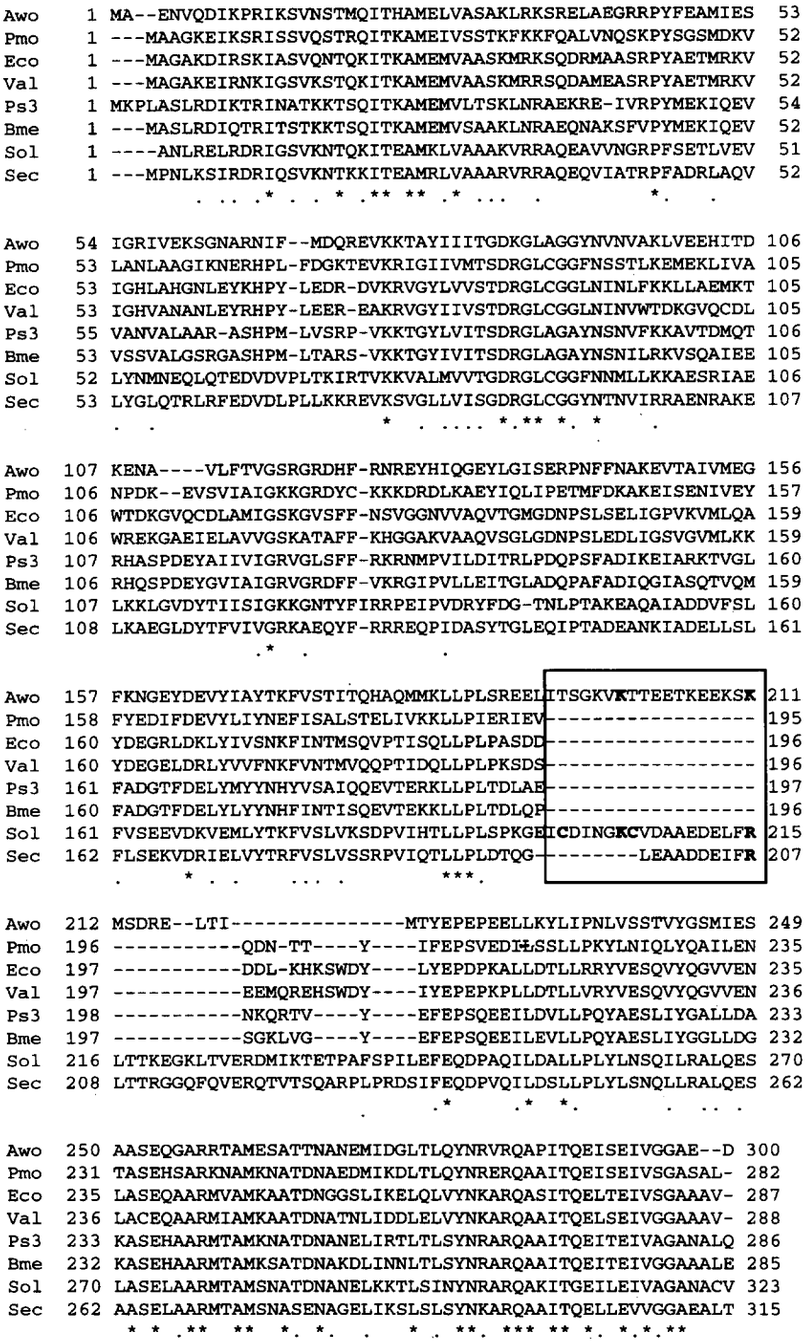

Fig. 3. Alignment of the deduced amino acid sequence of *uncG* with *uncG* from *P. modestum* (Pmo) [13], *E. coli* (Eco) [14], *Vibrio alginolyticus* (Val) [15], thermolphilic bacterium PS3 (PS3) [16], *Bacillus megaterium* (Bme) [17], Spinach chloroplasts [9] and *Synechocystis* 6803 [11]. Residues identical and homologous are indicated by asteriks and points, respectively. The region responsible for thiol modulation in CF_1F_O is boxed. Important residues in this region are highlighted.

Fig. 2. Nucleotide sequence of the 3′ end of the *unc* operon of *A. woodii* and deduced amino acid sequence of subunit γ, β and ϵ. Only basepairs 1–3203 of the *Eco*RI fragment are shown. The sequence of both strands was obtained by primer walking. Shine-Dalgarno sequences are underlined, promotor structures are boxed and stem-loop structures are marked by arrows. The sequence has been submitted to Genbank (accession number U10505).

hybridized with the homologous probe. Therefore, genomic DNA of *A. woodii* was restricted with *Eco*RI and size-fractionated by sucrose density centrifugation. Fragments ranging from 3 to 6 kbp were cloned into the *Eco*RI site of pUC18 and transformed into *E. coli* DH5α, and transformants were subjected to colony hybridization. One clone contained a plasmid with a 4.5 kbp *Eco*RI insert which in Southern blots hybridized with the probe. This plasmid was named pAF1 (Fig. 1).

The nucleotide sequence (4514 bp) of the *Eco*RI fragment in pAF1 was completely determined and the sequence relevant to the ATPase is shown in Fig. 2. Genes were identified by data bank searches which revealed that the fragment contained, in that order, the C-terminus of subunit α (*uncA*) as well as the complete coding sequence for the subunit γ (*uncG*), β (*uncD*) and ϵ (*uncC*) homologue of F_1F_0-ATPases. The coding sequence for each gene starts with the initiation codon ATG, except for the gene coding for subunit γ which uses GTG as the initiation codon. Each of these codons is preceded by a Shine-Dalgarno sequence [6]. Three basepairs downstream of the stop codon of *uncC* is an inverted repeat which most likely functions a transcriptional terminator. Downstream of the inverted repeat is a sequence resembling the -35 and -10 region, respectively, of a prokaryotic promoter which is followed by a Shine-Dalgarno sequence preceding ORF1 (of which only the start codon is shown in Fig. 2). ORF1 is 392 bp long and the deduced amino acid sequence has 53% sequence homology to the GDP-mannose-dehydrogenase (encoded for by *algD*) from *Pseudomonas aeruginosa* (sequence identity 29%) [7].

The deduced amino acid sequences were compared to subunits of F_1F_0-ATPases using the programm BESTFIT [8]. These comparisons revealed that subunit γ of *A. woodii* has 31 and 40% sequence identity to subunit γ from *E. coli* and *P. modestum*, respectively, subunit β 65 and 68% sequence identity to subunit β from *E. coli* and *P. modestum*, respectively, and subunit ϵ 33 and 32% sequence identity to subunit ϵ from *E. coli* and *P. modestum*. These data clearly assign the cloned genes as coding sequence for subunits of a F_1F_0-ATPase. A multiple alignment of the respective sequences did not reveal any significant differences between Na^+ and H^+-translocating enzymes. However, alignment of the γ subunits (Fig. 3) revealed the presence in *A. woodii* of a region otherwise present only in photosynthetic organisms and which is known to be responsible for "thiol modulation" in chloroplast F_1F_0-ATPase (CF_1F_0) [9,10]. Cys-199 and Cys-205 in CF_1F_0 are known to form a disulfide bridge in the dark which gives rise to an inactive enzyme; activation can be brought about by a ferredoxin-dependent reduction of the disulfide bond in the light. Artificially, activation can be achieved by trypsin cleavage at Lys-204 (site I) and Arg-215 (site II). In cyanobacteria, the region including the two cysteines and site I is not maintained but, site II is. The enzyme therefore corresponds to the reduced form of CF_1F_0, but maintenance of site II is sufficient to retain trypsin activation [11,12]. Interestingly, this regulatory region in its full extent is also present in *A. woodii*; the cysteine residues are not conserved, but the potential trypsin cleavage sites I and II are conserved. However, ATPase activity as catalyzed by the enzyme from *A. woodii* is not latent, but in light of this comparison it would be worthwhile studying the effect of trypsin on the ATPase from *A. woodii*.

Table 1
Comparison of N-terminal amino acid sequences of subunits of the Na^+-ATPase from *A. woodii* with those deduced from cloned genes

Subunits [a], gene products	N-terminal sequences [b]								
35 kDa subunit		(A)	E	N	V	Q	D	(I)	(K)
Subunit γ, deduced	M	A	E	N	V	Q	D	I	K
52 kDa subunit		X	Q	N	I	X	K	V	V
Subunit β, deduced	M	A	Q	N	I	G	K	V	V
16 kDa subunit		X	(E)	(T)	–	R	L	K	I
Subunit ϵ, deduced	M	A	E	T	F	R	L	K	I

[a] The experimentally derived N-termini of the subunits are from Ref. [2].
[b] X, no unequivocal signal was obtained; ( ), other amino acids were detected in minor amounts, –, no amino acid detected.

The identities of the genes are confirmed by a comparison of the deduced with the experimentally derived N-termini. The deduced N-termini of subunits γ, β and ϵ are identical to the experimentally derived N-termini of the 35, 52 and 16 kDa subunit of the Na^+-ATPase of *A. woodii*, respectively (Table 1) and the deduced molecular masses of subunit γ (33.8 kDa), β (51 kDa) and ϵ (17.5) correspond well to the experimentally derived values for the subunits of the purified protein. Interestingly, the *N*-formylmethionine has been cleaved off the polypeptides in all cases.

From the data reported in this communication it is now evident that the Na^+-ATPase of *A. woodii* is of the F_1F_0-type. The 52 kDa subunit corresponds to subunit β, the 35 kDa subunit to subunit γ and the 16 kDa subunit to subunit ϵ. So far, the organization of the genes (Fig. 1) is identical to the *unc* operon of *E. coli* but the unravelling of the complete subunit structure of this Na^+-ATPase has to await the cloning and sequencing of the entire operon which is now under way in our laboratory.

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