

Short Sequence-Paper

The Na⁺-translocating ATPase of *Acetobacterium woodii* is a F₁F₀-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 F₁F₀-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 F₁F₀-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 F₁F₀-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 F₁F₀-ATPase [2]. However, despite these similarities the ATPase from *A. woodii* differs from other F₁F₀-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 F₁-F₀ interaction is different and it is inhibited by nitrate [2]. Therefore, in order to clearly assign the Na⁺-ATPase from *A. woodii* as a F₁F₀-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 F₁F₀-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

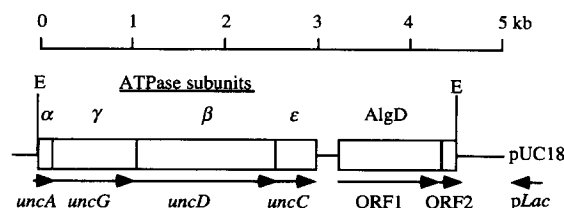


Fig. 1. Physical map of plasmid pAF1 and organization of genes in the *unc* operon of *A. woodii*. The *Eco*RI fragment was cloned into the *Eco*RI site of pUC18. Genes are indicated by boxes and the direction of transcription is indicated by the arrows. E, *Eco*RI.

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

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1	GAATTCGAAAAAGGACTAATTAATTTGCACAAAAGAAATACCCGTGAATCATGACTAAG	60	1141	TAAAAATGAGTGCAAAATTAATTTGTACAGGAGGTTAGTGGGAATGGCCCCAAATATAG	1200
	E F E K G L I K F A Q K K Y P E I M T K			β subunit $\rightarrow$ M A Q N I G	
61	GTTAAGGGTAAGGATGGCCTTCAGATGAGTCTGTCGACGCTTTTGCATGAATGATTTAG	120	1201	GAAGGTTGTTTCAGGTTATTGGACCGAGTAGTCGATGTTAAATTTCAAAAAGACAACTGCC	1260
	V K G K D G L S D E V V A A F A E C I E			K V V Q V I G P V D V K F D S Q K D K L P	
121	GCTTATAAAAAAGTTTTCAGTAAATCTGTATAGATTGTCACGCGAGGTGATTTTCAGGTG	180	1261	AAAATGAAACAACGAGTAAATATTGAGCTTTAATGGACACACCTGGTAATTGAGGTAGC	1320
	A Y K K V F S K S V			K L N N A V N I E L N G H T L V I E V A	
181	GCAGAGAAATGTACAAGATATAAAACCTCGGATAAAGAGTGTAAATAGTACAAATGCAATC	240	1321	TCAGCAACTGGGAGCAGCATTTGTCAGATGATTGCCATGGATTCAACAGACGGTTTGAT	1380
	A E N V Q D I K P R I K S V N S T M Q I			Q Q L G D D I V R C I A M D S T G D L M	
241	ACCCATGCCATGGAAATTCGGTGCATCGGCAAGCTGCGAAAAGTCGTGAGCTTCGGTAA	300	1381	GAGAAATCAGGAAGCTGTGGATACAGGGTCAGCGATTCAAGTACCCGTTGGGAAAGCAAC	1440
	T H A M E L V A S A K L R K S R E L A E			R N Q G E A V D T G S A I Q V P V G K A T	
301	GGTCGTGACCTTATTTTGGAGCTATGATAGAAAGTATTTGGCGGAGTTGTCGAGAAAAGT	360	1441	CCTAGGAAGAATGTTAACGTTTGGGTGAACCAATGATGGCAAGCAATTTGATACGAA	1500
	G R R P Y F E A M I E S I G R I V E K S			L G R M F N V L G E P I A M R K P F D T K	
361	GGAAACGCCCGCAATATTTTCATGGATCAGCGAGAAGTTAAAAAACTGCTTATATTATC	420	1501	AGATGTCGTTATGCATCGGATTCATCGCATCCACCCAGTTTGAAGAACAACAACGCA	1560
	G N A R N I F M D Q I V E V K K T A Y I I			D V V M H P I H R H P P S F E E Q Q T Q	
421	ATTACCGGAGATAAAGGCTTGGCTGGTGGATACAATGTAAATGTGCAAAATTTGGTTGAA	480	1561	ACCAGAAATTTTGAAACCGGAATTAAGTCGTTGACTTAATTTGTCTCTACGTTCTGG	1620
	I T G D K G L A G G Y N V N V A K L V E			P E M F T E T G I A V G P V D L I C P T V R G	
481	GAACATATAACCGATAAAGAGAATGCTGTTTATTACAGTTGGAATCAGGGGCGCGAT	540	1621	TGGTAAGATTGGCTTTTCGGTGGTCCCGGAGTTGGTAAACCGTATTGATTCAGGAATT	1680
	H I T D K E N A V L F T V G S R G R D			G K I G L F G G A G V G K T V L I Q E L	
541	CATTTTAGAAATCGTGAATACCATATTCAGGCTGAGTATCTCGAAATTTTCGGAACGCCA	600	1681	AATTAATAATTTGCAACCAACCCAGCGTGTTTATCGGTAATTTGCGCGTGTGGAGAAC	1740
	H F R N R E Y H I Q G E Y L G I S E R P			I N N I A T Q H G G L S V F A G V G E R	
601	AACTTTTTTAATGCCAAAGAAATTAACGCTATTGTTATGGAAGGTTTAAAAATCGGGAG	660	1741	GACCCGTGAAGGGAATGACCTTTTATATGAAATGATGAGAGTCTGCGCTTATTAAACAAAC	1800
	N F F N A K I F M D Q I V M E G F K N G E			T R E G N D L Y Y E M M E S G V I N K T	
661	TATGACGAAGTTTATATTGCTATACCAAGTTTGGTTTCGACCATTCACGACGATGCTCAA	720	1801	AGCGCTTTGCTTTGGTCAAAATGAATGAGCTCCTGAGAGCAAGATTCGCGATTGCATTAGC	1860
	Y D E V Y I A Y T K F V S T I T Q H A Q			A L C F G M N E P P G A R M R I A L A	
721	ATGATGAATTTGTTGCCCTTTTCGCGGAAGAGTTGATACATTCAGGTAAAGTGAACA	780	1861	TGGACTGACGATGGCTGAATATTTCGGGATGATGAAGGACAGGATGTTTATTGTTTAT	1920
	M M K L L P L S R E L I T S G K V K T			G L T M A E Y F R D D E G Q D V L L F I	
781	ACTGAAGAAACGAAAGAAAGAAATCAAAATGTCGGATCGAGAACTAAACATCATGACA	840	1921	TGATAACATTTTCGCTTTTACTCAAGCTGGTTCAGAAAGTTTCAGCACTTTTGGGTCCGAT	1980
	T E E T K E E K S K M S D R E L T I M T			D N I F R F T Q A G S E V S A L L G R M	
841	TATGAACCGGAACCGGAAGAACTTTTAAAGTATCTGATTCGGAATCTTGTCTAGTAGTACC	900	1981	GCCAACTGCCCTTGGTTACCAACCAACACTGGCAACTGAAATGGTGCTTTACAGGAACG	2040
	Y E P E P E E L L Q I V M E G F K N G E			P S A V G Y Q P T L A T E M G A L Q E R	
901	GTATATGGTTCAATGATTTGAAAGCGCGCCAGTGAACAAGGGCGCGCGGAACAGCGATG	960	2041	AATTACATCAACCAAGTAAAGGTTTCAATTAACCTGGTTACGCGCTTTACGATCCAGCGA	2100
	V Y G S M I E S A A S E Q G A R R T A M			I T S T S K G S I T S V Q A V Y D P A D	
961	GAATCGGCAACCCCAATGCCAATGAAATGATTGATGGGTAAACATCTCAATATAACCGT	1020	2101	TGACTTAACTGACCTGCTCCGCAACTACTTTTGGCATTTGGATGCAACGACAGATT	2160
	E S A T T N A N E M I D G L T L Q Y N R			D L T D P A P A T T F A H L D A T T V L	
1021	GTACGACAGCGCCCAATAACCCAGAAATATCGGAAATTTGTCGGTGTGCGGAAGATTAA	1080	2161	AAGTCGTGCCATTACGAAAAAGGATTTATTCGCGCTGTGATGCAATTTGATTTCACTTC	2220
	V R Q A P I T Q E I S E I V G G A E D			S R A I T E K G I Y P A V D P L D S T S	
1081	ACTATAAAATAAAACGATTTTGCAAAAAAGACTGAAGATTATTAGATAGAAGGTGAC	1140	2221	ACGTATTCTTGATCTTAAATAGTTTGGACAGGAACATTATGAAACAGCTCGAGAAAGTGA	2280
				R I L D P K I V G Q E H Y E T A R E V Q	

Awo	1	MA--ENVQDIKPRIKSVNSTMQITHAMELVASAKLRKSRELAEGRRPYFEAMIES	53
Pmo	1	---MAAGKEIKSRISVQSTRQITKAMEIVSSTKFKKFQALVNQSKPYSGSMDKV	52
Eco	1	---MAGAKDIRSKIASVQNTQKITKAMEMVAASKMRKSQDRMAASRPYAEATMRKV	52
Val	1	---MAGAKEIRNKIGSVKSTQKITKAMEMVAASKMRKSQDAMEASRPYAEATMRKV	52
Ps3	1	MKPLASLRDIKTRINATKKTSQITKAMEMVLTSKLNRAEKRE-IVRPYMEKIQEV	54
Bme	1	---MASLRDIQTRITSTKTSQITKAMEMVSAAKLNRAEQNAKSFVPYMEKIQEV	52
Sol	1	---ANLRELDRIGSVKNTQKITKAMKLVAACKVRAQEAUVNRPFSSETLVEV	51
Sec	1	---MPNLKSIRDRIQSVKNTKKITEAMRLVAAARVRAQEQVIATRPFDRLAQV	52
		 *	
Awo	54	IGRIVEKSGNARNIF--MDQREVKKTAYIIITGDKLAGGYNVNNAKLVEEHITD	106
Pmo	53	LANLAAGIKNERHPL-FDGKTEVKRIGIIVMTSDRGLCGGFNSSTLKEMEKLIVA	105
Eco	53	IGHLAHGNLEYKHPY-LEDR-DVKRVGYLVVSTDRGLCGGLNINLFKLLAEMKT	105
Val	53	IGHVANANLEYRHPY-LEER-EAKRVGYIIVSTDRGLCGGLNINVTDKGVQCCL	105
Ps3	55	VANVALAAR-ASHPM-LVSRP-VKKTGYLVITSDRGLAGAYNSNVFKKAVTDMQT	106
Bme	53	VSSVALGSRGASHPM-LTARS-VKKTGYIVITSDRGLAGAYNSNLRKVSQAIEE	105
Sol	52	LYNMNEQLQTEDVDVPLTKIRTVKKVALMVVTGDRGLCGGFNNMLLKAEARIAE	106
Sec	53	LYGLQTRLRFEDVDLPLKKREVSVGLLVISGDRGLCGGYNTNVIIRRAENRAKE	107
		 *	
Awo	107	KENA---VLFTVGSRRGRDHF-RNREYHIQGEYLGISERPNNFNAKEVTAIVMEG	156
Pmo	106	NPKD--EVSVAIGKKGRDYC-KKKDRDLKAEYIQLIPETMFDKAKEISENIVEY	157
Eco	106	WTDKGVQCCLAMIGSKGVSF-NSVGGNVVAQVTGMGDNPSLSELIGPVKVMQLQA	159
Val	106	WREKGAEIELAVVGSKATAFF-KHGGAKVAAQVSGLDNPSLEDLIGSVGVMKK	159
Ps3	107	RHASPDEYAIIVIGRVGLSFF-RKRNMPVILDIRLPDQPSFADIKEIARKTVGL	160
Bme	106	RHQSPDEYGVIAIGRVGRDFF-VKRGIPVLEITGLADQPAFADIQGIASQTVQM	159
Sol	107	LKKLGVDYTIISIGKKGNTYFIRPEIPVDRYFDG-TNLPTAKEAQAIADDFVSL	160
Sec	108	LKAEGLDYTFVIVGRKAEQYF-RRREQPIDASYTGLEQIPTADEANKIADDELLSL	161
		 *	
Awo	157	FKNGEYDEVYIAYTKFVSTITQHAQMMKLLPLSREELITSGVKVTEETKEEKSK	211
Pmo	158	FYEDIFDEVYLIYNEFISALSTELIVKLLPIERIEV-----	195
Eco	160	YDEGRDLKLYIVSNKFINTMSQVPTISQLLPLPASDD-----	196
Val	160	YDEGELDRLYVFNKFVNTMVQPTIDQLLPLPKSDS-----	196
Ps3	161	FADGTFDELYMYNHYVSAIQQEVTERKLLPLTDLAE-----	197
Bme	160	FADGTFDELYLYNHFINTISQEVTEKKLLPLTDLQF-----	196
Sol	161	FVSEEDKVEMLYTKFVSLVKSQDPVIHTLLPLSPKGEICDINGKCVDAAEDELFR	215
Sec	162	FLSEKVDRIELVYTRFVSLVSSRPVIQTLPLDQF-----LEAADDEIFR	207
		 *	
Awo	212	MSDRE--LTI-----MTYEPEPEELLKYLIPNLVSSTVYGSIMIES	249
Pmo	196	-----QDN-TT---Y---IFEPSVEDIESSLPKYLNILYQAILN	235
Eco	197	-----DDL-KHKSWDY---LYEPDPKALLDTLLRRYVESQVYQGVVEN	235
Val	197	-----EEMQREHSWDY---IYEPEPKLLDTLLVRYVESQVYQGVVEN	236
Ps3	198	-----NKQRTV---Y---EFEPSQEEILDVLLPQYAESLIYGALLDA	233
Bme	197	-----SGKLVG---Y---EFEPSQEEILEVLLPQYAESLIYGALLDG	232
Sol	216	LTTKEGKLTVERDMIKTETPAFSPILEFEQDPAQILDALLPLYLNSQILRALQES	270
Sec	208	LTTRGGQFQVERQTVTSQARPLPRDSIFEQDPVQILDLSLLPLYLNSQILRALQES	262
		 *	
Awo	250	AASEQGARRTAMESATTNANEMIDGLTLQYNVRVQAPITQEISEIVGGAE--D	300
Pmo	231	TASEHSARKNAMKNATDNAEDMIKDLTLQYNRRQAATQEISEIVSGASAL-	282
Eco	235	LASEQAARMVAMKAATDNGGSLIKELQLVYNKARQAATQEITEIVSGAAV-	287
Val	236	LACEQAARMIAMKAATDNATNLIDDELVYNKARQAATQEISEIVGGAAV-	288
Ps3	233	KASEHAARMTAMKNATDNANELIRTLTLNRRARQAATQEITEIVAGANALQ	286
Bme	232	KASEHAARMTAMKSATDNAKDLINNLTLNRRARQAATQEITEIVGGAAALE	285
Sol	270	LASELAARMTAMSNATDNANELKKTLSINYNRRARQAATQEITEIVAGANACV	323
Sec	262	AASELAARMTAMSNASENAGELIKSLSLSYNRRARQAATQEILEVVGAAEALT	315
		* * * * *	

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], thermophilic 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 CF₁F₀ 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, promoter 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 *EcoRI* and size-fractionated by sucrose density centrifugation. Fragments ranging from 3 to 6 kbp were cloned into the *EcoRI* site of pUC18 and transformed into *E. coli* DH5 α , and transformants were subjected to colony hybridization. One clone contained a plasmid with a 4.5 kbp *EcoRI* insert which in Southern blots hybridized with the probe. This plasmid was named pAF1 (Fig. 1).

The nucleotide sequence (4514 bp) of the *EcoRI* 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 program 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

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
52 kDa subunit	X	Q	N	I	X	K	V	V
Subunit β , deduced	M	A	Q	N	I	G	K	V
16 kDa subunit	X	(E)	(T)	–	R	L	K	I
Subunit ϵ , deduced	M	A	E	T	F	R	L	K

^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.

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*.

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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