Sequence of subunit *a* of the Na^+-translocating F_1F_0-ATPase of *Acetobacterium woodii*: proposal for residues involved in Na^+ binding

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Received 2 April 1999

Abstract **Na^+ transport through the F_0 domain of Na^+-F_1F_0-ATPases involves the combined action of subunits *c* and *a* but the residues involved in Na^+ liganding in subunit *a* are unknown. As a first step towards the identification of these residues, we have cloned and sequenced the gene encoding subunit *a* of the Na^+-F_1F_0-ATPase of *Acetobacterium woodii*. This is the second sequence available now for this subunit from Na^+-F_1F_0-ATPases. A comparison of subunit *a* from Na^+-F_1F_0-ATPases with those from H^+-translocating enzymes unraveled structural similarity in a C-terminal segment including the ultimate and penultimate transmembrane helix. Seven residues are conserved in this region and, therefore, likely to be involved in Na^+ liganding.**

Key words: ATPase; Na^+-F_1F_0; subunit *a*; DNA sequence; *Acetobacterium woodii*

1. Introduction

F_1F_0-ATPases are bipartite, membrane-bound enzymes which couple the electrochemical ion potential across the membrane to the synthesis of ATP. Ion flow through the membrane domain is converted to a rotational movement of subunits in the hydrophilic domain which leads to ATP synthesis [1,2]. However, the mechanism of ion transport as well as the coupling of ATP synthesis with ion flow is far from being settled. An alternative approach to elucidate the path of the ion through the membrane domain is to perform comparative studies with enzymes exhibiting altered ion specificity. The anaerobic bacteria *Propionigenium modestum* and *Acetobacterium woodii* are the only well established examples of organisms having ATPases which, as revealed by inhibitor studies, subunit composition, primary sequences and electron microscopy, are typical F_1F_0 enzymes but use Na^+ instead of H^+ as coupling ion [3–5]. The F_0 complex was shown to determine the ion specificity of the enzyme [6] and the 'active carboxylate' of subunit *c* was shown biochemically to contribute to Na^+ liganding [7,8]. By sequence comparisons as well as mutant studies a potential Na^+ binding site was identified on subunit *c* [9–11]. Apart from subunit *c*, a second subunit, subunit *a*, is required for ion transport; both are envisaged to make the ion channel of the ATPases [12]. Three residues of subunit *a* from H^+-F_1F_0-ATPases, R210, E219, and H245 (*E. coli* numbering), were indicated by mutant studies to be directly involved in H^+ transport [12] but recent studies demonstrated that E219 and H245 are not essential for H^+ transport [13,14]. However, the residues involved in Na^+ binding of subunit *a* from Na^+-F_1F_0-ATPases are unknown. As a further step towards a better understanding of Na^+ transport through the Na^+-F_1F_0-ATPase from *A. woodii* we cloned and sequenced the gene coding for subunit *a*. This sequence is now the second sequence available for subunit *a* from Na^+-F_1F_0-ATPases. A comparison of these two sequences to those from H^+-F_1F_0-ATPases revealed determinants likely to be important for Na^+ binding.

2. Materials and methods

2.1. Organism and plasmids

A. woodii (DSM 1030) was obtained from the 'Deutsche Sammlung für Mikroorganismen und Zellkulturen', Braunschweig, Germany, and grown under strictly anaerobic conditions on carbonate-buffered media supplied with 0.4% (w/v) glycine [15]. *E. coli* DH5α (*supE*44 Δ*lac*U169(Φ80*lacZ*ΔM15) *hsdr*17 *recA*1 *endA*1 *gyrA*96 *thi*1 *relA*1; [16]) was grown on LB at 37°C. Plasmids used were pSE420 [17], pHSG 398 and 399 [18], and pBluescriptII SK and KS (Stratagene, San Diego, USA).

2.2. Molecular procedures

Chromosomal DNA of *A. woodii* was isolated by a modified Marmur preparation as described [11,19]. The DNA was restricted, size fractionated by gradient centrifugation, cloned and sequenced as described elsewhere [20]. All procedures used were standard techniques [16]. DNA sequence was analyzed on a VAX computer using the Wisconsin genetics computer group sequence analysis software package, version 8.1 (University of Wisconsin Biotechnology Center, USA).

3. Results and discussion

atpB, the gene encoding subunit *a*, is part of the *atp* operon encoding the Na^+-F_1F_0-ATPase of *A. woodii*. Cloning and molecular characterization of the *atp* operon from *A. woodii* will be described elsewhere [20]. *atpB* starts with an ATG codon and is preceded by a well conserved Shine–Dalgarno sequence AATGAGGT nine base pairs upstream of the ATG codon. *atpB* codes for a strongly hydrophobic protein with 220 residues and a molecular mass of 24.5 kDa (Fig. 1). It is smaller than the corresponding subunits from *E. coli* and *P. modestum*, due to a shortage at the N-terminus and the loops connecting the transmembrane helices. A hydropathy profile predicts at least five transmembrane helices, similar to subunit *a* from other sources. Biochemical and immunological studies performed with subunit *a* from *E. coli* do not give an unambiguous topological model for subunit *a*; the models differ mostly with respect to the number of transmembrane helices at the N-terminus (first 100 residues), which are assumed to traverse the membrane once or twice. Starting from around position 100 (*E. coli* numbering) five transmembrane helices

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PII: S0014-5793(99)00576-1

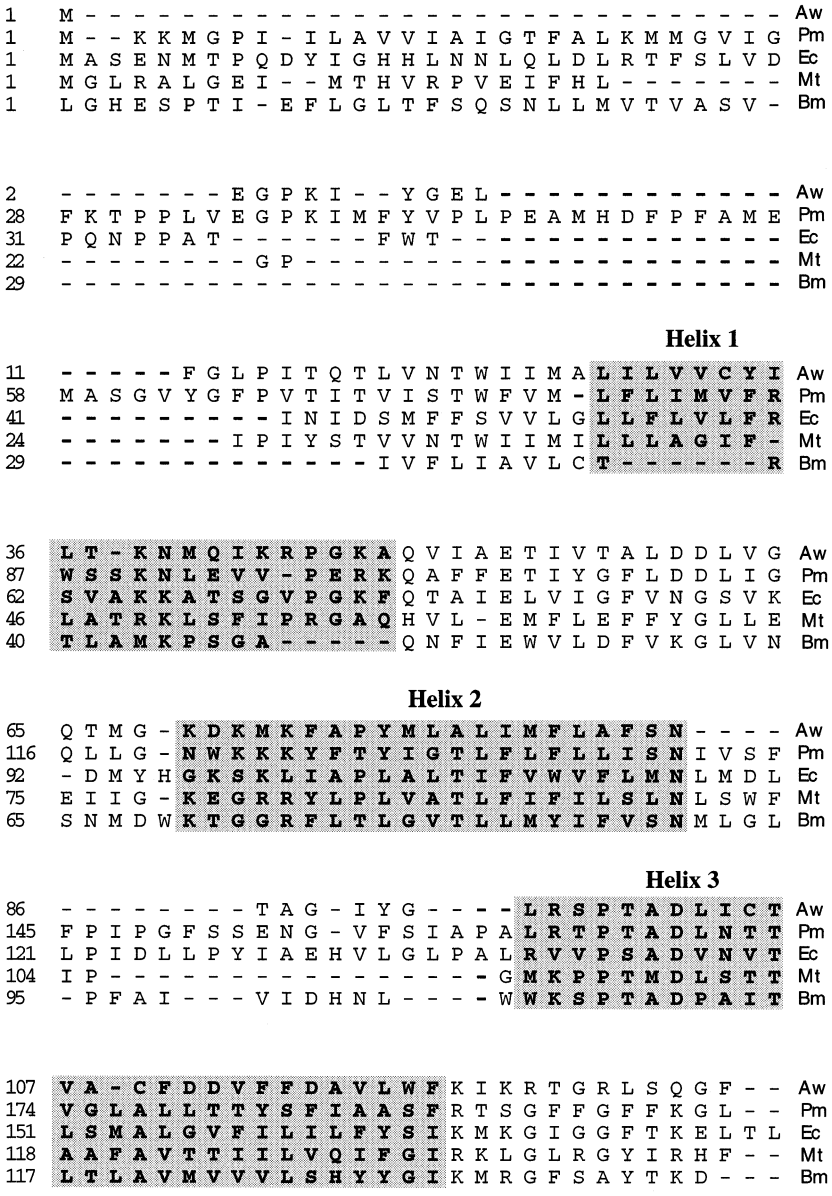

Fig. 1. Multiple alignment of subunit *a* from *A. woodii* (Aw), *P. modestum* (Pm) [27], *E. coli* (Ec) [28], *Moorella thermoacetica* (Mt) [29], and *Bacillus megaterium* (Bm) [30]. Residues located in transmembrane helices according to the model of Vik, Fillingame and Altendorf [21–23] are shadowed in gray. The sequence of *A. woodii* subunit *a* has been submitted to GenBank and is available under accession number U10505.

are predicted by any model, and the models are in general agreement with the assignment of residues to these helices. Therefore, subunit *a* is discussed to have five or six transmembrane helices [21–23]. A multiple alignment of subunit *a* indicating the five 'certain' transmembrane helices is presented in Fig. 1.

Whereas subunits *c* of the Na^+-F_1F_0-ATPases of *P. modestum* and *A. woodii* are more closely related to each other

Table 1
Comparison of deduced amino acid sequences of subunit *a* and predicted transmembrane helices thereof of *A. woodii* with the corresponding sequences from *E. coli*, *P. modestum*, *M. thermoacetica* and *B. megaterium*

aa sequence	% amino acid identity			
	E. coli	*P. modestum*	*M. thermoacetica*	*B. megaterium*
total *a*	41.2	45.3	37.4	32.7
Helix 1[a]	21.1	29.7	39.4	10.8
Helix 2	19.0	38.1	23.8	28.6
Helix 3	26.9	38.5	23.1	26.9
Helix 4	51.8	51.8	44.4	37.0
Helix 5	39.3	71.0	30.4	51.6

[a]For sequences of putative transmembrane helices, see Fig. 1.

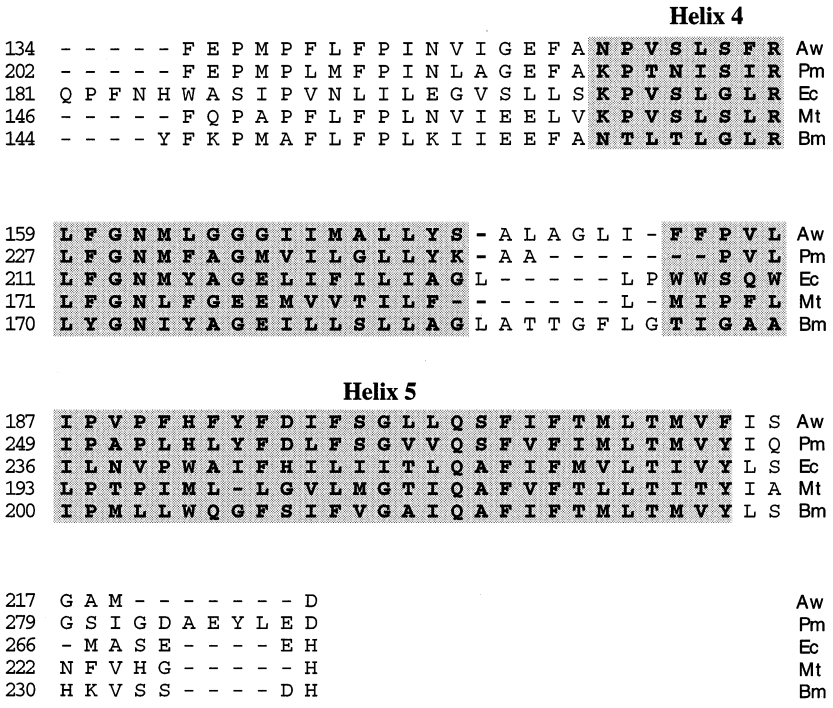

Fig. 1 (*continued*).

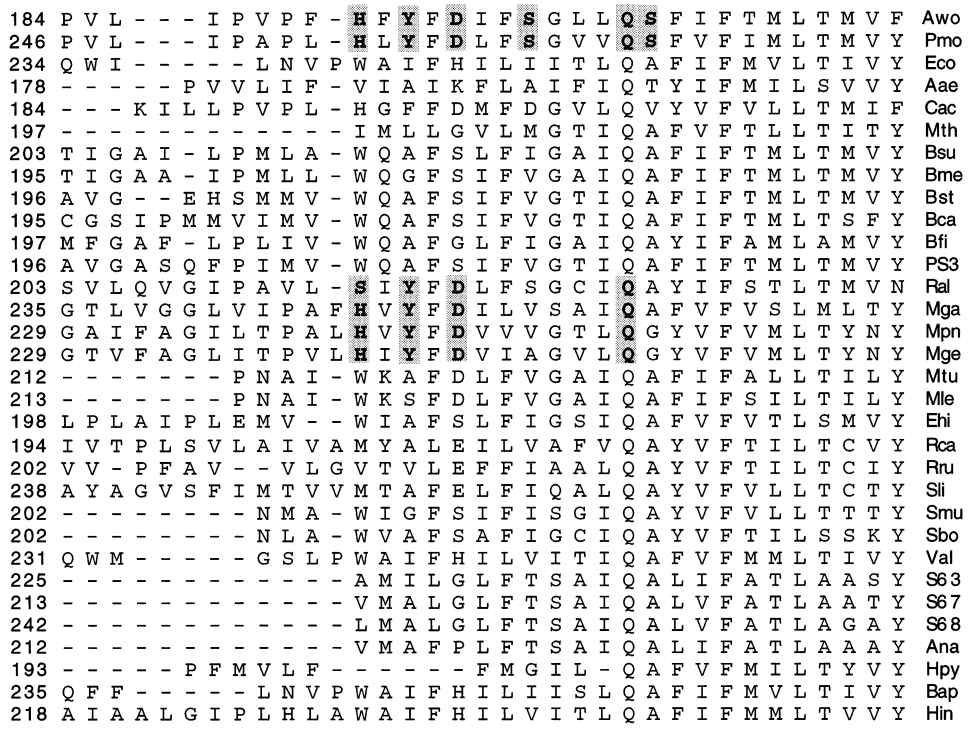

Fig. 2. Multiple alignment of residues located in the putative transmembrane helix 5 (see Fig. 1). Possible Na^+ liganding groups are shadowed in gray. Awo, *A. woodii*; Pmo, *P. modestum* [27]; Eco, *E. coli* [31]; Aae, *Aquifex aeolicus* [32]; Cac, *Clostridium acetobutylicum* (EMBL GenBank accession number 1905949); Mth, *M. thermoacetica* [29]; Bsu, *Bacillus subtilis* [33]; Bme, *B. megaterium* [30]; Bst, *Bacillus stearothermophilus* (EMBL GenBank accession number 1168556); Bca, *Bacillus caldotenax* (EMBL GenBank accession number 728914); Bfi, *Bacillus firmus* [34]; PS3, thermophilic bacterium PS3 [35]; Ral, *R. albus* (EMBL GenBank accession number 2662060); Mga, *M. gallisepticum* [36]; Mpn, *M. pneumoniae* [37]; Mge, *M. genitalium* [38]; Mtu, *M. tuberculosis* (EMBL GenBank accession number 1793479); Mle, *Mycobacterium leprae* (EMBL GenBank accession number 1168559); Ehi, *Enterococcus hirae* [39]; Rca, *Rhodobacter capsulatus* (EMBL GenBank accession number 1934975); Rru, *Rhodospirillum rubrum* [40]; Sli, *Streptomyces lividans* [41]; Smu, *Streptococcus mutans* [42]; Sbo, *Streptococcus bovis* (EMBL GenBank accession number 2662321); Val, *Vibrio alginolyticus* [43]; S63, *Synechococcus* 6301 [44]; S67, *Synechococcus* 6716 [45]; S68, *Synechocystis* PCC 6803 [46]; Ana, *Anabaena* PCC 7120 [47]; Hpy, *Helicobacter pylori* [48]; Bap, *Buchnera aphidicola* [49]; Hin, *Haemophilus influenzae* [50].

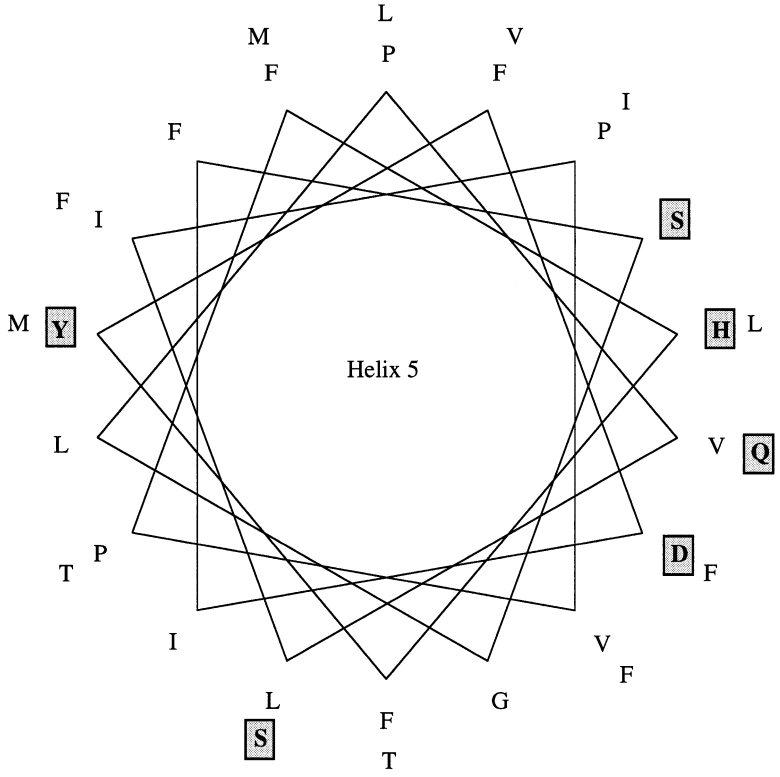

Fig. 3. Helical wheel projection of the putative transmembrane helix 5 of subunit *a* of *A. woodii*.

than to subunit *c* from H^+-F_1F_0-ATPases (60% identity versus 23% to *E. coli*; [11]), this is not the case with subunit *a*: 30–45% of the residues from *A. woodii* are identical with subunit *a* from other enzymes, irrespective of whether they transport Na^+ or H^+. However, the similarity increases dramatically if only single transmembrane helices are compared. It is evident from Table 1 that the degree of similarity increases towards the C-terminus. A striking 71% of the residues of helix 5 are identical in Na^+-F_1F_0-ATPases whereas the identity between H^+- or H^+- and Na^+-ATPases is only 30–50%. This high degree of structural conservation of helix 5 of Na^+-F_1F_0-ATPases is indicative for a conservation of function and could indicate a participation of helix 5 in Na^+ transport. The high degree of similarity of helix 5 of subunit *a* from Na^+-F_1F_0- compared to H^+-F_1F_0-ATPases might be due to the fact that the liganding of Na^+ requires more amino acids in a critical spatial organization than the binding of H^+.

A couple of residues potentially able to ligand Na^+ are present in helix 5 of subunit *a* of *A. woodii* (P184, P188, P190, F191, H192, F193, Y194, F195, D196, F198, S199, Q203, S204, F205, F207, T208, M209, T211, M212, F214, see Fig. 2). With the exception of F191, F193, T208 and F214 these residues are conserved in *P. modestum*. However, these residues are not exclusively found in Na^+-ATPases which makes the detection of the Na^+ binding site by sequence inspection very difficult and erroneous. H245 of *E. coli*, which was previously shown to be involved in H^+ transport but later questioned [14], is not conserved in the Na^+-ATPases. There is a strong bias towards histidine at position 192, tyrosine at position 194, aspartate at position 196, and serines at position 199 and 204 (*A. woodii* numbering) in Na^+-F_1F_0-ATPases which could indicate their involvement in Na^+ liganding. H192, D196, S199, and the strictly conserved Q203 are located at the same side of the α-helix whereas Y194 and S204 are located at the opposite side (Fig. 3). Is there another criterium to make the search for the Na^+ binding site more specific? Recently, an Na^+ binding site was described in subunit *c* of Na^+-F_1F_0-ATPases (P25, Q29, E62, S63 [9–11]) although P25 is not yet verified by experimental data. We inspected sequences available in databases for this motif and found it in subunit *c* of *Ruminoccus albus*, *Mycoplasma gallisepticum*, *Mycoplasma pneumoniae*, and *Mycoplasma genitalium*. It is not unlikely that these organisms have Na^+-F_1F_0-ATPases, for *M. gallisepticum* was shown to have an Na^+-ATPase [24], and *R. albus* is a typical member of the rumen bacteria which very often are sodium ion dependent [25]. Interestingly, H192, Y194, and D196, but not S199 and S204 are conserved in subunit *a* of these organisms. If it proves correct that they have Na^+-F_1F_0-ATPases this would make H192, Y194, and D196 prime candidates for Na^+ liganding groups. In addition, Q203 is a potential Na^+ liganding group, especially since it groups with H192, D196, and S199 on the same side of the helix.

Helix 4 (Fig. 4) of subunit *a* harbours two residues, one of which (R210) was shown to be absolutely essential for H^+ transport in *E. coli* [12]. This residue is strictly conserved among species. E219 was believed for a long time to be directly involved in H^+ transport but this was excluded recently [13]. E219 of *E. coli* is not conserved in a couple of organisms, including *A. woodii* and *P. modestum*. Inspection of the sequence reveals two positions with a strong bias for tyrosine (Y174) and serine (S175) in Na^+-F_1F_0-ATPases. These residues are also conserved in *R. albus*, *M. gallisepticum*, *M. pneumoniae*, and *M. genitalium*. A mutation of three residues of subunit *a* of the Na^+-F_1F_0-ATPase of *P. modestum* (K219, V263, I277 in Fig. 1) led to altered Na^+ binding. With the

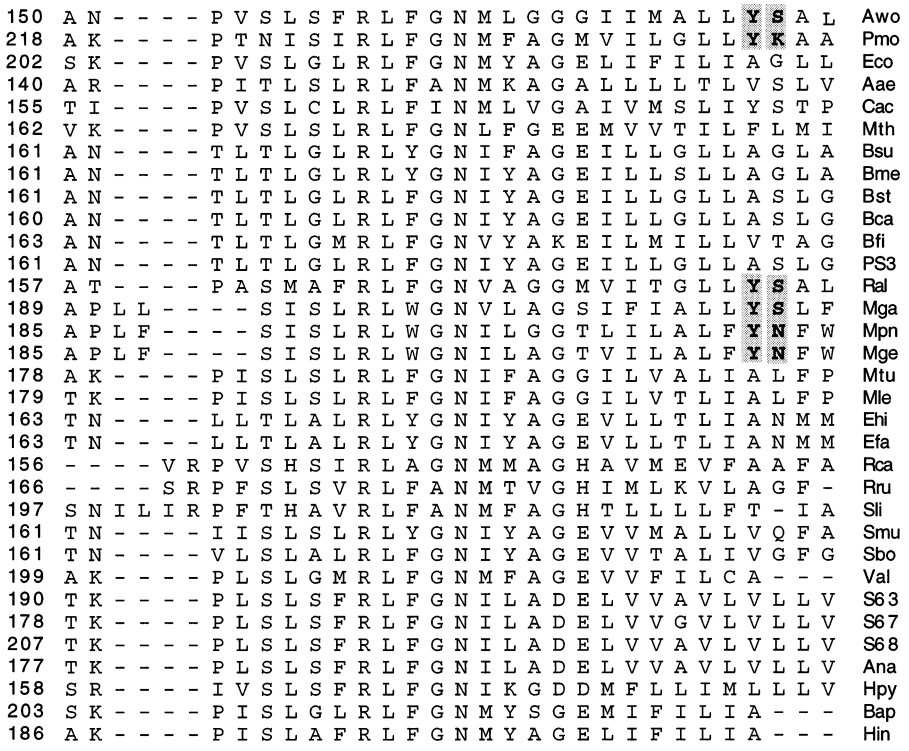

Fig. 4. Multiple alignment of residues located in the putative transmembrane helix 4 (see Fig. 1). Possible Na^+ liganding groups are shadowed in gray. For abbreviations and references, see Fig. 2.

exception of I278 these are not conserved in *A. woodii*. However, even in *P. modestum* they were not envisaged to be involved in Na^+ liganding but their mutation was seen to result in a more global structural change of subunit *a* leading to a perturbation of the Na^+ binding pocket [26].

In conclusion, with the cloning and sequencing of the gene encoding subunit *a* from the Na^+-F_1F_0-ATPase of *A. woodii* the primary amino acid sequence and secondary structure of subunit *a* can be predicted. Apart from *P. modestum*, *A. woodii* is the second organism in which the sequence for subunit *a* of the Na^+-F_1F_0-ATPase has been determined. Therefore, the delineation of an Na^+ binding motif in subunit *a* from Na^+-F_1F_0-ATPases by sequence comparisons can now be made more stringent. Likely candidates for Na^+ liganding reside in the ultimate and penultimate transmembrane helices. These are R158, Y174, S175, H192, Y194, and D196, but in addition S199 and S204 can not be excluded with certainty.

Acknowledgements: This work was supported by a grant from the Deutsche Forschungsgemeinschaft (Graduiertenkolleg 'Chemische Aktivitäten von Mikroorganismen'). We are indebted to Prof. G. Gottschalk, Göttingen, Germany, for generous support.

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