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Lolkema, JS; Boekema, EJ

Published in:
 FEBS Letters

DOI:
[10.1016/S0014-5793\(03\)00398-3](https://doi.org/10.1016/S0014-5793(03)00398-3)

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Document Version
 Publisher's PDF, also known as Version of record

Publication date:
 2003

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Citation for published version (APA):

Lolkema, JS., & Boekema, EJ. (2003). The A-type ATP synthase subunit K of *Methanopyrus kandleri* is deduced from its sequence to form a monomeric rotor comprising 13 hairpin domains. *FEBS Letters*, 543(1-3), 47-50. [https://doi.org/10.1016/S0014-5793\(03\)00398-3](https://doi.org/10.1016/S0014-5793(03)00398-3)

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The A-type ATP synthase subunit K of *Methanopyrus kandleri* is deduced from its sequence to form a monomeric rotor comprising 13 hairpin domains

Juke S. Lolkema^{a,*}, Egbert J. Boekema^b

^aMolecular Microbiology, Groningen Biomolecular Sciences and Biotechnology Institute, University of Groningen, Kercklaan 30, 9751 NN Haren, The Netherlands

^bBiophysical Chemistry, Groningen Biomolecular Sciences and Biotechnology Institute, University of Groningen, Groningen, The Netherlands

Received 4 March 2003; revised 8 April 2003; accepted 8 April 2003

First published online 28 April 2003

Edited by Stuart Ferguson

Abstract The *ntpK* gene of the archaeon *Methanopyrus kandleri* encodes the equivalent of the c subunit of ATP synthase. The gene product contains 1021 residues and consists of 13 homologous domains, each one corresponding to a single helical hairpin. The amino acid sequence of the domains is highly conserved, ranging between 50 and 80% sequence identity. Each of the 13 domains contains a conserved Gln and Glu residue in the N- and C-terminal helix, respectively, both of which are believed to be involved in cation binding. The protein is likely to form the monomeric rotor of the ATP synthase that consists of 13 hairpin domains.

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Key words: A-ATP synthase; Rotor; Subunit c; Ring structure; Hyperthermophile; *Methanopyrus kandleri*

1. Introduction

F-type ATP synthases, found in bacteria, mitochondria and thylakoids, and, by analogy, A-type ATPases, found in archaea, and V-type ATPases, found in vacuoles, are membrane-bound molecular motors that interconvert the free energy stored in the ATP molecule and the electrochemical gradient of cations, H⁺ or Na⁺, across the membrane. The ATP hydrolysis/synthesis and the ion translocation activities reside in different parts of the complex, termed F₁ (V₁, A₁) and F₀ (V₀, A₀), respectively. F₁, the headpiece, is soluble and connected to membrane-bound F₀ by a central stalk and by peripheral stalks, the number of which varies between the different types (e.g. [1–3]). F₁ consists of a hexagonal arrangement of alternating α and β subunits ($\alpha_3\beta_3$; F-type nomenclature). The elongated γ subunit, the main constituent of the central stalk, sticks in the central cavity of the $\alpha_3\beta_3$ complex. ATP synthesis/hydrolysis in F₁ is coupled to physical rotation of the γ subunit. The central stalk is connected to a multimer of subunit c that is part of F₀. The subunit c complex, or the rotor, rotates against the static part of F₀ that is fixed to F₁ via the peripheral stalk(s). Cation translocation takes place at the interface of the rotor and the static part of F₀.

While the structure of F₁ has been resolved to atomic resolution [4], much less is known about the structure of membrane-bound F₀. The rotor part of F₀ is a cylindrically shaped multimeric arrangement of c subunits. Subunit c is a small 8 kDa protein in F-ATPases, which forms a helical hairpin orientated perpendicular to the membrane. The C-terminal transmembrane α -helix contains the cation binding site. The c subunits interact to form a ring-shaped structure in the plane of the membrane. A low resolution crystal structure of the F₁c complex of yeast [5] and atomic force microscopy and cryo-electron microscopy of isolated bacterial [6,7] and chloroplast [8] rotors indicate that the rotor consists of an inner and an outer ring formed by the N-terminal and C-terminal transmembrane α -helices of the c subunits, respectively. This organization accounts for the cation binding site in the C-terminal helix to be in contact with the static part of F₀. The number of helical hairpins that form the rotor determines the ATP/cation stoichiometry during catalysis and was long believed to be a multiple of three, conforming to the rotational symmetry in F₁. Experiments proved otherwise. The rotor part of the F-type enzyme complexes of yeast, the bacteria *Ilyobacter tartaricus* and *Propiogenium modestum*, and spinach chloroplasts contained 10, 11, 11 and 14 c subunits, respectively [5–8]. The stoichiometry of subunit c in V-ATPases and A-ATPases has not been directly determined. Here, we provide strong evidence that the rotor of the A-type complex of the archaeon *Methanopyrus kandleri* consists of 13 helical hairpins.

2. Results and discussion

2.1. The *ntp* genes on the genome of *M. kandleri*

A-type ATPases/synthases consist of nine subunits G, I, K, E, C, F, A, B, D that usually are encoded in a single operon in the indicated order. The genome sequence of the archaeon *M. kandleri* revealed that the genes encoding the subunits of the ATP synthase (the *ntp* genes) were clustered in two separate groups [9]. The genes encoding subunits I, K, E, C, F, and A were clustered together in the indicated order, while the genes encoding subunits B and D were encoded elsewhere on the genome in the order B-D. A homologous gene encoding subunit G could not be detected by a BLAST search [10]. The number of residues of subunits I (656), E (200), C (375), F (113), A (593), and D (233) was very similar as observed for other A-ATPases/synthases. The *ntpB* gene encodes 991 resi-

*Corresponding author. Fax: (31)-50-3632154.

E-mail address: j.s.lolkema@biol.rug.nl (J.S. Lolkema).

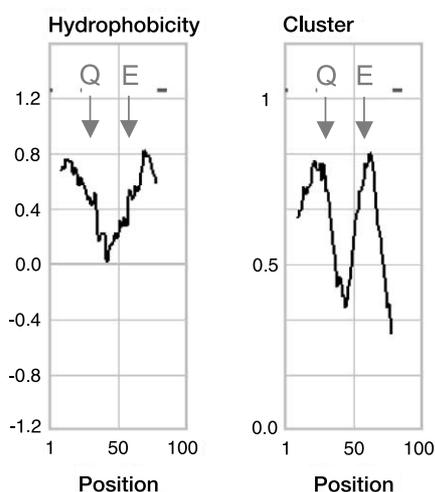


Fig. 3. Averaged hydropathy profile (left) and pairwise sequence identity profile (Cluster; right) of the 13 homologous domains of subunit K of *M. kandleri*. The cluster function measures the fraction of identical pairs at each position averaged over a window of 16 residues. The arrows indicate the positions of the conserved Gln and Glu residues in the first and second transmembrane segments, respectively. Bars in the upper part of the graph indicate positions of gaps in any of the sequences in the alignment.

phenylalanine residue that is conserved in all repeats. This is remarkable since the loop is believed to play an important role in the interaction with the central stalk [15]. Periodicity analysis of the region indicated that even though the sequence is poorly conserved, the loops have a strong and conserved hydrophobic moment when folded in a α -helical structure (Fig. 4). Possibly, the hydrophobic faces of the 13 putative loop helices, containing the conserved phenylalanine residue, form a kind of ‘base’ by which the rotor part connects to the c subunit that is intermediate between rotor and γ -like subunit in A- and V-type ATPases [16]. The hydrophilic face of the loop helices would contact the water phase.

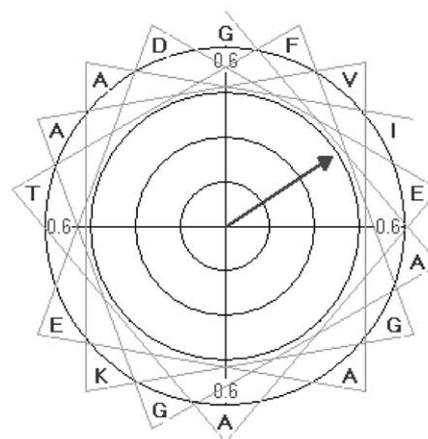


Fig. 4. Helical wheel representation of the loop region between the two transmembrane segments. Printed on the wheel is the sequence of domain NtpK3. The hydrophobic moment indicated by the arrow was calculated using the loop sequences of all 13 domains.

2.4. Comparison to other A- and V-type rotor subunits

Do all the rotors of A-type ATPases/synthases consist of 13 helical hairpins? Highest sequence identity between the domains of the *M. kandleri* subunit K is observed with the corresponding subunits of the related methanogens *Methanothermobacter thermautotrophicus* and *Methanocaldococcus jannaschii*. The c subunits of these organisms do not contain 13 hairpin domains, but two and three, respectively [17]. In fact, subunit K of *M. kandleri* is quite an exception in the archaeal kingdom (see Table 1). It is likely that the number of repeats that make a complete rotor in *M. thermautotrophicus* and *M. jannaschii* consists of a multiple of two and three, respectively, unless an additional gene encoding a single hairpin is encoded on the genome. A precedent for this would be the *atp* operon encoding the F-type ATPase of *Acetobacterium woodii* that contains multiple copies of genes encoding rotor subunits with one and two domains [18]. However, such an additional gene is not present in the operons encoding the

Table 1
Rotor subunits of A-type ATPases/synthases

Archaeon ^a	Gi ^b	Residues	Hairpin domains	Sites/hairpin ^c	Sequence motif
<i>Archaeoglobus fulgidus</i>	11498760	75	1	1/1	PET
<i>Pyrococcus abyssii</i>	14521964	158	2	1/2	PMT/PET
<i>Pyrococcus horikoshii</i>	14591716	162	2	1/2	PMT/PET
<i>Pyrococcus furiosus</i>	18976550	159	2	1/2	PMT/PET
<i>Methanocaldococcus jannaschii</i>	15668394	220	3	2/3	PQT/PET/PET
<i>Methanothermobacter thermautotrophicus</i>	15678977	162	2	1/1	PET/SET
<i>Methanosarcina acetivorans</i>	20092947	82	1	1/1	PET
<i>Methanosarcina mazei</i>	21226886	80	1	1/1	PET
<i>Methanosarcina barkeri</i>	23051714	81	1	1/1	PET
<i>Methanopyrus kandleri</i>	20094449	1021	13	1/1	PET
<i>Halobacterium</i> sp.	15790977	71	1	1/1	PET
<i>Halobacterium salinarum</i>	11362633	89	1	1/1	PET
<i>Thermoplasma volcanium</i>	13540879	75	1	1/1	PET
<i>Thermoplasma acidophilum</i>	16082468	75	1	1/1	PET
<i>Aeropyrum pernix</i>	14601995	131 ^d	1	1/1	GEG
<i>Sulfolobus solfataricus</i>	15897488	102 ^d	1	1/1	GEG
<i>Sulfolobus tokodaii</i>	15921729	101 ^d	1	1/1	GEG
<i>Pyrobaculum aerophilum</i>	18312157	87	1	1/1	AEA

^aThe upper half of the table lists the Euryarchaeota, the lower part the Crenarchaeota.

^bGi number of the sequence in the NCBI protein database at <http://www.ncbi.nlm.nih.gov/>.

^cA cation binding site is defined as a conserved glutamate residue in the C-terminal helix of the helical hairpin.

^dAdditional putative transmembrane helix at N-terminus.

subunits of the ATPase complex of the two methanogens. Also, BLAST searches [10] did not reveal a second gene encoded elsewhere on the genomes. In conclusion, it is unlikely that the rotor parts of the ATPase complexes of *M. thermotrophicus* and *M. jannaschii* are built of 13 repeats as well.

Duplication of the c subunit encoding gene is a characteristic of V-type ATPases of eukaryotic origin. The c subunits of V-ATPases have double the mass of those of F-ATPases. Importantly, only the second domain in the V-type c subunits contains the cation binding site, and, consequently, the cation/ATP stoichiometry is half that of an F-type enzyme with the same number of domains in the rotor [19]. The difference provides a mechanistic explanation for the different physiological functions of V- and F-types, ATP hydrolysis and ATP synthesis, respectively [18]. The archaeal complexes are evolutionarily closely related to the V-type ATPases, but their rotor subunits come in a great variety (Table 1). They differ both in the number of hairpin domains per subunit, the number of cation binding sites per hairpin, and, most likely, in the number of hairpins per rotor structure. The c subunit of all of the Crenarchaeota and many of the Euryarchaeota consists of a single hairpin domain. The *Pyrococcus* species and *M. thermotrophicus* have c subunits with two hairpin domains, but the first hairpin of the former does not contain a cation binding site. The *M. jannaschii* subunit is the only one with three domains, two of which contain a binding site [17]. Finally, the protein of *M. kandleri* contains 13 domains and 13 binding sites. In all cases, the binding site residues come as part of a conserved triad, PET in the Euryarchaeota and GEG in the Crenarchaeota. The neighboring residues are even conserved when the binding site Glu itself is not present. It should be noted that in a helical conformation, the neighboring residues are not likely to be part of the binding pocket. The variability in the archaeal rotors may reflect different physiological functions of the A-ATPases/synthases.

2.5. Conclusions

Analysis of the *ntpK* gene of *M. kandleri* results in two main conclusions. First, the rotor of the ATP synthase consists of a single protein, and second, the ring structure of the rotor is built of 13 homologous domains. *M. kandleri* is a strictly anaerobic methanogen isolated from a ‘black smoker’ at 2 km below sea level [20]. The hyperthermophile grows optimally at temperatures between 80 and 110°C. These harsh conditions will put high demands on the stability of the cellular proteins which may explain why the rotor consists of a single protein. An additional advantage would be that no regulatory mechanisms during biosynthesis of the complex are necessary to account for a higher stoichiometry of the rotor subunit in the complex. Recent structural data suggested that the stoichiometry of the rotor ring depends on the biological species

from which the ATP synthase originates. In the yeast *Saccharomyces cerevisiae* the rotor is a decamer, in the bacteria *I. tartaricus* and *P. modestum* an undecamer, and in chloroplasts a 14-mer. The 13-mer described here in *M. kandleri* appears to further add to the heterogeneity of the rotors. It is to be expected that this stoichiometry is not general in the archaeal kingdom. In general, rotor subunit stoichiometry may be determined conditionally, rather than evolutionally.

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