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F-type or V-type? The chimeric nature of the archaebacterial ATP synthase

G. Schäfer and M. Meyering-Vos

Institute of Biochemistry, Medical University of Lübeck, Lübeck (Germany)

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Archaebacterial plasma membranes contain an ATPase acting in vivo as a $\Delta\mu_{H^+}$-driven ATP synthase. While functional features and their general structural design are resembling F-type ATPases, primary sequences of the two large polypeptides from the catalytic part are closely related to V-type ATPases from eucaryotic vacuolar membranes. The chimeric nature of archaebacterial ATPase from *Sulfolobus* was investigated in terms of nucleotide interactions and related to specific sequence parameters in a comparison to well known F- and V-type ATPases. The study disclosed a general difference of F- and V-type ATPases at one class of the nucleotide binding sites.

Introduction

Several years ago in a systematic comparison of membrane ATPases two different but similar types of proton translocating membrane complexes were calssified as F-type and V-type ATPases [1,2]. Besides significant similarities their characteristic differences were related to their specific functions in cellular energetics, assigning F-type ATPases as functional ATP synthases in eucaryotic organelles or bacterial plasma membranes; in contrast, V-type ATPases were thought to occur exclusively in eucaryotic subcellular vesicles, generating a $\Delta\mu_{H^+}$ at the expense of ATP-hydrolysis. At that stage knowledge of archaebacterial H^+-ATPases was only fragmentary, and a classification amongst F-type ATPases appeared likely [3–5] based on their putative function in cellular energy conservation.

Meanwhile, genetically derived amino-acid sequences revealed an hitherto unexpected result, unmasking a surprising similarity on the level of primary structure between archaebacterial plasma membrane ATPases and eucaryotic vacuolar ATPases [6–10]. These data elicited a more critical comparison with respect to structural and functional parameters, e.g. nucleotide binding sites and their properties.

Quaternary structure

Generally, archaebacterial ATP synthases are composed of an F_1-like peripheral catalytic head piece linked to a membrane residing part which atleast contains a DCCD reactive small proteolipid and presumably other polypeptides, not unequivocally identified as yet. Thus, the total number of constituent subunits from various archaebacterial ATP synthases still appears inhomogeneous; a situation which might result from incomplete solubilization or from dissociation during isolation of the respective proteins. The catalytic moieties of all enzymes exhibit 2 large subunits (A and B; in the order of increasing M_r)) of comparable molecular mass, but differences exist in the number and size of observed small subunits [6–8]. On the genetic level an operon structure for the whole complex was shown [11], but not all structural genes could be assigned protein chemically so far. In contrast to mammalian vacuolar ATPases, archaebacterial ATPases do not contain the superficial large polypeptides of > 100 kDa [12] and hitherto unknown function. Also, no relation between the smaller polypeptides to any counterpart from known F-type ATPases could be established. Functionally, the A-polypeptides of vacuolar and archaebacterial ATPases are equivalent to the β-polypeptides of F-type ATPases [6–9], while an antiserum against *Sulfolobus*-β crossreacted specifically

Correspondence to: G. Schäfer, Institute of Biochemistry, Medical University of Lübeck, Ratzeburger Allee 160, D-2400 Lübeck, Germany.

Abbreviations: DCCD, *N,N'*-dicyclohexylcarbodiimide; F_1, membrane peripheral catalytic part of the ATP-synthase complex (EC 3.6.1.3).

with F-type β-subunits from eucarya or bacteria but also showed some reactivity with A and B from V-type ATPase [5].

Nucleotide binding

Classical F-type ATPases display 6 nucleotide binding sites distributed between a- and b-subunits, acting as 3 catalytic and 3 non catalytic sites [13]. While a precise and unifying characterization of the situation with V-type ATPases is still lacking, preliminary data [14] and more recent investigations from our laboratory with the archaebacterial ATPase from *Sulfolobus* were the first to elucidate obvious functional differences between these and F-type ATPases.

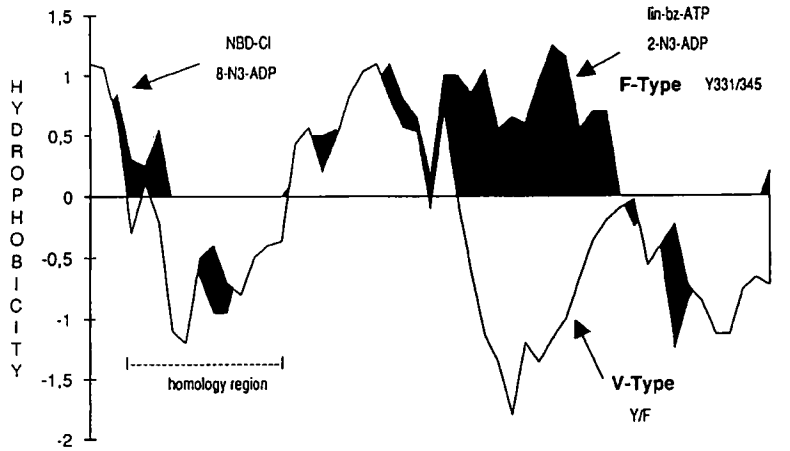
Table I summarizes nucleotide binding properties of isolated *Sulfolobus* ATPase, including the reversible binding of the covalently reacting photoaffinity probe 2-azido-ADP. Interestingly, the total number of sites is 6 as in F-type ATPases. Also in line with those is the clear distinction into two classes of different affinity. However, under the extreme conditions for the thermophilic enzyme (70–80°C) a possible cooperativity between the 'high affinity' sites as found in beef heart ATPase [13,15] could neither be demonstrated nor excluded. Notably, the total number of sites is accessible only at 70°C, whereas at lower temperatures the extremely thermophilic enzyme assumes a kind of a 'frozen state' where more than half of the sites appear to be occluded. Also, despite loading at high temperature after cooling to room temperature or below only a small fraction of bound nucleotides is retained

TABLE I

Nucleotide binding properties of ATPase from Sulfolobus acidocaldarius (DSM 639) and interaction with the photolabeling 2-azido-analogs

Data are from Ref. 14; experimental details are given in Ref. 18.

Endogenous nucleotides mol/mol enzyme	ADP 0, 1		ATP 0, 8-1
K_M (ATP)^{app}	180–200 μM		
Reversible sites:	at 4°	20°	70°C
n = [Mg-ADP/mol]	2, 5	4	6
K_d 1 [ADP]μM	–	–	0,2
K_d 2 [ADP]μM	–	–	1,8
K_M (2-N_3 ATP)^{app}	300–350 μM		
reversible sites:	–	–	6
n = (2-N_3 ATP/mol)	[no Mg^{2+} added]		
K_d 1 [μM]	–	–	1, 5
K_d 2 [μM]	–	–	170
% photoinact.	2-N_3 ATP		2-N_3 ADP
−5 mM ADP	30		40
+5 mM ADP	0		0
Labeled sites:	subunit A (B, trace)		
Incorporation:	1–1.5 mol/mol		

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M.barker.-A  AVSPPGGDFSEPVTQNTLRIVKVFWALDAKLSQRRHFPAI 383-422 (V)
H.halob. -A  AVSPPGGDFSEPVTQNTLRIVKTFWALDSDLAERRHFPAI 390-429 (V)
S.acidoc.-A  AVSPPGGDFTEPVTSNTLRFVRVFWPLDVSLAQARHYPAI 390-429 (V)
Dauc.car.-A  AVSPPGGDFSDPVTSATLSIVQVFWGLDKKLAQRKHFPSV 412-451 (V)
E.coli. -ß   AVYVPADDLTDPSPATTFAHLDATVVLSRQIASLGIYPAV 295-334 (F)
Bov.heart-ß  AIYVPADDLTDPAPAVTFAHLDATTVLSRAIAELGIYPAV 309-348 (F)
             *+  *  *++++    *+  +     *   +     +* +
                 ^                              ^
             NBD-Cl                        2-N3ADN
          8-N3-ADN                  lin-benzo ADP,FSBI, Bz-ATP
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Fig. 1. Aligned hydrophobicity profiles and sequences at a nucleotide binding sequence stretch of archaebacterial with distantly related F-type and V-type ATPases. The data refer to the A-subunits from archaebacterial or vacuolar ATPases, and the β-subunits from F-type ATPases. Identified insertion sites for covalent probes and the interaction site of *lin*-benzo-AD(T)P are indicated. Hydrophobocity profiles were constructed by averaging the closely similar profiles of each group (F- or V-type ATPases, respectively) within the shown region and centered according to the sequence alignment; the profiles of both groups were superimposed; they extend one to two window sizes (= 9) over the listed range of amino acids. Sequence data were accessed via the EMBL data bank.

(M.Meyering; unpublished) comparable to the endogenous nucleotide content of freshly prepared enzyme (Table I). This feature reflects the thermophilic nature of *Sulfolobus* and cannot be compared to properties of V-type ATPases, since those have never been described for a thermophilic eucaryotic organism. Recently, the eucaryote *Thermus thermophilus* was suggested to contain an ATPase with V-type like features [16]; however, so far no nucleotide binding data are known.

2-Azido-ATP, which serves as an effective substrate, was also suitable to label nucleotides sites of *Sulfolobus* ATPase covalently. In contrast to expectations from F-type ATPases, however, its inactivating effect upon ultraviolet irradiation as well as the labeling stoichiometry were poor. Even by repetitive illumination cycles at saturating concentrations inactivation never exceded 40–50%, while ADP clearly had a protective effect on label incorporation. Labeling was found almost exclusively within the A-subunit which according to primary sequence data is analogous to the catalytic subunits of V-type ATP-ases [6,9].

Structure at binding sites

Comparison of hydrophobicity profiles from various aligned F-type, V-type and archaebacterial ATPases revealed a surprising pattern identity at several regions considered to be functionally important; within these also theoretical secondary structure predictions coninCide fairly well [17].

Though being a crude simplification this approach clearly suggests that during evolution of functional domains the prerequisites to form a defined folding pattern were conserved rather than the identity of individual amino acids. Such an identity seems to be restricted merely to positions where residues involved in binding or catalysis are located within an environment providing the specifically required polarity and space. This is illustrated in the example given in Fig. 1.

lin-Benzo-ADP, a discriminating probe differentiating between catalytic and non catalytic sites of F-type ATPases [18] from beef heart or *E.coli*, did not respond by the expected fluorescence quench upon binding to the enzyme from *Sulfolobus*. Since by detailed mutagenesis studies with the *E.coli* enzyme [19] the structure at its binding site and the molecular quencher were identified (Y331 E.*coli*, Y345 bov.), a comparison with archaebacterial and V-typ ATPases became accessible as depicted in Fig. 1.

At the respective pocket interacting in F-type ATPase with *lin*-benzo-nucleotides, in archaebacterial or V-type ATPases the quencher *tyr* is located within a strongly polar sequence stretch (and/or is also replaced by *phe*), while in F-type ATPases this domain appears strongly hydrophobic. Notably this position is closely flanking an important nucleotide binding locus on its N-terminal side. In addition, this very locus plays a significant role within most hypothetical catalytic-site models proposed so far [20,21]. Moreover, it is situated right at the domain considered to confer cooperativity between sites or signal transmission between a- and b-subunits [22]. Therefore this structural pecularity is tentatively suggested to be responsible for the observed differences of nucleotide interactions between V-type and F-type ATPases, including the unusual response to the photoreactive agent 2-azido-ATP in *Sulfolobus* as an archaebactrial example.

Conclusions

The nature of archaebacterial ATPases is apparently chimeric with regard to properties and structure. With both types of ATPases they share the overall structure of a H^+-translocating complex built from a membrane residing base piece attached to a large peripheral catalytic head piece. Characteristics shared specifically with F-type ATPases are: the molecular size of the F_1-analogous peripheral part, the pseudo-hexagonal arrangement of A- and B-subunits [5,22] as an A_3B_3 array, the presence of 6 reversible nucleotide sites, the function as ATP synthases, the insensitivity versus bafilomycin, the occurrence of a 6–7 kDa DCCD reactive proteolipid [5,23] in the membrane integral part. With V-type ATPases they share: the arrangement of their largest subunits, significant homology of primary sequences, the largest subunit (A) being the catalytic one, immunological relation to A- and B-polypeptides, inhibitory action of nitrate, insensitivity towards azide. Evolutionarily they seem to originate from an archaic type of ATPases [24,25] which was modified later to form the eucaryotic V-type ATPases with different function and degree of coupling between proton translocation and ATP hydrolysis.

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