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The stimulating role of subunit F in ATPase activity inside the A_1-complex of the *Methanosarcina mazei* Gö1 A_1A_O ATP synthase

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ABSTRACT

A_1A_O ATP synthases couple ion-transport of the A_O sector and ATP synthesis/hydrolysis of the A_3B_3-headpiece via their stalk subunits D and F. Here, we produced and purified stable A_3B_3D- and A_3B_3DF-complexes of the *Methanosarcina mazei* Gö1 A-ATP synthase as confirmed by electron microscopy. Enzymatic studies with these complexes showed that the *M. mazei* Gö1 A-ATP synthase subunit F is an ATPase activating subunit. The maximum ATP hydrolysis rates (V_{max}) of A_3B_3D and A_3B_3DF were determined by substrate-dependent ATP hydrolysis experiments resulting in a V_{max} of 7.9 s^{-1} and 30.4 s^{-1}, respectively, while the K_M is the same for both. Deletions of the N- or C-termini of subunit F abolished the effect of ATP hydrolysis activation. We generated subunit F mutant proteins with single amino acid substitutions and demonstrated that the subunit F residues S84 and R88 are important in stimulating ATP hydrolysis. Hybrid formation of the A_3B_3D-complex with subunit F of the related eukaryotic V-ATPase of *Saccharomyces cerevisiae* or subunit ε of the F-ATP synthase from *Mycobacterium tuberculosis* showed that subunit F of the archaea and eukaryotic enzymes are important in ATP hydrolysis.

1. Introduction

Unlike energy conservation mechanisms in eukaryotes and bacteria, the metabolism in archaea, like methanogens, is coupled to the generation of an H^+- and/or Na^+-gradient across the membrane, and both ion gradients drive the synthesis of ATP [1]. The A_1A_O ATP synthase, catalyzing the synthesis of ATP, is composed of nine subunits in a proposed stoichiometry of A_3:B_3:C:D:E_2:F:G_2:*a*:c_x (Fig. 1A). Whereas the related F_1F_O ATP synthases in prokaryotes and eukaryotes catalyze ATP synthesis at the expense of an electrochemical ion gradient, the evolutionary related eukaryotic V_1V_O ATPases function as ATP-driven ion pumps, unable to synthesize ATP under physiological conditions [2–5]. Although the cellular function of archaeal ATP synthases is to synthesize ATP by ion gradient-driven phosphorylation, they also work as ATP-driven ion pumps to generate an ion gradient under fermentative conditions [1,2].

Like bacterial F-ATP synthases (α_3:ß$_3$:γ:δ:ε:*a*:b_2:c_{9-15}) and eukaryotic V-ATPase (A_3:B_3:C:D:E_3:F:G_3:H:*a*:c_X:c'_γ:c''_Z:*d*:*e*), the A-ATP synthases possess a water-soluble (A_1) sector, containing the catalytic sites, and an integral membrane (A_O) domain, involved in ion translocation [2–5] (Fig.1A). The catalytic A_3B_3-center of the A_1-sector is connected to the A_O-part by two peripheral stalks, composed of subunits E and G, and a central stalk which consists of the subunits C, D, and F [6,7]. Coupling of ATP synthesis/hydrolysis in the α_3ß$_3$- or A_3B_3-headpieces with ion-transport in the F_O- (F-ATP synthases) or V_O/A_O parts of V-ATPases and A-ATP synthases, respectively, occurs via the central stalk subunits γ and ε (F-ATP synthases) or the related subunits C, D, and F (V-ATPases, A-ATP synthases) [2–5].

The 101 amino acids subunit F of the *Methanosarcina mazei* Gö1 A-ATP synthase (*Mm*F) exhibits a distinct two-domain structure in solution, with the N-terminal globular region having 78 residues and the residues 79–101 forming the flexible and positively charged C-terminal part ([8,9]; Fig. 1). The flexible C-terminal tail enables this subunit to undergo up and down movements relative to the nucleotide-binding subunit B [8–11]. In the entire A-ATP synthase, subunit F can be cross-linked in a nucleotide-dependent way to subunits B through their C-terminal domains [8,12].

In the A-ATP synthase, subunit F is in close contact to the central stalk subunit D ([2,9]; Fig. 1A). The four-stranded ß-sheet in the N-terminal part forms a hydrophobic surface that mediates the interaction of both subunits ([9]; Fig. 1B). The positively and negatively charged surface at the bottom of the N-terminal domain of subunit F is oriented toward the central stalk subunit C – a subunit absent in F-ATP synthases – and thereby toward the membrane side [2,9]. As described recently, subunits D, C, and F form a cross-linked product inside the A_1 ATPase [8,12], giving the central stalk a significant length of 84 Å in solution [13].

Besides the structural and biochemical characteristics, detailed information about the mechanistic function of subunit F of methanogenic A-ATP synthases is lacking. Here, we show for the first time that subunit F activates ATP hydrolysis in the A_3B_3-headpice. Single mutation and

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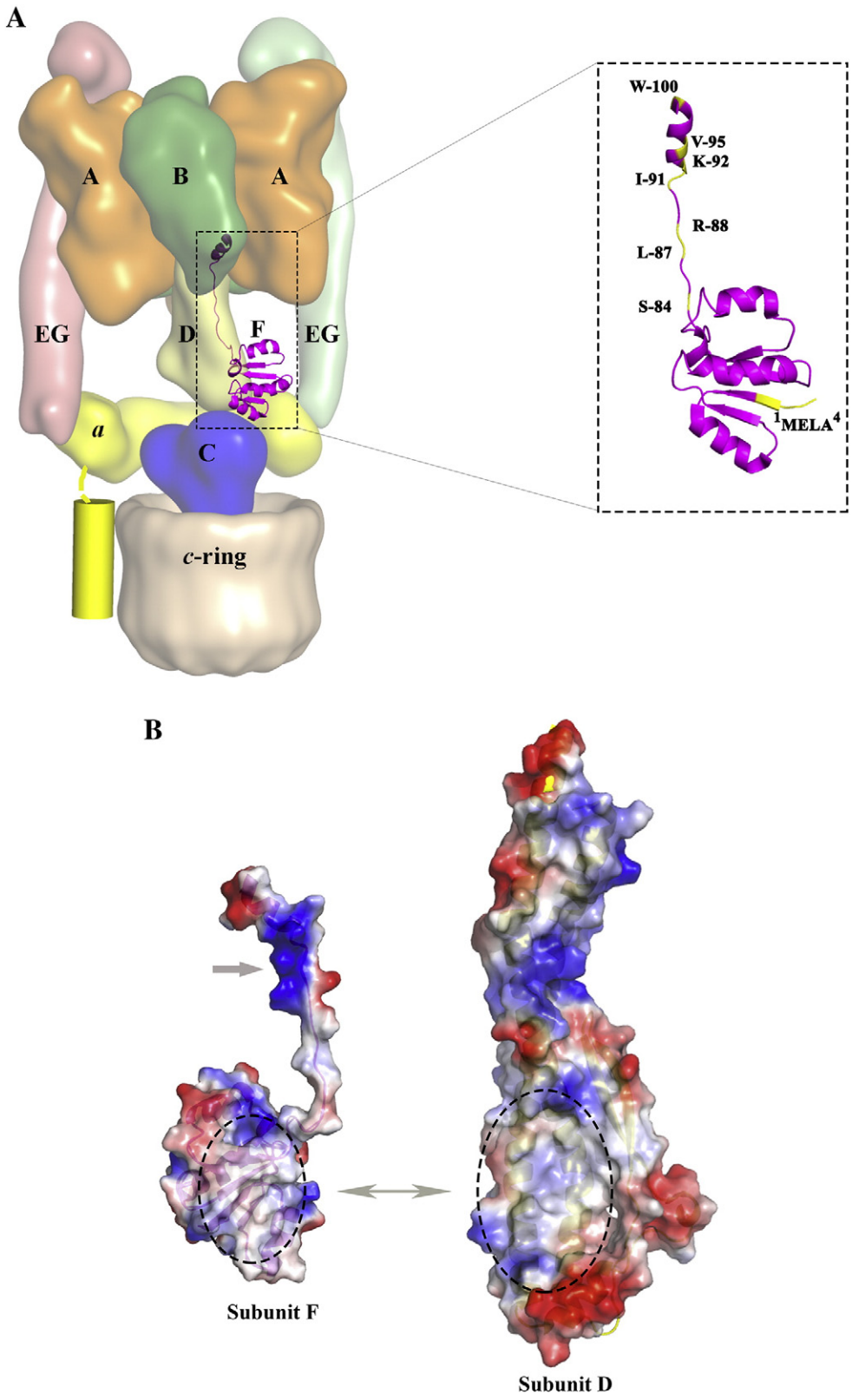

Fig. 1. *Arrangement of the atomic structures of individual subunits in A-ATP synthases shown as Gaussian surfaces.* (*A*) Subunits A (PDB ID 3I4L; *orange*) and B (PDB ID 2C61; *dark green*) alternate in the A_3B_3-hexamer. The NMR structure of subunit F ([9]; PDB ID 2OV6; *magenta*) and the crystal structures of subunits C (PDB ID 1R5Z; *blue*) and D (PDB ID 3AON; *light yellow*) form the central stalk. The structure of subunit *a* (*yellow*) was taken from PDB ID 3RRK and the *c*-ring (*wheat*) from PDB ID 2BL2. The *T. thermophilus* EG dimer (*green*) with straight peripheral stalk was taken from PDB ID 3K5B and the kinked second peripheral stalk (*red*) was modeled with the crystal structure of *P. horikoshii* OT3 subunit E (PDB ID 4DT0) and the NMR structure (PDB ID: 2KK7) along with the *T. thermophilus* G structure. The structurally unknown region of subunit *a* (345–668) is shown as yellow cylinder. On the right side, an enlarged picture of subunit F is shown. Important residues of the C-terminus of subunit F and the engineered mutations and truncations are labeled and shown in yellow. (*B*) The charge distribution on the surface of subunit F (PDB ID 2OV6) and subunit D ((PDB ID 3AON) of A-ATP synthases. Red and blue areas are negatively and positively charged areas, respectively. The arrow indicates the very positively charged C-terminus. The hydrophobic area can be seen in grey color. The dashed circle on the N-terminal domain of subunit F and D represents the hydrophobic amino acids involved in the binding of both subunits.