resolution. The structures reveal a modular architecture for subunit G and a highly complex E-G-coiled-coil interface made up of degenerate mixed repeating patterns and unexpected discontinuities. The high affinity EG-Chead interface, which is broken during regulated disassembly, is constituted by mainly hydrophobic contacts contributed by loop regions in the head domain. The EG heterodimer contains two flexible joints that allow for movement of the termini without disruption of the coiled-coil interface. Fitting of EG_Chead into a 3D EM map of V-ATPase revealed a mismatch for peripheral stalk EG3 and we propose that energy is required for incorporation of EG3 and subunit C during enzyme assembly, resulting in a spring loading mechanism that may facilitate breaking of protein interactions upon regulated enzyme dissociation.

References


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4P11

Beta barrels 9 and 10 are equally important for the gating properties of VDAC1 than the N-terminal domain

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VDAC (voltage dependent anion selective channel) is the pore that maintains the permeability of the outer mitochondrial membrane. The structure of its most abundant isoform called VDAC1 has been recently solved in mammals. Research studies are now aimed to define at a molecular level its peculiar gating property, the voltage-dependence, highly relevant in the bioenergetic metabolism. In VDAC1 the structure suspected to be in charge of the voltage dependent gating is the N-terminal domain (1-2). It has been reported as an incomplete amphipathic α-helix accessed to the inner side of the pore wall. Its mobility is candidate to cause alternative gating states. In this work we focus our attention onto the β-strands that take contact with the N-terminal domain. The exchange of the whole VDAC1 β-barrel with the homologous VDAC3 β-barrel shows that the chimeric protein in reconstituted systems loses completely voltage-dependence, despite the presence also in VDAC3 of V143 and L150 residues (2). VDAC1 mutants completely lacking either the β-strand 9 or both β-strands 9 and 10 were expressed, refolded and reconstituted in artificial bilayers. In these experiments the mutant lacking the β-strand 9 (where V143 is located) shows smaller pores but a normal voltage-dependence. The mutant lacking both β-strands 9 and 10 shows instead a peculiar voltage-dependence resulting in a fully asymmetric behavior. We used classical molecular dynamics simulations to model the protein missing β-strands 9 and 10. The results obtained support the experimental data. Our data point out the notion that the voltage dependent gating of VDAC1 is a complex phenomenon involving both the N-terminal moiety and some specific β-strands in the pore wall.

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References


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4P12

Regulation of ammonium transport proteins

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From prokaryotes to plants, the essential nutrient nitrogen is selectively taken up as ammonium/ammonia (NH₄⁺/NH₃) via ammonium transport (Amt) proteins [1]. Their regulation involves GlnK proteins, members of the P₆₀-protein family, via direct protein–protein interaction [2,3]. GlnK are central regulators of nitrogen assimilation and sense the intracellular energy, nitrogen and carbon levels by binding effector molecules such as ATP, ADP and 2-oxoglutarate (2-OG).

The genome of the hyperthermophilic archeon Archaeoglobus fulgidus encodes for three Amts each followed by a gene for a GlnK protein within an operon. We characterized the thermodynamics of ligand binding of GlnK-1, GlnK-2 and GlnK-3, using isothermal titration calorimetry (ITC). In combination with X-ray crystallography, we seek to elucidate the determinants that trigger a broad range of response modulations in GlnK such as conformational changes and cooperativity in ligand binding [4–6].

References


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4P13

The functional and structural analysis of the mitochondrial aspartate–glutamate carrier

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