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Membrane-integral pyrophosphatases (mPPases) are primary H^+- or Na^+-ion pumps directly energized by pyrophosphate, an abundant byproduct of anabolic reactions. mPPases are widespread in all domains of life and provide the host necessary energy reserves, particularly during stress and low-energy conditions. The enzyme holds promise in biotechnology insofar as agricultural plants that overexpress mPPase have salt- and drought-tolerant phenotypes.

Recent work has uncovered significant functional divergence among members of the mPPase protein family. Notably, mPPases differ in pumping specificity and sensitivity to K^+ ions [1,2]. All K^+-independent mPPases operate as H^+-pumps, whereas most K^+-dependent mPPases are primary Na^+-pumps. However, several types of K^+-dependent, H^+-pumping mPPases are known. One particular mechanism that allows a change in transport specificity from Na^+ to H^+ is spatial repositioning of a glutamate residue that forms part of the cytoplasmic gate in the ion transport channel [3].

The reconstructed evolutionary history of mPPases suggests that the ancestral enzyme operated as a Na^+-pump and the transition to H^+-pumping occurred in several independent enzyme lineages [3]. These data lend support to the hypothesis of primordial Na^+-based membrane bioenergetics [4]. Na^+- and H^+-pumping mPPases are structurally very similar [5], supporting the concept, first proposed for a rotating ATP-synthase/ATPase, that switching between Na^+ and H^+ transport specificities requires only subtle changes in structure [4].

References

[1] G.A. Belogurov, R. Lahti, J. Biol. Chem. 277 (2002) 49651–49654.
[2] A.M. Malinen, G.A. Belogurov, A.A. Baykov, R. Lahti, Biochemistry 46 (2007) 8872–8878.
[3] H.H. Luoto, G.A. Belogurov, A.A. Baykov, R. Lahti, A.M. Malinen, J. Biol. Chem. 286 (2011) 21633–21642.
[4] A.Y. Mulkidjanian, M.Y. Galperin, K.S. Makarova, Y.I. Wolf, E.V. Koonin, Biol. Direct 3 (2008) 13.
[5] S.M. Lin, J.Y. Tsai, C.D. Hsiao, Y.T. Huang, C.L. Chiu, M.H. Liu, J.Y. Tung, T.H. Liu, R.L. Pan, Y.J. Sun, Nature 484 (2012) 399–403.

4P9

Characterization and purification of the multi subunit type Na^+/H^+ antiporter from alkaliphilic *Bacillus pseudofirmus* OF4

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Mrp antiporters are monovalent cation/proton antiporters which exchange cytoplasmic Na^+, Li^+ and/or K^+ ions for extracellular H^+. They are widespread among bacteria and archaea. Mrp antiporters have seven or six hydrophobic proteins that are encoded in the *mrp* operons, in contrast to most of bacterial Na^+/H^+ antiporters which are single gene products. Interestingly, the entire Na^+/H^+ antiport activity requires all of these proteins, suggesting that Mrp antiporters function as a hetero-oligomeric protein complex in the cytoplasmic membrane. Purification and functional reconstitution of the Mrp antiporter have not been reported. Therefore, we purified and reconstituted the Mrp antiporter from alkaliphilic *Bacillus pseudofirmus* OF4, because purification of target proteins and their complex with the native conformation is required for further functional and structural research. The Mrp antiporter expressed in major Cation/H^+ antiporter-defective *Escherichia coli* strain KNabc cells was purified by immobilized metal ion adsorption chromatography (IMAC). The purified Mrp samples were reconstituted into artificial membrane vesicles (liposomes) with F_oF_1-ATPase from *Bacillus* sp. PS3 as the "power supply" to generate a proton motive force required for activation of the Mrp antiporter. The Na^+/H^+ antiport activity of the purified Mrp antiporter was measured in the constructed proteoliposomes (protein-inserted liposomes). Using TALON resin, all of the Mrp subunits could be purified from the *E. coli* membrane fraction expressing the Mrp antiporter and seemed to be present as predominantly a MrpABCDEFG complex dimer. Apparent Na^+/H^+ antiport activity was observed in the proteoliposomes into which purified Mrp and F_oF_1-ATPase were reconstituted. After elution of Mrp proteins, they remained as Mrp complexes and most of which were present as the MrpABCDEFG complex dimer. This suggested that MrpABCDEFG complexes detectable by BN-PAGE are the active forms. It was also speculated that the Mrp complex dimer is more stable than the complex monomer. This is the first report of the purification and functional reconstitution of a Mrp antiporter.

Reference

[1] T.H. Swartz, S. Ikewada, O. Ishikawa, M. Ito, T.A. Krulwich, The Mrp system: a giant among monovalent cation/proton antiporters? Extremophiles 9 (2005) 345–354.

4P10

Crystal structure of the heterotrimeric EGC$_{head}$ complex from yeast vacuolar ATPase

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The eukaryotic vacuolar ATPase (V-ATPase) is a rotary molecular motor and dedicated proton pump found on the endomembrane system of all eukaryotic cells and the plasma membrane of specialized cells in higher organisms [1]. The enzyme is composed of a soluble catalytic subcomplex (V1) and a membrane integral complex (Vo) involved in proton translocation. Linking the soluble and membrane sectors are the stator subunits (E, G, C, H and aNT) which absorb the torque generated during rotary catalysis. The unique mode of V-ATPase regulation, known as reversible dissociation, involves the release of V1-ATPase from the membrane integral Vo, and the activity of both domains is silenced [2]. Regulated release of V1-ATPase requires breaking of protein interactions mediated by three peripheral stalks, each composed of a heterodimer of subunits E and G. Two of the peripheral stalks (EG1 and EG2) connect the top of the V1 to the membrane bound a subunit while the third (EG3) is bound to subunit C, which is released from both V1 and Vo during enzyme dissociation. We have previously characterized and quantified the affinities of some of these interactions and have found that the globular "head" domain of subunit C (C$_{head}$) binds to one EG heterodimer with high affinity [3,4].

Here, we present X-ray crystal structures of two conformations of the EGC$_{head}$ complex from *Saccharomyces cerevisiae* at 2.91 and 2.82 Å

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-C$_{head}$ 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 EGC$_{head}$ 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
[1] M. Forgac, Nat. Rev. Mol. Cell Biol. 8 (2007) 917–929.
[2] P.M. Kane, Curr. Protein Pept. Sci. 13 (2011) 117–123.
[3] R.A. Oot, S. Wilkens, J. Biol. Chem. 285 (2010) 24654–24664.
[4] R.A. Oot, S. Wilkens, J. Biol. Chem. 287 (2012) 13396–13406.

4P11

Beta barrels 9 and 10 are equally important for the gating properties of VDAC1 than the N-terminus 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 apposed 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 b-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-terminus moiety and some specific β-strands in the pore wall.

References
[1] V. De Pinto, et al., Determination of the conformation of the human VDAC1 N-terminal peptide, a protein moiety essential for the functional properties of the pore, Chembiochem 8 (2007) 744–756.
[2] S. Reina, et al., Swapping of the N-terminus of VDAC1 with VDAC3 restores full activity of the channel and confers anti-aging features to the cell, FEBS Lett. 584 (2010) 2837–2844.

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 (NH_4^+/NH_3) via ammonium transport (Amt) proteins [1]. Their regulation involves GlnK proteins, members of the P_{II}-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
[1] S.L.A. Andrade, O. Einsle, Mol. Membr. Biol. 24 (2007) 357.
[2] T. Arcondeguy, R. Jack, M. Merrick, Microbiol. Mol. Biol. Rev. 65 (2001) 80.
[3] G. Coutts, G. Thomas, D. Blakey, M. Merrick, EMBO J. 21 (2002) 536.
[4] S. Helfmann, W. Lü, C. Litz, S.L.A. Andrade, J. Mol. Biol. 402 (2010) 165.
[5] S. Maier, P. Schleberger, W. Lü, T. Wacker, T. Pflüger, C. Litz, S.L.A. Andrade, PLoS One 6 (2011) e26327.
[6] C. Litz, S. Helfmann, S. Gerhardt, S.L.A. Andrade, Acta Crystallogr. F67 (2011) 178.

4P13

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

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