Moreover, we found that under a sufficiently strong torque in the opposite direction of ATP hydrolytic rotations, it rotated in the opposite direction, or the ATP synthetic direction, in a stepwise manner. The torque necessary for rotations in the synthetic direction times 120° was nearly equal to $\in'' \in 1/4$ under various conditions except for conditions at sufficiently low ADP concentrations.

869-Pos

Spatial Distribution of Elasticity in the F1 Motor of ATP Synthase Reveals the Microscopic Nature of the Coupling Between the Central Shaft and the Catalytic Subunit

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F_OF_1-ATPase is a rotary motor protein that synthesize ATP from ADP using the proton gradient across a membrane as a free energy source. The proton flow through the membrane-embedded portion, F_O, is thought to generate the rotary torque that drives the rotation of the asymmetric shaft in the cylinder of hexagonally arranged alpha and beta chains forming the catalytic subunit of the F_1 portion. Mechanical energy of the rotating shaft is used by the active sites of F_1 to synthesize ATP against thermodynamic potential gradient. The microscopic mechanism of this energy conversion is still not fully understood. It was suggested that elastic power transmission with transient storage of energy in some compliant part of the common shaftis required for the high turnover rate to occur [1].

Here we use fully atomistic molecular dynamics simulation to study the spatial distribution of torsional elasticity in the F_1 motor on the 500-ns timescale. The overall range of angular fluctuations of the central shaft with respect to the symmetry axis of the catalytic subunit is consistent with the results of the corresponding experimental study [1]. The detailed analysis of the rotational mobility reveals, however, that the measured range of fluctuations results from two different effects: the internal elasticity of the shaft itself and the effective load imposed on it by the catalytic subunit. Separation of these two effects has led to the detailed description of the dynamic coupling between the shaft and the catalytic subunit. We also propose a simple model of the F_1 motor that might be a useful tool in future studies of the energy transfer in F_OF_1-ATPase.

[1] Sielaff et al. PNAS 105:17760-17765 (2008)

870-Pos

Structure of CopA from Archaeoglobus Fulgidus by Cryoelectron Microscopy

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CopA, a bacterial transporter of Cu^+ and Ag^+ from *Archaeoglobus fulgidus* , was cloned, overexpressed, purified, reconstituted into lipid bilayers and crystallized into tubular crystals in the presence of Cu^+ chelator BCDS by dialysis at 50°C. Three Cryo-EM Structures were obtained with different constructs and lipids 1) dNdC-CopA with DOPC, N and C terminal cytoplasmic peptides truncated of CopA reconstituted into 1,2-dioleoyl-sn-glycero-3-phosphocholine, 2) dC-CopA with DOPC, C terminal cytoplasmic peptide truncated of CopA reconstituted into 1,2-dioleoyl-sn-glycero-3-phosphocholine, 3) dC-CopA with DMPC/DOPE, C terminal cytoplasmic peptide truncated of CopA reconstituted with 1,2-dimyristoyl-sn-glycero-3-phosphocholine and 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine. All reconstituted proteoliposomes retain their functionality with V_{max} ranging from 1.14 to 2.03 nmol/μg/min and $K_{0.5}$ ranging from 0.1 to 0.8 μM, depending on the reconstituted proteoliposomes and ion substrates. The optimal temperature for enzyme assays of these reconstituted proteoloposomes are located between 65°C to 75°C and these activity measurements were conducted at the temperature of 70°C. Three cryo-EM structures obtained by frozen-hydrated tubular crystals and Fourier processing have resolutions from 12.5 to 17.5 é…. Based on the difference map and the modeling between dC-CopA and dNdC-CopA, N-terminal metal binding domain (MBD) of CopA appears to lie between the ATP binding domain and Actuator domain and has an inhibitory role, which is relieved by receiving Cu^+ from the soluble metals chaperon. Efforts for higher resolution as well as computational modeling of CopA are underway in order to investigate the relative position of cytoplasmic domains with respect to transmembrane helices, in which the transport sites and ion gateway are located.

871-Pos

Sodium Pump A1 And A3 Subunit Isoforms Mediate Distinct Responses To Ouabain And Are Both Essential For Human Neuroblastoma

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The sodium pump (Na^+,K^+-ATPase) maintains the sodium gradient across plasma membranes of animal cells. By hydrolyzing ATP the enzyme transports 3 Na^+ ions out of the cell in exchange for 2 K^+ ions that are taken into the cytosol. This activity can be interrupted by cardiotonic steroids (CTS). Recent publications have, however, established that CTS not only inhibit the sodium pump but that they also induce signalling cascades that influence the physiology of cells in various ways.

Sodium pumps are composed of α and β subunits (and additionally in some tissues of γ subunits) that appear is several isoforms. In some cells different α subunit isoforms are coexpressed, giving rise to the question about the need for their co-existence.

Using human neuroblastoma cells SK-N-AS that co-express α1 and α3 isoforms of the sodium pump α subunit, we selectively silenced either the α1 or α3 subunit by means of small interfering RNA and investigated cell survival and the cellular response to ouabain, a widely used CTS. We find that both of the two α subunit isoforms are essential for cell survival, indicating that substitution of one subunit for the other is not sufficient. In the presence of both α subunits ouabain causes a sustained Erk1/2 activation. This activation is not affected when the α1 subunit is silenced. When α3 expression is silenced, ouabain-induced activation of Erk1/2 does not occur, even at a high concentration of ouabain (1 μM). Thus, ouabain-induced Erk1/2 activation is mediated in SK-N-AS cells by α3 only, and α1 does not participate in this event. This is the first demonstration of selective involvement of a specific sodium pump α subunit isoform in ouabain-induced signaling.

872-Pos

Ion-Selectivity of Externally Facing Na^+-Exclusive and Na^+/K^+-Shared Sites in the Na/K-Pump

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The Na/K-pump extrudes 3 Na^+ in exchange for 2 K^+ across the plasmalemma of animal cells. Two-out-of-three ion binding sites in the protein can be occupied by either Na^+ or K^+, whereas another site exclusively binds Na^+. At maximally activating [K^+_o], Na^+_o binding to the Na^+-exclusive site (first site to open in sequential Na^+ release) is manifested as [Na^+_o]- and voltage-dependent inhibition of outwardly-directed (due to the 3:2 stoichoimetry) pump current (I_P). Guanidinium$^+$ can also backward-transit this Na^+-release channel inhibiting I_P at negative voltages (Yaragatupalli et al. (2009) PNAS **106**:15107-15512). To study the ion-selectivity of this Na^+-release channel we measured voltage-dependent inhibition of I_P with external solutions containing different cations (120-125 mM). This inhibition followed the sequence: $Na^+_o > Li^+_o >$ guanidinium^+_o >aminoguanidinium^+_o > acetamidinium^+_o ~diaminoguanidinium^+_o ~formamidinium^+_o ~Cs^+_o ~K^+_o ~N-Methyl-D-Glucamine (NMG^+_o).

Ouabain-sensitive currents in the absence of Na^+_o and K^+_o were also measured. An inward current (possibly representing leakage through the Na-exclusive site when the shared sites are empty) was observed in NMG^+_o, guanidinium^+_o and aminoguanidinium^+_o. The other cations tested induced ouabain-sensitive outward currents at all voltages. Ouabain-sensitive current amplitude in 120 mM acetamidinium^+_o was similar to maximal K^+_o-induced I_P. Without Na^+_o, [acetamidinium ^+_o] of outwardly-directed current gave $K_{0.5acet+}$ ~10 mM indicating that this ion acts as a low-affinity K^+ surrogate. Consistently, in sheep kidney purified enzyme preparations, both acetamidinium$^+$ and formamidinium$^+$ induced ouabain-dependent ATPase activity ($K_{0.5acet}$ = 80 mM, $K_{0.5form}$ = 113 mM, $K_{0.5K+}$ = 0.9 mM). Transport was confirmed by means of ouabain-sensitive C^{14}-acetamidinium uptake. Our results indicate that acetamidinium$^+$ and formamidinium$^+$ can be transported like K^+ by the Na/K pump. Molecular dynamics simulations based on an atomic model are used to explain organic cation coordination in the occluded form. Supported by NIH DK083859 and GM062342.

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The Route and Mechanism of Uncoupled Current Flow through Na/K-ATPase Pumps Lacking the Two COOH-Terminal Tyrosines

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Na/K-ATPase pumps generate outward current during ATP-driven stoichiometric exchange of three intracellular Na ions for two extracellular K ions. At acidic pH, in the absence of extracellular Na and K ions, an uncoupled current flows through wild-type Na/K pumps at large negative membrane potentials, believed carried by protons. Both currents are abolished by the specific Na/K pump inhibitor ouabain. In *Xenopus*α1 pumps made less sensitive to ouabain by mutations Q120R/N131D or C113Y we observed a similar uncoupled current in the absence of extracellular Na and K ions even at physiological pH