decarboxylation-driven Na+ pump use Na+ as the coupling ion. Specific binding sites for the respective coupling ion are present in the oligomeric ring of c subunits. In the c ring from *Ilyobacter tartaricus*, each sodium ion is coordinated by side chain and backbone oxygens from the inner and outer helices of two neighboring c subunits and the binding pocket is firmly stabilized by hydrogen bonds donated to the conserved cE65residue. In this conformation the sodium ion is buried and cannot reach putative ion conducting channels in subunit a. Cysteine–cysteine cross-linking studies between subunits a and c indicated subtle but distinct conformational changes around the Na+ binding site that were elicited by the stator arginine. These experiments support a model in which the alkali ion is released from the binding site by adapting a conformation that favors arginine but not Na+ binding. pH profiles for DCCD inhibitor binding by the proton translocating ATP synthase of *Halobacterium salinarium* reflected the popular group protonation mechanism of the conserved carboxylates in the c ring. However, all other ATP synthases investigated yielded pH profiles that could be best explained by the coordination of a hydronium ion. Hence, three different modes of ion binding (Na+, H3O+, H+) have developed in different ATP synthases during evolution.

doi:10.1016/j.bbabio.2008.05.039

S1/2 Mechanistic insights of F_1-ATPase rotation from single-molecule measurements of the power stroke

Wayne D. Frasch
School of Life Sciences, Arizona State University, Tempe, AZ 85287-4501, USA
E-mail: Frasch@asu.edu

Single-molecule measurements of the rotation of the F_1-ATPase γ subunit from *E. coli* were made with a time resolution that allowed the power stroke (transition between dwells) to be resolved. The duration of transitions and dwells was measured as a function of the viscosity of the medium using PEG-400 with various sizes of gold nanorods attached to the γ subunit as a visible probe of rotation. In the absence of PEG, the power stroke was found to be viscous-limited when nanorods with dimensions of 91×45 nm were used as probes, but was kinetically-limited when 75×35 nm, 87×36 nm, and 90×46 nm were used. The dwell times matched ATPase turnover rates measured in bulk solution without an attached nanorod at low viscosities, and increased about 8 fold as a function of viscosity under conditions in which the power stroke was kinetically limited. When the power stroke became limited by the viscous drag, the dwell times became longer than the ATPase turnover time in the absence of a bound nanorod. Under these conditions, the increase in the transition time caused a 20 fold increase in the dwell time regardless of the size of the nanorod used. These results indicate that forcing the γ subunit to rotate more slowly than occurs by the intrinsic kinetically-limited mechanism causes the enzyme mechanism to deviate from its normal catalytic cycle, and provide insight into sequential conformational states of the enzyme during a catalytic cycle.

doi:10.1016/j.bbabio.2008.05.040

S1/3 The stator stalk of *Escherichia coli* ATP synthase

Stanley D. Dunn
Department of Biochemistry, Schulich School of Medicine and Dentistry, University of Western Ontario, London, Ontario, Canada, N6A 5C1
E-mail: sdunn@uwo.ca

The peripheral stator stalk of *E. coli* ATP synthase is formed by a dimer of helical, 156-residue *b* subunits that are anchored in the membrane by N-terminal transmembrane segments. b_2 reaches up the side of F_1 and binds near the top, interacting with the δ subunit. Most of this distance is spanned by the dimerization domain contained within residues 53–122. Sequence analysis reveals an unusual, alanine-rich 11-residue repeat pattern, consistent with a novel, two-stranded right-handed coiled coil (RHCC) structure. Disulfide formation studies, and analysis of the shapes and stabilities of disulfide-linked forms, support an RHCC structure with helices offset by 5–6 residues, making the structure intrinsically asymmetric. The RHCC is controversial and a left-handed coiled coil has also been proposed. Chimeric forms of *b* incorporating exogenous sequences containing a hendecad pattern similar to that of *b* into the region between positions 55 and 95 supported ATP synthesis *in vivo*, but those incorporating known left-handed coiled coil sequences failed to do so, even though ATP synthase still assembled. Single residue deletions within the dimerization domain also support assembly but not ATP synthesis, confirming that the stator stalk has a functional role beyond simply holding on to F_1. The significance of the proposed offset RHCC structure in binding to F_1 and resisting rotational torque will be discussed.

doi:10.1016/j.bbabio.2008.05.041

S1/4 Domain compliance and elastic power transmission in F_OF_1-ATPase

H. Sielaff[a], H. Rennekamp[a], A. Waechter[a], S. Dunn[b], S. Engelbrecht[a], W. Junge[a]
[a]*Department Biophysics, University of Osnabrück, 49069 Osnabrück, Germany*
[b]*University of Western Ontario, London, Ontario, Canada, N6A 5C1*
E-mail: Junge@biologie.Uni-Osnabrueck.de

F_OF_1-ATP synthase is composed from two stepping rotary motors coupled by a common rotary shaft. The electromotor, F_O, drives the chemical generator, F_1. Elastic power transmission between F_O is indispensible for their coupled operation at high speed. By fluctuation analysis the torsional spring constants of various enzyme domains were determined, engineered SS-bridges served to selectively stiffen others. Both, the central shaft in F_1 and, surprisingly, also the long eccentric bearing, were rather rigid. Only one domain of the rotor, namely where subunits γ and ε of F_1 contact the c-ring of F_O, was more flexible (50 pNnm) by order of magnitude. This elastic buffer, being located between the loci of torque delivery by F_O and by F_1, provides high kinetic efficiency to this twin engine under load, and it accounts for the ability of concerted action with different gears in different organisms.

doi:10.1016/j.bbabio.2008.05.042

S1/5 Photoaffinity labeling and photoaffinity cross-linking of ATP synthase complexes

Achim Schuhen, Renate Genswein, Hans-Jochen Schäfer
Institut für Biochemie, Johannes Gutenberg-Universität Mainz, Germany
E-mail: jschaef@uni-mainz.de

Photoaffinity labeling and photoaffinity cross-linking have been proven to be valuable techniques for the localization and characterization of ligand binding sites. In order to characterize nucleotide binding sites of ATP synthases we have synthesized various mono- and bifunctional photoactivatable ATP analogs. The six nucleotide binding sites – three catalytic and three noncatalytic – of ATP synthases are