S1.36 Solution structure of subunit *d*, E and G of the eukaryotic V-ATPase

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Vacuolar ATPases (V-ATPases) are abundant, ubiquitous proton pumps in eukaryotic cells. These pumps regulate pH and generate an electrochemical gradient that drives the transport of molecules across membranes of endosomes, golgi, secretory vesicles, vacuoles, lysosomes or plasma membrane of osteoclasts and kidney intercalated cells. Eukaryotic V-ATPases are composed of at least 14 different subunits in a stoichiometry of A_3:B_3:C:D:E_X:F:G_2:H_X:*a*:*c*:*c*′:*c*″:*d*:*e*. The enzyme is divided into V_1- and V_O parts. A critical point in V-ATPases is the structure of stalk subunits *d*, E and G. Here, we will present the first low resolution structure of subunit *d* of the *Saccharomyces cerevisiae* V_1V_O ATPase, determined from solution X-ray scattering data. The protein is a boxing glove-shaped molecule consisting of two distinct domains, with a width of about 6.5 nm and 3.5 nm, respectively. Furthermore, the 3D structure of E_{1-69} and G_{1-59} of subunit E and G, respectively, has been solved using NMR spectroscopy. Binding studies using ^{1}H–^{15}N heteronuclear single quantum coherence spectra of G_{1-59} show specific interactions only with the peptide E_{18-38} of subunit E and allow a clear assignment of interacting amino acids in the E–G assembly.

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S1.37 Changes in crosslink efficiency between *b* subunits in the peripheral stalk of F_1F_O ATP synthase

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Chimeric *b* subunits have been produced by substituting regions of the *b* and *b*′ subunits from the photosynthetic bacteria *Thermosynechococcus elongatus* into the *b* subunit of *Esherichia coli*. These chimeric subunits readily formed heterodimeric peripheral stalks when the region E39–I86 of the *E. coli* enzyme was replaced with *T. elongatus* sequences, producing chimeric subunits abbreviated *Tb* and *Tb*′. Cysteines were substituted for A83 or A90 in both *Tb* and *Tb*′ subunits and disulfide crosslink formation was used to probe for relative subunit position. Crosslinks formed readily between both Tb_{A83C}/Tb'_{A90C} and Tb_{A90C}/Tb'_{A90C} when the enzyme was at rest. However, addition of ATP greatly reduced the efficiency of crosslink formation. Additional constructs were made which expressed normal *E. coli b* subunit with cysteine substitutions at several positions. F_1F_O F_1F_0 ATP synthase with homodimeric $(b_{A90C})_2$ showed a similar ATP-dependent inhibition of crosslink formation while complexes with $(b_{I76C})_2$ and $(b_{R83C})_2$ did not.

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S1.38 Expression vectors for mutant peripheral stalk subunits in *Escherichia coli* and *Saccharomyces cerevisiae*

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The function of the peripheral stalk of F_1F_O ATP synthase is to hold the F_1 sector against the movement of the rotor stalk during catalysis. The aim of this study was to generate expression vectors to investigate the capacity of *E. coli* and *S. cerevisiae* peripheral stalk subunits to tolerate changes in length. *E. coli* plasmids containing the entire *unc* operon that included insertions and deletions in the peripheral stalk were constructed. ATP driven proton pumping assays in isolated membrane vesicles indicated approximately 50% of wild type activity. The eleven amino acid insertion was significantly more active than our previous studies with the mutant *b* subunit expressed individually from a plasmid. Examination of the high-resolution structure of the bovine peripheral stalk from the Walker laboratory suggested a site in the eukaryotic enzyme peripheral stalk that might accept insertions and deletions. Expression plasmids have been constructed for the wild type and an insertion into a synthetic ATP4 (*b* subunit) gene of *S. cerevisiae*. These constructs will be used to investigate the structure–function relationships within the eukaryotic peripheral stalk.

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S1.39 Quantum-mechanical approach to the description of proton diffusion in the membrane protein pore of ATP-synthase

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Diffusion of protons is a well-known key process in the coupling of oxidative phosphorylation in mitochondria. Nevertheless, the molecular mechanisms of H+ movement inside the protein pore remain still incomprehensible. We try to describe these processes using a quantum-mechanical model which can help us to estimate average time of both a proton jump from essential protonated residue (cAsp61) away to membrane surface and reverse movement as well. For this aim the internal space of each half-channel was approximated as a parallelepiped area which included all essential residues. Then wave functions were determined as the solutions of steady state Schrödinger equation for centrally symmetric field of pulse charge system. The localization of protons within the channel was assumed to be near oxygen or nitrogen atoms of both protein residues and water. It was shown that there were several ways for proton trace in the channel area and these traces had different types of energy emission. So we suppose that this difference can be the base for understanding of molecular mechanism of energy transformation of electro-chemical gradient into the energy of ATP bond.

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S1.40 Domain motions of the catalytic subunit β in F_1-ATPase revealed by single molecule observation

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