Structural model of cytosolic N-terminus of α2-subunit isoform of V-ATPase: Identification and characterization of two binding interfaces for cytohesin-2

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Abstracts

S10.P14

The V-type ATPase (V-ATPase) is a multi-subunit membrane proton-pumping enzyme that is evolutionary related to F- and A-type and ATP synthases [1,2]. In addition, it also serves as a pH-sensing and cytohesin-2 signaling receptor [3,4]. Here, we studied the molecular details of the interaction between the cytosolic N-terminus of the V-ATPase α2-subunit (α2N1–402) and cytohesin-2 (CTH2). To generate a structural model of the complete α2N1–402 we used a combination of in silico homology modeling and NMR data. First, using the crystal-structure of the bacterial M. ruber V-ATPase Ψα-subunit as a template, we built a model of the mammalian α2N1–352 fragment. Second, we determined the NMR structures of synthetic peptides α2N368–395 and α2N386–402 and combined them with our structural model of α2N1–352. Third, we mapped the distribution of six V-ATPase-derived and CTH2-interacting peptides [5], which are clustered in two binding sites on α2N1–402. Our data indicate that the proximal lobe sub-domain of α2N1–402 (α2Nα) is the major interacting site of the one CTH2 molecule (CHT2s) via its Sec7 domain, while the distal lobe of α2N4,402 (α2Nβ) most likely interacts with the PH-domain of a second CTH2 molecule (CHT2p). This model was further tested using a Sec7/Arf-GEF activity assay. We confirmed that two Sec7-binding peptides α2N17–35 and α2N34–49 form an interaction interface on α2Nβ, and are involved in regulation of the enzymatic GEF activity of CTH2. Further analysis revealed that the binding sites of CTH2s and CTH2p are close to the binding sites of S1- and S2-EG peripheral stalks of V-ATPase, located on α2Nα and α2Nβ, respectively [1]. We hypothesize that recruitment of CTH2 to V-ATPase during its function as a pH-sensing and CTH2-signaling receptor may also regulate assembly/disassembly and function of the V-ATPase. Thus, our study has revealed molecular details of the interactions between the V-ATPase and CTH2, which are important for regulation of their corresponding cell biological functions.

References


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Karsberg+: A tool to predict pKa values and study proton transfer pathways in proteins using electrostatic energy calculations

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The software Karsberg+ (http://agknapp.chemie.fu-berlin.de/karsberg/) has been developed to be an easy to use tool for the prediction of pKa values in proteins using electrostatic energy calculations. A special feature of the software is the generation of pH adapted conformations. These are automatically modeled structures, representing the protein in a specific pH interval. The modeling includes relaxation of the hydrogen bond network, sampling of salt bridge geometries and in its latest version also a generalized approach that uses molecular dynamic simulations to sample structural conformations. This procedure has demonstrated to predict pKa values reliably [1,2]. The recent development of the software is focused on new features providing tools to analyze the detailed energetic and interaction of chargeable residues, based on the results of the electrostatic energy calculations. A special focus is set the identification and study proton transfer pathways in proteins.

References


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S10.P16

Mitochondrial iron regulation: Interaction of Mitoferrin-2 (Mfrn2) and the electrogenic mitochondrial calcium uniporter (MCU)

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MCU catalyzes electrogenic mitochondrial uptake of both Ca2+2 and Fe2+ (JBC 1975;250:6433). More recently, Mfrn1 and 2 were identified to mediate mitochondrial iron uptake in erythroid and non-erythroid cells, respectively (Mol Cell Biol 2009;29:1007), whereas the CCDC109A gene product (MUC) was discovered to be the core protein of the uniporter complex (Nature 2011;476:336&341). Here, our aim was to determine the role of Mfrn2 in uniporter function. Respiration-driven Ru360-sensitive mitochondrial Fe2+ and Ca2+ uptake was measured in rat liver mitochondria (RLM) and permeabilized UMCC1 and UMCC22A head and neck squamous carcinoma cells. Overexpression and siRNA knockdown of Mfrn2 were performed in UMCC1 and UMCC22A cells, respectively. Pull down assays were performed in MCU-V5 and Mfrn2-GFP expressing HeLa cells. In RLM, Ca2+ and Fe2+ (250 μM) each stimulated respiration to a nearly identical degree, an effect that was completely blocked by Ru360. In UMCC22A cells, mRNA and protein expression of Mfrn2 was 2–3 times higher than in UMCC1 cells. High Mfrn2-expressing UMCC22 cells had 3-fold greater rates of mitochondrial Ca2+ and Fe2+ uptake. After Mfrn2 knockdown (55% decrease), rates of mitochondrial uptake of both Ca2+ and Fe2+ decreased by 75%, whereas Mfrn2 overexpression increased Ca2+ and Fe2+ uptake by 56%. All uptakes were completely blocked by Ru360.
HeLa cells co-transfected with MCU-V5 and Mfrn2-GFP, anti-GFP beads pulled down MCU-V5, and anti-V5 beads pulled down Mfrn2-GFP. COX-IV was not pulled down by either bead, indicating that the interaction between MCU and Mfrn2 was specific. In conclusion, Mfrn2 positively modulates Ru360-sensitive respiration-driven mitochondrial uptake of both Ca\(^{2+}\) and Fe\(^{2+}\). Mfrn2 physically interacts with MCU.

Under physiological conditions, Na\(^+-\)PPases are strictly specific for Na\(^+\), whereas Na\(^+\)/H\(^+\)-transporting ATPases function as funnel-like transporters that form Na\(^+\) or H\(^+\) concentration gradients (or both) using the energy they release upon PPI hydrolysis [1]. H\(^+-\)PPases have been discovered in prokaryotes, plants and protists; Na\(^+-\)PPases are found in bacteria and archaeabacteria; and Na\(^+\),H\(^+-\)-PPases are mainly observed in bacteria of the human gastrointestinal tract [2]. mPPases overexpression in archaebacteria; and Na\(^+,\)H\(^+-\)-PPases are mainly observed in bacteria of the human gastrointestinal tract [2].

Membrane pyrophosphatases (mPPases) are primary transporters that form Na\(^+\) or H\(^+\) concentration gradients (or both) using the energy they release upon PPI hydrolysis [1]. H\(^+-\)PPases have been discovered in prokaryotes, plants and protists; Na\(^+-\)PPases are found in bacteria and archaeabacteria; and Na\(^+\),H\(^+-\)-PPases are mainly observed in bacteria of the human gastrointestinal tract [2]. mPPases function as funnel-like homodimers of ~75-kDa monomers [3]. mPPase overexpression in plants confers resistance to abiotic stresses, such as salinity and drought.

V-ATPases are conserved ATP-driven proton pumps that acidify organelles and energize secondary transport systems essential for lysosomal/vacuolar and endosomal functions. V-ATPase assembly and activity are glucose-dependent in yeast. Glucose depletion causes V-ATPase disassembly and its inactivation; glucose re-addition triggers reassembly and resumes proton transport and organelle acidification. Phosphofructokinase-1 is a key enzyme in the glycolytic pathway. The yeast ortholog of human phosphofructokinase-1 consists of two tetramers, each made of two subunits, Pfk1p and Pfk2p. We investigated the roles of the phosphofructokinase-1 subunits Pfk1p and Pfk2p for V-ATPase function. The pfk1Δ and pfk2Δ yeast mutants grew on glucose, indicating that they can metabolize glucose. In the wild-type cells, the subunits Pfk1p and Pfk2p co-immunoprecipitated with V-ATPase. Upon deletion of one subunit the other subunit retained binding to V-ATPase and the pfk1Δ and pfk2Δ cells assembled wild-type levels of V-ATPase pumps. The vacuolar lumen was alkalized and the cytosol acidified in pfk1Δ and pfk2Δ cells, suggestive of impaired V-ATPase proton transport in vivo. These pH alterations were more dramatic in pfk2Δ, which also exhibited a partial vma growth phenotype. Binding of disassembled V-ATPase (V1 domain) to its assembly factor RAVE (subunit Rav1p) was 5-fold enhanced in pfk2Δ. RAVE-assisted glucose-dependent reassembly and/or glucose signals were disturbed; V-ATPase reassembly was significantly reduced after re-addition of glucose to glucose-deprived pfk2Δ cells. Normal V-ATPase function and regulation were rescued after increasing the concentration of glucose, which stimulated glycolysis in pfk2Δ cells. We concluded that V-ATPase proton transport, at steady state, and V-ATPase reassembly and reactivation, after glucose re-addition, are controlled by the glycolytic flow in yeast.

Yeast phosphofructokinase-1 subunit Pfk2p is necessary for pH homeostasis and glucose-dependent V-ATPase reassembly: The role of glycolysis
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V-ATPases are conserved ATP-driven proton pumps that acidify organelles and energize secondary transport systems essential for lysosomal/vacuolar and endosomal functions. V-ATPase assembly and activity are glucose-dependent in yeast. Glucose depletion causes V-ATPase disassembly and its inactivation; glucose re-addition triggers reassembly and resumes proton transport and organelle acidification. Phosphofructokinase-1 is a key enzyme in the glycolytic pathway. The yeast ortholog of human phosphofructokinase-1 consists of two tetramers, each made of two subunits, Pfk1p and Pfk2p. We investigated the roles of the phosphofructokinase-1 subunits Pfk1p and Pfk2p for V-ATPase function. The pfk1Δ and pfk2Δ yeast mutants grew on glucose, indicating that they can metabolize glucose. In the wild-type cells, the subunits Pfk1p and Pfk2p co-immunoprecipitated with V-ATPase. Upon deletion of one subunit the other subunit retained binding to V-ATPase and the pfk1Δ and pfk2Δ cells assembled wild-type levels of V-ATPase pumps. The vacuolar lumen was alkalized and the cytosol acidified in pfk1Δ and pfk2Δ cells, suggestive of impaired V-ATPase proton transport in vivo. These pH alterations were more dramatic in pfk2Δ, which also exhibited a partial vma growth phenotype. Binding of disassembled V-ATPase (V1 domain) to its assembly factor RAVE (subunit Rav1p) was 5-fold enhanced in pfk2Δ. RAVE-assisted glucose-dependent reassembly and/or glucose signals were disturbed; V-ATPase reassembly was significantly reduced after re-addition of glucose to glucose-deprived pfk2Δ cells. Normal V-ATPase function and regulation were rescued after increasing the concentration of glucose, which stimulated glycolysis in pfk2Δ cells. We concluded that V-ATPase proton transport, at steady state, and V-ATPase reassembly and reactivation, after glucose re-addition, are controlled by the glycolytic flow in yeast.

Quantifying the importance of each site along the structure of mitochondrial carriers by monitoring single-nucleotide evolution
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Mitochondrial carriers (MCs) are proteins embedded in the inner mitochondrial membrane consisting of a tripartite structure, a three-fold pseudo-symmetry, related sequences, and similar folding whose main function is to catalyze the transport of various metabolites [1,2]. In this study, the evolutionary rate in vertebrates was screened at each of the approximately 50,000 nucleotides corresponding to the amino acids of the 53 human MCs. Using this information as a starting point, a scoring system was developed to quantify the evolutionary pressure acting on each site of the common MC structure and estimate its functional or structural relevance [3]. The degree of evolutionary selection varied greatly among all sites, but it was highly similar among the three symmetric positions in the tripartite structure, known as