Recombinant Production of Human Aquaporin-1 to an Exceptional High Membrane Density in Saccharomyces Cerevisiae

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porin-OmpPst1 of Providencia stuartii is investigated in its susceptibility for two carbapenem antibiotics imipenem and meropenem. Electrolytically measured and deduced kinetic parameters of porin and the channel. Activity of antibiotics against bacteria determined by microbiological assays correlates with the results obtained from liposome swelling assay and planar lipid bilayer measurements. In addition, the effect of divalent and trivalent cations on antibiotic affinity to the channel is investigated. To follow the exact translocation pathway, molecular modeling provides atomicistic details on the interaction of specific side chains of the two antibiotics with the channel residues and the position of affinity sites. Further, mutation of these specific sites in the channel and recording ion current fluctuation in presence of antibiotics reveal the rate limiting interaction for its translocation. Here, we have concluded on the relation between permeation of the antibiotics and resistance acquired by Providencia stuartii. Moreover, employing a multidisciplinary approach from MD simulations to protein engineering helps to get a picture of this information. This further might give insights for rational drug design, for effective uptake of antibiotics through porins: check-point of the cell.

References:

2016-Pos Board B508
Antibiotic Transport through Porins
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The emergence and dissemination of coordinated resistance mechanism (MDR) underline the importance to understand the rate limiting steps in antibiotic action. Here OmpF porin from E. coli is used as an example to demonstrate what limits the penetration of carbapenems. Bacteriological kinetic killing assays, temperature dependent ion conductance measurements, and all-atom computer simulations were combined to study interaction and translocation of clinically relevant β-lactam antibiotics through wild type OmpF and two mutants D113A and D121A, where the key residues at the constriction region have been substituted. Expression of these various OmpF mutants in an otherwise porin-null bacterial strain revealed an increase of bacterial susceptibility for the mutants. High-resolution conductance measurements and modulating the temperature indicates lower energy barriers for mutant porin correlating with the microbiological assays. All atom modeling provided a most probable molecular picture. This information further might give insights for rational drug design, for effective uptake of antibiotics through porins: check-point of the cell.

References:

2017-Pos Board B509
Characterization of a Cyanobacterial Outer Membrane Protein: An E. Coli Tole Homologue from Synechocystis Sp. Pcc 6803
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E. coli TolC (tolerance to colicins) represents an interesting class of outer membrane (OM) proteins, as it has an α-helical periplasmic tunnel and β-barrel membrane region, providing a conduit for export of metabolites and xenobiotics from cell interior to exterior, and import of colicin E1 (1). A TolC homologue, Slr1270 from Synechocystis 6803, cloned and expressed in E. coli, has > 40% similarity and ~16% identity to E. coli and Pseudomonas counterparts, and has a similar domain organization. Homology modeling using Pseudomonas OmpM as template modeled 93% of Slr1270 sequence. The 1581bp slr1270 gene was cloned and overexpressed in E. coli. Protein from inclusion bodies, refolded through step-wise dialysis showed major bands at ~55kDa and >150 kDa on SDS-PAGE corresponding to the monomer and trimer respectively and ~300 kDa on CN-PAGE. Purified protein displays a far-UV CD spectrum characteristic of E. coli TolC with >50% α-helix, and formed channels in planar lipid bilayers with a characteristic single channel conductance of ~50 pS in 0.1M NaCl. The intact protein mass spectrum (LC-MS) with a major peak at 54,489 probably represents a mixture of two species, the TolC product with 1-40 removed and an intact 6-His tag (calculated mass 54,457.1 Da), and a product with 1-38 removed and a 5-His tag (calculated mass 54,490.2 Da) after a single carboxypeptidase event. The small peak at 54,638 Da probably corresponds to TolC product with 1-38 removed and an intact 6-His tag (calculated mass 54,627.3 Da). Peptides 39-76 and 41-76 were recovered from trypsin digestes confirming N-terminal homology.

References:

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Electrophysiological Analysis of PapC Mutants Provides Insights into the Mechanism of Plug Displacement
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The PapC usher is a twin beta barrel pore of the outer membrane of urapathogenic E. coli used for the assembly of the P pilus, a key virulence factor in bacterial colonization of human kidney cells. The usher catalyzes the translocation and ordered addition of folded pilus subunits delivered by the chaperone PapD to the growing pilus. Each PapC monomer is a 24-stranded beta barrel, flanked by N- and C-terminal globular domains and occluded by a large plug domain (PD). The alpha helix and the beta 5-6 hairpin loop are additional structural components that may play a role in controlling plug dynamics. Indeed, structural studies have revealed that the PD is released to the periplasmic side during pilus biogenesis, but the exact mechanism for PD displacement has not yet been elucidated. Several key residues that may be critical for plug stabilization have been proposed. They cluster in regions at the interface of the plug, the barrel, the alpha helix and the hairpin, and appear to belong to either electrostatic or allosteric networks. In order to assess the roles of these residues in plug displacement, we have used planar lipid bilayer and patch-clamp electrophysiology to compare the activity of the wildtype channel with that of mutant channels with either single or multiple alanine substitutions at these sites. Many mutants showed an increased propensity at plug displacement, as witnessed by openings with a conductance similar to that of the previously characterized plugless mutant (~ 3 nS). Others displayed quieter than WT activity. In addition, evidence of modal gating was observed in WT and some mutants. Together, these mutants provide insight into the molecular mechanism of PD displacement for pilus assembly and translocation through the PapC usher.

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Recombinant Production of Human Aquaporin-1 to an Exceptional High Membrane Density in Saccharomyces Cerevisiae
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Due to the lipid nature of cellular membranes preventing transport of most solutes between the cytosol and the extracellular environment as well as from the cytosol to the interior of organelles, cellular homeostasis relies on integral membrane proteins allowing selective trans membrane movement of solutes. Aquaporins constitute a family of physiologically very important integral membrane proteins that are found in all three kingdoms, eubacteria, archaea and eukaryotes. As protein channels, they facilitate passive transport of water and small molecules at high rates across cells. In addition, they take part in a number of specific biological processes. Aquaporin-1 (Aqp1) is a non-selective water channel constituting up to 8.5% of total membrane protein content in vivo and is present in almost all types of mammalian cells. The exact mechanism for PD displacement has not yet been elucidated. Several key residues that may be critical for plug stabilization have been proposed. They cluster in regions at the interface of the plug, the barrel, the alpha helix and the hairpin, and appear to belong to either electrostatic or allosteric networks. In order to assess the roles of these residues in plug displacement, we have used planar lipid bilayer and patch-clamp electrophysiology to compare the activity of the wildtype channel with that of mutant channels with either single or multiple alanine substitutions at these sites. Many mutants showed an increased propensity at plug displacement, as witnessed by openings with a conductance similar to that of the previously characterized plugless mutant (~ 3 nS). Others displayed quieter than WT activity. In addition, evidence of modal gating was observed in WT and some mutants. Together, these mutants provide insight into the molecular mechanism of PD displacement for pilus assembly and translocation through the PapC usher.
Plant aquaporins regulate water fluxes across membranes by enhancing membrane water permeability ($P_w$). In particular, the plant plasma membrane holds PIPs, one of the largest groups of aquaporins. PIPs are divided in two clusters (PIP1 and PIP2) that disclose intriguing aspects: i) the potential of modulating $P_w$ by PIP1-PIP2 co-expression, distinguished for each PIP showing differential capacity to reach the PM and ii), the facility to reduce water permeation through the pore after cytosolic acidification, as a consequence of a gating process. Our working hypothesis is that cytosolic pH (gating) and PIP co-expression (trafficking) enhance plasticity to the membrane water transport capacity as a consequence of a PIP1-PIP2 cooperative interaction. Thus, PIP1 cellular trafficking and its effect in water permeability emerge as playing a key role as a regulatory mechanism. To analyze this interaction we used PIP1-PIP2 pairs from different species (Fragaria ananassa and Beta vulgaris). Our experimental approach included i) designing mutants to alter the PIP1-PIP2 interaction by means of site directed mutagenesis; ii) tracking aquaporin localization at internal structures or expressed at the level of the PM; and iii) analyzing water transport capacity in control and inhibited (medium acidification) conditions by means of measuring $P_w$ in Xenopus oocytes. Our finding support evidences in agreement with the concept that PIP2 and PIP1 interact to form functional heterooligomeric assemblies, and thus the composition of these PIP assemblies determines their functional properties. As PIP1 alone is not able to reach the plasma membrane its contribution to enhance water permeability is associated to its translocation and interaction with a PIP2. This regulatory mechanism seems to be present in different vascular plants. This information is integrated in a proposal for water transport pathways including the organs where these PIPs are present.

Selective Filter Scanning of the Human Voltage Gated Proton Channel Hv1

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Selectivity Filter scanning revealed that CYMAL-5 was superior in solubilizing recombinant Aquaporin-1 and generated a monodisperse protein preparation. A single Ni-affinity chromatography step was used to obtain almost pure Aquaporin-1.

The Permeation Pathway Mechanism in Ciona Intestinalis Hv Channel Ester Otarola David E. Baez-Nieto, Gustavo Contreras, Osvaldo Yañez, Karen Castillo, Peter Larsson, Ramon Latorre, Carlos Gonzalez.

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Voltage-gated proton (HV) channels are expressed by different cell types including immune cells, microglia, among others. In other voltage-gated cation channels, the pore-forming domains are S5, S6, and the P loop connecting S5 and S6. Since Hv channels do not contain a S5-S6 region, other parts of the channel must form the pore domain. Interestingly, Hv channel present three gating charges in the S4 followed by an asparagine, N264, highly conserved among all Hv channel. Using non-stationary fluctuation analysis we establish the conductance for the dimer and the monomer in 200 and 100 fS, respectively. Mutations at position S191 (S2) and N264 (S4) modified the unitary conductance of Hv channel. Furthermore, mutants S191E/D and N264R removed the H⁺ current remaining the S4 functioning unaltered, according to voltage-clamp biophysics experiments. The introduction of an arginine or lysine at position 264 or negative residue at position S191, drastically reduced or abolished the proton currents. Interestingly, mutations of the analogue position S191 of Ciona VSOP, in the voltage sensor domain of a non-conductor Shaker K⁺ channel produce fully functional voltage gated H⁺ channels. In the same way, mutation resembling the position N264 of Ciona Hv in the VSD of the voltage-dependent phosphate Ci-VSP spontaneously entail the formation of a voltage-gated H⁺ channel. Thus, S191 and N264 form the molecular determinants of permeation pathway in Hv channel. Supported by Fondecyt ACT 1104 and Fondecyt 1120802 to CG and 1110430 to R.L.