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Permeation Of Beta-lactam Antibiotics Through E. Coli Ompf Altered By Constriction Zone Mutations

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Accelerated growth of resistance of pathogenic gram-negative bacteria to various antibiotics makes evaluation of highly efficient assay for antibiotic screening an important task. Control of the outer membrane permeability and/or increase of the antibiotic efflux via various efflux pumps prevent the antibiotic penetration into the cell. It was found earlier that beta-lactam antibiotics translocate into bacteria mainly via nonspecific porins like E. coli OmpF. Some of the recent studies showed that the mutations in the OmpF alter the translocation rate of antibiotics and this mechanism is still vigorously investigated. Data indicate that the structural and functional effect of each mutation should be taken into account separately and is individual for each antibiotic. It is expected that the constriction region of the porin play a very important role in antibiotic passage. This zone is characterized by a strong electric field, where negatively charged residues Asp113, Glu117 face a cluster of positively charge residues Arg42, Arg82, and Arg132. In the present study OmpF mutants were incorporated into the planar lipid bilayer and ionic current through the channels was analyzed in the presence of beta-lactams. As an additional method to investigate the process of permeation we employed liposome swelling assay technique that has been applied previously to such problems with success. The advantage of this technique is that penetration rate of antibiotic in proteoliposomes generally mimic that into the intact cells and swelling rates are directly proportional to the permeability of antibiotic in vivo. Finally, molecular dynamic simulations were used to study the event of translocation through OmpF in molecular level. Thus is become possible to obtain the objective picture of permeation mechanism which later can be applied in evaluation of highly efficient antibacterial drugs.

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Calculation of Current-Voltage properties of Biological Ion Channel, using Poisson-Nernst-Planck (PNP) Theory

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Biological ion channels are integral membrane proteins that are usually formed by relatively large proteins. Studying current-voltage (I-V) characteristics of channels is commonly used to understand how channels function and estimate effectiveness of existing and potential drugs. The current - voltage characteristics of a channel depend on the channel's structure and conformation. Current conduction through a channel in the protein is a slow process, which makes the traditional methods for theoretical modeling of proteins (such as molecular dynamics or Monte-Carlo simulations) practically inapplicable for direct modeling of channel function. Therefore other simplified theoretical methods have been developed. One such method is the Poisson-Nernst-Plank (PNP) theory of electrodiffusion, where a set of partial differential equations (the Poisson and the Nernst-Plank equations) are solved self-consistently. We are developing the PNP equations solver (PNPS) for calculating current-voltage properties of ion channel proteins. To improve computational efficiency the solver was parallelized. The new solver has been applied to predict ion conductance properties of the α-Hemolysin channel, a robust and well studied pore forming heptameric protein complex. Because of the asymmetry of the protein structure its position with respect to the lipid bilayer has not been well determined. We performed a - series of calculations in which the membrane position has been varied. pKa calculations show that the protonation state of some residues depends on the membrane position. The results of PNP calculation are compared with experimental data on channel conductance, ion selectivity, reverse potential, rectification properties. Such detailed analysis allowed us to pinpoint position of the protein in the membrane. Several methods for setting diffusion coefficients were tested. We have also investigated models for interaction of a permeant ion with the protein and its mobility in the constricted environment of the protein pore.

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Computational Study on the Ion Selectivity of Modified Alpha-Hemolysin Channels

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Alpha-hemolysin (AHL) monomers are secreted by the bacterium Staphylococcus aureus. They self-assemble into heptameric beta-barrel AHL channels, which act as exotoxins by forming wide pores in the outer membrane of cells. AHL channels can be successfully engineered for applications in biotechnology such as stochastic sensing of molecules and DNA sequencing. Ion permeation through AHL can be manipulated via mutations and by lodging molecular adapters such as the cyclic polysaccharide beta-cyclodextrin (BCD) in the pore. In order to clarify how BCD and BCD derivatives can alter the permeability and ion selectivity of AHL, we have performed potential of mean force (PMF) calculations and grand canonical Monte Carlo/Brownian dynamics (GCMC/BD) simulations on the basis of the x-ray structures of wild type AHL and two AHL mutants. The computed current-voltage curves and reversal potentials with and without BCD bound reproduce the experimentally observed increase in anion selectivity after adding BCD. The PMF free energy profiles of single and multiple ions along the channel axis show that BCD reduces the ionic and dielectric shielding of positively charged residues and, thus, amplifies the weak anion selectivity of AHL. Our results for a positively charged BCD derivative predict a further increase in anion selectivity and also more current compared to BCD.

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Vectorial Ion Transport by Channelrhodopsin-2

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Channelrhodopsins represent a third member of microbial-type rhodopsins and have gained considerable attention in neurobiology as a tool to control the excitability of neurons. The other photoreceptors act either as light-driven ion pumps like bacteriorhodopsin (BR) or as part of a relay system like the sensory rhodopsins (SR). In contrast, channelrhodopsins are light-gated ion channels that allow the passive permeation of cations over the membrane barrier after light activation. Previously, we could follow the spectral characteristics of different photointermediates and relate them to the functional states of the channel. In the gating process, the Schiff-base undergoes a deprotonation reaction before it relaxes into a red-shifted species. The red-shifted photointermediate is characteristic for the open state with a lifetime of 10 ms.
So far, the role of the protonation reactions is not fully understood. Channelrhodopsins possess a glutamate residue at the homologue position of the proton acceptor D85 in BR. The main difference among the microbial-type rhodopsins is found in the nature of the proton donor at the opposite side of the retinal moiety towards the cytoplasm: In BR, an aspartic acid reprotonates the Schiff-base in a fast manner as expected for an efficient proton pump, while in SR an aromatic residue leads to a long-lived deprotonated state of the Schiff-base. In channelrhodopsins, the homologue residue is a histidine that could allow a reprotonation of the observed deprotonated Schiff-base from the cytoplasmic side. As a net result, one proton would be transported per photocycle. Here we show that one can indeed observe such a vectorial ion translocation under illumination of purified and proteoliposome reconstituted channelrhodopsin-2. Therefore, the mechanism of channelrhodopsin-2 shows similar features as other rhodopsins, i.e. control of the accessibility to the Schiff-base by light-induced isomerization of the retinal and vectorial ion transport.

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Directional Ion Selectivity In An Ion Channel With Bipolar Charge Distribution

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The selectivity of the bacterial porin OmpF from E. coli to small inorganic ions has been investigated by single channel experiments. In a recent study, we showed that the OmpF channel may function as a pH-regulated, biological, nanofluidic diode (J. Phys. Chem. B 110 (2006) 21205). Here we show that Reversal potential measurements done under asymmetric conditions of pH and salt concentration provide valuable information about the channel fixed charge distribution that cannot be extracted from the rectification displayed in current-voltage curves. We find that the pH gradient imposed across the pore induces an asymmetric fixed charge distribution that resembles the structure of a synthetic bipolar membrane (a composite of an anion-exchange membrane and a cation-exchange membrane used to split water under reverse polarization conditions). This particular arrangement demonstrates that the ionic selectivity of a non-uniformly charged pore is not an intrinsic property of the system but depends crucially on several external factors. Amazingly, changing the direction of the salt concentration gradient can turn a cation selective channel into an anion selective one.

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Relative Dielectric Permittivity And Resting Membrane Potential In Living Cells Suspensions: An Experimental Approach

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