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-lactam antibiotics. As hitherto not available for the investigation of the method we employed liposome swelling assay technique that has been previously suggested to study problems with success. The advantage of this technique is that it allows to investigate the current-voltage characteristic of the antibiotic in proteoliposomes generally mimicking the intact cell and swelling rates are directly proportional to the permeability of the antibiotic in vivo. Finally, molecular dynamic simulations were used to study the structure of the porin. The results of PNP calculation are compared with experimental data on channel conductance, ion selectivity, reverse potential, rectification properties, and its mobility in the constriction environment of the protein pore.

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, nanofluodieorimeter (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.
We present a method to obtain the resting membrane potential ($\Delta W$) from the dielectric behavior of a suspension of living cells by the use of dielectric spectroscopy. Since cells behave as conducting particles surrounded by low-conducting shells with surface charge densities, we can apply this technique to record the dielectric permittivity $\varepsilon$ and conductivity $\sigma$ of the suspension as a function of frequency. A previous theoretical model has correlated the relative dielectric permittivity $\varepsilon$ of the suspension with resting membrane potential in the very low radio frequency regime (alpha). We use this model with our experimental results to obtain $\Delta W$ for bacteria (E.Coli K12) and mammalian cell suspensions and compare our values with the traditional methods-voltage sensitive dyes and patch clamping. For E. Coli measurements, resting membrane potential is changed by KCl addition to the suspension bath. As for mammalian cells, $\Delta W$ changes are triggered by the use of various pharmaceutical compounds that act as HERO $K^+$ channel blockers and $I_{\text{C}}$ values are computed for each compound. Precise measurements of the dielectric permittivity $\varepsilon$ and conductivity $\sigma$ of live cells suspensions in the alpha frequency regime require prior elimination of the polarization errors. Polarization errors are caused by the ionic content of a buffer, and they affect the total impedance in the low frequency interval. We hereby present our approach of measure the polarization impedance then remove it by fitting both real and imaginary experimental curves with an ideal impedance $Z = \text{Re} + \text{Im} \cdot \frac{1}{\text{Sr}}$, where $\text{Re} + \text{Im} = 1 + i \cdot \text{Sr}$.

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Impact Of Na$_\text{1.7}$-PEPD Missense Mutations That Slow The Rate Of Inactivation On Sensory Neuronal Resurgent Sodium Currents
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Voltage-gated sodium (Na$_\text{1.1-9}$) channels are dynamic transmembrane proteins that, in response to changes in the potential across the lipophilic cell membrane, undergo specific conformational (gating) modifications, between ion-conducting (open) and non-conducting (closed and inactivated) states, to selective ion-conduction. Sodium ions permeate through their aqueous pore. Importantly, changes in these voltage-dependent gating properties can impact action potential (AP) characteristics. TTX-sensitive sodium channels in cerebellar neurons can produce resurgent currents (Raman & Bean, 1997), intriguing currents that are re-activated during intermediate repolarizations following strong, but short, depolarizations. We observe resurgent currents in some DRG neurons and found that wild-type Na$_\text{1.7}$ but not wild-type Na$_\text{1.7}$ channels can generate resurgent currents in DRG neurons (Cummins et al., 2005). It has been demonstrated that, in cerebellar neurons from Na$_\text{1.6}$-null mice, slowing inactivation of the remaining Na$_\text{1.7}$ currents can induce resurgent currents (Grieco & Raman, 2004). Interestingly, single-point missense mutations in the SCN9A gene that encode for Na$_\text{1.7}$, implicated in paroxysmal extreme pain disorder (PEPD), slow the rate of Na$_\text{1.7}$ inactivation (Jarecki et al., 2008). Therefore, we hypothesized that slowing of Na$_\text{1.7}$ by PEPD mutations might induce abnormal resurgent currents, thus altering AP properties. To explore this hypothesis, we transiently transfected adult rat DRG neurons with a TTX-resistant form of human Na$_\text{1.7}$-wild type or PEPD mutant cDNA and rat Na$_\text{1.8}$-targeted shRNA. Voltage-dependent properties were observed using whole-cell voltage-clamp electrophysiology and AP generation was tested using current-clamp electrophysiology. Recordings were made in the presence and absence of extracellular TTX. These experiments should yield insight into (1) the mechanism of resurgent sodium current generation in DRG neurons, (2) a potential additive effect in channel dysfunction observed in PEPD, and (3) how these mutant channels contribute to alterations in AP characteristics.