Membrane Transporters & Exchangers I

2605-Pos

Determination of the Number of Molecules Involved in Different Types of Amphotericin B Channels

Javier Iñiguez-Stahl, Javier González-Damián, Iván Ortega-Blake.
Universidad Nacional Autónoma de México, Cuernavaca, Mexico.

Electrophysiological properties of Amphotericin B (AmB) pores in lipid bilayers have been studied extensively with the purpose of understanding membrane transport phenomena[1]. Also because AmB has a clinical relevance, it is the drug of choice for treating systemic fungal infection in spite of having strong collateral toxicity. One of the interests has been to determine the number of molecules involved in the formation of the ion channels reported. Reports in the literature present values that go from 4 to 12. This has been explained because channels with different conductivities are formed[2] and therefore the determination with macroscopic currents corresponds to an average of the population of different channels. In this work, we determined the number of molecules of AmB involved in the formation of each one of these pores by single channel methods[3]. Our results show that different types of channels are formed by the same number of AmB molecules, which is very interesting, considering that these channels have certainly different radius. This suggests a model in which AmB pores are not the only component of the pores, having either lipids in a toroidal structure or sterols in a barrel structure.

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2606-Pos

Double Packaged System for Localized Drug Delivery for Ovarian Cancer

Eva Christabel Williams, Ryan Toomey, Norma Alcantar.
University of South Florida, Tampa, FL, USA.

This research aims at developing a drug delivery system that will provide a plethora of benefits such as cost effectiveness, reduction of toxicity and a control over the release of chemotherapeutic drugs in Ovarian Cancer patients. We have designed a model drug delivery system consisting of non-ionic surfactant vesicles (niosomes) packaged within a biodegradable, temperature and pH sensitive hydrogel (chitosan) network. Optimization of the release rates were accomplished by altering the condition of its two components, chitosan and niosome. Two ovarian cancer drugs Paclitaxel and Carboplatin were used for encapsulation. It was found that medium molecular weight chitosan with a crosslinker:chitosan ratio of 4:1 which corresponded to a pH of 7.4 resulted in the finest controlled release. Surface characteristics, such as the interaction between the niosomes and chitosan were determined using Surface Forces Apparatus. The system was also tested in-vivo in mice models. Xenogen was used to study the release of drugs in-vivo. Our results will help in the development of a low cost and improved method for drug delivery with application to intracavitary ovarian cancer treatment and other cancer types.

2607-Pos

Acid-Activation, Proton Transport Rate Saturation, and pH-Dependence of Amantadine Block for Influenza a M2 Protein Truncate (22-62)

Emily Peterson[1], Myunggi Yi[2], Huan-Xiang Zhou[2], Mukesh Sharma[2], Timothy A. Cross[2], David Busath[1].
[1]Brigham Young University, Provo, UT, USA, [2]Florida State University, Tallahassee, FL, USA.

A novel truncate (residues 22-62) of the influenza A M2 protein was tested with pH-sensitive proteoliposome proton uptake assays. At six different pHs, from 6.5 to 3.8, the proton flux into M2-containing liposomes (0.1 mg protein: 20 mg E. coli polar lipid:1 mL) increased to a maximum at pH 4.9, and then decreased at the lowest measured pH levels. The lack of proportionality to [H+] and saturation of flux indicate protein activity consistent with transporter function, rather than a traditional ion "channel." To gain mechanistic insight, the transport data are analyzed within a kinetic model in which the four His34 residues are obligatory binding sites for transported protons, and related to some results from molecular dynamics simulations. Application of the antiviral drug amantadine (0.1 mM) to the assay blocked ~80% of the M2 function at pH 6.5, as expected when compared to published block efficiency in other, similar systems. However, amantadine block became less effective as the pH of the assay dropped, in keeping with observations by others from analytical centrifugation and Xenopus oocyte transport studies.

2608-Pos

Can Passive Asymmetric Vacant Carrier Distributions Counterbalance Asymmetric Carrier Affinities?

Richard J. Naftalin.
King's College London, London, United Kingdom.

There is a popular fallacy that asymmetric vacant carrier distributions account for asymmetric transporter affinities, e.g. GLUT1 and SGLT1. An alternating site transporter can be passively asymmetrically distributed, because of differences in standard free energy, μ'_c, or activity coefficients, γ_c of the vacant carrier isoforms, c^i between the opposing membrane sides; i.e. either $\mu'_c{}^{in} < \mu'_c{}^{out}$ or $\gamma_c{}^{in} < \gamma_c{}^{out}$. Thus, at equilibrium the Gibbs free energy difference, ΔG_c of the vacant carrier distribution, $\Delta G_c = \Delta\mu_c' + RTLn(c_c{}^{out}/c_c{}^{in}) = 0$. Alternatively, if only the activity coefficients are unequal, $\Delta G_c = RTLn((c_c{}^{out}.\gamma_c{}^{out}) /(c_c{}^{in}.\gamma_c{}^{in})) = 0$. In both cases, at equilibrium, when $\Delta G_c = 0$, $c_c{}^{out} < c_c{}^{in}$.The carrier partition coefficient between the inside and outside membrane phases $P_{in/out} = c_c{}^{in}/c_c{}^{out}$ hence $\Delta\mu'_c{}^{out\text{-}in} = -RTLn(P_{in/out})$ and $\Delta G_c = RTLn(c_c{}^{out}/(P_{in/out}.c_c{}^{in})) = 0$ Since at equilibrium, unidirectional vacant carrier flows $k_{ij}.c^i$ are equal, $k_{out\text{-}in}.c_c{}^{out} = k_{in\text{-}out}.c_c{}^{in}$, hence $c_c{}^{in}/c_c{}^{out} = k_{out\text{-}in}/k_{in\text{-}out} = P_{in/out}$ The flux ratio equation, $\Delta G_c = RTLN(k_{out\text{-}in}/k_{in\text{-}out})$, applicable to reactions in homogeneous membrane phases, requires a correction for asymmetric carrier equilibration between heterogeneous membrane phases, becoming: $\Delta G_c = RTLn(k_{out\text{-}in}/(P_{in/out}.k_{in\text{-}out}) = 0$, or $\Delta G_c = RTLn(\gamma_c{}^{out}.k_{out\text{-}in}/(\gamma_c{}^{in}.k_{in\text{-}out}) = 0$. However, when these corrections are applied, neither the asymmetric flux ratios, nor vacant carrier distributions compensate for the energetic difference existing from the asymmetric ligand affinities, i.e. if $K_D{}^{out} < K_D{}^{in}$ then $\Delta G_c{}^{affinities} = RTLn(K_D{}^{out}/K_D{}^{in}) < 0$. Since a passively acquired asymmetric distribution at equilibrium generates no force, no energy is available from the vacant carrier distribution to counterbalance any energy difference between the asymmetric ligand affinities. Therefore, asymmetric transport needs another rationalization.

2609-Pos

Characterization of Mutant hCTR1 Copper Transporters Truncated by Proteolytic Cleavage in the Absence of O-Linked Glycosylation at Thr27

Ed B. Maryon, Jack H. Kaplan.
Univ. of Illinois at Chicago, Chicago, IL, USA.

The human copper transporter hCTR1 is a plasma membrane protein of 190 amino acids that contains three transmembrane segments. Three hCTR1 polypeptides form symmetrical homotrimeric complexes that contain a permeation pathway for copper transport across the plasma membrane that is formed by the transmembrane segments (1). Little is known about the role(s) of the 65 amino acid extracellular amino terminus.

In previous studies (2-4) we showed that extracellular terminus of hCTR1 contains both N-linked (at Asn15) and O-linked (at Thr27) sites of glycosylation. If O-glycosylation at Thr27 is prevented by mutation or by expressing wild type hCTR1 in mutant cells that cannot initiate O-glycosylation, hCTR1 is efficiently cleaved, removing 31-32 amino acids from the amino-terminus. The cleaved amino terminal peptide accumulates in punctate structures in the cytoplasm that overlap compartments containing Rab9, indicating that hCTR1 cleavage occurs in a late golgi or late endosomal compartment. The amino-terminal truncated hCTR1 transporters are delivered to the plasma membrane, where they exhibit about 50% of the 64Cu uptake of full-length (wild type) hCTR1 transporters.

We have further examined the truncated hCTR1 transporters for defects in copper transport and copper-stimulated endocytosis, and we have characterized wild type and amino terminal truncated hCTR1 transporter complexes in native gels. The truncated transporters retain some copper regulatory and transport characteristics, such as copper-stimulated endocytosis. Analysis of full-length and amino-terminal truncated complexes in native gels indicates that the truncated hCTR1 transporters may lack non-hCTR1 components present in wild type hCTR1 complexes.

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