761-Pos Board B640

Roles Of The Membrane Protein CD36 In Fatty Acid Transport And Metabolism

Su Xu, James A. Hamilton.
Department of Physiology&Biophysics, Boston University, Boston, MA, USA.

Obesity has become a major public health concern and represents a predisposition factor for the development of cardiovascular-related diseases and non-insulin dependent diabetes mellitus. Therefore, deciphering the molecular mechanisms of fatty acid (FA) uptake will provide new insights for dietary and other therapeutic interventions for managing diseases associated with obesity. FA uptake into cells occurs by multiple mechanisms, including transport and metabolism, beginning at the plasma membrane. Understanding the contributions of passive diffusion and facilitated transport by plasma membrane proteins, such as FAT/CD36, requires experimental approaches that separate biophysical and metabolic mechanisms. In previous experiments with protein-free lipid bilayers, we have used multiple fluorescence assays to show that FA bind to, and diffuse through, phospholipid bilayers very rapidly but desorb more slowly from the lipid into the aqueous phase. Here we apply these methods to HEK293 cells engineered to stably express CD36. FA movement across the plasma membrane occurred rapidly (within sec) with or without expression of CD36. HEK293 cells without CD36 exhibit very slow conversion of FA into acetylated products. However, incorporation of 14C-labeled oleic acid into triglycerides occurred more rapidly and was significantly increased in HEK293 cells overexpressing CD36. Formation of small lipid droplets was observed after incubation with a fluorescent FA analog BODIPY-FA. Thus, FA transport through plasma membranes occurred by the mechanism of diffusion without a requirement for CD36. It appears that this protein enhanced metabolism by an as yet unknown mechanism.

762-Pos Board B641

Analytical Description Of The Diffusion Coefficient And Current Of A Single Particle On A Two-dimensional Corral Model

Hernan L. Martinez.
California State University, Dominguez Hills, Carson, CA, USA.

The study of lateral dynamics in corrals has attracted atention since 1983 when Sheetz introduced the corral model. In this project, we propose a simple model consisting of a two-dimensional lattice containing periodically distributed flashing walls. These walls are represented by finite asymmetrical flashing potentials which isolate a portion of the lattice to represent semipermeable corrals. Contigous corrals share a wall creating an arrangement of similar compartments on the lattice. We included the presence of a constant external field to account for the effect of gradients, adding another degree of complexity to the dynamics of a single particle in this medium. We derive general analytical expressions to describe the diffusion coefficient (D) and the current (J) of a single particle moving on this medium. We use a formulation based on a single particle microscopic model and a diffusion relaxation condition to derive our equations as a function of the corral's size (and concentration), the time between flashes, and the strength of the external field. We compare our theory against Monte Carlo simulations.

763-Pos Board B642

Phase Transitions in Single Nano-Vesicles

Poul M. Bendix, Peter P. Wibroe, Dimitrios Stamou.
Bio-Nanotechnology Laboratory, Department of Neuroscience and Pharmacology & Nano-Science Center, University of Copenhagen, Copenhagen, Denmark.

Phase transitions in nano-scale lipid vesicles are believed to be influenced by the morphology as well as the finite size of the system. Both the effect of finite size and the asymmetric lateral stresses in the bilayer are believed to broaden the phase transition in small vesicles which has been confirmed by bulk studies. However, it still remains to be resolved whether the measured phase transition of the ensemble results from a broadening of the phase transition in individual vesicles or to heterogeneities among single vesicles exhibiting sharp phase transitions. We investigate the transition from gel (ordered) to fluid (disordered) state in single nano-sized lipid vesicles, composed of only ~10^4 molecules, by measuring the anomalous permeability which peaks at the phase transition. We study single vesicles by immobilizing them on functionalized substrates. The vesicle size and efflux/influx for each vesicle is accurately quantified which allows us to study the effect of curvature on phase transitions. To assess the width of the phase transition we probe the pore size at different temperatures using chromophores of different molecular radii. Our data suggest a revaluation of the currently accepted concepts about the nature of permeation at Tc and of phase transitions in nano-scale systems.

764-Pos Board B643

Intracellular Water Lifetime Depends on Cellular Energetic State

Yajie Zhang[1], Marie Poirer-Quinot[1], Charles Springer[2], James Balschi[1].
[1]Brigham and Women's Hospital, Boston, MA, USA, [2]Advanced Imaging Research Center, Oregon Health Science University, Portland, OR, USA.

Intra- and extracellular water undergoes equilibrium exchange *via* mechanisms that include passive diffusion across the membrane and movement through channels, like aquaporin. Longitudinal ^1H_2O NMR relaxography can employ extracellular relaxation agent $GdDTPA^{2-}$ to distinguish intra- and extracellular ^1H_2O signals by creating a difference in their relaxation time constant (T_1) values. Inverse Laplace transform of relaxation decay produces the relaxogram, the T_1 distribution, which reports *apparent* water populations, p_i' and p_e'. The *true* water fractions, p_i and p_e - measures of the volume fractions v_i and v_e, are determined if the kinetics of transcytolemmal water exchange (the mean intracellular water life time τ_i) are quantified using two-site-exchange analysis. The ''well-mixed'' kinetic expression is: $\tau_i^{-1} = P_W(A/V)$, where P_W is the water permeability coefficient; A and V are the *individual* cell surface area and volume, respectively. Furthermore: $v_i/\tau_i = P_W S$, where S is the *total* cell surface area. We determined the relationship between τ_i and cellular energetics in suspensions of *Saccharomyces cerevisiae*. Under aerobic conditions τ_i was 333 ($\pm$ 4) ms; under anaerobic conditions τ_i was 670 ($\pm$ 62) ms. Changing from aerobic to anaerobic conditions also resulted in a decrease of ATP content. In the yeast strain MR6, which requires adenine supplementation to grow, ATP content increased with medium adenine concentration. As ATP was thus increased, the τ_i was found to inversely correlate with ATP content ($R^2 = 0.96$). Therefore, τ_i is a sensitive indicator of the cellular energetic state and energetics affects the kinetics of transcytolemmal water exchange. The aerobic $P_W S$ was greater than the anaerobic $P_W S$. The underlying mechanism(s) are not yet clear. Experiments with baker's yeast stored in an anaerobic state found that inhibition of protein synthesis prevented the anaerobic-to-aerobic τ_i decrease and that aquaporin is not responsible.
1

765-Pos Board B644

Interaction Of Enrofloxacin With Model Membrane Systems. Implications In The Permeation Pathways, Revealed By Fluorescence And Conductance Studies

Paula Gameiro[1], Isabel Sousa[1], Tivadar Match[2], Mathias Winterhalter[2].
[1]REQUIMTE, Faculdade de Ciências, PORTO, Portugal, [2]Jacobs University, Bremen, Germany.

With the actual increasing menace of bacterial resistance, the antibiotic permeation mechanisms have been the centre of attention, in many research fields. OmpF is a pore forming protein, found in Gram-negative bacteria's outer membrane, known to have an important role in the uptake of nutrients and antibiotics (e.g. quinolones), towards the interior of the bacterial cell. Although several studies have been published of protein-drug interaction in the complex microbiological environment, an understanding of this mechanism at a molecular level is still lacking. Quinolones are one of the most prescribed classes of antibiotics, both in human and veterinary medicine, and this wide use seems to be the main cause for bacterial resistance. Fluoroquinolones were developed to increase quinolone antibacterial activity by changes in the quinolone structure. Enrofloxacin is a second generation fluoroquinolone being the first one to be introduced in Veterinary medicine. Its human analogue is the well known Ciprofloxacin. The main purpose of this work was to study Enrofloxacin permeation pathways. Permeation or interaction of antibiotics with lipid bilayers (liposomes) is of great importance in order to clarify the lipid component function's in membrane permeation. A first approach was the determination of partition coefficients Enrofloxacin/liposomes, which allowed the quantification of this interaction. After, OmpF was reconstituted in liposomes (proteoliposomes) by two different reconstitution methods (Gel exclusion chromatography and using detergent adsorbing beads) in order to compare size, homogeneity and protein orientation in proteoliposomes. Proteoliposomes quenching studies, using water soluble quencher KI and acrylamide, as well as ion conductance measurements were performed to establish the site of interaction/translocation of Enrofloxacin.

766-Pos Board B645

The Porin passport control - Conductance measurements and biological relevance

Mahendran R. Kozhinjampara[1], Tivadar Mach[1], Andrey N. Bessonov[1], Helge Weingart[1], Chloe E. James[2], Jean-Marie Pages[2], Eric Hajjar[3], Amit Kumar[3], Matteo Ceccarelli[3], Mathias Winterhalter[1].
[1]Jacobs University Bremen, Bremen, Germany, [2]Universite de la Mediterranee, Marseille, France, [3]Universita degli Studi di Cagliari, Monserrato, Italy.