Platform BC: Membrane Physical Chemistry II

3253-Plat

Interaction of DNA-PAMAM Dendrimers with a Model Biological Membrane

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The systemic delivery of DNA for gene therapy requires control of DNA compaction by an agent, such a lipid, surfactant or a polymer (e.g. cationic dendrimers). The crucial step here is to make the DNA cross the membrane to gain entry into the cell. Poly (amido amine) (PAMAM) dendrimers show great promise as synthetic gene-transfection agent. We have studied the structure of the complexes formed between DNA and PAMAM dendrimers as well as their interaction with model membranes, using a range of biophysical experimental techniques and simulation. We noted that the structure of the complex formed strongly depends on the generation of the dendrimer. The results show that generation 2 (G2) and 4 (G4) PAMAM dendrimers are able to penetrate surface deposited bilayers, consisting of palmitoyl oleoyl phosphatidyl choline as shown by neutron reflectometry (NR). The ability of the dendrimers to penetrate lipid bilayers is confirmed by coarse-grained simulations. The experimental and simulation data show that the DNA-dendrimer complex has a reduced ability to penetrate the bilayer, compared to the naked dendrimers. We will discuss these results in relation to the design of efficient transfection mediators with membrane permeating ability.

3254-Plat

Solid State NMR Studies on Acylated Transmembrane Peptides Driving Membrane Fusion

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The fusion of biological membranes is mediated by integral membrane proteins with α-helical transmembrane segments (TMSs). Additionally, those proteins are often modified by the covalent attachment of hydrocarbon chains. Previously, a series of *de novo* designed α-helical peptides with mixed Leu/Val sequences was presented, mimicking fusogenic TMSs in model membranes (Hofmann et al., Proc. Natl. Acad. Sci. USA 101 (2004) 14776-14781). From this series, we have investigated the peptide LV16 (KKKWLVLVLVLV LVLVLVLVKKK), which was synthesized presenting either a free N-terminus or an N-acylation of 2, 8, 12, or 16 carbons. We used ^{2}H and ^{31}P NMR spectroscopy to investigate the structure and dynamics of those peptide lipid modifications in POPC and DLPC bilayers and compared them to the hydrocarbon chains of the surrounding membrane. Except for the C-2 chain, all peptide acyl chains were found to insert well into the membrane. This can be understood from the high local lipid concentration, which the N-terminal lipid chains experience. The insertion of these peptides did not influence the membrane structure and dynamics as seen from ^{2}H and ^{31}P NMR. In spite of the fact that the longer acyl chains insert into the membrane, there is no perfect length adaptation. Even the C-16 chain on the peptide, which could match the length of the POPC palmitoyl chain exhibited lower order parameters in the upper chain, which get closer and finally reach similar values in the lower chain region. ^{2}H NMR square law plots reveal a slightly more dynamic characteristic of the peptide acyl chains compared to the surrounding phospholipids. In spite of the significantly different chain lengths of the acyl chains, the fraction of *gauche* defects in the inserted chains is constant, suggesting similar entropies of the inserted chains.

3255-Plat

Effect of PEG-based Biocompatible Polymers on the Response of Lipid Vesicles under External Stimuli

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Cell membrane dysfunction due to loss of structure integrity is the pathology of tissue death in trauma, muscular dystrophies, reperfusion injuries and common diseases. It is now established that certain PEG-based biocompatible polymers, such as Poloxamer 188, Poloxamine 1107 and PEG, are effective in sealing of injured cell membranes, thus can prevent acute necrosis if delivered timely after injury. Despite these broad applications of PEG-based polymers for human health, the fundamental mechanisms how PEG-based polymers interact with cell membranes are still under debate. Here, the effects of PEG-based biocompatible polymers on phospholipid membrane integrity under external stimuli (osmotic stress and oxidative stress) were explored using model cell membranes - giant unilamellar vesicles. Through fluorescence leakage assays and time-lapse fluorescence microscopy, we directly monitored the loss of structural integrity of single fluorescent dye-loaded GUVs under different stimuli, and observed the effects of triblock copolymers on these damaged membranes. We find that the interaction of the polymers with the lipid membrane involves two stages: an adsorption (I) and an insertion (II) state. We propose that the adsorption of the polymers on the membrane surface is responsible for the cell membrane resealing process due to its corralling effect, which is evidenced by slow-down of surface hydration dynamics upon adsorption of the polymers. In the insertion state, on the other hand, the polymers disturb the packing of phospholipids due to the mismatch in size and hydrophobicity of the PPO block with the lipid hydrocarbon tails, increasing the membrane permeability. Our results indicate that the biomedical application of PEG-based polymers, either as cell membrane resealing agents or as accelerators for drug delivery, is directed by the delicate balance between the adsorption and insertion of the polymers to the cell membranes.

3256-Plat

Detergent Effects on Membranes at Sub-Solubilizing Concentrations: Transmembrane Lipid Motion, Bilayer Permeabilization and Vesicle Lysis/reassembly are Independent Phenomena

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Soluble amphiphiles, or detergents, are known to produce a number of structural and dynamic effects on membranes, even at concentrations below those causing membrane solubilization, i.e. at the so-called stage I of detergent-membrane interaction. The main sub-solubilizing detergent effects on membranes are: transmembrane lipid motion (flip-flop), breakdown of the membrane permeability barrier leakage, and vesicle lysis / reassembly.

For a proper understanding of membrane solubilization by detergents it is important to assess whether the various effects seen at sub-solubilizing surfactant concentrations occur independently from each other, or else they are interconnected by cause-effect relationships, so that they can be interpreted as necessary steps in the overall process of solubilization. In order to answer this question we have explored the three above-mentioned effects, i.e. flip-flop, leakage and lysis / reassembly, apart from solubilization, in model (large unilamellar vesicles) and cell (erythrocyte) membranes. Five structurally different surfactants, namely chlorpromazine, imipramine, Triton X-100, sodium dodecylsulfate and sodium deoxycholate have been used. Each of them behaves in a unique way. Our results reveal that lipid flip-flop, vesicle leakage and vesicle lysis/reassembly are independent phenomena between them and with respect to bilayer solubilization, so that they can not be considered as necessary stages of a higher-order unified process of membrane solubilization by detergents.

3257-Plat

Effect of Cations on the Nanomechanical Response of Phospholipid Model Membranes. A Force Spectroscopy Study

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How do metal cations affect the stability and structure of phospholipid bilayers? Which role does ion binding play in the insertion of proteins and in the overall mechanical stability of biological membranes? To characterize such effects, several theoretical and microscopic approaches have been proposed in the past to study the mechanical properties of lipid bilayers. While providing crucial information, molecular dynamics simulations can not completely deal with the extraordinary complexity of biological membranes. Experimental techniques also have problems when it comes to test ion binding to lipid bilayers in an accurate way. Hence, a new perspective from the nanometric scale [1,2], where most of the specific molecular phenomena are sensitive, was introduced being Atomic Force Spectroscopy an essential tool to examine the lipid bilayers structure and behaviour. So, we used Force Spectroscopy to quantitatively characterize the nanomechanical resistance as a function of the electrolyte concentration and composition thanks to a reliable molecular fingerprint that reveals itself as a repetitive jump in the approaching force curve. By systematically testing two model membranes, DPPC and DPPE, immersed in an electrolyte containing a series of either one monovalent (Li^{+} to Cs^{+}) or divalent cation (Mg^{2+} and Ca^{2+}) we provide a wealth of information which unambiguously proves an independent contribution of each ion to the gross mechanical resistance, reporting quantitative measurements for the membrane elastic modulus and also for its