stoichiometries of 2:1 or higher. At pH 7, the folding kinetics of OmpA into lipid membanes was well described by double-exponential time courses, both in the presence and in the absence of FkpA. While the presence of FkpA did not affect the rate constants of the faster OmpA folding phase, both the relative contribution of the faster rate to the overall folding kinetics and the rate constants of the slower folding phase were increased when FkpA was present. Our results provide direct evidence that the periplasmic chaperones Skp and FkpA support folding of OMPs into lipid membranes independently of each other.

[1] Patel, G. J., Behrens-Kneip, S., Holst, O., Kleinschmidt, J.H., 2009 Biochemistry, in press.
[2] Qu, J., Behrens, S., Holst, O., Kleinschmidt, J.H., 2007 J. Mol. Biol. 374, 91-105.

483-Pos

Fusion Peptide Effects on Epitope Recognition At Membrane Surfaces by the Broadly Neutralizing Anti-HIV-1 2f5 Monoclonal Antibody

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The HIV-1 glycoprotein fusogenic subunit gp41 is the target for 2F5, a broadly neutralizing monoclonal antibody (MAb2F5) isolated from asymptomatic infected individuals. The 2F5 epitope locates close to the membrane interface within the membrane proximal external region (MPER) that connects the HIV-1 envelope gp41 ectodomain with the transmembrane anchor. Here evidence is presented indicating that the conserved amino-terminal fusion peptide (FP) increases the affinity of this antibody for its membrane-inserted epitope. Structural characterization by circular dichroism together with membrane-disrupting activity measurements suggests the formation of FP-MPER complexes at the surface of lipid bilayer vesicles. MAb2F5 associated more efficiently with lipid vesicles containing FP and MPER peptides as compared to those containing only MPER, or MPER in combination with FPctl, a scrambled version of the FP. Moreover, the N-terminal FP sequence had almost no effect on membrane-inserted epitope binding by MAb4E10, a neutralizing antibody that has been shown to extract its C-terminal MPER epitope from the membrane interface. In combination with recently reported crystallographic data (Julien et al. (2008) J. Mol. Biol., 384, 377-392), these results support a ''catch-and-hold'' mechanism for the process of MAb2F5-epitope binding at membrane surfaces.

484-Pos

Effect of the Conjugation of Peg to the PLL on the Micro- and Mesoscopic Properties of a POPC Bilayer

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Recent studies have demonstrated that the conjugation of poly(ethylene glycol) (PEG) to poly(L-lysine) (PLL) allows these copolymers to be adsorbed to the surfaces of pancreatic islets with minimal toxicity. The molecular mechanism for the effect of the conjugation of PEG to PLL on attenuation of cytotoxicity remains unknown. This knowledge is important for the design of optimized PLL-*g*-PEG copolymers for the design of nanoscale immunoisolation barriers. In this study, we investigated the conformational and electrostatic changes of PLL by the grafting of PEG chains, and the interaction changes of the PLL-*g*-PEG copolymer with a POPC bilayer compared to that of the PLL polymer with molecular dynamics simulations. The results showed that the grafting of PEG chains to PLL resulted in a change in the conformation of PLL from a randomly coiled structure to a structurally more stable globular conformation, and a significant change in electrostatics distribution. The interactions of PLL and PLL-*g*-PEG copolymers with a POPC bilayer showed that conformational and electrostatic changes resultant from the conjugation of PEG to PLL further affected the interaction of the polymer with the lipid bilayer. In addition to resulting in a structural change of the lipid bilayer, the permeability, compressibility, and bending modulus of the lipid bilayer are also affected by PEG grafting to PLL. The results will provide molecular insight for the experimental observation about the effect of the conjugation of PEG to PLL on the reduced cototoxicity.

Keywords: PEG; PLL; conjugation; conformation; electrostatics; lipid bilayer; micro-mesoscopic properties.

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Observation of Backbone C_α-Deuteron Signals in Solid-State NMR Spectra of Labeled Alanines in Oriented Transmembrane Peptides

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Solid-state deuterium NMR spectra combined with the Geometric Analysis of Labeled Alanines (GALA) method provide information about the average apparent tilt and dynamics of membrane-spanning peptides in oriented, hydrated lipid bilayer membranes. When Ala-d_4 labels are used, the side-chain methyl (CD_3) signals are readily detected, but the weaker backbone C_α-D resonances are often problematic to observe (van der Wel, Biophys J, 83, 1479) for peptides of the WALP family [GWW$(LA)_n$LWWA]. In spite of this difficulty, we recently have begun to observe surprisingly intense backbone C_α-D resonances in the ^{2}H NMR spectra for some oriented transmembrane peptides, particularly in cases where the $(LA)_n$ core sequence of WALP is interrupted by a ''guest'' residue such as Pro, Lys or Arg. Examples will be discussed. In some cases, the C_α-D signal intensities appear to depend upon the macroscopic orientation of the lipid bilayers with respect to the external magnetic field. The sequence and orientation dependence of the backbone C_α-D signals may therefore reflect the local and global peptide dynamics in ways that are incompletely understood. A selection of spectral examples will be presented and interpreted.

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Charged and Aromatic Anchoring Amino Acids Affect the Orientation of Transmembrane Peptides: A Deuterium NMR Study

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In integral proteins, the membrane-spanning segments are often flanked by aromatic and/or charged amino acids, which serve as anchoring residues, promoting protein-lipid interactions. These residues have specific polarity preferences, which are reflected in their typical immersion depths. Namely, tryptophan (W) is localized primarily to the carbonyl region of lipid acyl chains, while charged lysine (K) and arginine (R) prefer more polar regions. Although such patterns are widely recognized, little is known regarding the contributions of these residues for the orientations of transmembrane segments.

Solid-state NMR spectroscopy offers a way to approach this problem. Peptides of the ''WALP'' family, GWW$(LA)_n$LWWA, have served as useful models. Membrane-spanning proteins, nevertheless, typically contain more diverse sets of anchoring amino acids, which may act together to influence molecular geometry and orientation. To address these issues, we have designed the $\underline{X}^{2,22}W^{5,19}$ALP23 peptides (acetyl-G$\underline{X}$ALW^5(LA)$_6$LW^{19}LA$\underline{X}$A-[ethanol] amide) to encompass two different anchor residues. In these peptides the *inner* anchor is kept constant (W), while the *outer* ''anchor'' (separated by a short Leu-Ala spacer) is varied to either W, K, R or the control residue G.

Solid-state ^{2}H NMR spectra were recorded for peptides having Ala CD_3 groups in aligned bilayers of DLPC, DMPC and DOPC. The ''Geometric Analysis of Labeled Alanines'' provided a means to deduce the apparent average peptide orientations. Introduction of charged anchors ($\underline{X}$ = K or R) resulted in a 2-5° increase in the apparent tilt angle compared to $\underline{X}$ = G. Conversely, for $\underline{X}$ = W, the apparent tilt angle decreases 2-9°, accompanied by a significant change in the tilt direction.

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Influence of WALP Peptides on Phase Behavior of Cholesterol Containing Ternary Lipid Mixtures

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Partitioning of lipid bilayer membrane components plays a crucial role in biological processes. For example, cellular mechanisms such as signaling, adhesion and transport are often dependent on and regulated by inhomogeneities in the biological membranes. Ternary lipid systems containing cholesterol provide an opportune model for characterizing phase behavior, such as separation between liquid disordered and liquid ordered ("raft") phases. In these model systems one can incorporate (non-perturbing) deuterium labels and use ^{2}H NMR methods to establish the phase behavior of the system as a function of temperature. It is of interest to investigate also the possible influence that membrane-spanning peptide components may have on the segregation of lipids.

In some experiments, we have used the saturated fluid model lipid diphytanoylphosphatidylcholine (DPhPC). We have investigated both DPhPC:DPPC-d_{62}:Cholesterol and DOPC:DPPC-d_{62}:Cholesterol (35:35:30) mixtures, with and without incorporation of WALP peptides of different lengths (acetyl-GWW$(LA)_n$LWWA-ethanolamide).

When WALP peptides are introduced into DPhPC:DPPC-d_{62}: Cholesterol, a peptide length-dependent appearance of non-bilayer phase is observed. The non-bilayer phase is apparent in both ^{2}H and ^{31}P NMR spectra. A prominent isotropic lipid peak has disqualified this system as a model for investigations of longer WALP peptides.

The DOPC:DPPC-d_{62}:Cholesterol model system is better behaved. Upon addition of WALP peptides, no apparent formation of non-bilayer phase is evident. Incorporation of WALP peptides of various lengths results in a slight increase in phase transition temperature, from a range of about 288-293 K with no peptide present, to a range of about 293-298 K when a WALP peptide is present at 1/160 (peptide/total lipid). Results for different length WALP peptides will be discussed.