necessary for activity. Also, innate-immunity-like peptides were described that contained multiple histidine residues. Although these peptides consisted of 12 to 15 amino acids, these were less toxic to the host, and were lytic to numerous pathogens and cancer cells at slightly acidic environments. Here we report the design of an ultrashort histidine containing peptide whose antifungal activity could be significantly increased in a covalent trimeric form. Low micromolar activity was observed for Aspergillus fumigatus and Cryptococcus neoformans but not Candida albecans. Using transmission electron microscopy, we observed that this trimeric ultrashort histidine containing peptide formed distinct and differing nanostructures at pH 5 and 7, which could explain the activity differences. Since various organs or areas of the human body have a slightly acidic pH environment such as tumors, gastric lumen and lung-lining fluids in cystic fibrosis and asthma, understanding the importance of nanostructure-activity relationships of these pH dependent ultrashort peptides could lead to improvements in the delivery and administration of the peptides.

1459-Pos

Spectroscopic Studies of the Interaction of Native and TOAC-Labeled Peptide Hormones with Model Membranes: Angiotensin II

Nélida Marín[1], Erick Poletti[2], Clóvis Ryuichi Nakaie[2], Shirley Schreier[1].
[1]University of São Paulo, São Paulo, Brazil, [2]Federal University of São Paulo, São Paulo, Brazil.

The peptide hormone angiotensin II (DRVYIHPF, AII) plays an important role in the renin-angiotensin-aldosterone system. AII derivatives containing the paramagnetic amino acid 2,2,6,6-tetramethylpiperidine-1-oxyl-4-amino-4-carboxylic acid (TOAC) replacing residues 1 ($TOAC^1$-AII) and 3 ($TOAC^3$-AII) were synthesized and their conformational properties, as well as those of the native peptide, were examined in the presence of model membranes - micelles of 1-palmitoyl-2-hydroxy-phosphatidylcholine (LPC) and 1:1 mol:mol LPC: 1-palmitoyl-2-hydroxy-phosphatidylglycerol (LPG) and large unilamellar vesicles (LUV) of 1-palmitoyl-2-oleoyl phosphatidylcholine (POPC) and 1:1 mol:mol POPC:1-palmitoyl-2-oleoyl phosphatidylglycerol (POPG). Experiments were conducted at pH 4.0, 7.0, and 10.0 to evaluate the effect of peptide charge on peptide-membrane interaction. Fluorescence spectra showed that the peptides bound to negatively charged micelles to a much larger extent than to zwitterionic micelles. CD spectra of AII and $TOAC^1$-AII showed acquisition of secondary structure upon binding to LPC:LPG micelles at pH 4.0; the changes occurred to a lesser extent at the higher pHs. In the case of $TOAC^3$-AII, binding had a small effect on peptide conformation since the TOAC ring imposes a more constrained conformation already in solution. In the case of bilayers, the peptides interacted only with POPC:POPG LUV, especially at pH 4.0. Line broadening of EPR spectra of the labeled peptides also provided evidence for interaction of the labeled peptides with negatively charged micelles and bilayers. In several cases, two-component spectra were obtained, one due to the peptides in solution and the other to the bilayer-bound population, allowing for the calculation of partition coefficients. The rigidity of the TOAC-labeled analogue is very likely responsible for its inability to acquire the correct receptor-bound conformation, leading to loss of biological activity. These data show that spectroscopic studies can provide relevant information regarding peptide-membrane interaction.

1460-Pos

Unraveling the Molecular Basis of the Selectivity of the HIV-1 Fusion Inhibitor Sifuvirtide Towards Phosphatidylcholine-Rich Rigid Membranes

Henri G. Franquelim, A. Salomé Veiga, Nuno C. Santos, Miguel A.R.B. Castanho.
Instituto de Medicina Molecular, Lisbon, Portugal.

Sifuvirtide, a 36 amino acid anionic peptide, is a novel HIV-1 fusion inhibitor with improved antiretroviral activity. The selective ability of this peptide to interact with lipid bilayers has already been identified (Franquelim et al, *J Am Chem Soc* 2008, **130**, 6215-23) and the aim of this work is to evaluate the interaction of sifuvirtide with several biomembrane model systems, retrieving details of its mode of action at the membrane level. Since this peptide has aromatic residues, fluorescence spectroscopy techniques were mostly used. The interaction was assessed by partition and fluorescence quenching experiments. Results showed no significant interaction with large unilamellar vesicles composed by sphingomyelin and ceramide. In contrast, sifuvirtide presented selectivity towards vesicles composed by phosphatidylcholines (PC) in the gel phase, in opposition to fluid phase PC vesicles. The interaction of this peptide with gel phase PC (zwitterionic) membranes ($K_p = 1.2\times10^2$) is dependent on the ionic strength, which indicates the mediation of electrostatic interactions at an interfacial level. The effects of sifuvirtide on the lipid membranes' structural properties were further evaluated using dipole potential membrane probes, zeta-potential, dynamic light scattering and atomic force microscopy measurements. The results show that sifuvirtide does not cause a noticeable effect on lipid bilayer structure. Altogether, one can conclude that sifuvirtide presents a specific affinity towards rigid PC membranes (in agreement with the adsorption model previously proposed), and the interaction is mediated by electrostatic factors, not affecting the membrane architecture. Because saturated PC lipids are found in high concentration in lipid rafts, but mainly in the viral envelope, the efficacy of sifuvirtide may be related to its screening ability towards those regions, allowing an increased concentration of this peptide drug near the fusion site.

1461-Pos

Fusion Peptide of Gp41 Self Associates in the Model Membrane and then Interacts with its Trans-Membrane Domain

Hirak Chakraborty[1], David G. Klapper[2], Barry R. Lentz[1].
[1]Biochemistry and Biophysics Department & Molecular and Cellular Biophysics Program, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA, [2]Microbiology & Immunology Department, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA.

We have examined the peptide structure and membrane packing when the fusion peptide (FP) of gp41 was added either in the membrane alone or in the membrane containing gp41 the trans-membrane domain (TMD). Circular Dichroism (CD) measurements showed that FP is mostly in a beta sheet conformation independent of FP concentration. TMD has ~ 30% helical, ~25% beta sheet and the complex of TMD and FP has less alpha helix than the TMD itself, which indicates the TMD looses its alpha helical structure upon interacting with FP. DPH and TMA-DPH fluorescence anisotropy revealed that FP alone increased the interior packing of the membrane, but FP in the presence of TMD increased the interior packing at lower concentrations of FP and then decreased it at higher concentrations. FP alone increased membrane surface packing at lower concentrations but increased it at higher concentrations. In the presence of TMD, FP addition decreased surface packing cooperatively. From the lifetime of TMA-DPH in H2O and D2O we documented water penetration into the membrane. FP alone increases water penetration slightly whereas FP in presence of TMD significantly increased water penetration into the interface region of the membrane in a cooperative fashion. The fluorescence lifetime of C6NBDPC revealed that FP alone fills more space than the FP in the presence of TMD. In summary, our results clearly demonstrate that gp41 FP forms a complex with the gp41 TMD to alter both TMD structure and membrane structure. It remains to be seen whether this complex promotes membrane fusion. Supported by NIGMS grant 32707 to BRL.

1462-Pos

HIV Fusion Peptides Significantly Soften Lipid Bilayers

Pavlo Shchelokovskyy[1], Stephanie Tristram-Nagle[2], Reinhard Lipowsky[1], Rumiana Dimova[1].
[1]Max Plank Institute of Colloids and Interfaces, Potsdam, Germany, [2]Carnegie Mellon University, Pittsburgh, PA, USA.

The fusion peptide (FP) of the human immunodeficiency virus (HIV) is found on N-terminus of the viral envelope glycoprotein gp41 and is believed to play an important role in the virus entry process. In order to understand the immediate effect of this peptide on the cell membrane we have studied the influence of the synthetic fusion peptide residue FP-23 on the mechanical properties of model lipid bilayers. For this purpose, giant unilamellar vesicles (GUV) were prepared by electroformation from the unsaturated lipid dioleoylphosphatidylcholine mixed in various ratios with the fusion peptide. The bending stiffness of the vesicles was measured with two different methods: fluctuation analysis and aspiration with micropipettes. The data obtained from both of these approaches show that the bending stiffness of the membrane decreases gradually with increasing the concentration of the fusion peptide in the bilayer. Even low concentrations of only a few mol % FP-23 are sufficient to decrease the bending stiffness of the lipid bilayer by more than a factor of two. This observation is in agreement with previous results obtained with X-ray scattering on stacked lipid layers; see Tristram-Nagle and Nagle, Biophys. J. 93: 2048 (2007). Ongoing research is carried out to investigate the effect of FP-23 on the spontaneous fusion of GUVs.

1463-Pos

Augmentation of Single Channel Water Permeability by Modification of Membrane Anchoring

Florian Zocher[1], Tanya Polupanowa[2], Danila Boytsov[1], Guillem Portella[3], Bert de Groot[4], Ulf Diederichsen[2], Peter Pohl[1].
[1]Universitaet Linz, Linz, Austria, [2]Universitaet Göttingen, Göttingen, Germany, [3]Institute for Research in Biomedicine, Barcelona, Spain, [4]Max Planck Institute for Biophysical Chemistry, Göttingen, Germany.

Water transport through very narrow channels occurs according to the single file mechanism. While entering the channel, every water molecule loses most of its neighbouring water molecules. The energetic costs are thought to be