Membrane Active Peptides and Toxins II

2771-Pos  Board B201
The Presence of Antiparallel Beta-Sheets in Toxic Fibrils Formed by Aβeta on GM1 Clusters
Yuki Okada, Hiroshi Ueno, Yoshiaki Yano, Masaru Hoshino, Hiroki Itoh-Watanabe, Akira Naito, Katsumi Matsuzaki.

Graduate School of Pharmaceutical Sciences, Kyoto University, Kyoto, Japan; Yokohama National University, Yokohama, Japan.

The abnormal aggregation of amyloid β-protein (Aβ) is considered to be central in the pathogenesis of Alzheimer’s disease. We have focused on membrane-mediated amyloidogenesis because Yangagisawa et al. identified a specific form of Aβ that was bound to monosialoganglioside GM1 in brains exhibiting the early pathological changes associated with the disease. We have found that amyloid fibrils formed on GM1 clusters were more toxic than those formed in solution [1, 2]. The less toxic fibrils formed in solution are considered to be composed of in-resister parallel β-sheets, whereas the structure of the toxic fibrils is unknown, although FTIR spectra suggested the presence of antiparallel β-sheets [1, 2].

In this study, we investigated the structure of the toxic fibrils in detail. Solid-state NMR measurements using site-specifically [15N, 1-13C]-labeled Aβs suggested that the fibrils contained both parallel and antiparallel β-sheet structures. Chemical cross-linking experiments using Cys-substituted Aβs also supported this conclusion. Thus, the toxic fibrils were found to possess a novel unique structure.


2772-Pos  Board B202
Structural Transformation of Amyloid Peptides Interacting with Lipid Membranes
Yen Sun, Huey W. Huang.

Physics and Astronomy, Rice University, Houston, TX, USA.

The inherent cytotoxicity of protein aggregates implies a common mechanism for amyloid diseases (Bucciantini et al). However, accumulated evidence suggests that the insoluble fibrils or aggregates are not the culprit. On the other hand, amyloid peptides in soluble form do interact with lipid bilayers, suggesting that the cell plasma membranes are a target of amyloid pathogeneses. In particular, Keyed et al. have demonstrated that amyloid peptides all increase membrane ion conductance without any evidence of discrete channel or pore formation. In this study we try to find the common molecular process of soluble amyloid peptides interacting with lipid membranes that might induce membrane conductivity. It is difficult to study this molecular process for most amyloid peptides because of its propensity to fibrillize at relatively low solution concentration. P/L=106-126 is a random coil in its soluble form. We study its kinetics of structural transformation in the presence of lipid vesicles. The time dependence of the structural changes was analyzed as a function of the lipid concentration. We demonstrate that the soluble peptides transform from random coils to alpha-helices upon binding to lipid bilayers. The helical state is stable, as long as the bound peptide to lipid ratio P/L on the lipid vesicle is below a critical value P/L*. But as P/L exceeds P/L*, the peptides transform from the helical state to beta-aggregates. This is consistent with previous studies of penetration and hIAPP in multiple lipid bilayers. Thus we found the common fibrillization process of amyloid peptides interacting with lipid bilayers. Our proposal is that the process of peptides’ transformation from random coils to helices and then to beta-aggregation creates defects in the membranes that allow ion permeation to occur as observed by Keyed et al.

2773-Pos  Board B203
Alpha-Synuclein Stabilizes Small Unilamellar Vesicles by Reducing Both Membrane Surface Tension and Rrigidity
Anthony R. Braun, Jonathan N. Sachs.

Biomedical Engineering, University of Minnesota, Minneapolis, MN, USA.

Alpha-Synuclein (αSyn) stabilizes small unilamellar vesicles (SUV) by reducing the vesicle’s surface tension and membrane rigidity. Using coarse-grained molecular dynamics simulations we explored the membrane remodeling characteristics of αSyn bound to DPPC lipid vesicles. By combining our recently developed Spherical Harmonics fluctuation analysis with 3-dimensional pressure tensor calculations we were able to characterize αSyn’s effect on both the bulk and mechanical properties of DPPC vesicles. Our findings highlight a dramatic reduction in membrane surface tension and increased membrane fluctuations, suggesting a less rigid bilayer, for the αSyn vesicle system relative to pure DPPC.

2774-Pos  Board B204
Membrane binding of lytic peptides, and the resulting peptide-induced lipid extraction (membrane solubilization) are influenced by the nature of lipids forming the membranes. However, the impact of various lipid species on these two phenomena cannot be directly inferred one from the other; distinct peptide/lipid interactions are pivotal in these distinct events. Melittin is a small cationic peptide with a secondary amphipathic helical character. It interacts spontaneously with lipid bilayers, and induces lipid extraction. We investigated how phosphatidylserine (PS), cholesterol, and phosphatidylethanolamine (PE) modulate melittin binding to phosphatidylcholine (PC) membranes as well as their impact on lipid extraction.

First, PS, a negatively charged phospholipid, increases melittin affinity for membranes but inhibits melittin-induced lipid efflux. It is proposed that the attractive electrostatic interactions between melittin and PS, which are responsible for the increased affinity, anchor melittin at the bilayer interface and prevent its relocation required for lipid extraction. Second, cholesterol reduces melittin membrane affinity. In parallel, it inhibits the extent of lipid extraction induced by melittin and LC-MS analysis indicates that lipid/melittin particles resulting from the extraction are cholesterol-depleted relative to the cholesterol content of the original membranes. It is proposed that the phospholipid ordering caused by cholesterol is unfavorable to peptide binding and inhibits the overall melittin action. Third, PE decreases the affinity of melittin for PC-based bilayers, an inhibition more significant in the gel than in the fluid phase. The limited amount of melittin that can be accommodated in PE-containing gel-phase bilayers appears to produce bilayers more susceptible to lipid extraction. As a consequence, PE can act as a promoter or an inhibitor of melittin-induced lipid extraction, depending on its proportion. These findings demonstrate that membranes can tune peptide-induced lipid extraction by altering their lipid composition.

2775-Pos  Board B205
Mechanism of Action of β-Hairpin Antimicrobial Peptides
Richard B. Lipkin, Themis Lazaridis.

Chemistry, City College, New York, NY, New York, NY, USA.

Previous work showed that the β-hairpin antimicrobial peptide (AMP) protegrin forms stable octameric β-barrels and tetrameric arcs (half barrels) in both implicit and explicit membranes. Here, we extend this investigation to several AMPs of similar structure: tachyplesin, androctonin, polyphemusin, gomesin, and the retrocyclin α-defensin. We also examine synthetic β-sheet peptides selected from a combinatorial library for their ability or inability to form pores in lipid membranes. When heptameric, octameric, and decameric β-barrels and tetrameric arcs of these peptides were initially embedded in preformed neutral and anionic lipid pores, a variety of behaviors and membrane binding energies were observed. The synthetic peptides bound very strongly and formed stable barrels and arcs in both neutral and anionic pores. The natural AMPs exhibited unfavorable or marginally favorable binding energy and kinetic of neutral pores, consistent with the lower hemolytic activity of some of them compared with protegrin. Binding to anionic pores was more favorable, but significant distortions of the barrel or arc structures were sometimes noted. These results are discussed in light of the available experimental data. The diversity of behaviors obtained makes it unlikely that the barrel and arc mechanisms are valid for the entire family of β-hairpin AMPs.

2776-Pos  Board B206
Attack on Single Escherichia Coli Spheroplasts by Antimicrobial Peptides
Tru-Lin Sun, Yen Sun, Huey W. Huang.

Physics, Rice University, Houston, TX, USA.

Studies of the molecular mechanisms of AMPs are mostly performed with lipid bilayers. Thus there is a persistent question as to whether the action of AMPs on bacterial membranes can be reproduced on lipid bilayers. Recently Weisshaar and coworkers have studied the actions of AMPs on E. coli and Bacillus subtilis by time-lapse fluorescence microscopy. The direct observation of the action of AMPs on bacteria revealed two key steps. The first is growth halting due to direct interference of AMP with cell wall synthesis and is recoverable. The second is permeabilization of the cytoplasmic membrane which is not recoverable. Here we study the direct action of AMPs on the cytoplasmic membranes by using E. coli spheroplasts, the cell form from which the outer membranes have been removed. The purpose is to compare the action on bacterial membranes with that on lipid bilayers and to answer the key question is how to reveal the mechanisms of AMPs on bacterial membranes. First we observe the action of AMPs on giant unilamellar vesicles (GUVs) made of E. coli total lipid extract. We used the aspiration method to hold the GUV in a solution containing a soluble dye.
molecule and a fluorescence labeled melittin to obtain the lipid bilayer response to an AMP. The same method was applied to spheroplasts to observe the action of AMPs on model membranes. In both cases, we found first the binding of peptides expanded the membrane area. As the membrane area increased to ~2-3%, the dye molecules began to leak in. After a time, we found the spheroplasts lost their phase contrast indicating the loss of the cell content. The same experiments were repeated with LL37 and magaB. We found that sytox green is not a good indicator of membrane permeability.

2777-Pos Board B207
Insights into the Effects of Antimicrobial Peptides on Live E. coli Cells using Time-Lapse Fluorescence Microscopy
Ranga Rajan, James C. Weisshaar.
Chemistry, University of Wisconsin Madison, Madison, WI, USA.
Time-lapse fluorescence microscopy enables detailed observations of the effects of natural and synthetic antimicrobial peptides (AMPs) on live E. coli cells. Apart from tracking membrane permeabilization caused by AMPs, we also observe the effect of AMPs on intracellular components such as the nucleoid and the ribosome.

Using our assays, we have uncovered differences in the mechanisms of action of synthetic peptides known to permeabilize LUVs. Our single-cell, real-time data is rich in spatial and temporal resolution, and is biologically relevant.

2778-Pos Board B208
Histidine-Rich Designer Peptides with pH-Dependent Membrane Topology, Antimicrobial, Nucleic Acid Transfection and Viral Transduction Capabilities
Christopher Aisenbrey1, Philippe Bertani2, Andrea Ferrari3, David Fenard4, Anne Galy5, Elise Glattard6, Arnaud Marquette7, Jesus Raya8, Evgeniy S. Salnikov1, Joachim Seelig9, Lorenzo Stella2, Louic Vermeer10, Nathalia Voievoda11, Burkhard Bechinger12.
1Institute of Chemistry UMR7177, University of Strasbourg/CNRS, Strasbourg, France; 2Dipartimento di Scienze e Tecnologie Chimiche, University Tor Vergata, Roma, Italy; 3UMR_S951, Genethon, INSERM, University of Evry, Evry, France; 4Biocenter, University of Basel, Basel, Switzerland.

The synthetic LAH4 peptides were designed to investigate the interactions that determine the membrane topology of helical peptides (1). Their core consist of alanines, leucine and four histidines arranged to form an amphipathic helix, as well as two lysines at each terminus. Through protonation of its histidines (pKs between 5.4 and 6.0) the alignment of the helices is transmembrane at neutral pH and in-plane at pH <5.5 (1).

The LAH4 peptides exhibit membrane pore-formation and antimicrobial action at both neutral and at acidic pH including against clinical isolates where the low pH configuration is more active (2). The LAH4 peptides have been found to also exhibit potent DNA and siRNA transfection activities (3). LAH4 can therefore act as a non-viral vector and has indeed been used for the delivery of quantum dots or protein-based vaccines. Furthermore, transduction by adeno-associated viruses or lentiviruses is enhanced by LAH4 peptides (4).

Ongoing biological and structural investigations will be reported which aim to understand these activities at atomic resolution (5-8).


2779-Pos Board B209
Effect of Histidine Rich Antimicrobial Peptides, Gad-1 and Gad-2, On Membrane Model at Different pH Values
Physics and Physical Oceanography, Memorial University of Newfoundland, St. John’s, NL, Canada.
Antimicrobial polypeptides (AMPs) are an important component of the innate immune system of many different organisms and are generally amphipathic and cationic in nature. Electrostatic interactions between positively charged residues of the AMP and negatively charged lipids in pathogen membranes are an important component of AMP mechanism and specificity. AMPs such as Gad-1 and Gad-2 that are rich in histidine, an amino acid that is uncharged at neutral pH and positively charged at slightly acidic pH, can thus exhibit pH dependent activity. 1H solid state NMR spectroscopy was used to study the effect of Gad-1 and Gad-2 on model membranes at different pH values. The addition of peptides to the model membrane system POPE/POPG/G3 (1:3) results in a change in the splitting of spectrum both at pH 7 and pH 5. Further investigation of peptide effects on lipid acyl chain motion were determined through calculations of orientational order parameter values (S_C2). The order parameter profile demonstrated that Gad-1 causes more lipid acyl chain disordering than Gad-2, at both pH 7 and pH 5. These results are consistent with Gad-1’s greater activity in assisting for bacterial growth inhibition. On the other hand, while Gad-2 is more active at inhibiting bacterial growth at low pH than at neutral pH, its effect on lipid acyl chain disordering is the same at pH 7 and pH 5.

2780-Pos Board B210
The Transmembrane State of Antimicrobial Piscidin in Bacterial Cell Membrane Mimics Dramatically Alters the Polar-Nonpolar Segregation of the Bilayer
Myriam Cotten1, Jorge I. Hernandez2, Kimberly Bogardus3, Mihaela Mihailasescu4.
1Chemistry, Hamilton College, Clinton, NY, USA, 2Chemistry, Clemson University, Clemson, SC, USA, 3Institute for Bioscience and Biotechnology Research, University of Maryland, Rockville, MD, USA.
Piscidin 1 (P1) and piscidin (P3) are twenty-two-residue-long antimicrobial peptides that are membrane-active. While their amphipathic structures lend themselves to forming pores by the carpet/toroidal pore mechanism, direct evidence for their mode of action is lacking. Previously, we determined the high resolution structures of helically packed, head-to-head helices mimics constituted of 3:1 phosphocholine (PC)/phosphoglycerol (PG). In a comprehensive analysis to decipher their mechanism of action, we combined dye leakage assays, oriented CD (OCD), X-ray diffraction (XRD), and neutron diffraction (ND) in 3:1 PC/PG to simultaneously investigate at various peptide-to-lipid (P/L) ratios the permeabilization capability of the peptides, the physical state of the bilayer, and the bilayer orientations and locations of the peptides. The OCD results show that P1 adopted an inserted state at a lower P/L than P3 (1:25 versus 1:16) in agreement with its stronger permeabilization capability. XRD showed that the bilayer gradually thinned from 52.5 Å in the peptide-free state to 48.5 Å in the presence of piscidin at 1:1 P/L. ND experiments at 1:25 P/L confirmed the OCD and XRD results. Because we calculated the ND profiles for samples containing peptides that were 2H-labeled at either the C-end or at both the N- and C-ends, we could determine the bilayer orientation and location of each peptide. Importantly, the combined OCD and ND data revealed that P1 has a transmembrane orientation almost parallel to the bilayer normal. Further investigation of this state at a 1:1 P/L demonstrated that it induces a dramatic broadening of the lipid headgroup distribution indicating that it abolishes the strict nonpolar-polar segregation of the bilayer. The implications for piscidin’s mode of action will be discussed.

2781-Pos Board B211
Antimicrobial Peptide Piscidin Permeabilizes Bacterial Membranes and Binds Intracellular Targets at Sub-Lethal Concentrations
Mason Schoeneck, Robert Bihorel, Gina Goldberg, Bryan Ferguson, Michael McCormick, Herman Lehman, Myriam Cotten.
Chemistry, Hamilton College, Clinton, NY, USA.
Piscidins 1 (P1) and 3 (P3) are cationic α-helical membrane active antimicrobial peptides originally isolated from the hybrid striped seabass. The peptides, which possess broad-spectrum activity against bacteria, fungi, and viruses, bind to anionic membranes and lyse them once a sufficient concentration has been reached. In this study, we used confocal microscopy and FITC-labeled piscidins to show that at sub-inhibitory concentrations (0.75 μM), P1 and P3 are able to translocate across the membrane of Gram-positive and -negative bacteria. The FITC signal of the peptides was co-localized with the DAPI signal of the nucleoid region of the bacteria. CD using a 15 base pair piece of DNA and a gel retardation assay using a 1782 base pair piece of DNA confirmed that P1 and P3 could bind to DNA in vitro. It appears that P3 binding was more disruptive to the structure of DNA, and this may be due to the extra arginine residue in P3’s sequence. However, these results did not indicate whether the peptides translocated through the membrane or entered the cells via pores formed in the membrane. To probe this question, we used the bacterial strain E. coli ML35 in a permeabilization assay to determine at which peptide concentration the membrane became permeabilized. We ran a dose response test at the lowest concentration tested (0.1 μM) the membrane of ML35 cells became permeabilized by P1 and P3. Our results agree with prior findings that permeabilization of bacterial cell membranes can occur prior to lysis and cell death. The implications of piscidin’s ability to translocate into bacterial cells and bind to intracellular targets at sub-lethal concentrations will be discussed in terms of its mechanism of action, which was previously believed to be based on its membrane activity.