S1h1-3

Structures of tryptophan-rich antimicrobial peptides bound to micelles and their interactions with phospholipid bilayers

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Trp-rich antimicrobial peptides play important roles in the host innate defense mechanisms of many plants, insects, and mammals. Indolicidin, a 13-residue antimicrobial peptide-amide (ILPWKWPWWPWR-NH₂), is isolated from the cytoplasmic granules of bovine neutrophils. NMR structure of indolicidin in aqueous solution was a globular and amphipathic conformation, differing from the wedge shape adopted in lipid micelles. It is suggested that indolicidin mainly reduces synthesis of DNA and the inhibition of DNA synthesis contributes to the antimicrobial activity of indolicidin. We found that indolicidin indeed binds with DNA by using gel retardation, fluorescence quenching and surface plasmon resonance experiments. A model illustrating the DNA-binding mechanism of the antimicrobial action of indolicidin and explaining the roles of the peptide when interacting with lipid bilayers was hence suggested. By contrast, a newly designed peptide Ac-KWRRWVRWI-NH₂, designated Pac-525 contains an alpha helix at Trp2-Arg4 and form an amphipathic structure. Interestingly, the reverse sequence of Pac-525 also demonstrates similar antimicrobial activity and structure in micelles and vesicles. A variety of biophysical and biochemical experiments showed that Pac-525 interacted strongly with negatively charged phospholipids vesicles and induced efficient dye release from these vesicles, suggesting that the antimicrobial activity of Pac-525 may be due to interactions with bacterial membranes, which is different from that of indolicidin.

S1h1-5

Interactions of two kinds of arthropods-derived antimicrobial peptides, pandinins and oxyopinins, with model lipid membranes

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The interaction of two kinds of arthropods-derived antimicrobial peptides, pandinins (pin1 and pin2) and oxyopinins (oxki1 and oxki2), with lipid membranes were studied mainly using solid-state NMR spectroscopy. The interaction of the pin1 with the lipid membrane at various temperatures was first examined by the ³¹P NMR measurements. It was found that the pin1 induces various lipid phase behaviors depending on the acyl chain length of the lipid molecule. The pin1 was suggested to be located at the membrane-water interface using ¹H magic angle spinning (MAS) NMR chemical shift measurements. Moreover, ¹³C chemical shift values for the pin2 labeled at Gly3, Gly8, Leu12, Phe17, or Ser18 under slow MAS conditions indicate that the pin2 rapidly rotates around the average helical axis which is inclined at about 45° to the lipid long axis. Differential scanning calorimetry measurements showed that the pin2 induces negative curvature strain in lipid bilayers. Amide H-D exchange experiments of ¹⁵N-Ala4, Gly8, and Ala9 triply-labeled pin2 suggested that this peptide forms oligomers whose N-terminal region creates membrane pores. On the other hand, unlike the pandinins, the oxyopinins were found to induce positive curvature strain in lipid bilayers on the basis of ³¹P NMR measurements. Furthermore, High-sensitivity titration calorimetry measurements of the oxyopinins indicated that the bindings of the oxyopinins were exothermic, and the binding enthalpies for the oxki1 and oxki2 to POPC SUVs were -18.1 kcal/mol and -15.0 kcal/mol, respectively.

S1h1-4

An epididymis-specific antimicrobial peptide has dual functions in sperm maturation

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Epididymis plays an important role in sperm maturation and sperm storage by secreting different proteins and ions to create a unique and gradually changed microenvironment along this convoluted tubule. Therefore, it is an idea research target organ in better understanding the molecular mechanism of sperm maturation in epididymis and providing new thoughts in male fertility control drug design. Since 1998, a number of epididymis-specific and programmatically expressed novel genes have been identified in rodents and primates by our lab. We found at least 4 novel proteins so far which even belong to different protein super-families but displayed somehow antimicrobial activity in various degree. The question was why an organ like epididymis needs so many antimicrobial peptides? In another words, how they share out the work with individual responsibility? A caput region-specific, principle cell exclusively expressed gene named Bin1b was identified as a natural antimicrobial peptide belongs to the beta-defensin family. It secreted into the epididymal lumen and bound with sperm. This binding makes the sperm uptake Ca++ through the L-type ion channel initiating the sperm motility but not in the motility maintenance. These results implied that to be an epididymis-specific beta-defensin, is not limited to bacteria killing, but also involved in sperm maturation in the epididymis. Over-expressing Bin1b transgenic mouse showed a significant antimicrobial ability in epididymitis but no harm for its fertility. Knock out experiment is on the way.

S1c2-0

Introductory Remark

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The three-dimensional structure of a protein molecule in solution is undergoing fluctuations under thermal equilibrium. Proteins can function generally by utilizing these fluctuations. These fluctuations include small fluctuations within the same topology of folding as well as those between different topologies of folding. The former fluctuations can be visualized as changes in structure (i.e., compaction) largely by applying force (i.e., pressure) to the system, while the latter type of fluctuations can also be visualized as structural changes (i.e., shift of conformational equilibrium or changing populations among conformers of different topology) also by applying pressure. Thus pressure can be used to probe a wide range of fluctuation in protein structures, as it is a mechanical perturbation as well as a thermodynamic perturbation to a protein system in solution. The utility of this unique aspect of pressure perturbation has been recognized in combination with a microscopic observation of protein structure, such as NMR, X-ray and Molecular Dynamics calculation. The mini-symposium will start with the actual examples how pressure affects protein function in an example of enzymes. Then, the experimental studies using high pressure NMR will be reported. Finally, newly emerging M.D. calculations will be presented. With these modern techniques, the classical parameters such as volume and compressibility will be shined new light from structure fluctuations in atomic level.