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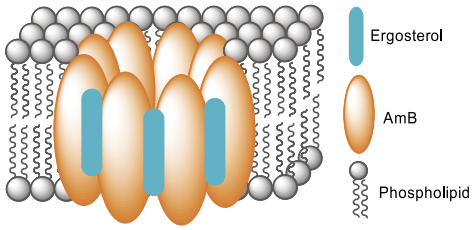
Structure of Membrane-bound Molecular Assemblies

Amphotericin B (AmB), an antibiotic produced by actinomycetes, has been the drug of choice for the treatment of systemic fungal infections. This drug forms an ion channel assembly in the presence of membrane lipids, where fungal specific ergosterol plays a key role in its selective toxicity. We prepared isotope-labeled AmB for structure elucidation of this channel assembly using solid-state NMR. ^{13}C-, ^{19}F-, and ^{2}H-labeled AmBs were prepared by biosynthetic or chemical synthetic methods. These labeled AmBs were then mixed with POPC and subjected to solid-state NMR measurements. The ^{13}C{^{19}F}REDOR spectra of ^{19}F- and ^{13}C-AmBs showed that the interatomic interactions were significantly weakened in the ergosterol-containing membrane, as compared to the sterol-free membrane. These results suggest that ergosterol enhances the mobility of AmB assemblies or increases intermolecular distances in AmB aggregates. Solid-state ^{2}H-NMR was measured using ^{2}H-labeled AmB, indicating that ergosterol significantly increases the mobility of AmB while AmB is almost immobilized in sterol-free membranes. These observations imply that in sterol-free membranes AmBs are aggregated to undergo phase separation while, in ergosterol-PC membranes, AmBs form small assemblies which may correspond to ion-permeable channels.



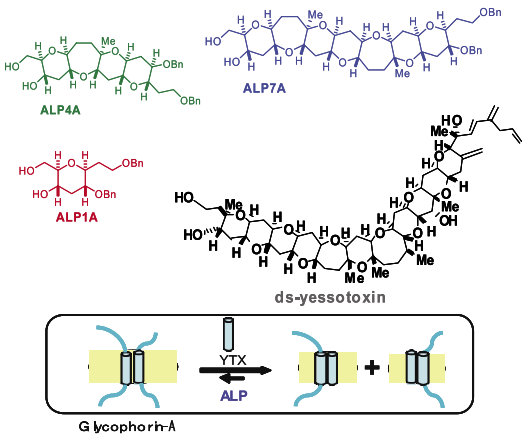
Ion Channel Model Formed by AmB and Lipds

Sphingomyelin and cholesterol are known to form micro-domains (lipid rafts) in biological membranes, and to play a role in numerous biological functions such as signal transduction. Details of the molecular recognitions in lipid rafts, however, have yet to be revealed. We prepared ^{13}C- or ^{19}F-labeled sphingomyelin and ^{19}F-labeled cholesterol, and measured intermolecular distances in lipid rafts using the ^{13}C-^{19}F REDOR method. In addition, ^{2}H-labeled sphingomyelin was also synthesized and subjected to ^{2}H solid-state NMR to determine the mobility and orientation of sphingomyelin when forming lipid rafts.

Interaction between Polycyclic Ether Toxins and Membrane Peptides

Ladder-shaped polyether (LSP) toxins are characteristic metabolites of unicellular algae and are thought to bind to transmembrane (TM) proteins, as shown by brevetoxins and ciguatoxins, both of which are known to be extremely powerful toxins. LSPs are thought to interact with a ubiquitous motif in membrane proteins. We assessed the interactions between polycyclic ethers and the structural motif using surface plasmon resonance (SPR), SDS-PAGE and NMR experiments. SPR experiments showed that membrane proteins containing transmembrane α-helices had significant affinity for yessotoxin, which is another well-known toxin [associated][found in association] with seafood poisoning cases, and a desulfated derivative (dsYTX) with dissociation constants of about 10-100 μM. In contrast, water-soluble proteins and a membrane protein forming a β-sheet showed very weak interactions with YTX and dsYTX. These results suggest that LSPs recognize membrane proteins, particularly transmembrane α-helix.

During the course of our efforts to elucidate the interactions of LSPs with TM proteins, artificial ladder-shaped polyethers (ALPs) containing [the] 6/7/6/6 tetracyclic [system] (ALP4A, as shown in the Figure below), 6/7/6/6/7/6/6 heptacyclic (ALP7A), and 6/7/6/6/7/6/6/7/6/6 decacyclic system, have been synthesized using the convergent method via α-cyano ethers. The simple iterative structure of the ALPs possessing different number of rings would be useful for structure-activity relationship (SAR) studies on the molecular length, which is supposed to be important when naturally occurring LSPs elicit their toxicity. Preliminary SAR studies on the interaction of these ALPs with TM proteins have revealed that the ALPs dissociated dimers and/or oligomers of glycophorin A (GpA) into monomers (as shown in the Figure). The heptacyclic ether (ALP7A) elicited the most potent activity, whereas the decacyclic ether exhibited an intriguing phenomenon, inducing precipitation of GpA in a dose-dependent manner. The differences in activities among the ALPs can be correlated with the concept of 'hydrophobic matching' *i.e.* the length of the hydrophobic region (including the side chains of ALP7A) is ca. 25Å, which matches the lengths of the hydrophobic region of α-helical TM proteins, as well as the hydrophobic thickness of lipid bilayer membranes. These findings could provide a clue to understanding the molecular interactions underlying the potent biological activities of LSPs.



Polycyclic Ethers Interacting with Integral α-Helix

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