Rhombohedral trap for studying molecular oligomerization in membranes: application to daptomycin

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Abstract

A persistent problem in the studies of membrane-active peptides, including antimicrobial peptides and pathogenic amyloidal peptides, is the lack of methods for investigating their molecular configurations in membranes. These peptides spontaneously bind to membranes from solutions, and often form oligomers that induce changes of membrane permeability. For antimicrobials, such actions appear to relate to the antimicrobial mechanisms, but for amyloidal peptides, the oligomerization has been linked to neurodegenerative diseases. In many cases, no further understanding of such oligomerization has been achieved due to the lack of structural information. In this article, we will demonstrate a method of trapping such peptide oligomers in a rhombohedral (R) phase of lipid so that the oligomers can be subjected to 3D diffraction analysis. The conditions for forming the R phase and the electron density distribution in the rhombohedral unit cell provide information about peptide–lipid interactions and the molecular size of the trapped oligomer. Such information cannot be obtained from membranes in the planar configuration. For illustration, we apply this method to daptomycin, an FDA-approved antibiotic that attacks membranes containing phosphatidylglycerol, in the presence of calciumions. We have successfully used the brominated phosphatidylglycerol to perform bromine-atom anomalous diffraction in the rhombohedral phase containing daptomycin and calcium ions. The preliminary results apparently exhibit diffraction data related to daptomycin oligomers. We believe that this method will also be applicable to the difficult problems related to amyloidal peptides, such as amyloid beta of Alzheimer's disease.

Keywords - membrane-active peptides, oligomerization, rhombohedral (R) phase, anomalous diffraction, daptomycin.