Determination of the Conformation and Geometry of Bacterial Pilin Proteins in an Artificial Membrane

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Introduction

The aim of this collaborative research is to determine the architecture of *P. aeruginosa* PAK pilin and *C. perfringens* PilA2 in the cytoplasmic membrane. For understanding the cell adhesion and motility functions of type IV pili (TFP) in the membrane, the membrane-associated structure and dynamics of TFP will be investigated by solid-state NMR methods. We expect that our study can lead to the fundamental understanding of the twitching motility, self-aggregation, adhesion to host cells, genetic transformation and bacteriophage attachment of TFP in the cell membrane. Pilins have a tennis racket-like structure, with a conserved N-terminal α-helix (α1) which is hydrophobic, topped with a C-terminal globular head.[1] The α1 helix may serve as the core of the polymerization domain in the assembled pilus filament, holding subunits together in a hydrophobic helical array.[2] The globular domains at the C-terminus form the outer face of the pilus and comprise the interacting groups that would mediate pili functions. According to the known electron microscope image,[2] filaments are held together almost entirely by hydrophobic interactions among the N-terminal α-helices, but the precise positioning of these helices is not known. Subunits are related along a right-handed 1-start helix by an axial rise of 8-10 Å and an azimuthal rotation of ~100°. A dominant feature of TFP is a left-handed three-start helix, where the subunits follow 3 helical strands (starts) that form a left-handed twist along the filament axis (Fig. 1).

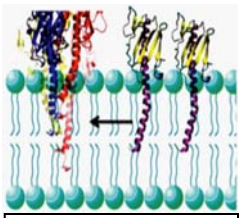

Fig. 1. Schematic diagram showing the first step in pilin polymerization.

Experimental

The orientation and insertion profile of the membrane-bound PilA2 subunit in oriented lipid bilayers were investigated by the polarization inversion spin-exchange at the magic angle (PISEMA) technique.[3] ^{15}N-labelled pilin subunits were prepared by growing *P. aeruginosa* cells in M9 minimal media supplemented with $^{15}NH_4Cl$. Pilin protein was reconstituted into a 3:2 molar mixture of POPE-PE and POPG-PG and the detergent removed by dialysis. Oriented lipid bilayers were prepared by dissolving the protein:lipid solution in trifluoroethanol (TFE), spreading the mixture on glass slides and evaporating the TFE.

Results and Discussion

Fig. 2 shows the PISEMA spectrum of oriented TFP in POPE(3)/ POPG(2) membranes prepared between coverglass plates. Overlaid on the spectrum are the tilt angle of the inserted N-terminal helix in the membranes. The average tilt angle is 25 ± 5° according to this preliminary simulations. The strong dipolar-CSA correlation peak intensity spanning over 70-140 ppm (^{15}N chemical shift anisotropy) and -4 ~ 4 kHz (^{1}H-^{15}N dipolar coupling) is due to the presence of the globular part of the protein that does not insert into the membrane. Based on Fig. 2, it is clear that the N-terminal α-helix, α1N, is immobile in the lipid bilayer even in the presence of the mobile, globular C-terminal domain. The C-terminal sit on the surface of the lipid bilayer, orienting α1N such that all these α-helices are aligned uniaxially in the applied magnetic field. Our future study will be the investigation of the depth profile of TFP in the membrane by utilizing various other solid-state NMR methods and sample labeling methods.

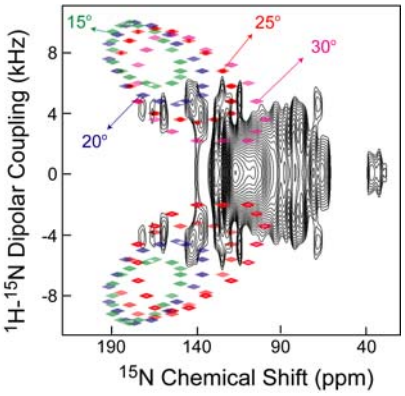

Fig 2. PISEMA spectrum of TFP aligned in oriented POPE (3)/POPG (2) measured at 40 °C.

Acknowledgements

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References

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