Characterization of Hydrogen Bonding In Amphiphilic Peptide Helices via REDOR

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Introduction

KLLLLKLLLLKLLLLKLLLLK (KL_4), in combination with phospholipids, can serve as a substitute lung surfactant in premature infants and reduce the incidence of respiratory distress syndrome. How KL_4 affects the phase properties of the lipids, the structure and dynamics of the peptide in the lipid membrane, and the molecular level interactions between KL_4 with the phospholipids are questions remaining to be answered. The focus of this project is to obtain NMR data to characterize hydrogen bonding patterns in KL_4 when interacting with lipid bilayers. This data will be combined with other ssNMR structural measurements, EPR measurements of partitioning, and molecular dynamics simulations to obtain a high resolution structure of the interaction between KL_4 and lipids found in pulmonary surfactant and develop a high-resolution molecular model to explain complex biophysical properties of lung surfactant.

Experimental

The unusual periodicity of hydrophobic and hydrophilic residues in KL_4 has lead to some controversy regarding both its backbone secondary structure and its orientation in lipid bilayers. Previously, Long *et al*. have measured peptide backbone torsion angles and peptide sidechain dynamics which have led to a model of KL_4 partitioning in the plane of lipid bilayers with an unusual helical structure that places the charged lysine residues on one side of the helix [1-2]. This model, combined with molecular dynamics simulations based on NMR constraints, suggests both i→i+4 and i→i+5 hydrogen bonding may stabilize the peptide conformation in a lipid bilayer. We have also found that the peptide can change helical conformation as a function of either lipid acyl chain saturation or pH. We are using homo- and hetero- dipolar recoupling techniques to measure the relative populations of these helical conformers and hydrogen bonding patterns at various points in the KL_4 helix. These studies will not only answer fundamental questions regarding the interaction between KL_4 and lipid bilayers, but will also provide constraints for a comprehensive model of structure-function relationships in lucinactant (the clinical formulation of KL_4 with PS lipids).

Results and Discussion

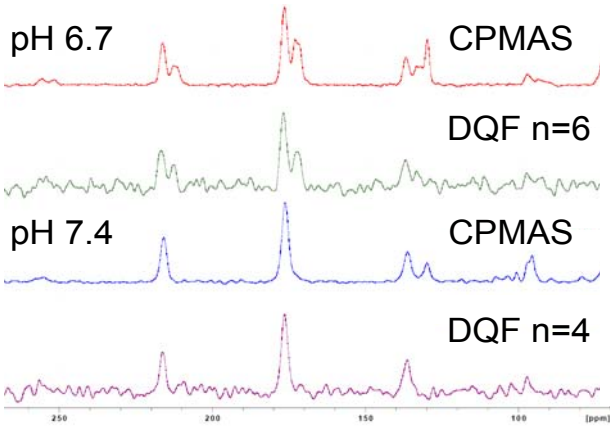

Figure 1. Data collected for KL4 $^{13}C'$ labeled at positions L4 and L5 in POPC/POPG lipid bilayers as a function of pH.

MD simulations of KL_4 constrained with $^{13}C'$-$^{13}C'$ distances from DQDRAWS experiments predict a helical structures intermediate between a classical □-helix and a □-helix. Two distinct helical conformers, with isotropic chemical shifts of 172 and 176 ppm, can be seen at lower pH; at higher pH a single conformer is observed. Using REDOR to measure distances between amino acids four and five residues apart, we have been able to confirm the predictions of the MD simulations based on the shorter distance constraints. We are continuing to collect further NMR restraints to develop a high resolution model of helical plasticity as a function of lipid composition and pH relevant to the lung environment.

Acknowledgements

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References

[1] F.D. Mills, et al., *Biochemistry* **47**:8292-8300 (2008).
[2] J. R. Long, et al., *BBA-Biomembranes*, **1798**:216–222 (2010)