Oriented Circular Dichroism Spectroscopy (OCD)

OCD is a fast and sensitive spectroscopic method for analyzing the secondary structure and orientation of membrane-embedded peptides and proteins in lipid bilayers that are macroscopically aligned with respect to the light beam. It helps, e.g., to understand the mechanisms during formation of transmembrane pores by antimicrobial peptides. The method is complementary to solid-state NMR structure analysis using the same oriented samples and exhibits characteristic features:

- very high sensitivity, minimum amount of peptide required ~1 µg / sample
- relative fast measurement (typically 3 hours / sample)
- no isotope labeling required (wt peptide can be used)
- simple peptide preparation (similar to solid-state NMR)
- exact control of temperature and humidity
- low resolution method: only global information on alignment and secondary structure of peptides
- at present theory is restricted to α-helical peptides

Experimental set-up OCD cell

Schematic of the developed rotatable OCD cell, which was manufactured in-house to measure peptide alignment in lipid bilayers at constant temperature and humidity. OCD cell mounted on rotation stage in JASCO J-810 spectropolarimeter; rotational averaging of spectra diminishes spectral artifacts caused by linear dichroism of the solid sample.

OCD reveals secondary structure, re-orientation and aggregation of membrane-active peptides in lipid bilayers

Most organisms use antimicrobial peptides as a first line of defense against bacterial invasion. A peptide found in the skin of the African frog Xenopus laevis is PGLa (GMASKAGAIGKALKALKALKNH2). MSI-103 (KIAKGIAIANH2), is a peptide designed based on the sequence of PGLa, and has a higher antimicrobial activity. MAP (KIAKGIAIANKALKNH2) is also a designer-made peptide, which can penetrate cell membranes. They all have amphiphilic properties, bind to lipid bilayers and should form α-helices in membranes. We have used OCD to study their structure and orientation in DMPC bilayers for understanding structure / function relationships.

OCD spectra of PGLa in DMPC bilayers, showing its re-arrangement from a surface-bound α-helical S- to a helical T-state induced by increasing the peptide/lipid ratio.

Results:

- PGLa, MSI-103 and the D-epimer of a MAP analogue exhibit mostly α-helical conformation and re-arrangement in DMPC
- For low peptide concentration the S-state predominates, at threshold P/L the T-state starts to appear, and above a higher threshold P/L all peptides are in the T-state
- For all three peptides P/L was about four times P/L, but the value of P/L varies strongly in the order MSI-103 > MAP > PGLa (same order found in NMR11)
- The P/L threshold is inversely correlated with the charge and hydrophobic moment of the peptides

Conclusion

OCD allows to screen and identify conditions where functionally relevant changes in peptide structure and orientation occur as a function of concentration, lipid environment, temperature, and humidity. These conditions can then be used in high-resolution solid-state NMR structure and alignment analysis of such systems.

References