Molecular Insight on the Molecular Mechanisms for the Biological Activity of Dendritic Amphiphiles on Biological Membranes

A. K. Sum¹, R. D. Gandour², J. Karlovská³, S. S. Funari⁴, P. Balgavý³

¹Department of Chemical Engineering, ²Department of Chemistry, Virginia Polytechnic Institute and State University, Blacksburg, Virginia, USA

³Department of Physical Chemistry of Drugs, Faculty of Pharmacy, Comenius University, Odbojárov 10, SK-832 32 Bratislava, SLOVAKIA

⁴HASYLAB, DESY, Hamburg, GERMANY

48th International Conference on the Bioscience of Lipids

Turku, FINLAND

4-8 September, 2007

Introduction

Lipid bilayers are central in the study and understanding of biological membranes. Their structure, function, and activity are directly related to the composition and molecular properties of the membrane constituents. In an effort to understand the antimicrobial activity of a new class of dendritic amphiphiles (**3CAmn**)—potentially safe, effective, acceptable, and affordable topical microbicides with anti-HIV, anti-STD pathogens, antibacterial, and antifungal activities [1], we combine experimental and computational techniques to obtain insight into how these amphiphiles interact with lipid bilayers. Because **3CAmn** are lipid-like compounds, their interactions with the lipid bilayer of biological membranes result in biophysical changes in the structure and dynamics of the membranes. Wide-angle (WAXD) and small-angle (SAXD) X-ray diffraction on model **DPPC** membranes show systematic changes to the membrane structure and, consequently, the phase transition due to interaction with these dendritic amphiphiles. Molecular dynamics simulations of these structures reveal the specific molecular mechanisms associated with the changes to the membrane structure. This study represents a multi-scale modeling approach to design, develop, and understand the biological activities of this homologous series of dendritic amphiphiles that include very long chains.

[1] Williams, A. A.; Sugandhi, E. W.; Macri, R. V.; Falkinham, J. O., III; Gandour, R. D. *Antimicrobial Activity of Long-Chain, Water-Soluble, Dendritic Tricarboxylate Amphiphiles*. *J. Antimicrob. Chemother.* 51, 451–458 (2007).

Dendritic Tricarboxyl Amphiphiles

Dipalmitoylphosphatidylcholine (DPPC)

- Diffraction peaks fitted with Lorentzians and linear background to determine maxima, intensities, integral intensities, and half-width of peaks
- Pre-transition and main transition were present for pure **DPPC** and 0.05:1 **3CAmn:DPPC**
- Pre-transition was shifted to lower temperature for the mixtures
- Data used to obtain main gel-liquid crystalline phase transition temperature

Typical RDFs between 3CAmn and DPPC

- **3CAmn** amide group hydrogen bonds to **DPPC** carbonyl group, mainly O35
- **3CAmn** acid groups hydrogen bond to **DPPC** phosphate headgroup

Experimental: Materials and Methods

Long chained amphiphile and **DPPC** (Avanti) were mixed in desired molar ratio in chloroform + methanol, the solvent was evaporated under a stream of gaseous nitrogen and its traces removed by an oil vacuum pump. Redistilled water was added at the H₂O:**DPPC** = 5:1 weight ratio and the mixture was homogenized mechanically at 60 °C. SAXD and WAXD experiments were performed at beam A2 in HASYLAB at DESY using a monochromatic radiation of wavelength 0.15 nm. During temperature scans, the samples were heated from 20 to 60 °C in 40 min. and the diffractograms were recorded for 10 sec. every min. Data reduction, normalization and analysis were done with the programs STAF0, OTOKO, PEAKFIT and GRAPHER.

Simulation Methods

- NPT simulations
- Berendsen thermostat
- Berendsen barostat
- 2 fs time-step
- Over 80 ns of simulation time
- Short-range cutoff: 1.0 nm
- PME for long-range electrostatics
- All-bonds constrained (LINCS and SHAKE)
- Water: SPC model
- **DPPC**: Berger *et al.* and Chiu *et al.*
- **3CAmn**: OPLS
- United-atom for methyl and methylene groups
- GROMACS 3.3.1 (single precision in parallel)

All systems simulated at:
T = 27 °C and 52 °C
P = 1 bar

All systems contained 40 water per total **3CAmn** + **DPPC**

Snapshot of 3CAmn:DPPC bilayer (water not shown)

- **3CAmn** aligns with lipid along the bilayer
- At 325 K, **3CAmn** induces a slight order in the bilayer
- At 300 K, **3CAmn** induces significant order on the carbon atoms near the headgroups
- In the liquid-crystalline state, most **3CAmn** molecules have the end tail bent
- In the gel state, most **3CAmn** molecules have the end tail straight penetrating the opposing leaflet
- On average, four **DPPC** molecules surround each **3CAmn**

Acknowledgements

The experimental portion of this study was supported by the European Community – Research Infrastructure Action under the FP6 “Structuring the European Research Area” Programme Contract RI3-CT-2004-506008 (HASYLAB project II-05-018 EC) and by the VEGA 1/3029/06 and APVV 51-027404 grants.

- Beam-time for SAXD and WAXD experiments made available in HASYLAB at DESY
- Computational resources provided by Virginia Tech Advanced Computing Research facilities
- Travel financial assistance provided by VT Chemical Engineering and VT Research Office