Structure of Lipopolysaccharide Multilayers in a Presence of External Counterions.

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Lipopolysaccharides (LPS) are a major class of macromolecules populating the surface of Gram-negative bacteria. They contribute significantly to the bacteria's surface properties and play a crucial role in regulating the permeability of its outer membrane. They are found exclusively on the outer face of the outer membrane, forming an asymmetric bilayer. The location of LPS molecules at the cell's surface then contributes to the overall cell surface properties and its interactions with the outside components with the cell.

There have been many studies on LPS in an attempt to establish its structure and function in bacterial membranes. An interesting result was recently observed by us using "smooth" LPS [1]. The structural results, based on neutron scattering contrast variation, revealed water penetration into the hydrocarbon region up to and including the center of liquid crystalline LPS bilayers [1]. This unexpected outcome has far-reaching impact on the understanding of how small molecules penetrate the outer membrane of Gram-negative bacteria. It was also believed that this permeability to water would be modulated by the type of cations which bind to the LPS. In other words, specific LPS-salt interactions can affect the stability of the outer membrane, resulting in limited or enhanced permeability [2].

Neutron diffraction experiments were conducted using the N5 spectrometer located at the National Research Universal (NRU) reactor (Chalk River, ON). The oriented multilayers were constructed from LPS molecules that included Na^+, Ca^{2+} or Mg^{2+} counterions and were hydrated through the vapor of different D_2O vs. H_2O mixtures (e.g.: 100, 50 and 0 % D_2O). Relative humidity (RH) was controlled by saturating the various water solutions with KCl salt (81% RH), while temperature was maintained at 50 ^oC. LPS is expected to form a lamellar phase at this hydration and temperature, which was confirmed by the characteristic diffraction pattern. Up to 6 lamellar Bragg reflections were collected for well-aligned samples, while only 3 peaks were collected for the Mg^{2+}-LPS sample. Scattering length density (SLD) profiles were reconstructed from the corrected peak intensities using the Fourier transform. The phases of diffraction peaks were determined from H_2O/D_2O contrast variation resulting in the SLD profiles of LPS bilayers shown in a Figure 1.

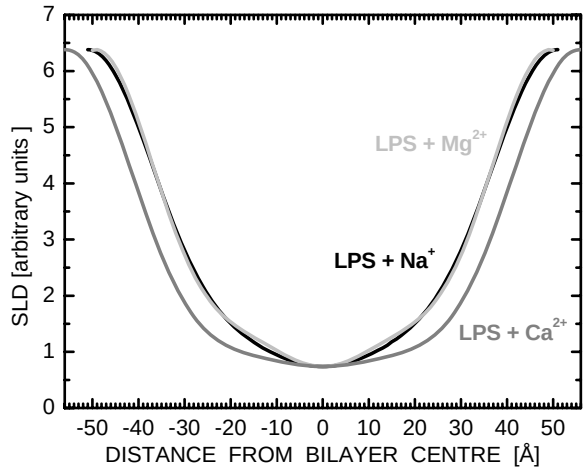

Figure 1: 1D scattering length density (SLD) profiles obtained from the Fourier reconstruction of diffraction data from oriented multilayers of LPS with Na^+, Ca^{2+} and Mg^{2+} counterions and hydrated with 100 % D_2O. The profiles correspond to the results reconstructed from 3 diffraction peaks.

Features of the one dimensional SLD profiles can be linked to the chemical structure of LPS molecules. The bilayer is formed by two LPS monolayers, with their hydrophilic groups residing in the water region and exemplified by the highest SLD (as it corresponds to 100 % D_2O). The SLD values decrease continuously to their minima corresponding to the hydrophobic acyl chains in the middle of the bilayer. However, this slow decrease in SLD suggests that significant amounts of D_2O molecules penetrate deep into the bilayer. It was previously reported [1], that such water molecules penetrate up to and including the hydrophobic core center. Although, the presently obtained SLD profiles do not provide sufficient resolution for such conclusions, their behavior is consistent with this observation. The absence of the characteristic central trough in the middle of the bilayer indicates the presence of D_2O molecules in this region. This result is due to a combination of a highly disordered hydrocarbon bilayer center and D_2O molecules occupying the space of displaced lipid chains.

The effect of external counterions on the structure of LPS bilayers was examined for Na^+, Mg^{2+} and Ca^{2+} cations. We have observed a substantial structural change in a presence of Ca^{2+} as compared to the other two ions (see Figure 1). The region of water penetration is shifted further from the bilayer center, implying less water molecules located inside LPS hydrophobic core. This is most likely a result of more compact organization of LPS molecules, inhibiting water from filling the defects which occur in a case of more disordered structures in a presence of Na^+ and Mg^{2+} cations. Our observation agrees nicely with the increased stability of the outer membrane of Gram-negative bacteria in the presence of metal cations Ca^{2+} [2], and contributes to the understanding of properties and mechanism of how small molecules penetrate this membrane.

References

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