Effect of Cations on the Structure of Bilayers Formed by Lipopolysaccharides Isolated from *Pseudomonas aeruginosa* PAO1

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Bacterial lipopolysaccharides (LPSs, Figure 1) are the major lipid component making up the outermost leaflet of the asymmetric outer membrane (OM) of Gram-negative bacteria [1,2]. LPS contributes to the OM's structural integrity and also protects the bacteria from a variety of toxic molecules, such as certain antibiotics (e.g. penicillin), digestive enzymes (e.g. lysozyme), detergents, heavy metals, bile salts, and some dyes. On the other hand, the OM's inner leaflet is predominantly composed of common lipids such as phosphatidyl-ethanolamine (PE), phosphatidyl-choline (PC), phosphatidylglycerol (PG) and cardiolipin (diphosphatidylglycerol) [3]. The passage of nucleotides, disaccharides, amino acids, vitamins, and iron for nutritional growth are usually transported through the OM by porin proteins, but it is LPS that provides the bacteria with its remarkable selectively permeable membrane that is resistant to a variety of deleterious agents. In particular, *P. aeruginosa* is well-noted for its recalcitrance to conventional antibiotic therapy, partly as a result of its unique surface chemistry [4]. For this reason, and also due to the ubiquity of *P. aeruginosa* and its impact upon health as both an opportunistic and nosocomial pathogen, this organism represents an attractive candidate for study.

Here we report of the effects of three different cations (i.e. Na^+, Ca^{2+}, Mg^{2+}) on the multilamellar structure of bilayers formed using LPS isolated from *P. aeruginosa* PAO1. From the different one-dimensional neutron scattering length density (1D SLD) profiles, we find lower penetration of water molecules through Ca^{2+}-LPS bilayers, when compared to Na^+- or Mg^{2+}-LPS bilayers.

Features in the 1D SLD profile (Figure 2) can be associated with identifiable LPS chemical moieties. The bilayer is formed by two LPS monolayers, with their hydrophilic polysaccharide chains residing in the inter-layer water region, which in the 100% D_2O case has the highest SLD. From the water region, the SLD decreases in a continuous fashion to the bilayer centre. The central bilayer region is made up of Lipid A hydrocarbon chains, which are calculated to extend about 12.5 Å on either side of the bilayer centre [5].

The hydrocarbon chains are attached to the Lipid A headgroup and the inner core, further extending the LPS molecule (from 12 to 24 Å on either side of the bilayer). The SLD profile is slightly higher in this region as it contains chemical groups with high neutron SLDs (e.g. phosphates and carboxylates). The SLD region associated with these groups is much more distinct in the case of Ca^{2+}-LPS bilayers, while in the case of Na^+-LPS bilayers this region is somewhat obscured by a presence of high SLD D_2O. Further increases to the SLD profile reflect the ever-increasing amounts of D_2O into the outer core and O-side chain regions.

For the most part, the SLD profiles of Ca^{2+}- and Na^+-LPS exhibit the same structural features (Figure 2). Nevertheless, where they differ is in the outer/inner core region. In Ca^{2+}-LPS bilayers, compared to Na^+-LPS, the amount of water penetrating this region is substantially less. But they cannot be compared directly as a result of different scaling factors associated with the two SLD profiles. The differences in hydration between the two bilayers are better appreciated by referring to the water distribution profiles shown in Figure 3.

In the region ±14 Å from the bilayer centre, water molecules are seemingly distributed similarly in both Ca^{2+}- and Na^+-LPS bilayers, but differ outside of this region. In the case of Na^+-LPS bilayers the amount of water begins to increase at approximately ±14 Å (inset to Figure 3), while this increase is not seen until approximately ±22 Å (inset to Figure 3) in Ca^{2+}-LPS bilayers, indicating that the outer/inner core region of Ca^{2+}-LPS is substantially less hydrated. Interestingly, although the amount of water differs in the two systems, the shape of the water distribution functions are fundamentally similar, implying that the O-side chains are similarly hydrated.

In addition to Ca^{2+}- and Na^+-LPS bilayers, we studied the effect of Mg^{2+} on LPS. Unfortunately, the Mg^{2+}-LPS bilayers yielded lower resolution diffraction patterns and as a consequence, less detailed 1D SLD profiles. Figure 4 compares the 1D SLD profiles for the three different systems reconstructed using only three Bragg reflections, thus ensuring a direct comparison between the various bilayers. Interestingly, there is little difference between the Na^+- and Mg^{2+}-LPS bilayers, implying that these two counterions have a similar effect on LPS, despite their different valence number.

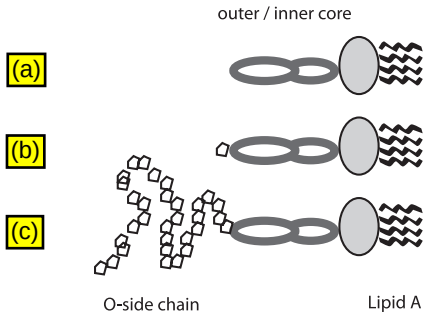

Fig. 1 *Schematic of LPS architectures. LPSs consist of a hydrophobic region formed by lipid A, and an extensive hydrophilic region made up of the inner and outer cores, with additional O-side specific chains: (a) "Rough" LPS does not contain O-side chains. (b) "Semirough" LPS has an O-side chain with only one repeat unit. (c) "Smooth" LPS with an O-side chain made up of up to 50 trisaccharide repeat units.*

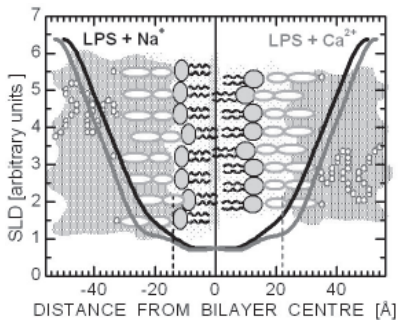

Fig. 2 *Arbitrary scale 1D SLD profiles obtained from the Fourier reconstruction of diffraction data from oriented LPS bilayers hydrated in 100% D2O. The solid black line corresponds to Na+-LPS bilayers ~~(calculated from structure factors shown in Table 1)~~, whereas the solid grey line corresponds to Ca2+-LPS bilayers ~~(structure factors in Table 2)~~. The dashed lines demarcate the borders of regions that are highly accessible to water.*

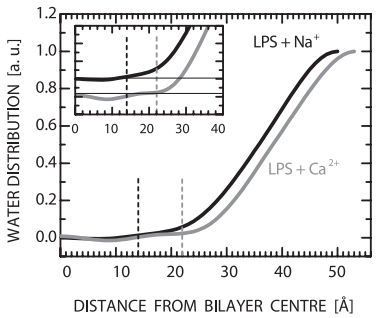

Fig. 3 *Water distribution profiles of Na+- and Ca2+-LPS bilayers (only half of the bilayer is shown). The water distribution functions in the O-side chain region are similar for the two systems, whereas they differ in the bilayer core. The amount of water begins to increase at ± 14 Å in Na+-LPS, whereas this increase is not seen until ± 22 Å in the case of Ca2+-LPS bilayers, as emphasized in the inset (vertical offset was introduced for clarity purposes).*

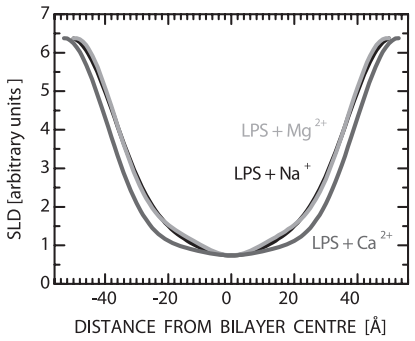

Fig. 4 *1D SLD profiles calculated for oriented multilayers of Na+-, Mg2+-, and Ca2+-LPS bilayers hydrated with 100% D_2O. All profiles were reconstructed using three Bragg reflections.*

References

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