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ATR-FTIR study of lipopolysaccharides at mineral surfaces

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Abstract

Lipopolysaccharides (LPS) are ubiquitous in natural aqueous systems because of bacterial cell turnover and lysis. LPS sorption and conformation at the mineral/water interface are strongly influenced by both solution and surface chemistry. In this study, the interaction of LPS with various surfaces (ZnSe, GeO_2, α-Fe_2O_3, α-Al_2O_3) that vary in surface charge and hydrophobicity was investigated using attenuated total reflectance Fourier transform infrared (ATR-FTIR) spectroscopy. The presence of Ca^{2+} (versus Na^+) in LPS solutions resulted in aggregate reorientation and increased sorptive retention. ATR-FTIR spectra of Na-LPS systems are consistent with reduced surface affinity and are similar to those of solution phase LPS. Ca-LPS spectra reveal hydrophobic interactions of the lipid A region at the ZnSe internal reflection element (IRE). However, pH-dependent charge controls Ca-LPS sorption to hydrophilic surfaces (GeO_2, α-Fe_2O_3, and α-Al_2O_3), where bonding occurs principally via O-antigen functional groups. As a result of accumulation at the solid–liquid interface, spectra of Ca-LPS represent primarily surface-bound LPS. Variable-angle ATR-FTIR spectra of Ca-LPS systems show depth-dependent trends that occur at the spatial scale of LPS aggregates, consistent with the formation of vesicular structures.
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Keywords: LPS; ATR-FTIR spectroscopy; Depth profiling; Endotoxins

1. Introduction

Bacterial adhesion to surfaces is a complex function of the full array of macromolecules resident on the cell surface (e.g., LPS, EPS, teichoic acids, surface proteins, flagella), substratum surface chemistry (e.g., hydrophobicity, surface charge), aqueous environmental conditions (e.g., pH, ionic strength), and the distribution and composition of conditioning films. No single factor exerts full control, and therefore deconvolution of various factors requires a model systems approach. In the case of Gram negative bacteria, the surface interaction of free- and membrane-bound lipopolysaccharides (LPS) certainly represents one of the important molecular-level controls over bacterial adhesion [1–4]. LPS are amphiphilic molecules with a hydrophobic lipid A region embedded in the outer membrane of Gram negative bacteria [5]. Beyond the lipid A is a "core sugar" region, and the O-antigen (Fig. 1). The portion of the molecule comprising the O-antigen is present in "smooth" LPS, whereas it is absent from "rough" LPS. The O-antigen is hydrophilic and extends outward from the intact cell into aqueous solution. It is composed of 20–70 repeating units of three to five sugars [5]. Some bacteria, such as *Pseudomonas aeruginosa*, possess LPS with O-antigens extending up to 40 nm from the cell surface [6]. Since cell turnover and lysis results in the presence of both "cell-bound" and "free" LPS in natural aquatic systems [7], LPS may promote bacterial adhesion by sorption of either free LPS molecules to surfaces during conditioning film formation, or through cell adhesion mediated by membrane bound LPS [1,3]. It has been suggested that during cell adhesion to negatively charged surfaces, the O-antigen may extend beyond the electrostatic energy barrier and become adsorbed in a secondary minimum in close proximity to the surface [3].

Adhesion of both rough and smooth LPS has been observed to occur on metal oxides [2,8], crystalline calcium silicate hydrate [9,10], ZnSe [11], GeO_2, positively charged lipids and polymers [12], and to bovine lung and tracheal tissue samples [13]. LPS of *P. aeruginosa* ser 10 LPS (in ultrapure water) was found to bond more strongly to positively charged (aminopropyltriethoxysilane polymers) than to hydrophilic (GeO_2) or hydrophobic (dipalmitoylphosphatidic acid monolayer) surfaces [12].

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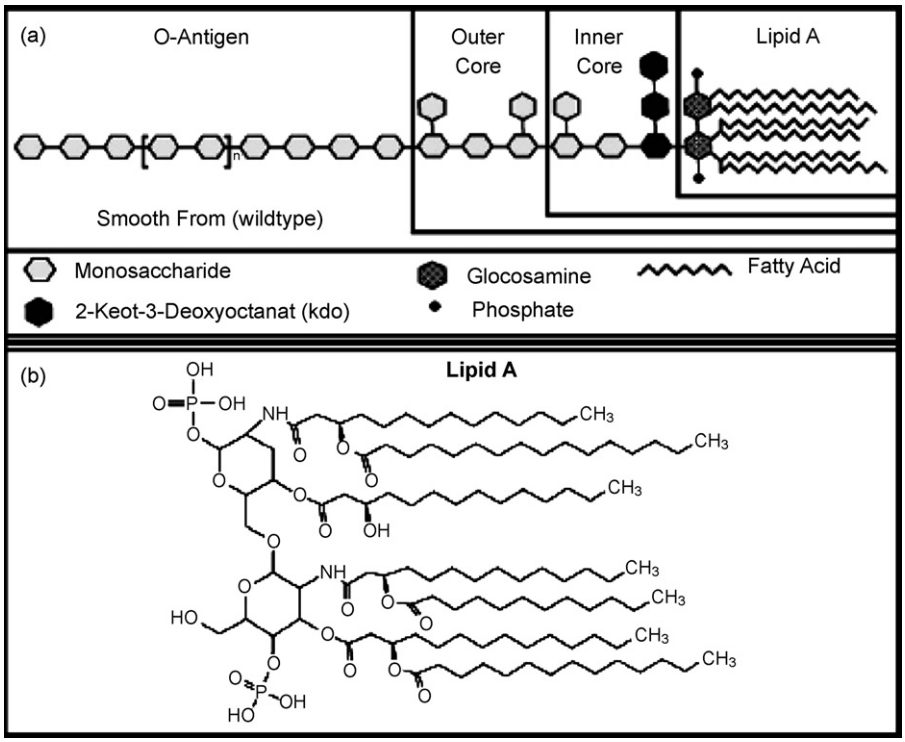

Fig. 1. Schematic diagram of (a) smooth lipopolysaccharide and (b) lipid A [5]. Republished with permission from Parikh and Chorover [11].

Adsorption of free LPS may be mediated by functional groups associated with either hydrophilic or hydrophobic portions of the molecule. However, in free LPS, exposure of the lipid A is limited by LPS amphiphilic properties that promote intermolecular associations and the formation of supramolecular structures above a critical aggregation concentration (CAC) [14–17]. Dynamic light scattering measurements indicate that LPS aggregate sizes (4 mg mL^{-1} LPS, *I* of 10 mM, pH 6) range from 325 to 400 nm for Na-LPS and from 400 to 475 nm for Ca-LPS [18]. LPS aggregates have been used above the critical aggregate concentration (CAC) to represent cell-bound forms under the assumption that only the O-antigen is exposed for interaction with environmental surfaces. For example, Jucker et al. [2] measured the adsorption of phosphate-buffered LPS aggregates in various ionic strengths and electrolytes (NaCl, KH_2PO_4, K_2HPO_4) to surfaces of TiO_2, Al_2O_3, and SiO_2. They found greater adhesion to TiO_2 and Al_2O_3 surfaces. In some cases irreversible adhesion was observed, particularly for LPS with long O-antigen regions. However, the possible surface interactions of monomeric LPS (in thermodynamic equilibrium with aggregates) and/or the potential restructuring of LPS aggregates that may occur upon association with a surface were not investigated.

The relation between surface hydrophilicity and LPS structure also plays a role. For example, *P. aeruginosa* (PAO1) cells with primarily long O-antigen preferentially adhere to hydrophilic surfaces, whereas cells with shorter O-antigen have a higher affinity for hydrophobic surfaces [1]. Thus, the capability of a cell to mediate O-antigen length might confer a capacity to influence adhesion in dynamic environments. However, adhesion of free-LPS to surfaces may be quite different, particularly if LPS aggregates are disrupted and interaction between the lipid A region and a substratum is favorable.

Toward the goal of building a molecular-level understanding of initial bacterial cell adhesion at mineral surfaces, the current work involves isolation of the LPS component for in-situ spectroscopy studies. This approach allows us to investigate the influence of substratum surface composition (charge and hydrophobicity), solution chemistry, and LPS aggregation. Specifically, it has been suggested that an increase in LPS aggregation [19–22] might result when Ca^{2+} – rather than Na^+ – is present because of the high stability of Ca^{2+} complexation with phosphate moieties of the lipid A region. If so, that would be expected to affect surface interactions of the macromolecules [14,19,23,24]. Our previous studies using attenuated total reflectance (ATR) Fourier transform infrared (FTIR) spectroscopy do indeed provide direct evidence of Ca^{2+} binding to LPS phosphate groups, but also suggest a more complex effect on aggregate structure [11]. The spectra showed increased intensity of phosphate and fatty acid absorbances relative to carbohydrate for Ca-LPS versus Na-LPS samples. These results suggested that Ca^{2+} ion bonding to LPS phosphate groups in the lipid A region resulted in disruption of LPS aggregates. Our data support the hypothesis of Wang et al. that Ca^{2+} may disrupt LPS aggregates causing reorientation on calcium silicate hydrate surfaces [9]. Therefore, the objective of this work was to examine the LPS aggregation and reorientation in the presence of surfaces with varying surface chemistry.