LIPOPOLYSACCHARIDES ASSOCIATION ON THE SURFACE OF A SILICON CRYSTAL ADDRESSED BY THz SPECTROSCOPY AND MOLECULAR MODELLING

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Abstract. Gram-negative bacteria lipopolysaccharides (LPS) are lipids with a chemical structure comprising a lipidic region with several hydrocarbonate chains and a large saccharide region. Due to their structure, LPS can associate through hydrophobic forces, but also can dissolve in water. Here we investigated the association of LPS molecules on the surface of a silicon crystal by terahertz (THz) spectroscopy. We measured the THz absorption of LPS molecules directly deposited on the crystal or deposited on the crystal coated with a $CaCl_2$ solution. Resulting spectra present a non-linear increase of THz absorption, with two absorption shoulders at 1.05 THz (~35 cm^{-1}) and 1.37 THz (~45 cm^{-1}). The presence of the $CaCl_2$ solution does not influence the shoulders localization, suggesting a similar association of LPS molecules in both situations. Based on the comparison with previous THz studies on phospholipid bilayers, we suggested a layer ordering of LPS molecules in our samples that was validated through molecular modelling methods. We modelled LPS monolayers and simulated the THz spectra by normal modes analysis (NMA) and molecular dynamics (MD). The NMA spectrum of a LPS monolayer resulted in agreement with experiments, allowing us to validate the proposed LPS layer organization and the spectra simulation method.

Key words: lipopolysaccharides, THz spectroscopy, spectra simulation.

INTRODUCTION

LPS are glycolipids specific to the external leaflet of Gram-negative bacteria outer membrane. LPS molecules represent the endotoxins responsible for the toxicity of these bacteria, as they are released when the bacterial cell walls are damaged. By interacting with Toll-like receptors from immune cells, LPS stimulate

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the host immune system and trigger dose-dependent immune responses that vary from mild to very intense, up to septic shock [1].

The chemical structure of LPS molecules is significantly different from that of eukaryotic phospholipids. They comprise three regions: (i) a hydrophobic internal region, lipid A, comprising up to seven fatty acids bound to a disaccharide backbone, (ii) an intermediary region, the oligosaccharide core, formed by sugar units bound to lipid A, and (iii) an external polysaccharide region, the O antigen, formed by a variable length chain of repeating oligosaccharide units [7]. While lipid A and the core region have a more conserved structure between different bacteria strains, the structure of the core region being associated with the genus specificity [8], the O antigen is very variable and its structure is associated with the serologic specificity of bacteria from the same group [14]. The minimal LPS structure that presents endotoxic activity is represented by lipid A [14].

LPS molecules have marked amphipathic properties: they readily associate through hydrophobic forces or cross-links between glycans, but they also easily dissolve in aqueous solutions [15]. In the present work we investigated the association of LPS molecules on the surface of a silicon crystal using Terahertz (THz) spectroscopy. THz spectroscopy, using radiation in the 0.3–3 THz frequency range, addresses the sub-picosecond to picoseconds dynamics of molecules that gives information on the dielectric relaxation of lipid bilayers and their hydration water [19]. To our best knowledge, there are no THz spectroscopy studies performed on LPS molecules and only a few THz spectroscopy studies were performed on phospholipids.

THz time-domain spectroscopy was used for investigating the hydration and temperature effects on the low frequency spectra of lipids bilayers constituted as lipid films [2, 4, 19], multilamellar [4] or stacked membranes [17]. Yamamoto N. et al. showed that the THz spectrum of a 1,2-dimyristoyl-sn-glycero-3-phosphoryl-3′-rac-glycerol (DMPG) bilayer in gel phase presents a monotonic increase of absorption coefficients with frequency, presenting two wide absorption bands at 40 cm^{-1} and 60 cm^{-1} [19]. The location of these bands does not change upon hydration since hydration does not change the packing of the DMPG bilayer, as proven by X-ray diffraction and Fourier-transformed infrared spectroscopy [19]. In contrast, in the case of 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC) bilayers, hydration changes lipid packing and consecutively shifts the frequency of the THz absorption bands [2, 19].

Here we considered both dry and hydrated LPS molecules deposited on the ATR silicon crystal. Resulting spectra show an absorption band at a frequency independent of hydration. The packing of LPS molecules was addressed by molecular modelling, as we simulated THz spectra of different LPS structures and compared them with experiments. Our results point toward a layer association of the analysed LPS samples.

MATERIALS AND METHODS

EXPERIMENTAL METHODS

Samples for spectroscopy measurements were prepared using LPS molecules from *Escherichia coli* O111:B4 (further called LPS molecules, unless a different *E. coli* strain is specified) purchased from Sigma-Aldrich (product code L2630). LPS molecules (2 mg/mL) were dissolved into a 7:3 chloroform-methanol mixture [16].

THz spectra were recorded using a TPS spectra 3000 spectrometer (TeraView Ltd., United Kingdom) in attenuated total reflection (ATR) setup under continuous nitrogen purge. The ATR module comprises a 35 degrees cut silicon crystal on which samples are deposited. The resulting spectra had a resolution of 1.2 cm^{-1} and represent the averaging over 1000 spectra acquired with 30 scans/second rate. A three-term Blackman-Harris apodization function symmetrical around the peak, available in the spectrometer software, was applied for improving data quality.

Two sets of measurements were performed. In the first set, LPS-solvent mixtures were deposited directly on the silicon crystal and spectra were acquired only after the complete evaporation of solvents, considering as reference the ATR spectrum of air. A second set of measurements involved coating the silicon crystal with 10 μL of a 100 mg/mL $CaCl_2$ solution. LPS-solvent mixtures were added afterwards and the organic solvents were left to evaporate prior to measuring the spectrum. The reference spectrum was considered that of the $CaCl_2$ solution layer. In both cases, we considered that solvents evaporated completely when the consecutively acquired spectra did not show any variation.

MOLECULAR MODELING METHODS

The structure of *E. coli* O111:B4 lipid A and core oligosaccharide regions were identified from [5, 12, 14]. The molecule was built based on the structure of *E. coli* K12 modelled in a previous study [11] by retaining the common parts and by *de novo* modelling of the different parts using Discovery Studio [2]. The same software was used for structure optimization and for deriving CHARMM force field parameters necessary for the simulation of LPS molecules. The resulting 3D structure of the LPS molecule and a comparison between *E. coli* K12 and *E. coli* O111:B4 core oligosaccharide regions are presented in Figure 1.

A monolayer comprising 16 *E. coli* O111:B4 LPS molecules was modelled by replacing the *E. coli* K12 LPS molecules from a membrane leaflet built and equilibrated in a previous study [11]. The monolayer was neutralized with 64 Ca^{2+} ions and hydrated with 2745 water molecules. The molecular dynamics (MD) of the monolayer was simulated with NAMD [13] using in plane periodic boundary

conditions, at 300 K and 1 atm. After system heating at 300 K, we simulated 1 ns of MD for equilibration followed by 5 ns of production run. The THz spectrum of the monolayer was calculated based on the 5 ns MD trajectory as the auto-correlated Fourier transformed total dipole moment from MD trajectories using the infrared spectral density calculator plugin of VMD [6].

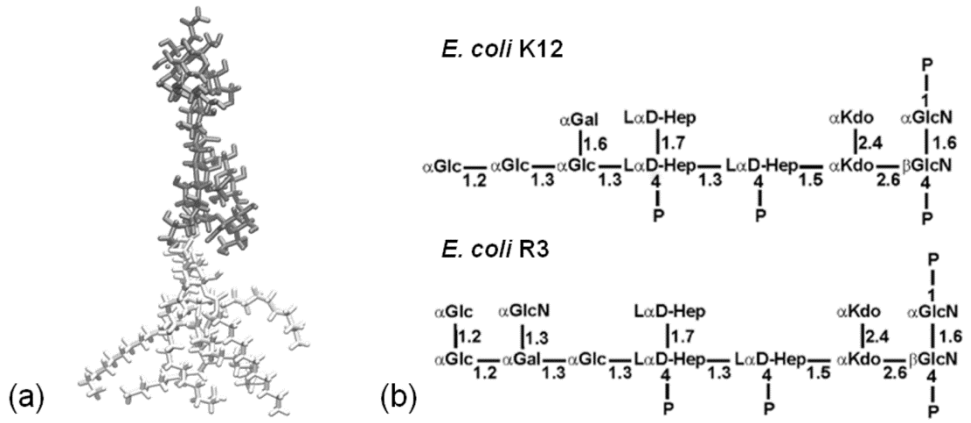

Fig. 1. (a) The 3D structure of *E. coli* O111:B4 LPS molecule. Lipid A is represented with white and the oligosaccharide region is represented with grey. (b) Structure of LPS oligosaccharide core of *E. coli* O111:B4 in comparison to *E. coli* K12.

A single LPS molecule and a patch of five LPS molecules were extracted from the monolayer structure. Their THz spectrum was calculated based on the information derived from NMA, as presented in previous studies [9, 10]. NMA was performed after thorough energy minimization with CHARMM [3, 18].

RESULTS AND DISCUSSIONS

LPS ASSOCIATION ADDRESSED EXPERIMENTALLY

The association of LPS molecules on the silicon crystal of the THz spectrometer ATR module was investigated in two situations. Firstly, the LPS-solvent mixture was deposited directly on the crystal in order to observe the ordering of LPS molecules in the absence of water. Secondly, we coated the ATR crystal with a thin layer of $CaCl_2$ aqueous solution on which we deposited the LPS-solvents mixture. The choice of coating solution thickness was based on the facts that: (i) aqueous solutions heavily absorb THz radiation, implying that a thicker solution could hide LPS contribution to the spectrum and (ii) the penetration depth

of THz radiation decreases with frequency (a discussion is given in [10]), possibly resulting into missing LPS contribution to the spectrum at higher frequencies in the case of a thicker coating. The purpose of the coating solution was to create a water-air interface on which LPS molecules can arrange as lipid films.

The spectra measured in the 0.3–1.5 THz frequency range are presented in Figure 2. In the figure we present only a spectrum for each condition, as repeating the measurement with samples from the same LPS-solvents mixture led to similar results (data not shown).

The spectrum acquired on LPS molecules from the LPS-solvents mixture deposited directly on the silicon crystal presents a THz absorption that increases non-linearly with frequency. While the spectra that we previously measured on proteins and protein solutions were linear [9, 10], the monotonic, non-linear increase of THz spectra measured in the case of LPS molecules was also identified in the case of phospholipids THz spectra [2, 19]. We can observe absorption shoulders around 1.05 THz (~35 cm^{-1}) and 1.37 THz (~45 cm^{-1}). Such absorption shoulders/bands were previously identified on the THz spectra of: (i) phospholipids in gel phase, at these exact frequencies in the case of DMPC bilayers [2] or at a intermediate frequency (40 cm^{-1}) in the case of DMPG bilayers [19], or (ii) of crystalline DMPG, at 36 cm^{-1} [19]. We expect the similarity with DMPG in crystalline phase to be a coincidence, as Yamamoto N. et al. determined that DMPG in crystalline phase were found in the purified powder, while the treatment with chloroform−methanol leads to the emergence of gel phase [19].

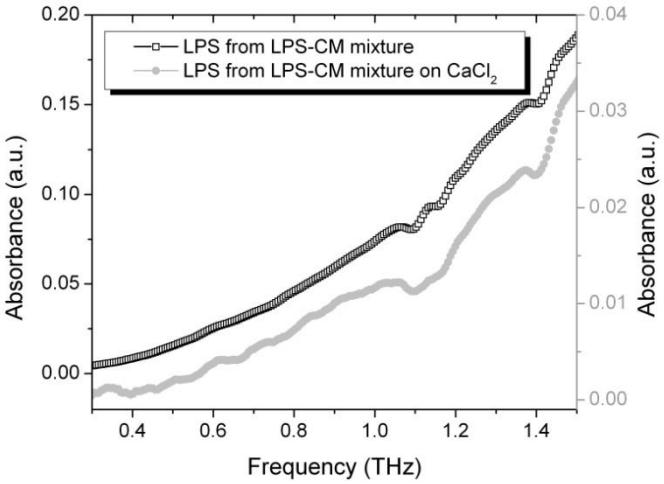

Fig. 2. The THz spectra of LPS molecules on the surface of the ATR crystal resulting from the LPS-solvents mixture deposited directly on the crystal and from the LPS-solvents mixture deposited on the crystal coated with $CaCl_2$.

When the LPS-solvents mixture was added on the ATR crystal coated with $CaCl_2$ solution, the resulting THz spectrum had a similar shape to that measured on the LPS molecules deposited directly on the crystal (Fig. 2). The overall lower absorption can be explained by the subtraction of the $CaCl_2$ solution layer which itself presents a high THz absorption. The spectrum of LPS molecules deposited on the $CaCl_2$ solution presents absorption shoulders at the same frequencies as dry LPS molecules, supporting that hydration does not change the THz response of LPS molecules. A similar hydration dependency of THz spectra was observed in the case of DMPG bilayers, where hydration did not change the ordering of lipids [19]. This can be explained by the fact that both DMPG and LPS are anionic lipids, DMPG having Na^+ and LPS having Ca^{2+} as counterions.

The similarity of measured LPS spectra when directly deposited on the ATR crystal and on the ATR crystal coated with the $CaCl_2$ solution suggests a similar association of LPS molecules in both samples. Additionally, the agreement between our THz spectra and those measured on DMPG bilayers [19] would point toward a layer organization of the LPS molecules in both dry and LPS-$CaCl_2$ solution samples. In order to test this hypothesis, we simulated the THz spectra of a LPS monolayer and a monolayer patch using two methods and compared the results with experiments.

SIMULATED SPECTRA OF LPS STRUCTURES

We simulated the THz spectra of a patch of a single LPS molecule (Figure 1(a)), of five LPS molecules (Fig. 3(a)) and of a LPS monolayer (Fig. 3(b)) in order to obtain theoretical spectra of different LPS arrangements.

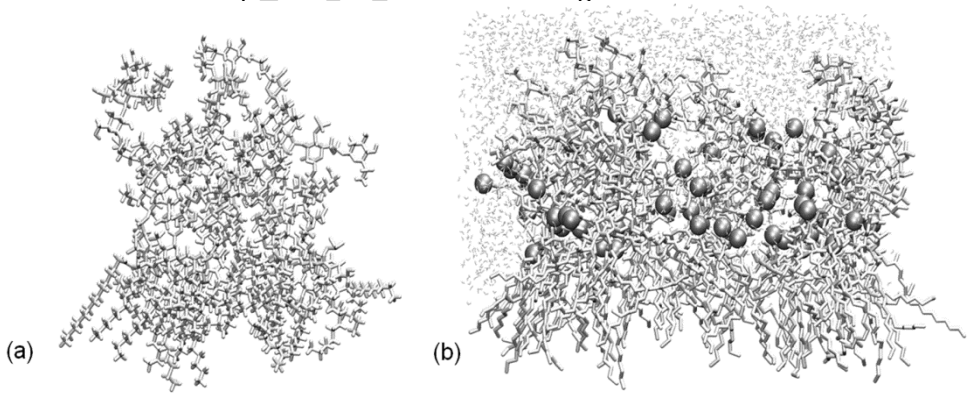

Fig. 3. (a) The LPS monolayer formed by a patch of five LPS molecules that are represented as white licorice. (b) The LPS monolayer used in MD simulations. LPS molecules are represented as white licorice, Ca^{2+} ions are represented as silver spheres and oxygen atoms from water molecules are represented with grey dots.

The THz spectra of one and that of five LPS molecules were simulated using NMA. As presented in the Methods section, the structure of the five LPS molecules patch was obtained from a 16 LPS molecules monolayer. Therefore, we consider this patch to be a coarse monolayer model. Due to the exponential increase in NMA computation times with the number of considered atoms, we couldn't consider a larger LPS patch for NMA calculations. The spectra of LPS molecules resulting from NMA are presented in Figure 4(a). As shown below, the simulated spectrum of a single LPS molecule is similar with that of a patch of five LPS molecules, both of them presenting a non-linear increase of THz absorption with frequency which is in agreement with the experimental spectra. In contrast to the simulated spectrum of one LPS molecule, the simulated THz spectrum of the monolayer patch presents the absorption shoulders at 1.05 THz and 1.37 THz that were observed on the experimental spectrum. This similitude validates the layer association of LPS molecules in both LPS samples deposited directly on the crystal or in the $CaCl_2$ solution. Moreover, the similitude with the experiment validates the force field parameters used for spectra simulation.

The spectrum of the LPS monolayer derived from MD is presented in Figure 4(b). The spectrum of the monolayer presents a linear increase of the THz absorption, a shape dissimilar to that of the experimental spectrum. Linear spectra were also obtained when using the MD trajectory for calculating the absorptions of a single LPS molecule or of the same five LPS molecules considered for NMA (data not shown).

The comparison of results obtained using NMA and MD show that a good correlation with the experiments occurs when simulating the spectra with NMA. This could suggest that the low frequency vibrations of LPS layers are rather harmonic as a consequence of motion restrictions due to lipid packing.

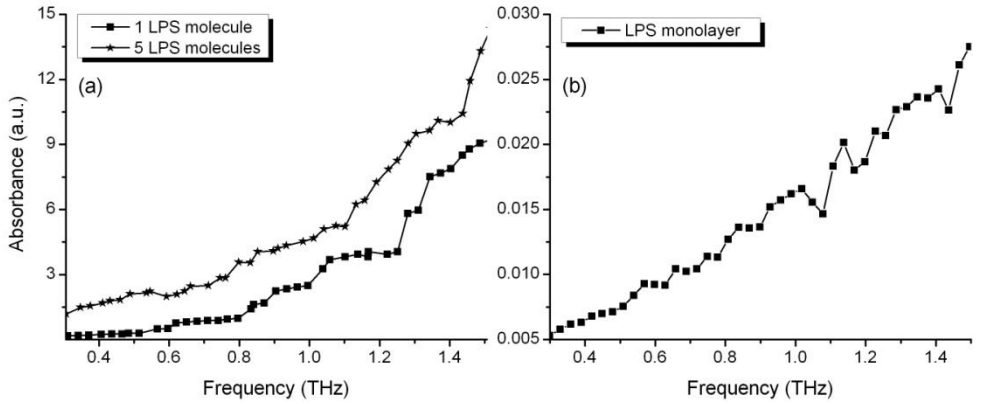

Fig. 4. (a) THz spectra of a single LPS molecule and of five LPS molecules simulated by NMA. (b) THz spectrum of a LPS monolayer (16 LPS molecules) simulated by MD.

CONCLUSIONS

Here we addressed the packing of LPS molecules when deposited directly on an ATR silicon crystal or when deposited on the crystal coated with a CaCl2 solution using THz spectroscopy. The measured spectra, in agreement with spectra previously recorded on phospholipids [2, 19], present a non-linear increase of THz absorption at increasing frequencies, with two absorption shoulders at 1.05 THz and 1.37 THz. The similarity of acquired THz spectra in both experimental conditions suggests a common organization of LPS molecules, regardless of hydration. Based on the agreement between our results with THz spectra previously recorded on anionic lipid bilayers [19], we hypothesized that LPS molecules adopt a layer arrangement in the samples that we analyzed. To test the hypothesis, we modelled LPS monolayers and simulated the THz spectra by NMA and MD. Only the NMA spectra of a LPS monolayer comprising five LPS molecules resulted in good agreement with the experiments, which allows us to validate the layer arrangement of LPS molecules in our experiments. This allows us to validate the simulation parameters of LPS molecules that we used and to conclude that NMA is better suited for the simulation of LPS monolayers THz spectra.

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