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Heterogeneous Binding of Lipoteichoic Acid to the Surface of Titanium Dioxide as Determined with ^{31}P Solid-State NMR Spectroscopy

Charles V. Rice* and Jason R. Wickham

Department of Chemistry and Biochemistry, University of Oklahoma, 620 Parrington Oval, Room 208, Norman, Oklahoma 73019

Received November 11, 2004; E-mail: rice@ou.edu

Bacterial biofilms have been implicated in post-surgical chronic infections and in the formation of dental cavities.[1,2] The formation of biofilms is essentially a four-step process.[3−5] First, planktonic cells attach to the surface, followed by the production of extracellular polymeric substances (slime), the development of a biofilm architecture, and finally biofilm spreading through bacteria release. For most Gram-positive bacteria, the first step involves lipoteichoic acid (LTA) binding to the surface.[6,7] In this communication, we provide evidence that the interaction of LTA with titanium dioxide is heterogeneous.

LTA consists of alkyl tails, a disaccharide of D-glucose headgroup, and an extended chain of teichoic acid (Figure 1). Teichoic acid is a long chain (40−50 repeat units)[8−11] of phosphodiesters (anionic) with glucosamine (neutral), hydroxyl (anionic), and D-alanine branches (cationic). Charge neutralization is incomplete,[6] allowing teichoic acid to form ionic bonds with surrounding fluids, dissolved ions, and the substrate. The disaccharide headgroup can bind to the peptidoglycan of the cell wall. When located at the outer edge of the cell wall, the teichoic acid chain extends away from the cell.[12] In this way, teichoic acid is one of the first cellular components to come in contact with surfaces. Using a mutant of *Staphylococcus aureus*, Gross et al.[6] have shown that the ionic component of teichoic acid can be altered by removing alanine branches. The increased anionic character of the chain inhibited bacterial adsorption on polystyrene and glass surfaces. The presence of D-alanine is also necessary for the in vitro adhesion of *Listeria monocytogenes* onto mammalian cells.[13] ^{1}H and ^{13}C NMR have been used to characterize the teichoic acid structure,[8,10,11,14,15] and we report the first NMR studies of teichoic acid adsorption. Phosphorus-31 solid-state NMR provides information about chemical environment and molecular motion through measurement of the chemical shift anisotropy (CSA), asymmetry parameter (η), and rotating-frame spin−lattice relaxation ($T_{1\rho}$).[16]

LTA extracted from *Staphylococcus aureus* was obtained as a dry powder from Invivogen, Inc. and used as received. LTA (10 mg) was transferred into a 5 mm zirconia rotor on the benchtop. The rotor was fitted with a Torlon drive tip and airtight Teflon spacers and sealed with an airtight Teflon cap. LTA was also adsorbed onto titanium dioxide (amorphous powder, Kerr McGee, Inc.) because titanium is used in orthopedic surgery and dental reconstruction.[17,18] TiO_2 (200 mg) was added to a solution of 1 mg of LTA in 1 mL of doubly distilled water. The slurry was agitated with a vortex mixer for 10 min and allowed to rest for 24 h at 10 °C. Centrifugation at 3000 Hz formed a pellet, and the supernatant was drawn off with a syringe. The LTA/TiO_2 was redispersed in 1 mL of water, agitated with a vortex mixer, and pelleted with centrifugation. The pellet was isolated and dried in a desiccator for 2 h and transferred to a 5 mm zirconia rotor. The rotor was also fitted with airtight Teflon spacers and cap. The amount of LTA on the TiO_2 surface has not been measured, but comparison of NMR spectra intensities suggests that the LTA/TiO_2 signal is about 300 times weaker than that of the pure LTA sample.

Solid-state NMR experiments were performed using a three-channel NMR spectrometer (^{1}H = 300 MHz, UnityInova, Varian Inc.) and a 5 mm, three-channel, magic-angle-spinning NMR probe (Varian APEX design). CSA data for pure LTA were collected with 1000 scans, 2 s repetition rate, and 3000 Hz spinning rate. The CPMAS contact time was 1000 μs with ^{1}H decoupling at an rf power level of 50 kHz. Data for LTA/TiO_2 were taken under the same conditions, but the lower amount of LTA in the sample rotor required the acquisition of 100 000 scans. To measure $T_{1\rho}$, the spinning speed was increased to 9000 Hz to improve the signal-to-noise ratio. Drive and bearing gas were provided by dry, compressed air. ^{31}P chemical shift data are referenced to an external standard of 85% phosphoric acid (0 ppm). The temperature was 25 °C. Data acquisition and analysis were accomplished using VnmrJ 1.1D and the STARS software packages provided by Varian Inc.

The ^{31}P CPMAS spectrum of pure LTA is shown in Figure 1a. The isotropic peak at 0 ppm (fwhm = 553 Hz) is surrounded by a series of spinning sidebands. For LTA on TiO_2, two isotropic peaks (I, 0 ppm and II, −6 ppm) are seen in Figure 1b and are similar to that of the direct polarization spectrum (see Supporting Information). The lack of significant differences indicates that there are no highly mobile species that would give a signal from direct polarization but not give a CPMAS signal. Deconvolution of the direct polarization ^{31}P NMR spectrum of LTA on the surface of TiO_2 produces two peaks (species I and II) at 0 and −6 ppm. The relative integral areas are 1.0 and 0.95 and indicate nearly equal amounts of I and II. The line width of II (fwhm = 1091 Hz) is twice that of I (fwhm = 543 Hz). The cause of line broadening could be homogeneous exchange or the result of heterogeneous chemical shift variations. The later is the likely line broadening mechanism. The ^{31}P chemical shift and line width of species I are similar to those of pure LTA powder, which implies that any exchange must be in the fast-exchange limit. 2D exchange experiments are possible,[19] but the low concentration of LTA and the large number of scans make this difficult. Evidence against an exchange mechanism is provided by measurement of $T_{1\rho}$ (below), which is a measure of molecular dynamics. $T_{1\rho}$ values for species I and II are nearly 10 times slower than that of pure LTA, a difference that suggests a quenching of molecular motions on the TiO_2 surface and that fast-exchange processes are improbable.

Although pure LTA and species I have a similar chemical shift, species I is not free LTA within the sample rotor, and their respective values of $T_{1\rho}$ and CSA tensors differ. Rotating frame spin−lattice relaxation ($T_{1\rho}$) is commonly used to evaluate molecular dynamics.[20,21] The $T_{1\rho}$ exponential decays for pure LTA, species I, and species II have a time constant of 3.5, >30, and >30 ms, respectively (see Supporting Information). Molecular motion

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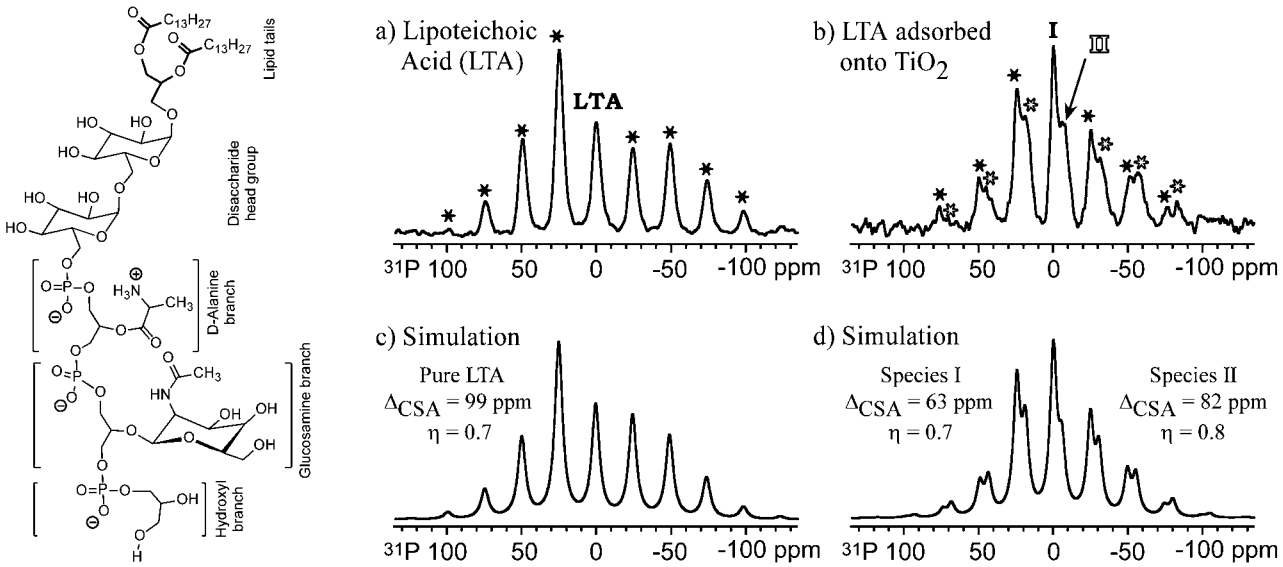

Figure 1. Structure of lipoteichoic acid (LTA) from *S. aureus*. It has 40−50 repeat units, distributed as 50% hydroxyl, 25% glucosamine, and 25% alanine. ^{31}P CPMAS spectra of (a) lipoteichoic acid powder and (b) lipoteichoic acid adsorbed onto titanium dioxide. Contact time = 1 ms, spinning speed = 3000 Hz. The isotropic peaks are denoted as LTA, I, and II, with asterisks above the spinning sidebands. Spectrum (a) was acquired with 10 mg of LTA and 1000 scans (35 min). The spectrum of LTA/TiO_2 required 100 000 scans (2.3 days). CSA tensor and asymmetry parameter (η) values were determined by simulations with the STARS program (spectra c and d).

increases relaxation and $T_{1\rho}$ will be short (fast relaxation rate). Likewise, long $T_{1\rho}$ values are indicative of hampered molecular motion. These data suggest that on the TiO_2 surface LTA molecular motion is slowed due to adhesion. Similar $T_{1\rho}$ values for species I and II suggest that, although their chemical shifts are different, the molecular dynamics are similar.

Another difference between pure LTA, species I, and species II is the relative heights of the isotropic peak and the −1 spinning sideband at ∼25 ppm. The heights of the other sidebands also differ. The spinning sidebands map out the chemical shift anisotropy (CSA) tensors,[19,20,22] and spectral simulations yield Δ_{CSA} values of 99, 63, and 82 ppm for pure LTA, species I, and species II. For species I and II, the reduction in Δ_{CSA} and reduced motions (reflected in $T_{1\rho}$ values) suggest significant structural changes upon surface attachment.

From the simulations, the chemical shift tensor asymmetry parameter (η) is 0.7, 0.7, and 0.8 for pure LTA, species I, and species II, respectively. The asymmetry parameter is a measure of line shape deviation from an axially symmetric tensor; $\eta = [(\sigma_{xx} - \sigma_{yy})/\sigma_{zz}]$.[19,20,22] An increase in η for species II shows that the electron distribution around the phosphorus has changed and become more distorted from axial symmetry. These data support the changes in structure upon surface adsorption.

Heterogeneous binding of LTA on TiO_2 is possible given the different types of phosphodiesters that make up the large polymeric material. The glucosamine side chain can participate in hydrogen binding, while the alanine side chain can have ionic bonds, both of which can interact with the phosphate and affect its bonds with the surface. We have shown that on the TiO_2 surface LTA has two separate chemical environments, which differ in their molecular dynamics from a sample of pure LTA. These observations suggest that on the surface about 50% of the phosphodiesters within LTA interact with the surface. The remaining phosphodiesters also have limited motion, but are in a chemical environment similar to pure LTA. We attribute this fraction to non-surface-bound phosphodiesters. Their motion is also restricted, perhaps due to surface bonds through the alanine/glucosamine/hydroxyl substituents. The complex nature of LTA requires additional solid-state NMR studies to understand the molecular interactions that are responsible for the surface binding. These experiments are currently underway.

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Supporting Information Available: ^{31}P direct polarization spectra, deconvolution to quantify species I and II, and $T_{1\rho}$ data (PDF). This material is available free of charge via the Internet at http://pubs.acs.org

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