Nuclear Magnetic Resonance Spectroscopic Studies of Lipoteichoic Acid Adhesion on Biomaterials

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Biofilms are responsible for persistent clinical infections and dental cavities. A major component of gram-positive bacterial-biofilms is lipoteichoic acid (LTA). When located at the outer edge of the cell wall, the LTA chain extends away from the surface and is the first cell component to come in contact with surfaces. The prevention of bacterial attachment could include therapies to repel LTA and these efforts will benefit from understanding the molecular-level interactions that promote LTA adsorption.

LTA is a long chain of phosphodiesters (anionic) with glucosamine (neutral) and alanine branches (cationic). Charge neutralization is incomplete, allowing LTA to form ionic bonds with water molecules and the substrate. The adsorption mechanism is governed by ionic interactions, but the nature of the surface bonds is unknown.

High-resolution magic-angle-spinning nuclear magnetic resonance (HRMAS-NMR) spectroscopy was used to study LTA on silica and hydroxiapatite. NMR data are characteristic of the chemical environment and because each LTA component is chemically unique, we were able to collect sidechain-specific data. The chemical environment is altered by LTA-surface interactions, which are revealed in the NMR data. In this fashion, we are able to follow LTA adsorption on different surfaces.

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Introduction

Recent studies have shown that lipoteichoic acid (LTA) is the binding mechanism of gram-positive bacteria. Although full functions of LTA are unknown; gram-positive bacteria are responsible for numerous diseases and cavity-causing germs. Teichoic acids are embedded in the surface of gram-positive peptidoglycan and attached to the head groups of lipid molecules. When located at the outer edge of the cell wall, the lipid head group resides at the edge with the LTA chain extending away from the surface. Because of this, the LTA chain is the first component to come in contact with other surfaces.

Our hypothesis was that, when LTA interacts with a surface, the chemical environment changes. NMR is sensitive to changes in chemistry, and we were able to detect LTA adsorption with NMR. LTA, a cell wall component found in all gram-positive bacteria, is a long chain of phosphodiesters (anionic) with glucosamine (neutral)and alanine branches (cationic).

Charge neutralization is incomplete, allowing LTA to form ionic bonds with water molecules and the substrate. Additionally, NMR can determine if the glucosamine and/or the alanine branches are the primary contact/bond to the surface. Knowledge of the interactions of LTA is essential for understanding the functions of LTAs in bacterial physiology as well as in experimental responses.

Methods

The method used to collect data was high-resolution magic-angle spinning nuclear magnetic resonance (HRMAS-NMR) spectroscopy, with the spectrometer operating at 300 MHz. The NMR data shows changes in the chemical structure and molecular motion of materials, so if the results of the NMR change from one test to another, then the chemicals have changed. For LTAs that are on a surface, some parts may move faster than others (ie, the "velcro" does not move). NMR data identifies which part is sticking to what, and how well it is sticking.

Using a pipette, we accurately drew up 20μL of CD_3OD (methanol), and inserted it into the NMR tube. The two solutions mix on their own. The LTA/water/methanol sample was then inserted into the NMR probe and the machine was started. Data was taken for the LTA/water/methanol mixture, an LTA/water/methanol on silica mixture, and an LTA/water/methanol/hydroxyapatite.

Results

We were interested in the glucosamine and alanine peaks because they represent branches in the teichoic acids. These peaks are NMR signals that can be used to follow the chemical environments of the branches in teichoic acid. When LTA is placed in water with silica, the NMR spectrum is identical to the spectrum of the LTA in water. The chemical shift values do not change and the peak shapes do not change. The water peak does change shape, but this is a result of the NMR experiment. We concluded that the silica does not alter or affect the chemical environment of LTA.

On the other hand, the NMR of LTA in water with hydroxyapatite is dif-

ferent from the spectrum of LTA in water alone. Most obvious is that the alanine peak moves from 1.5 ppm to 2.15 ppm, while the glucosamine peak remains unchanged. This indicates that the chemical environment of the alanine branch has been changed. Therefore, it is likely that LTA has adsorbed onto the surface of hydroxyapatite. Interestingly enough, though, because the glucosamine CH_3 does not have a change in ppm value, it is not in contact with the surface. Thus, the alanine branches are acting as the "velcro" for LTA.

CONCLUSION

In conclusion, our hypothesis was correct. The NMR spectra did, indeed, show changes in the chemical environment of the lipoteichoic acid and the included solvent. It did not change anything in the water/silica mix, because silica does not form any ionic bonds with LTA, but LTA does adsorb to the bonelike qualities of hydroxyapatite.

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