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Frequency-selective REDOR and spin-diffusion relays in uniformly labeled whole cells

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ABSTRACT

Solid-state NMR is a powerful and non-perturbative method to measure and define chemical composition and architecture in bacterial cell walls, even in the context of whole cells. Most NMR studies on whole cells have used selectively labeled samples. Here, we introduce an NMR sequence relay using frequency-selective REDOR (fsREDOR) and spin diffusion elements to probe a unique amine contribution in uniformly ^{13}C- and ^{15}N-labeled *Staphylococcus aureus* whole cells that we attribute to the D-alanine of teichoic acid. In addition to the primary peptidoglycan structural scaffold, cell walls can contain significant amounts of teichoic acid that contribute to cell-wall function. When incorporated into teichoic acid, D-alanine is present as an ester, connected via its carbonyl to a ribitol carbon, and thus has a free amine. Teichoic acid D-Ala is removed during cell-wall isolations and can only be detected in the context of whole cells. The sequence presented here begins with fsREDOR and a chemical shift evolution period for 2D data acquisition, followed by DARR spin diffusion and then an additional fsREDOR period. fsREDOR elements were used for ^{13}C observation to avoid complications from ^{13}C–^{13}C couplings due to uniform labeling and for ^{15}N dephasing to achieve selectivity in the nitrogens serving as dephasers. The results show that the selected amine nitrogen of interest is near to teichoic acid ribitol carbons and also the methyl group carbon associated with alanine. In addition, its carbonyl is not significantly dephased by amide nitrogens, consistent with the expected microenvironment around teichoic acid.

1. Introduction

1.1. The bacterial cell wall

The bacterial cell wall is essential to cell viability and is a major target of antibiotics [1]. The cell wall of Gram-positive bacteria such as *Staphylococcus aureus* (*S. aureus*) has two primary components: peptidoglycan (Fig. 1, left), and teichoic acid (Fig. 1, right) [2]. The peptidoglycan is a mesh-like network of polymerized N-acetyl glucosamine (GlcNAc) and muramic acid (MurNAc) disaccharides that are highly cross-linked by short peptide stems attached to the lactyl moiety of MurNAc. In *S. aureus*, the peptide stems include a mixture of four or five amino acids that are cross-linked to an adjacent glycan strand through a pentaglycine bridge, providing mechanical strength and elasticity to the cell wall. The peptidoglycan provides the major structural and mechanical framework of the cell wall. The inhibition of cell-wall synthesis results in cell-wall thinning and cell lysis. Teichoic acids are long polymers that decorate and are appended to the peptidoglycan. Teichoic acid can contribute to virulence in *S. aureus* by aiding in adhesion and colonization. In addition, the inhibition of final steps of teichoic acid synthesis, after synthesis has been initiated, arrests cell growth [3,4].

Quantifying alterations in cell-wall composition are important in evaluating drug modes of action, particularly important for human pathogens that are now resistant to multiple antibiotics such as *S. aureus*. Harsh degradative methods are needed to generate digested cell walls for analysis by traditional biochemical methods, including HPLC and mass spectrometry [5–7]. Yet, for Gram-positive bacteria with the very thick cell wall present outside the cell membrane, complete digestion is usually not possible, compromising quantification. Solid-state NMR has emerged as a powerful tool to examine cell-wall composition in intact isolated cell walls, also known as sacculi, void of cytoplasmic contents, and in whole cells themselves [8,9].

1.2. Selective labeling and REDOR approaches for bacterial cell walls

Bacterial peptidoglycan contains unique compositional linkages that are not found intracellularly. *S. aureus* use D-Ala in the cell wall (Fig. 1) which results in D-Ala–Gly crosslinks, for example, that exist only in that particular site out of all the cellular components.

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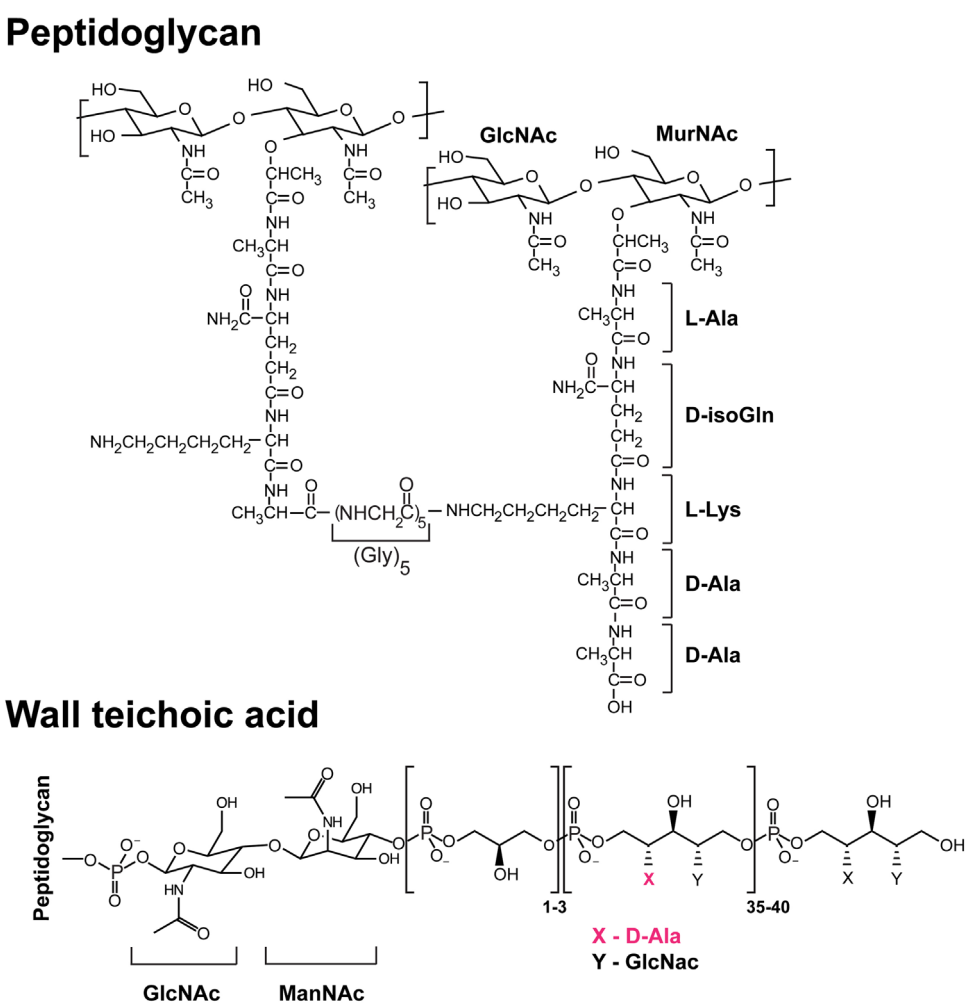

Fig. 1. Chemical structures of major cell-wall components peptidoglycan and teichoic acid. Wall teichoic acids are covalently attached to peptidoglycan and are substituted with D-Ala through an ester linkage.

Thus, specific labeling strategies have been immensely valuable in measuring features like these crosslinks using selective D-[1-^{13}C]Ala and [^{15}N]Gly labels, for example, during growth in defined medium to select the unique one-bond C-N pairs [10]. [^{15}N]Gly and L-[ε-^{15}N]Lys labels have been valuable in quantifying bridgelinks where the pentaglycine bridge is attached to the lysine sidechain, converting the sidechain amine to an amide [7,10]. Rotational-echo Double Resonance (REDOR) experiments have been useful for these selections and for measurements of internuclear distances (e.g. from D-Ala–Gly crosslink site to an antibiotic ^{19}F label) to determine drug modes of action and to generate atomic-level models for cell-wall antibiotic complexes [8,11–13].

1.3. Non-selective natural abundance and uniform labeling NMR approaches for bacterial cell walls

We recently reported that one-dimensional spectra of either unlabeled ^{13}C or uniformly ^{13}C and ^{15}N enriched cell walls and whole cells provide valuable compositional profiles of cell-wall and whole-cell samples [14]. We were even able to determine that the polysaccharide contributions to whole cells primarily arise from the cell wall by examining protoplasts in which much of the cell wall is removed. Furthermore, the polysaccharide signatures in whole cells were able to reflect the influence of two antibiotics with different modes of action. A cell-wall inhibitor, fosfoymicn, resulted in whole-cell spectra that differed in having reduced peak intensities in the polysaccharide region. Chloramphenicol, on the other hand, inhibits protein synthesis and during the time of treatment has little effect on cell-wall assembly. Whole-cell ^{13}C spectra revealed an altered balance of ^{13}C contributions, where cell-wall polysaccharides were preferentially increased as protein synthesis was inhibited and protein contributions were reduced [14]. We have been working to extend this approach to other bacterial organisms and to explore the extent of atomic-level compositional detail that can be extracted from uniformly labeled samples, with one of the goals being to examine the full repertoire of cellular changes that accompany treatment with old and new antibiotics. Uniform labeling is typically simpler to implement than specific-labeling strategies. One does not have to quantify scrambling and isotopic dilution due to endogenous synthesis. Uniform ^{13}C labeling also provides the opportunity to use homonuclear spin-diffusion mixing to select other nearby resonances, in a manner similar to that widely used for assignment and structure determination of proteins.

1.4. A new approach using relayed frequency-selective REDOR and spin diffusion steps

In this contribution, we report the design and implementation of a relayed frequency-selective REDOR experiment (fsREDOR–DARR–fsREDOR) in one and two dimensions with uniformly labeled whole cells. The experiment was designed to provide atomic-level specificity regarding teichoic acid in *S. aureus* whole cells. The first application of fsREDOR was implemented to identify