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Molecules in focus

O-Acetylated peptidoglycan: Controlling the activity of bacterial autolysins and lytic enzymes of innate immune systems

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ABSTRACT

The *O*-acetylation of peptidoglycan is now known to occur in 50 different bacterial species, both Gram positive and Gram negative, including a number of important human pathogens. This modification to the essential cell wall component of bacteria provides both a level of control over endogenous autolysins and protection from the lysozymes of innate immune systems. In this review, we describe the details of the pathways for peptidoglycan *O*-acetylation that are now beginning to emerge and we explore the possibility that the associated enzymes may present new candidates for antibacterial targets.

1. Introduction

Peptidoglycan (PG) is comprised of alternating *N*-acetylglucosamine (GlcNAc) and *N*-acetylmuramic acid (MurNAc) residues linked periodically by short peptides at the C-3 lactyl moiety of Mur*N*Ac (Fig. 1). The resulting macromolecule (sacculus) envelopes the entire cell contributing to shape and providing essential strength against the cell's turgor pressure. Given the importance of this structure, it is not surprising that there exist enzymes, both endogenous and host derived, which are active on nearly all of the major linkages of PG. Lysozymes, a class of hydrolases produced by numerous organisms as a component of their innate immune systems, hydrolyze the β-1,4-glycosidic linkage between GlcNAc and MurNAc. This linkage is also the site of action of the lytic transglycosylases (LTs), enzymes endogenous to bacterial cells for the growth and maintenance of the PG sacculus (Scheurwater et al., 2007). However, despite the same substrate specificity, their reaction mechanisms and products differ. Unlike the hydrolytic reaction of lysozymes, LTs catalyze an intramolecular rearrangement resulting in the production of 1,6-anhydromuramic acid and GlcNAc (Fig. 1). Their catalytic mechanism thus requires a free C-6 hydroxyl moiety on MurNAc. As one level of control over the activity of both of these and other lytic enzymes, many bacteria modify their PG in a variety of ways (reviewed in Vollmer, 2008). One such modification is *O*-acetylation.

2. Structure

The *O*-acetylation of PG occurs specifically at the C-6 hydroxyl group of muramoyl residues in PG of most bacteria that perform this modification generating the 2,6-*N*,*O*-diacetyl derivative (Fig. 1). The *O*-acetylation of GlcNAc residues was postulated to occur also in *Bacillus anthracis* to account for the results of mutagenesis studies (Laaberki et al., 2010), and recently such was demonstrated in another Gram-positive bacterium *Lactobacillus plantarum* (Bernard et al., 2011). As the greatest diversity in PG structure is found in Gram positive bacteria (Schleifer and Kandler, 1972), it is likely that more examples of GlcNAc *O*-acetylation will be discovered. The modification to both aminosugars occurs non-stoichiometrically with levels typically ranging between 20% and 60% relative to the concentration of the respective aminosugar, being both strain specific and dependent on culture age (Clarke, 1993; Clarke and Dupont, 1992; Clarke et al., 2002; Pfeffer et al., 2006). For example, increases of 10–40% *O*-acetylation are observed with cultures of *Enterococcus faecalis* upon entering stationary phase and a

Abbreviations: Ape, O-acetylpeptidoglycan esterase; GlcNAc, N-acetylglucosamine; LT, lytic transglycosylase; MurNAc, N-acetylmuramic acid; Oat, O-acetylpeptidoglycan transferase; Pat, peptidoglycan O-acetyltransferase; PG, peptidoglycan.

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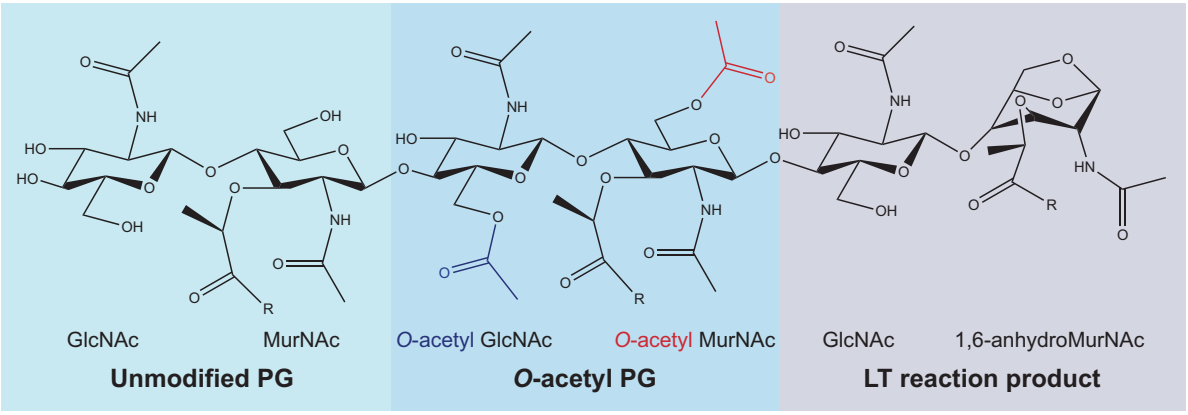

Fig. 1. Structure and modification of a PG glycan chain. PG is a macromolecule of both glycan and peptide chains which are covalently linked together to form the 'sacculus' that completely surrounds a bacterial cell. Depicted is a hypothetical hexasaccharide of GlcNAc-β-1,4-MurNAc repeats from the non-reducing end of a single glycan chain identifying the C-6 hydroxyl residues involved in *O*-acetylation and the formation of a terminal 1,6-anhydromuramoyl residue. R denotes the stem peptides involved in the cross-linking of neighboring glycan chains.

further 10–16% when cells become viable but non-culturable (VBNC) (Pfeffer et al., 2006). Similar observations have been made with *B. anthracis* where the levels of PG *O*-acetylation of these cells growing in batch culture increase with time (unpublished data).

O-AcetylPG was discovered more than 50 years ago in the cell walls of *E. faecalis* (Abrams, 1958) and the modification has since been found in 49 other species of bacteria, both Gram positive and Gram negative, including species of *Staphylococcus, Neisseria*, *Campylobacter*, *Helicobacter*, *Proteus* and *Bacillus* (including *B. anthracis*) (Table 1) (Bera et al., 2006; Clarke, 1993; Clarke and Dupont, 1992; Clarke et al., 2002; Pfeffer et al., 2006). Indeed, to date only a few organisms have been shown to not O-acetylate their PG; notable exceptions include *Escherichia coli* and *Pseudomonas aeruginosa* (Clarke, 1993; Clarke et al., 2010).

Table 1
Bacteria demonstrated to produce *O*-acetylated PG.

Gram positive	
Bacillus anthracis	*Listeria monocytogenes*
Bacillus cereus	*Macrococcus caseolyticus*
Bacillus megaterium	*Micrococcus luteus*
Bacillus subtilis	*Ruminococcus flavefaciens*
Enterococcus durans	*Staphylococcus aureus*
Enterococcus faecium	*Staphylococcus epidermidis*
Enterococcus feacalis	*Staphylococcus haemolyticus*
Enterococcus hirae	*Staphylococcus hyicus*
Lactobacillus acidophilus	*Staphylococcus lugdunensis*
Lactobacillus casei	*Staphylococcus saccharolyticus*
Lactobacillus fermentum	*Staphylococcus saprophyticus*
Lactobacillus plantarum	*Streptococcus pneumoniae*
Lactococcus lactis	
Gram negative	
Acinetobacter lwoffi	*Photorhabdus luminescens*
Acintobacter anitratus	*Proteus mirabilis*
Bacteroides fragilis	*Proteus myxofaciens*
Bacteroides thetaiotamicron	*Proteus penneri*
Bradyrhizobium japonicum	*Proteus vulgaris*
Campylobacter jejuni	*Providencia alcalifaciens*
Chromobacterium violaceum	*Providencia heimbachae*
Helicobacter pylori	*Providencia rettgeri*
Morganella morganii	*Providencia rustigianii*
Neisseria gonorrhoeae	*Providencia stuartii*
Neisseria lactamica	*Pseudomonas alcaligenes*
Neisseria meningitidis	*Synechocystis* sp.
Neisseria perflava	

3. Biosynthesis and control

3.1. Pathways for PG O-acetylation

Until 2005, very little was known about the pathway(s) for PG *O*-acetylation. Early biochemical evidence indicated that it is a maturation event occurring after the assembly (*viz.* transglycosylation and transpeptidation) of PG precursors into the existing sacculus (reviewed in Clarke et al., 2002). Thus, it was recognized that the source of acetate for this modification must be transported from the cytoplasm to the PG sacculus through the cytoplasmic membrane but efforts to delineate the process were hindered by technical challenges of working with integral membrane proteins that act on a totally insoluble PG substrate. Progress was finally made with the release of the genome sequences of *S. aureus* and *N. gonorrhoeae* in 2005 which led to the identification of two distinct systems specific to Gram-positive and Gram-negative bacteria, respectively.

Bera et al. (2005) identified the *oatA* gene as being responsible for *O*-acetylation of muramoyl residues in *S. aureus*. Homologs of OatA have been identified in several other Gram-positive bacteria, including species of *Streptococcus* (Crisóstomo et al., 2006), *Bacillus cereus* and *B. anthracis* (Laaberki et al., 2010), *Lactococcus lactis* (Veiga et al., 2007) and *L. plantarum* (Bernard et al., 2011). It encodes a hypothetical integral membrane protein that is predicted to be bimodular. The N-terminal half is classified as a member of the membrane-bound *O*-acetyltransferase (MBOAT) family of acyltransferases and it possesses 11 hypothetical transmembrane helices. The C-terminal module appears to be globular and it is predicted to be surface exposed. A lysine rich region in this module has been proposed to be the site of catalytic activity (Bera et al., 2006) but closer analysis suggests that it has the fold of SGNH/GDSL hydrolases complete with the signature catalytic triad of Asp, His and Ser residues. Preliminary data support the importance of the Asp and Ser in conferring *O*-acetylation (Bernard et al., 2011). A paralog of OatA, named OatB, was identified in *B. anthracis* by Laaberki et al. (2010) and it was demonstrated to contribute to the overall level of *O*-acetylation of PG, however no obvious phenotype was observed with a deletion mutant. It was speculated that OatB was modifying GlcNAc although this was not confirmed experimentally. Bernard et al. (2011) identified a different enzyme, also named OatB, in *L. plantarum, Lactobacillus sakei* and *Weissella paramesenteroides* and it was demonstrated phenotypically to function as a GlcNAc *O*-acetyltransferase.

No biochemical analysis of either enzyme has been reported; in fact, until recently no *in vitro* assay had been developed to monitor