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Characterization of *O*-Acetylation of *N*-Acetylglucosamine

A NOVEL STRUCTURAL VARIATION OF BACTERIAL PEPTIDOGLYCAN[*] [S]

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Abstract

Peptidoglycan (PG) *N*-acetyl muramic acid (MurNAc) *O*-acetylation is widely spread in Gram-positive bacteria and is generally associated with resistance against lysozyme and endogenous autolysins. We report here the presence of *O*-acetylation on *N*-acetylglucosamine (GlcNAc) in *Lactobacillus plantarum* PG. This modification of glycan strands was never described in bacteria. Fine structural characterization of acetylated muropeptides released from *L. plantarum* PG demonstrated that both MurNAc and GlcNAc are *O*-acetylated in this species. These two PG post-modifications rely on two dedicated *O*-acetyltransferase encoding genes, named *oatA* and *oatB*, respectively. By analyzing the resistance to cell wall hydrolysis of mutant strains, we showed that GlcNAc *O*-acetylation inhibits *N*-acetylglucosaminidase Acm2, the major *L. plantarum* autolysin. In this bacterial species, inactivation of *oatA*, encoding MurNAc *O*-acetyltransferase, resulted in marked sensitivity to lysozyme. Moreover, MurNAc over-*O*-acetylation was shown to activate autolysis through the putative *N*-acetylmuramoyl-L-alanine amidase LytH enzyme. Our data indicate that in *L. plantarum*, two different *O*-acetyltransferases play original and antagonistic roles in the modulation of the activity of endogenous autolysins.

Keywords: Bacteria, Bacterial Metabolism, Carbohydrate, Cell Wall, Lactic Acid, Autolysis, Lactobacillus plantarum, Lysozyme, Peptidoglycan O-Acetylation, Glucosaminidase

Introduction

Peptidoglycan (PG),[6] the major constituent of the cell envelope of Gram-positive bacteria, is a polymer of the disaccharide *N*-acetylmuramic acid-(β-1,4)-*N*-acetylglucosamine (MurNAc-GlcNAc) associated with a peptidic stem linked to MurNAc (1, 2). The composition of the peptidic stem varies from one bacterium to another and, in *Lactobacillus plantarum*, is composed of L-alanine, D-glutamic acid, *meso*-diaminopimelic acid, D-alanine, and a D-lactate as last moiety (see Fig. 1) (3–6). The different glycan strands are cross-linked between the fourth amino acid of the donor stem and the third amino acid of the acceptor peptide forming a three-dimensional network around the cell called the sacculus. The main functions of this sacculus are to ensure cell integrity against the internal osmotic pressure and to provide the shape of the bacteria (1, 2, 7).

Because of its rigidity, PG has to be remodeled by the PG hydrolases (PGH) to allow cell growth and cell division. PGH are divided into different classes according to their cleavage site: the carboxy- and endopeptidases that cleave the peptidic stem, the *N*-acetylmuramoyl-L-alanine amidases that cleave