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Carbohydrate recognition and lysis by bacterial peptidoglycan hydrolases

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The major component of bacterial cell wall is peptidoglycan (PG), a complex polymer formed by long glycan chains cross-linked by peptide stems. PG is in constant equilibrium requiring well-orchestrated coordination between synthesis and degradation. The resulting cell-wall fragments can be recycled, act as messengers for bacterial communication, as effector molecules in immune response or as signaling molecules triggering antibiotics resistance. Tailoring and recycling of PG requires the cleavage of different covalent bonds of the PG *sacculi* by a diverse set of specific enzymes whose activities are strictly regulated. Here, we review the molecular mechanisms that govern PG remodeling focusing on the structural information available for the bacterial lytic enzymes and the mechanisms by which they recognize their substrates.

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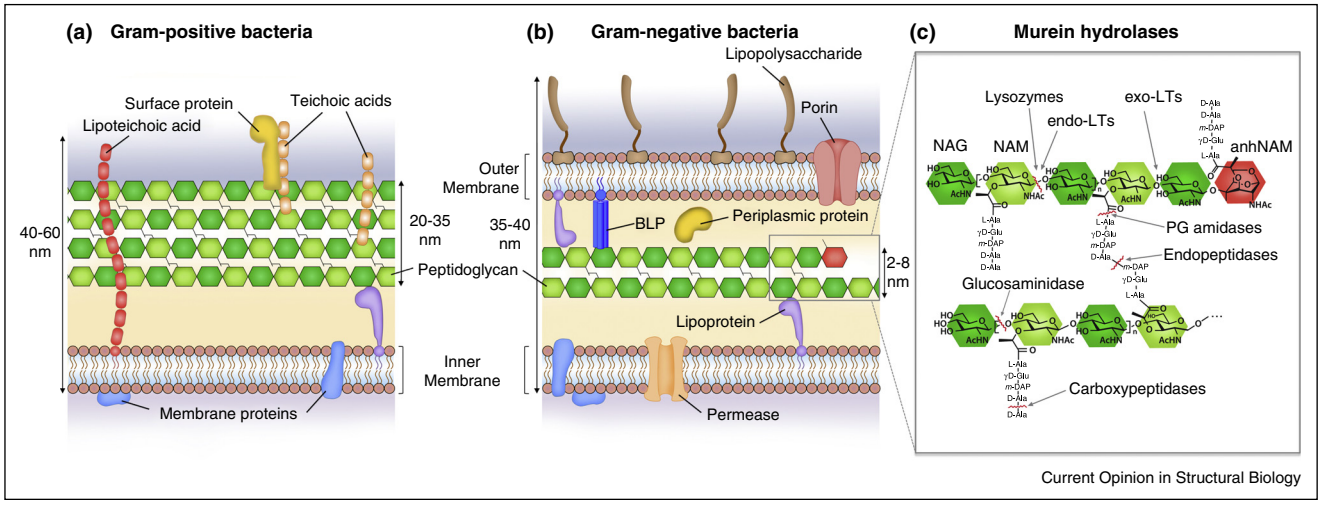
Introduction

Peptidoglycan (murein) is an essential and specific component of the bacterial cell wall preventing cell lysis as a result of the cell's high intracellular osmotic pressure. PG is intimately involved in the processes of cell growth and cell division, contributes to the maintenance of a defined shape and also serves as a scaffold for anchoring other cell envelope components such as proteins [1] and teichoic acids [2] (Figure 1). PG is composed by linear glycan strands cross-linked by short peptides. The glycan strands are made up of alternating *N*-acetylglucosamine (NAG) and *N*-acetylmuramic acid (NAM) residues linked by β(1–4) bonds. The D-lactoyl group of each NAM residue is substituted by a peptide stem. Although several variations in the structure of the peptide stem are known, the core structure of the non-crosslinked stem attached is L-Ala-γ-D-Glu-m-DAP-D-Ala-D-Ala in Gram-negative bacteria (m-DAP for 2,6-diaminopimelic acid) and L-Ala-γ-D-Glu-L-Lys-D-Ala-D-Ala in most Gram-positive bacteria [2]. This conserved core of the PG is constantly edited, by chemical modification (*e.g.*, *O*-acetylation, *N*-deacetylation or amidation) or by incorporation of Gly residues or non-canonical D-amino acids at the peptide-stem, in response to different environmental stresses (reviewed in Ref. [3]).

Bacterial murein hydrolases contribute to this PG plasticity and form a vast and highly diverse group of enzymes capable of cleaving bonds in *sacculi* and/or its soluble fragments. The growing body of data generated by massive genome sequencing is progressively shedding more light on the number and variety of the enzymes involved in PG metabolism in different bacteria. However, identification and classification of novel PG hydrolases from genomic and metagenomic data is difficult due to their lack of homology with the previously well-characterized PG hydrolases. Efforts have been made in the development of computational tools to aid in the identification and classification of these novel PG hydrolases [4]. Fifty years of research work have led to the discovery of more than 35 PG hydrolases in *Escherichia coli* that have been classified into 12 families (reviewed in Ref. [5]). However, present knowledge on the regulation mechanism of these activities is still fragmentary and limited to a few examples. Hydrolases are involved in several critical functions [6], including PG maturation, turnover, recycling, autolysis, cleavage of the septum during cell division [7], and antibiotic resistance [8]. PG degradation products also act as critical activators of the mammalian immune system [9].

Hydrolases can cleave glycosidic (glycosidases) or amide bond (peptidases) in the PG network (Figure 1). Regarding glycosidases, three different subgroups, glucosaminidases, lysozymes and lytic transglycosylases, can be distinguished. While glucosaminidases hydrolytically cleave the β(1–4) glycosidic bond between NAG and NAM, lysozymes and lytic transglycosylases (LTs) cleave the β(1–4) glycosidic bond between sequential NAM and NAG (Figure 1). Differences between lysozymes and LTs arise from their specific catalytic mechanisms. While lysozymes are hydrolytic enzymes, the reaction catalyzed by LTs is the non-hydrolytic cleavage of the glycan strand of PG as first demonstrated by Holtje *et al.* in 1975 [10],

Figure 1



Gram-positive **(a)** and Gram-negative **(b)** cell wall organization scheme. The PG layer comprises long polymers of the repeating disaccharide *N*-acetylglucosamine-*N*-acetylmuramic acid (NAG–NAM, dark and pale green respectively) that are linked via peptide bridges. BLP refers to Braun's lipoprotein (dark blue) attached to the outer membrane and covalently crosslinked with the PG. **(c)** Detailed scheme of the crosslinked PG and the different bacterial peptidoglycan hydrolases involved in the cell wall processing.

with formation of the non-reducing *N*-acetyl 1,6-anhydromuramic and the *N*-acetylglucosamine as the two termini (Figure 1). LTs can be classified as exolytic, when the cleavage is performed at the end of the glycan strands, or endolytic if the cleavage is carried out in the middle of the PG chain. Recent analysis of the reactions products of all LTs from *E. coli* have revealed that almost all LTs are able to exert, in different degree, both exolytic and endolytic activities, and even, although to a lesser extent, muramidase activity [11].

Regarding peptidases, two different groups, PG amidases and peptidases, can be distinguished depending on the nature and localization of amide bond to be hydrolyzed (Figure 1). While PG amidases are NAM-L-Ala amidases, which cleave the amide bond between L-Ala residue of the stem peptide and NAM, peptidases cleave the amide bonds within the stem peptide itself. Depending on the position of the amide bond to be cleaved, peptidases are subdivided into carboxypeptidases that remove C-terminal residues, and endopeptidases that cleave within the peptide, with prefixes DD-, LD- or DL referring to the stereochemistry of the two amino acid residues constituting the cleavage site. Recent results on the structural and functional characterization of the different PG-hydrolysing enzymes are summarized below.

Relevant databases for PG hydrolases classification

Different databases are now available providing updated information on both the enzymes processing PG and different carbohydrate-binding modules. The PG hydrolases can be analyzed based on sequence similarity and structure motifs through three different databases (see Table 1). (i) The Carbohydrate-Active enZymes (CAZy) database (http://www.cazy.org/) and its extension CAZypedia (http://www.cazypedia.org/) for glycosidic hydrolases; this is an encyclopedic resource on structurally-related enzymes that degrade, modify, or create glycosidic bonds. These carbohydrate-active enzymes are classified into different glycoside hydrolase (GH) families according to their catalytic mechanism, enzyme active site residues or three-dimensional structure. For each GH family, a description of its known activity and catalytic mechanism is provided, in addition to the GenBank, Uniprot and PDB accession codes of its members. (ii) *MEROPS* is an on-line database (http://merops.sanger.ac.uk/) for peptidases (also known as proteases, proteinases or proteolytic enzymes) and their inhibitors. The summary page describing a given peptidase can be reached by using an index under its name, a *MEROPS* identifier or the source organism. The *MEROPS* database uses a hierarchical, structure-based classification of peptidases. Each peptidase is assigned to a family on the basis of statistically significant similarities in the amino acid sequence, and families that are thought to be homologous are grouped together in a Clan. The summary page for each family provides links to supplementary pages with further information. (iii) The Pfam is a database containing a large collection of protein families (http://www.xfam.org/), each represented by multiple sequence alignments and hidden Markov probabilistic models (HMM) used for the statistical inference of homology sequences. A high-quality seed alignment provides the basis for the position-specific amino-acid frequencies, gap and length