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Mini review

Peptidoglycan perception—Sensing bacteria by their common envelope structure

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ABSTRACT

Most *Eubacteria* possess peptidoglycan (PGN) or murein that surrounds the cytoplasmic membrane. While on the one hand this PGN sacculus is a very protective shield that provides resistance to the internal turgor and adverse effects of the environment, it serves on the other hand as a major pattern of recognition due to its unique structure. Eukaryotes harness this particular bacterial macromolecule to perceive (pathogenic) microorganisms and initiate their immune defence. PGN fragments are generated by bacteria as turnover products during bacterial cell wall growth and these fragments can be sensed by plants and animals to assess a potential bacterial threat. To increase the sensitivity the concentration of PGN fragments can be amplified by host hydrolytic enzymes such as lysozyme or amidase. But also bacteria themselves are able to perceive information about the state of their cell wall by sensing small soluble fragments released from its PGN, which eventually leads to the induction of antibiotic responses or cell differentiation. How PGN is sensed by bacteria, plants and animals, and how the antibacterial defence is modulated by PGN perception is the issue of this review.

Introduction

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The cytoplasmic membrane of most eubacteria is surrounded by the peptidoglycan (PGN) sacculus. It is a unique structure present in almost all bacteria that can be isolated as a whole and its shape corresponds to the form of the original cell (Weidel and Pelzer, 1964). The main purpose of the PGN sacculus is indeed to maintain the bacterial shape and to counteract the internal pressure of the bacterial cell. In Gram-negative bacteria this mostly single layered macromolecule is located within the periplasm between the cytoplasmic membrane and the outer membrane. In Gram-positive bacteria a PGN sacculus up to forty layers thick forms the outermost part of the cell (Litzinger and Mayer, 2010). Debris of PGN, the so called muropeptides, act as PAMPs/MAMPs (pathogen or microbe associated molecular patterns) in plants and animals as well as signalling molecules in bacteria themselves.

In this mini review, only a compendious overview of PGN signalling and perception in bacteria, animals and plants can be given and surely not all relevant primary literature on this topic can be covered. For more detailed information we refer to excellent recent reviews (Boudreau et al., 2012; Dworkin, 2014; Johnson et al., 2013; Mark et al., 2011; Wheeler et al., 2014). We will first summarize the current knowledge on PGN structure, PGN isolation and compositional analysis, and subsequently address the PGN perception machineries in different organisms, and antibacterial defences such as PGN hydrolases.

Peptidoglycan-types

At first glance the structure of PGN looks very uniformly being composed of the main building blocks: glycan strand, peptide subunit or stem peptide and the interpeptide bridge. However, looking closer into the structure there is an enormous variability within the structure in the various bacterial species as shown in the classical review by Schleifer and Kandler (1972).

Abbreviations: anhMurNAc, 1,6-anhydro MurNAc; mDAP, meso-diaminopimelic acid; i-D-Glu, iso-D-glutamine; DS, disaccharide; GlcNAc, N-acetylglucosamine; LysM, lysin motif; MK, keratinocytes from murine oral epithelium; MurNAc, N-acetylmuramic acid; NOD, nucleotide-binding oligomerization domain containing; PAMP/MAMP, pathogen/microbe-associated molecular pattern; PBPs, penicillin binding proteins; PGN, peptidoglycan; PGRP/PGLYRP, PGN recognition protein; PRR, pattern recognition receptor; TCT, *Bordetella persussis* tracheal cytotoxin; TLR, Toll-like receptor.

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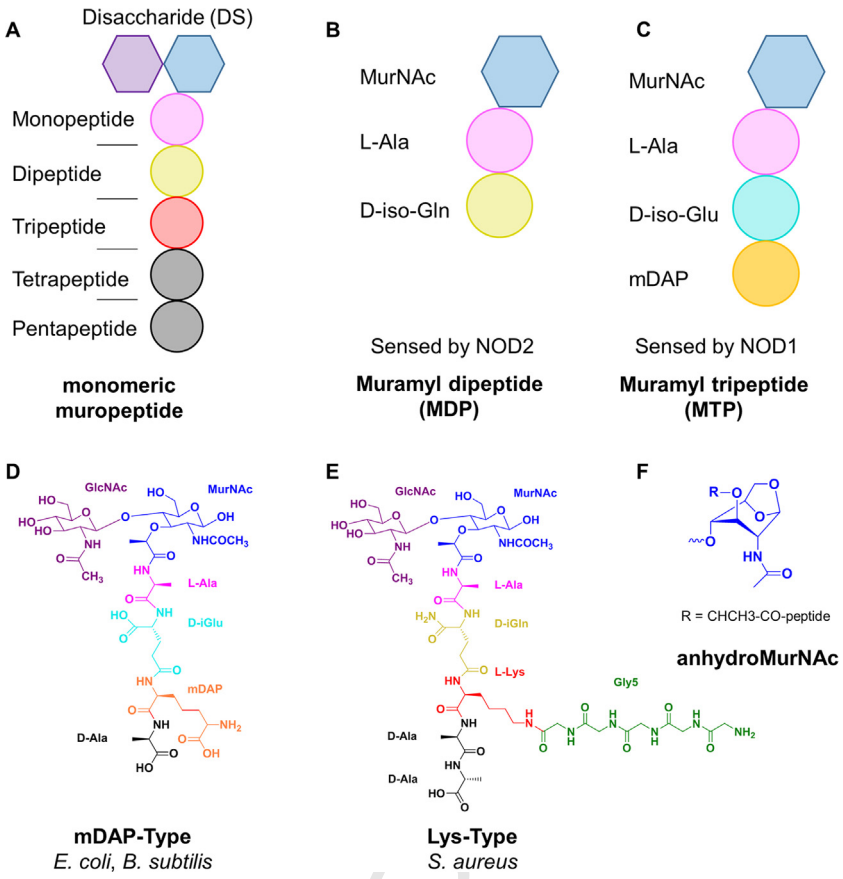

Fig. 1. Muropeptide structures. (A) Schematic drawing of a monomeric muropeptide consisting of the disaccharide GlcNAc and MurNAc and a stem peptide of five amino acids. (B) Muramyldipeptide (MDP), as structure sensed by NOD2. (C) Muramyltripeptide (MTP) containing mDAP as structure sensed by NOD1. (D) Chemical structure of an mDAP-type monomer. In *E. coli* and *B. subtilis* most remaining pentapeptides within the PGN sacculus are degraded into tetrapeptides by a D,D-carboxypeptidase. (E) Chemical structure of a Lys-type monomer with a pentaglycine interpeptide bridge. (F) Chemical structure of the 1,6-anhydro-form of MurNAc (anhMurNAc).

The glycan strand is composed of alternating β-1,4 linked N-acetylglucosamine (GlcNAc) and N-acetylmuramic acid (MurNAc), which are cross-linked by short peptides attached by an amide linkage to the lactyl group of MurNAc (Fig. 1). The glycan strands can be modified, including O-acetylation, O-deacetylation and N-deacetylation (Moynihan et al., 2014; Vollmer et al., 2008), frequently conferring resistance towards lysozyme (one of the first host defence mechanisms, see also below) (Bera et al., 2005; Clarke and Dupont, 1992). A prominent modification of the PGN of Gram-negative bacteria is an intramolecular glycosidic bond between C_1 and C_6-OH of MurNAc yielding the 1,6-anhydro-form of MurNAc (anhMurNAc) (Fig. 1F) (van Asselt et al., 1999).

An unusual feature of the peptide moieties is the presence of D-amino acids, which are generally absent in eukaryotes. The most common sequence of the stem peptide is L-Ala-D-iso-Glu-mDAP/L-Lys-D-Ala-D-Ala, with a dibasic amino acid (mostly either meso-diaminopimelic acid (mDAP) or L-Lys) residing at position three. The γ-carboxyl-group of the second amino acid forms an amide bond with the α-amino-group of the third amino acid: mDAP is used in most Gram-negatives as well as in Bacilli and Mycobacteria (Fig. 1D) compared to Lys in most Gram-positives (Fig. 1E). However, position three is the most variable one of the stem peptide (Vollmer et al., 2008). A common feature of Lys-type peptidoglycan is the existence of an interpeptide bridge tethered to the ε-amino group of the dibasic amino acid and typically composed of one to seven amino acids (Fig. 1E). In most species the fourth and fifth amino acid of the stem peptide is D-Ala. Incorporation of D-Ser (*Enterococcus gallinarum*) or D-Lactate (*Lactococcus casei* and other Enterococci) at position five confers resistance against vancomycin (Healy et al., 2000). In *E. coli* and *B. subtilis* but not *S. aureus*, most remaining pentapeptides within the PGN sacculus are trimmed into tetrapeptides by a D,D-carboxypeptidase, the penicillin binding protein (PBP) 5 (Potluri et al., 2010). The structure of the L,D-carboxypeptidase LdcB, that further degrades tetrapeptides into tripeptides in Streptococcus *pneumoniae*, *Bacillus anthracis*, and *Bacillus subtilis* was solved just recently (Hoyland et al., 2014).

Peptidoglycan isolation and analysis

To study structural features and immunogenic activity of PGN, its purification is required. Isolation of PGN relies on the fact that it is insoluble in SDS and does not get degraded while boiling. To generate PGN fragments for structural analyses by HPLC or applications such as the stimulation of host immune responses the isolated, macromolecular PGN is usually digested with a muramidase such as mutanolysin or an endo-N-acetylglucosaminidase. Both enzymes hydrolyse the β-1,4 glycosidic bond between MurNAc-GlcNAc or GlcNAc-MurNAc of the glycan strands, respectively. The resulting muropeptides contain a disaccharide (DS) and a peptide part (Fig. 1A) that can be cross-linked to other muropeptides thereby forming multimeric compounds such as dimers, trimers, or tetramers. Within the last decades the muropeptide composition of several bacterial genera has been elucidated by HPLC (Desmarais et al., 2013).

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