# Chapter 1

# Introduction

## 1.1 Peptidoglycan

Almost all bacterial cells are surrounded by a layer of peptidoglycan (PG; murein) on the outside of the cytoplasmic membrane. This major component of the cell wall functions primarily to maintain cell strength and integrity to withstand internal turgor pressure (reviewed in Schleifer and Kandler, 1972). PG forms a mesh-like 'sacculus' surrounding the bacterial cell, serving as a scaffold to anchor other cell envelope components including proteins (Dramsi *et al.*, 2008) and teichoic acids (Neuhaus and Baddiley, 2003). Because this heteropolymer is both essential and exclusive to bacteria, its metabolism is the target of several important antibiotics; inhibition of its biosynthesis or its specific degradation results in cell lysis.

## 1.2 Peptidoglycan structure and arrangement

In Gram-positive organisms, peptidoglycan is multi-layered, and for those bacteria characterized it is observed to be 15–80 nm thick (Figure 1.1) (Touhami *et al.*, 2004). In Gram-negative organisms, the PG layer is localized to the periplasm, sandwiched between the cytoplasmic and outer membranes, and is comparatively thin at 2.5–7.5 nm (Labischinski *et al.*, 1991; Murray *et al.*, 1965).

Peptidoglycan consists of linear glycan chains of alternating units of β-1,4-linked N-acetylglucosamine (GlcNAc) and N-acetylmuramic acid (MurNAc) residues, which are cross-linked by short peptides (Figure 1.2) (reviewed in Schleifer and Kandler, 1972). Stem peptides are attached to the C3 lactyl moiety of MurNAc, most often consisting of L-alanine, D-glutamic acid, *meso*-diaminopimelic acid (*m*-$A_2$pm), D-alanine, and D-alanine. This pentapeptide stem is present in nascent PG; however, in the mature macromolecule the terminal D-alanine residue is lost. The stem peptide serves as the point of covalent attachment of cell envelope proteins to PG (Braun and Rehn, 1969; Braun and Sieglin, 1970), and peptides from neighbouring glycans can form cross-links with each other. Cross-

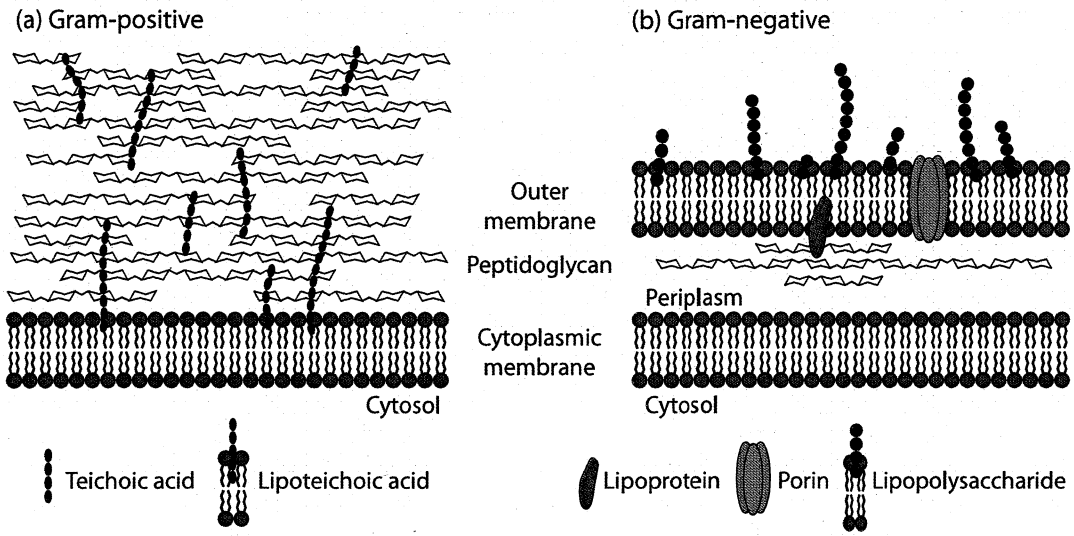


**Figure 1.1** – The Gram-positive and Gram-negative cell envelope. (a) Gram-positive bacteria have a thick layer of peptidoglycan (15–80 nm) external to the cytoplasmic membrane, embedded with teichoic acids. (b) Gram-negative bacteria have a relatively thin layer of peptidoglycan (2.5–7.5 nm) located between the inner and outer membranes, anchored by lipoproteins to the outer membrane.

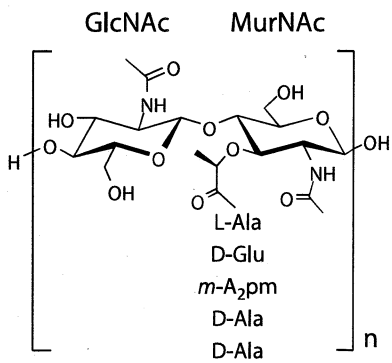


**Figure 1.2** – The peptidoglycan monomer. Peptidoglycan consists of linear glycan chains of alternating units of β-1,4-linked N-acetylglucosamine (GlcNAc) and N-acetylmuramic acid (MurNAc), with short stem peptides covalently bound to the lactyl moiety of MurNAc. In *Escherichia coli*, the peptide stem consists of L-alanine, D-glutamic acid, *meso*-diaminopimelic acid, D-alanine, and D-alanine.

**Table 1.3** – Family archetypes of lytic transglycosylases in *Escherichia coli*. LTs are either membrane-bound (Mlt) or soluble (Slt), and have either exo- or endo-specificity. Molecular weights are given for the unprocessed form.

| Family | Gene | Enzyme | Amino acids | MW (Da) | Specificity | Reference(s) |
|---|---|---|---|---|---|---|
| 1A | *sltY* | Slt70 | 645 | 73,353 | exo | Höltje *et al.* (1975) |
| 1B | *yfhD* | MltF (YfhD) | 518 | 58,302 | **n.d.** | Scheurwater and Clarke (2008) |
| 1C | *mltC* | MltC | 359 | 40,113 | exo | Dijkstra and Keck (1996) |
| 1D | *mltD* | MltD | 452 | 49,417 | exo | Kajie *et al.* (1991) |
| 1E | *mltE* | MltE (EmtA) | 203 | 22,227 | **endo** | Kraft *et al.* (1998) |
| 2 | *mltA* | MltA | 365 | 40,411 | exo | Ursinus and Höltje (1994); Lommatzsch *et al.* (1997) |
| 3 | *mltB* | MltB | 361 | 40,256 | exo | Engel *et al.* (1992); Ehlert *et al.* (1995) |

1995), *E. coli* MltA (van Straaten *et al.*, 2005), *N. gonorrhoeae* MltA (Powell *et al.*, 2006), *E. coli* Slt35 (a soluble product of MltB) (van Asselt *et al.*, 1999a), and bacteriophage lambda LT (Leung *et al.*, 2001). Both amino acid sequences and overall tertiary structures lack similarity between members of respective families, although family 1A, 3, and 4 LTs are mostly α-helical and possess a lysozyme-like fold at their catalytic domains. Family 2 enzymes are structured as a β-barrel. Aside from these differences, the active site clefts of Slt70, MltA, and MltB are comprised of four substrate-binding subsites that can accommodate the aminosugar residues of a tetrasaccharide substrate. The exo-activity of these LTs (Table 1.3) is supported by this subsite architecture, as these LTs catalyze the release of disaccharide anhydromuropeptides from either the reducing or non-reducing ends of PG. In contrast, the endo-acting bacteriophage LTs (Table 1.3) can accommodate hexasaccharide substrates within their active site clefts (Leung *et al.*, 2001). Although not yet solved, it will be interesting to compare these structures to the putative endo-acting *E. coli* MltE (Kraft *et al.*, 1998) and MltF (Scheurwater and Clarke, 2008).

### 1.7.2 Mechanism of LT action

The MurNAc and GlcNAc residues are shown to bind to the -1 and +1 subsites, respectively, indicating the substrate specificity of the LTs. For example, the amino acid residues at subsite -1 interact with both the lactyl moiety of MurNAc and its associated peptide, and this interaction is critical for the proper orientation of substrate for lytic activity (Reid *et al.*, 2004c, 2006). The proposed intramolecular reaction involves a single catalytic acid residue, often a glutamic acid, positioned at the centre of the active site clefts between subsite -1 and +1. This residue is thought to function as the catalytic acid/base for bond cleavage by substrate-assisted catalysis (Thunnissen *et al.*, 1994; van As-