2.3: The Peptidoglycan Cell Wall

Skills to Develop

1. State the three parts of a peptidoglycan monomer and state the function of peptidoglycan in bacteria.
2. Briefly describe how bacteria synthesize peptidoglycan, indicating the roles of autolysins, bactoprenols, transglycosylases, and transpeptidases.
3. Briefly describe how antibiotics such as penicillins, cephalosporins, and vancomycin affect bacteria and relate this to their cell wall synthesis.

The mycoplasmas are the only bacteria that naturally lack a cell wall. Mycoplasmas maintain a nearly even pressure between the outside environment and the cytoplasm by actively pumping out sodium ions. Their cytoplasmic membranes also contain sterols that most likely provide added strength. The remaining bacteria in the domain *Bacteria*, with the exception of a few bacteria such as the Chlamydiases, have a semirigid cell wall containing peptidoglycan. (While bacteria belonging to the domain *Archaea* also have a semirigid cell wall, it is composed of chemicals distinct from peptidoglycan such as protein or pseudomurein. We will not take up the *Archaea* here.)

**Function of Peptidoglycan**

Peptidoglycan prevents **osmotic lysis**. As seen earlier under the cytoplasmic membrane, bacteria concentrate dissolved nutrients (solute) through active transport. As a result, the bacterium's cytoplasm is usually hypertonic to its surrounding environment and the net flow of free water is into the bacterium. Without a strong cell wall, the bacterium would burst from the osmotic pressure of the water flowing into the cell.
Structure and Composition of Peptidoglycan

With the exceptions above, members of the domain *Bacteria* have a cell wall containing a semirigid, tight knit molecular complex called peptidoglycan. Peptidoglycan, also called murein, is a vast polymer consisting of interlocking chains of identical peptidoglycan monomers (Figure 1). A peptidoglycan monomer consists of two joined amino sugars, N-acetylglucosamine (NAG) and N-acetylmuramic acid (NAM), with a pentapeptide coming off of the NAM (Figure 2). The types and the order of amino acids in the pentapeptide, while almost identical in gram-positive and gram-negative bacteria, show some slight variation among the domain *Bacteria*.

*Figure 1*: Peptidoglycan is composed of cross-linked chains of peptidoglycan monomers (NAG-NAM-pentapeptide). Transglycosylase enzymes join these monomers together to form chains. Transpeptidase enzymes then cross-link the chains to provide strength to the cell wall and enable the bacterium to resist osmotic lysis. (left) In a peptidoglycan
monomer of S. aureus, the pentapeptide coming off the NAM is composed of the amino acids L-alanine, D-glutamine, L-lysine, and two D-alanines. The peptide cross-link forms by formation of a short peptide interbridge consisting of 5 glycines. In the process the terminal D-alanine is cleaved from the pentapeptide to form a tetrapeptide in the peptidoglycan. (right) In a peptidoglycan monomer of E. coli, the pentapeptide coming off the NAM is composed of the amino acids L-alanine, D-glutamic acid, meso-diaminopimelic acid, and two D-alanines. The peptide cross-link forms between the diaminopimelic acid of one peptide chain with the D-alanine of another and in the process the terminal D-alanine is cleaved from the pentapeptide to form a tetrapeptide in the peptidoglycan.

The peptidoglycan monomers are synthesized in the cytosol of the bacterium where they attach to a membrane carrier molecule called bactoprenol. As discussed below, The bactoprenols transport the peptidoglycan monomers across the cytoplasmic membrane and work with the enzymes discussed below to insert the monomers into existing peptidoglycan enabling bacterial growth following binary fission.
Figure 2: (left) A peptidoglycan monomer consists of two joined amino sugars, N-acetylglucosamine (NAG) and N-acetylmuramic acid (NAM), with a pentapeptide coming off of the NAM. In E. coli, the pentapeptide consists of the amino acids L-alanine, D-glutamic acid, meso diaminopimelic acid, and two D-alanines. (right) A peptidoglycan monomer consists of two joined amino sugars, N-acetylglucosamine (NAG) and N-acetylmuramic acid (NAM), with a pentapeptide coming off of the NAM. In S. aureus, the pentapeptide consists of the amino acids L-alanine, D-glutamine, L-lysine, and two D-alanines.

Once the new peptidoglycan monomers are inserted, glycosidic bonds then link these monomers into the growing chains of peptidoglycan. These long sugar chains are then joined to one another by means of peptide cross-links between the peptides coming off of the NAMs. By linking the rows and layers of sugars together in this manner, the peptide cross-links provide tremendous strength to the cell wall, enabling it to function similar to a molecular chain link fence around the bacterium (see Figure 1).

Synthesis of Peptidoglycan

In order for bacteria to increase their size following binary fission, links in the peptidoglycan must be broken, new peptidoglycan monomers must be inserted, and the peptide cross links must be resealed. The following sequence of events occur:

Step 1. Bacterial enzymes called autolysins:

- a) Break the glycosidic bonds between the peptidoglycan monomers at the point of growth along the existing peptidoglycan (see Figure 3, steps 1-3); and

- b) Break the peptide cross-bridges that link the rows of sugars together (see Figure 3, steps 1-3).
Step 2. The peptidoglycan monomers are synthesized in the cytosol (see Figure 4, step-1 and Figure 4, step-2) and bind to bactoprenol. The bactoprenols transport the peptidoglycan monomers across the cytoplasmic membrane and interacts with transglycosidases to insert the monomers into existing peptidoglycan (see Figure 4, step-3, Figure 4, step-4, Figure 4, step-5, and Figure 4, step-6).

Step 3. Transglycosylase (transglycosidase) enzymes insert and link new peptidoglycan monomers into the breaks in the peptidoglycan (see Figure 5, step 1 and Figure 5, step 2).

Step 4. Finally, transpeptidase enzymes reform the peptide cross-links between the rows and layers of peptidoglycan to make the wall strong (see Figure 6, step 1 and see Figure 6, step 2).

In *Escherichia coli*, the terminal D-alanine is cleaved from the pentapeptides to form a tetrapeptides. This provides the energy to bond the D-alanine of one tetrapeptide to the diaminopimelic acid of another tetrapeptide (see Figure 1B). In the case of *Staphylococcus aureus*, the terminal D-alanine is cleaved from the pentapeptides to form a tetrapeptides. This provides the energy to bond a pentaglycine bridge (5 molecules of the amino acid glycine) from the D-alanine of one tetrapeptide to the L-lysine of another (see Figure 1A).

Exercise: Think-Pair-Share Questions

1. As we will see in Unit 2, the antibiotic bacitracin binds to bactoprenol after it inserts a peptidoglycan monomer into the growing bacterial cell wall.

   Explain how this can lead to the death of that bacterium.

2. As we will see in Unit 2, the penicillin antibiotics binds to the bacterial enzyme transpeptidase.

   a. Explain how this can lead to the death of that bacterium.

   b. Could this antibiotic be used to treat protozoan infections such as giardiasis and toxoplasmosis?

In the center of the bacterium, a group of proteins called Fts (filamentous temperature sensitive) proteins interact to form a ring at the cell division plane. These proteins form the cell division apparatus known as the divisome and are directly involved in bacterial cell division by binary fission (see Figure 1 and Figure 2).
The divisome is responsible for directing the synthesis of new cytoplasmic membrane and new peptidoglycan to form the division septum.

**Antimicrobial Agents that Inhibit Peptidoglycan Synthesis Causing Bacterial Lysis**

Many antibiotics work by inhibiting normal synthesis of peptidoglycan in bacteria causing them to burst as a result of osmotic lysis. As just mentioned, in order for bacteria to increase their size following binary fission, enzymes called autolysins break the peptide cross links in the peptidoglycan, transglycosylase enzymes then insert and link new peptidoglycan monomers into the breaks in the peptidoglycan, and transpeptidase enzymes reform the peptide cross-links between the rows and layers of peptidoglycan to make the wall strong.

Interference with this process results in a weak cell wall and lysis of the bacterium from osmotic pressure. Examples include the penicillins (penicillin G, methicillin, oxacillin, ampicillin, amoxicillin, ticarcillin, etc.), the cephalosporins (cephalothin, cefazolin, cefoxitin, cefotaxime, cefaclor, cefoperazone, cefixime, ceftriaxone, cefuroxime, etc.), the carbapenems (imipenem, metropenem), the monobactems (aztreonem), the carbacephems (loracarbef), and the glycopeptides (vancomycin, teichoplanin).

- For example, penicillins and cephalosporins bind to the transpeptidase enzymes (also called penicillin-binding proteins) responsible for resealing the cell wall as new peptidoglycan monomers are added during bacterial cell growth. This blocks the transpeptidase enzymes from cross-linking the sugar chains and results in a weak cell wall and subsequent osmotic lysis of the bacterium (see Figure 8).

Antimicrobial chemotherapy will be discussed in greater detail later in Unit 2 under Control of Bacteria by Using Antibiotics and Disinfectants.

For More Information: Preview of Chemotherapeutic Control of Bacteria from Unit 2.

For More Information: Preview of Using Chemical Agents to Control of Bacteria from Lab 19.
Gram-Positive, Gram-Negative, and Acid-Fast Bacteria

Most bacteria can be placed into one of three groups based on their color after specific staining procedures are performed: Gram-positive, Gram-negative, or acid-fast.

- **Gram-positive Bacteria**: These retain the initial dye crystal violet during the Gram stain procedure and appear purple when observed through the microscope. Common Gram-positive bacteria of medical importance include *Streptococcus pyogenes*, *Streptococcus pneumoniae*, *Staphylococcus aureus*, *Enterococcus faecalis*, and *Clostridium* species.

![Gram Stain of Staphylococcus aureus](https://bio.libretexts.org/Bookshelves/Microbiology/Book%3A_Microbiology_(Kaiser)/Unit_1%3A_Introduction_to_Microbiology_and_Prokaryotic_Cell_Anatomy/2%3A_The_Prokaryotic_Cell_-_Bacteria/2.3%3A_The_Peptidoglycan_Cell_Wall)

(Left) Gram Stain of *Staphylococcus aureus* which are gram-positive (purple) cocci in clusters. (Right) Gram Stain of *Escherichia coli* which are Gram-negative (pink) bacilli.
• **Gram-negative Bacteria**: These decolorize during the Gram stain procedure, pick up the counterstain safranin, and appear pink when observed through the microscope. Common Gram-negative bacteria of medical importance include *Salmonella* species, *Shigella* species, *Neisseria gonorrhoeae*, *Neisseria meningitidis*, *Haemophilus influenzae*, *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus* species, and *Pseudomonas aeruginosa*. Also see gram stain of a mixture of gram-positive and gram-negative bacteria.

![A Gram Stain of a Mixture of Gram-Positive and Gram-Negative Bacteria. Note Gram-negative (pink) bacilli and Gram-positive (purple) cocci.](https://bio.libretexts.org/Bookshelves/Microbiology/Book%3A_Microbiology_(Kaiser)/Unit_1%3A_Introduction_to_Microbiology_and_Prokaryotic_Cell_Anatomy/2%3A_The_Prokaryotic_Cell_-_Bacteria/2.3%3A_The_Peptidoglycan_Cell_Wall)

• **acid-fast Bacteria**: These resist decolorization with an acid-alcohol mixture during the acid-fast stain procedure, retain the initial dye carbolfuchsin and appear red when observed through the microscope. Common acid-fast bacteria of medical importance include *Mycobacterium tuberculosis*, *Mycobacterium leprae*, and *Mycobacterium avium-intracellulare* complex.

![Acid-Fast Stain of Mycobacterium tuberculosis in Sputum. Note the reddish acid-fast bacilli among the blue normal flora and white blood cells in the sputum that are not acid-fast.](https://bio.libretexts.org/Bookshelves/Microbiology/Book%3A_Microbiology_(Kaiser)/Unit_1%3A_Introduction_to_Microbiology_and_Prokaryotic_Cell_Anatomy/2%3A_The_Prokaryotic_Cell_-_Bacteria/2.3%3A_The_Peptidoglycan_Cell_Wall)

These staining reactions are due to fundamental differences in their cell wall as will be discussed in Lab 6 and Lab 16. We will now look at each of these three bacterial cell wall types.
The S-layer

1. Structure and Composition

The most common cell wall in species of *Archaea* is a paracrystalline surface layer (S-layer). It consists of a regularly structured layer composed of interlocking glycoprotein or protein molecules. In electron micrographs, it has a pattern resembling floor tiles. Although they vary with the species, S-layers generally have a thickness between 5 and 25 nm and possess identical pores with 2-8 nm in diameter. Several species of *Bacteria* have also been found to have S-layers.

To view electron micrographs of S-layers see the following:

- S-Layer Proteins, the Structural Biology Homepage at Karl-Franzens University in Austria.
- Characteristic Properties of S-layer Proteins, at Foresight Nanotech Institute in Austria.

2. Functions and Significance to Bacteria Causing Infections

The S-layer has been associated with a number of possible functions. These include the following:

a. The S-layer may protect bacteria from harmful enzymes, from changes in pH, from the predatory bacterium *Bdellovibrio*, a parasitic bacterium that actually uses its motility to penetrate other bacteria and replicate within their cytoplasm, and from bacteriophages.

b. The S-layer can function as an adhesin, enabling the bacterium to adhere to host cells and environmental surfaces, colonize, and resist flushing.

c. The S-layer may contribute to virulence by protecting the bacterium against complement attack and phagocytosis.

d. The S-layer may act as a coarse molecular sieve.

Summary

1. The vast majority of the domain Bacteria have a rigid cell wall composed of peptidoglycan.
2. The peptidoglycan cell wall surrounds the cytoplasmic membrane and prevents osmotic lysis.
3. Peptidoglycan is composed of interlocking chains of building blocks called peptidoglycan monomers.
4. In order to grow following binary fission, bacteria have to synthesize new peptidoglycan monomers in the cytoplasm, transport those monomers across the cytoplasmic membrane, put breaks in the existing cell wall so the monomers can be inserted, connect the monomers to the existing peptidoglycan, and cross-link the rows and layers of peptidoglycan.
5. Many antibiotics inhibit peptidoglycan synthesis in bacteria and lead to osmotic lysis of the bacteria.
6. Most bacteria can be placed into one of three groups based on their color after specific staining procedures are performed: Gram-positive, Gram-negative, or acid-fast. These staining reactions are due to fundamental differences in the bacterial cell wall.
7. Gram-positive bacteria stain purple after Gram staining while Gram-negative bacteria stain pink.
Questions

Study the material in this section and then write out the answers to these questions. Do not just click on the answers and write them out. This will not test your understanding of this tutorial.

1. A monomer of peptidoglycan consists of _____________, _____________, and _______________. (ans)
2. State the function of peptidoglycan in bacteria. (ans)
3. State the role of the following enzymes in peptidoglycan synthesis:
   a. autolysins (ans)
   b. bactoprenols (ans)
   c. transpeptidases (ans)
   d. transglycosylase (ans)
4. A penicillin is used to treat a bacterial infection. Describe the mechanism by which this antibiotic eventually kills the bacteria. (ans)
5. Gram-positive bacteria stain ____________ (ans) after Gram staining while Gram-negative bacteria stain ____________ (ans).
6. Bacteria normally live in a hypotonic environment. Since water flows into a cell in an environment that is hypotonic, why don't the bacteria burst from osmotic pressure? (ans)
7. Multiple Choice (ans)

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