SECTION OVERVIEW

8.4: Oligosaccharides

An oligosaccharide is a carbohydrate whose molecule, upon hydrolysis, yields two to ten Monosaccharid molecules. Oligosaccharides are classified into subclasses based on the number of monosaccharide molecules that form when one molecule of the oligosaccharide is hydrolyzed.

Oligosaccharides are not commonly found free in cells, but instead are found **covalently attached to proteins**, which are then said to be glycosylated. Oligosaccharides attached to proteins may be **N-linked** (through **asparagine**) or **O-linked** (though **serine or threonine**). O-linked sugars are added only in the Golgi apparatus while N-linked sugars are attached starting in the endoplasmic reticulum and then completed in the Golgi.

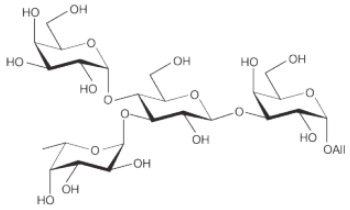

Figure 8.4.1: *An oligosaccharide.*

Glycoproteins

Membrane proteins are often covalently linked to **oligosaccharides**, which are branched *glycoside-linked* sugars (averaging around 15 sugar residues). As **glycans**, they are the sugars linked to **glycoproteins**. Glycoproteins are rare in the cytosol, but common on secreted and membrane proteins. Oligosaccharides are typically linked to proteins via the hydroxyl group on *serine* or *threonine*. Occasional linkages are to modified amino acids like hydroxylysine or hydroxyproline (*O-glycosylation*), and to the amide nitrogen on asparagine (*N-glycosylation*). The oligosaccharide domains of glycoproteins often play a major role in membrane protein function. For example, the glycoproteins, along with the polar domains of integral and peripheral proteins and glycolipids, are a major feature of the glycocalyx.

Glycosylation

Sugars are commonly attached to proteins in a process called **glycosylation.** Typically the attachment is to a hydroxyl or other functional group. The majority of proteins synthesized in the endoplasmic reticulum are glycosylated. Five classes of glycosylated products (called glycans if multiple carbohydrates are attached via glycosidic bonds) are known. They include:

• N-linked glycans - carbohydrate attached to N group of asparagine or arginine side chain

• O-linked glycans - carbohydrate attached to OH of serine, threonine, tyrosine, hydroxyproline, hydroxylysine, or lipids.

• Phosphoglycans - attachment to a phosphoserine

• Glypiation - linkage of a phosphatidyl inositol to link proteins to lipids via glycan linkages

• C-linked glycans - sugar attached to a carbon on a tryptophan side chain.

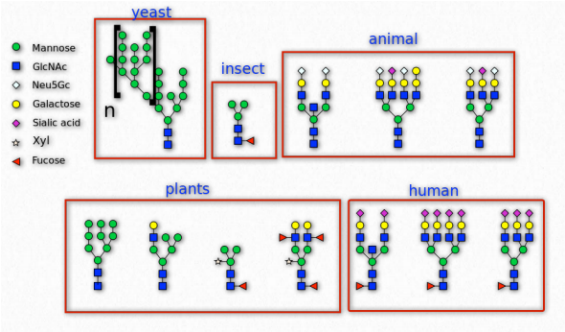

Figure 8.4.2: - *N-linked glycosylation in various organisms. (*https://en.wikipedia.org/wiki/N-linked_glycosylation)

Glycosylation has several molecular/ cellular functions. Some proteins require glycosylation to **fold properly** or to be stable. Glycosylated proteins on the plasma membrane serve as **cellular identifiers**. Blood types, for example, arise from differential glycosylation of a blood cell membrane protein. Glycosylation can also play an important role in **cell-cell adhesion** - important in the immune system.

Glycoprotein Function

The role of CHO in glycoprotein structure/function is slowly being determined. The most important seems to involve their role in directing proper folding of proteins in the endoplasmic reticulum (ER) which accounts for the observations that glycan addition to proteins in the ER is a cotranslational event. When inhibitors of ER glycosylation are added to cells, protein misfolding and aggregation are observed. The extent of misfolding depends on the particular protein and particular glycosylation sites with the protein. The polar CHO residues help promote solubility of folding intermediates, similar to the effects of many chaperone proteins.

The glycan moieties of the folding glycoprotein also lead to binding of the protein to lectins in the ER which serve as molecular chaperones. The most studied of these chaperones are involved in the calnexin-calreticulin cycle, and facilitate correct disulfide bond formation in the protein. After two glucose residues are removed by glucosidase I and II, the monoglucosylated protein binds to calnexin (CNX) and/or calreticulin (CRT), two homologous ER lectins specific for monoglucosylated proteins. Once bound, another protein, ERp57, a molecular chaperone with a disulfide bond (shown in diagram) interacts with the protein. This protein has protein disulfide isomerase activity.

If a glycoprotein has not folded completely, it is recognized by a glycoprotein glucosyltransferase, which adds a glucose to it. This then promotes reentry into the calnexin/calreticulin cycle.

Ideally, unfolded or misfolded proteins would be targeted from degradation and elimination from cells. The ER has evolved a system to accomplish this. Since folding occurs in the ER, to prevent misfolding and aggregation, the ER also contains chaperones and folding catalysts. Stress (such as through heat shock) stimulates ER chaperone activity. As a final defense mechanism, unfolded or aberrantly-folded proteins are degraded by the cytoplasmic proteasome complex. Nonnative forms of some proteins that "escape" this surveillance system can accumulate and result in disease (for example neurodegenerative diseases like Alzheimers and Parkinson's disease.

Glycosylation versus Glycation

The sugars on glycoproteins have been placed there by **glycosylation** — a precise enzymatic activity that makes a product which would otherwise not function correctly. However, sugars can also spontaneously form covalent links to proteins (and lipids) — a process called **glycation**. No enzymes are involved so the process is quite random. The products are apt to have reduced, or even no, function. The glycation of proteins and lipids is an inevitable outcome of aging. It is hastened in diabetics with their high blood sugar (glucose) levels. In fact, measuring the amount of glycation of hemoglobin is an important test for determining how well diabetes is being controlled.

Peptidoglycan

Peptidoglycan, also known as murein, is a polymer consisting of sugars and amino acids that forms a mesh-like layer outside the plasma membrane of bacteria (but not Archaea), forming the cell wall. The sugar component consists of alternating residues of β-

(1,4) linked N-acetylglucosamine (NAG) and N-acetylmuramic acid (NAM). Attached to the N-acetylmuramic acid is a peptide chain of three to five amino acids. The peptide chain can be cross-linked to the peptide chain of another strand forming the 3D mesh-like layer.

The peptidoglycan layer is substantially thicker in Gram-positive bacteria (20 to 80 nanometers) than in Gram-negative bacteria (7 to 8 nanometers), with the attachment of the S-layer. Peptidoglycan forms around 90% of the dry weight of Gram-positive bacteria but only 10% of Gram-negative strains. Thus, presence of high levels of peptidoglycan is the primary determinant of the characterization of bacteria as gram-positive. In Gram-positive strains, it is important in attachment roles and stereotyping purposes. For both Gram-positive and Gram-negative bacteria, particles of approximately 2 nm can pass through the peptidoglycan. Gram-positive and Gram-negative bacteria are sensitive to different types of antiobiotics.

Structure and Composition of Peptidoglycan

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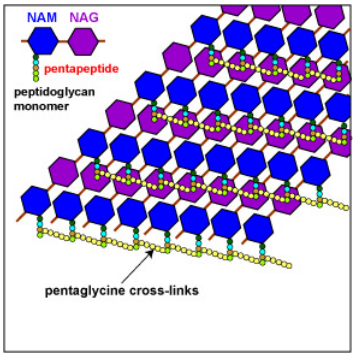

Figure 8.4.3: *Peptidoglycan is composed of cross-linked chains of peptidoglycan monomers (NAG-NAM-pentapeptide). Transglycosylase enzymes join these monomers join together to form chains. Transpeptidase enzymes then cross-link the chains to provide strength to the cell wall and enable the bacterium to resist osmotic lysis.*

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.

Peptidoglycan serves a structural role in the bacterial cell wall giving it strength, as well as counteracting the osmotic pressure of the cytoplasm. A common misconception is that peptidoglycan gives the cell its shape. However, it is actually the MreB protein that facilitates cell shape. Peptidoglycan is also involved in binary fission during bacterial cell reproduction.

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. 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.

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.

Topic hierarchy

Thumbnail: Structure of galactooligosaccharide. Image used with permission (Public Domain; Klaas1978)