CHAPTER 7

Carbohydrates and Glycobiology

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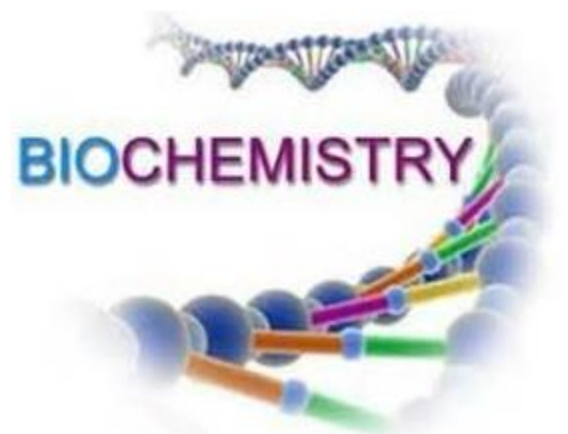
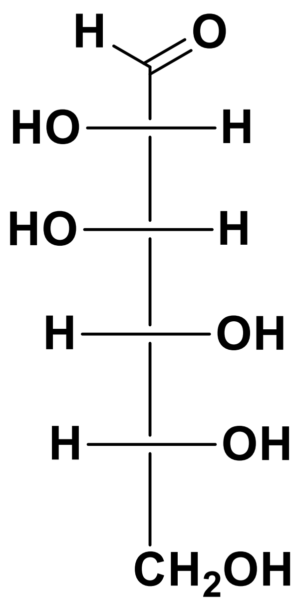
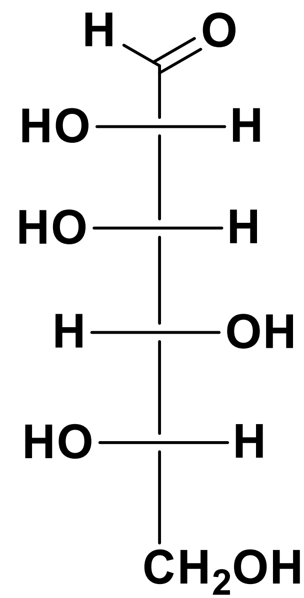


Quiz



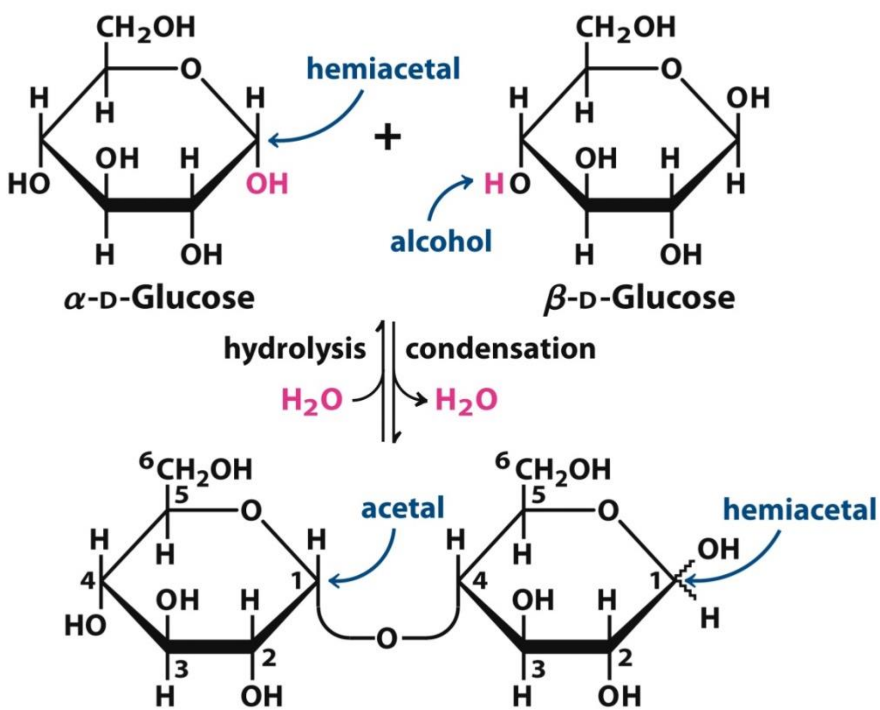
(A)



(B)

1) Name the chemical structures.
2) Draw the cyclic form of the above structures.

Review Disaccharides

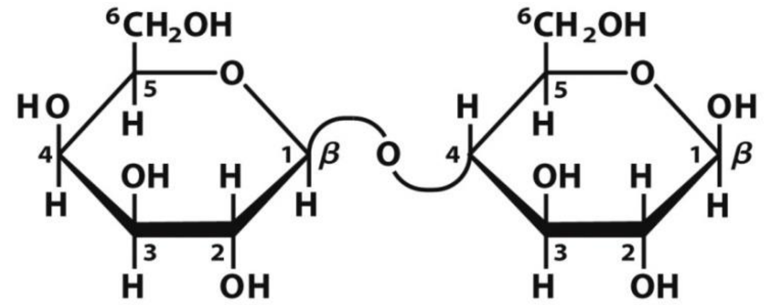


Maltose

α-D-glucopyranosyl-(1→4)-D-glucopyranose

Glc(α1→4)Glc

Reducing sugars

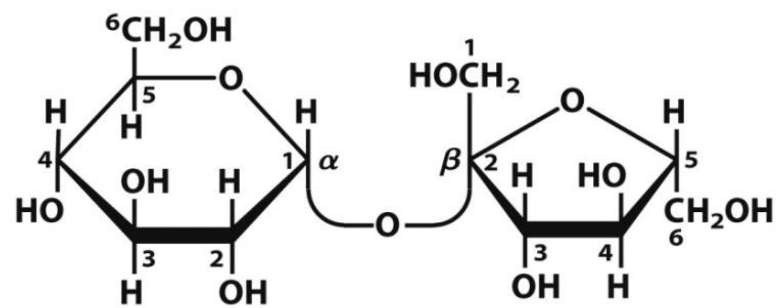


Lactose (β form)

β-D-galactopyranosyl-(1→4)-β-D-glucopyranose

Gal(β1→4)Glc

Reducing sugars



Sucrose

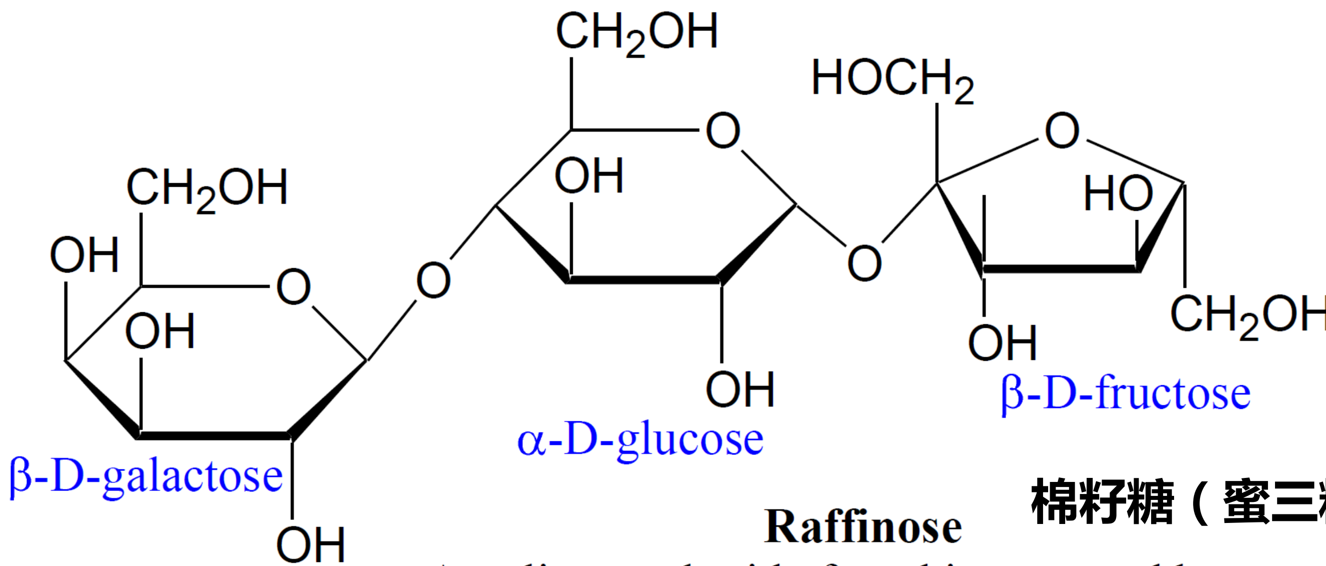
β-D-fructofuranosyl α-D-glucopyranoside

Fru(2β↔α1)Glc ≡ Glc(α1↔2β)Fru

Nonreducing sugars

Oligosaccharides

- Oligosaccharides contain **3 to 10** monosaccharide units.



Raffinose **棉籽糖（蜜三糖）**

An oligosaccharide found in peas and beans; largely undigested until reaching the intestinal flora in the large intestine, releasing hydrogen, carbon dioxide, and methane)

7.2 Polysaccharides

➢ **Polysaccharides**

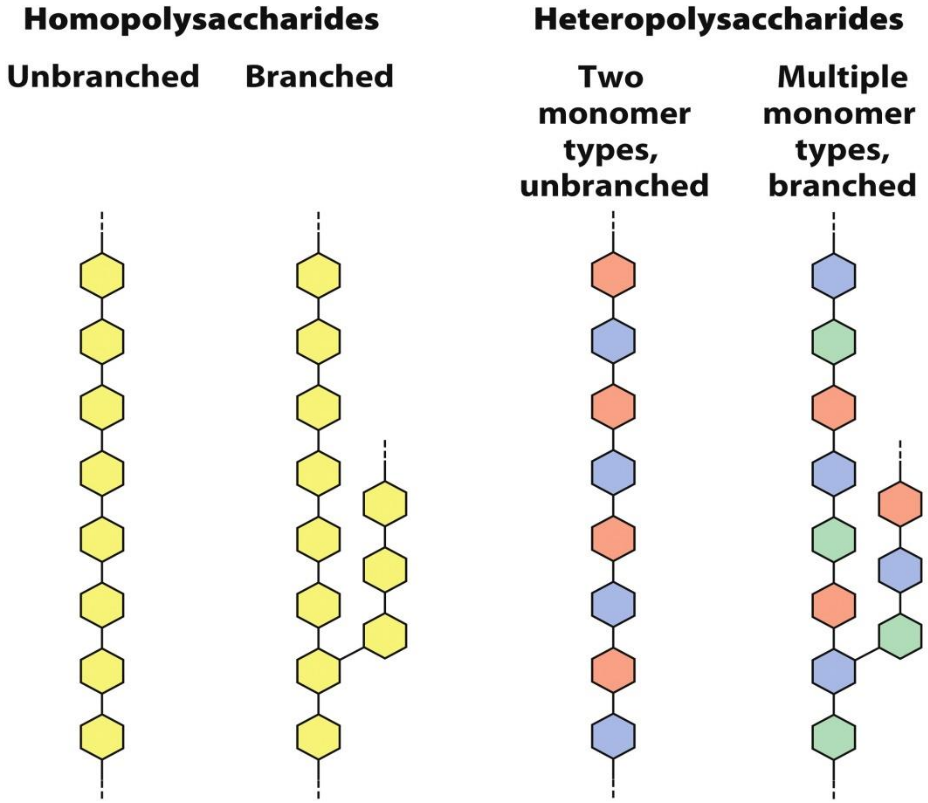
- Most carbohydrates found in nature occur as polysaccharides with high molecular weights (Mr >20,000). Polysaccharides contain **hundreds or thousands** of monosaccharide units,

- Polysaccharides **are not reducing sugars**, since the anomeric carbons are connected through glycosidic linkages.

- We will consider three kinds of polysaccharides, all of which are polymers of glucose: ***starch, glycogen, and cellulose.***

淀粉、糖原、纤维素

- **Homopolysaccharides and heteropolysaccharides**



Polysaccharides may be composed of one, two, or several different monosaccharides, in **straight** or **branched** chains of varying length.

Homopolysaccharides contain only a single monomeric species;

heteropolysaccharides contain two or more different kinds

Unlike proteins, polysaccharides generally do not have defining molecular weights. There is no template for polysaccharide synthesis, and there is no specific stopping point in the synthetic process; the products thus vary in length.

➢ Biological functions of polysaccharides

- **Storage**

  Some homopolysaccharides serve as storage forms of monosaccharides that are used as fuels; **starch** and **glycogen**

- **Structural elements**

  Other **homopolysaccharides** (for example **cellulose** and **chitin**) serve as structural elements in plant cell walls and animal exoskeletons.

  Heteropolysaccharides provide extracellular support for all organisms.

  Bacterial cell envelope (peptidoglycan)

  Extracellular matrix in animal tissues

➢ Homopolysaccharides as stored forms of fuel

The most important storage polysaccharides are **starch** in plant cells and **glycogen** in animal cells.

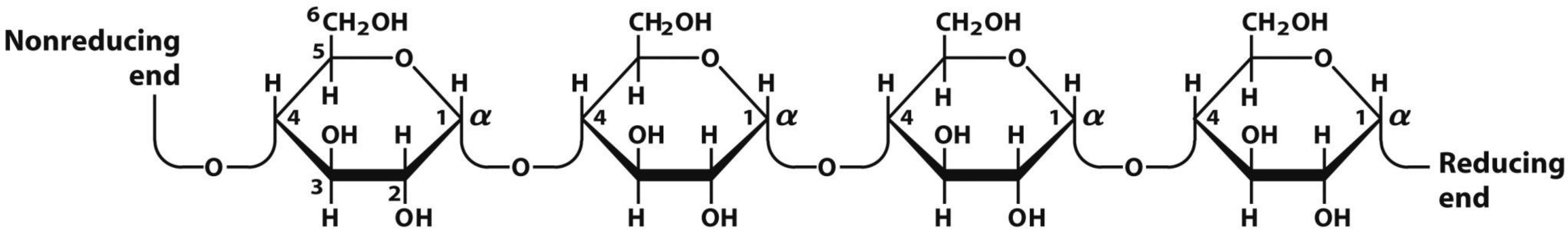
- **Starch** contains two types of D-glucose polymer, **amylose** (unbranched) and **amylopectin** (highly branched, occurring every 24 to 30 residues)

- **Glycogen** is similar to amylopectin, is more extensively branched (every 8 to 12 residues)

Starches and other glucose polymers are usually insoluble in water because of the high molecular weight. Because they contain large numbers of OH groups, some starches can form thick colloidal dispersions when heated in water (e.g., flour or starch used as a thickening agent in gravies or sauces).

- **Starch--Amylose**

Amylose consists of long, unbranched chains of D-glucose residues connected by (α1→4) linkages. Such chains vary in molecular weight from a few thousand to more than a million.

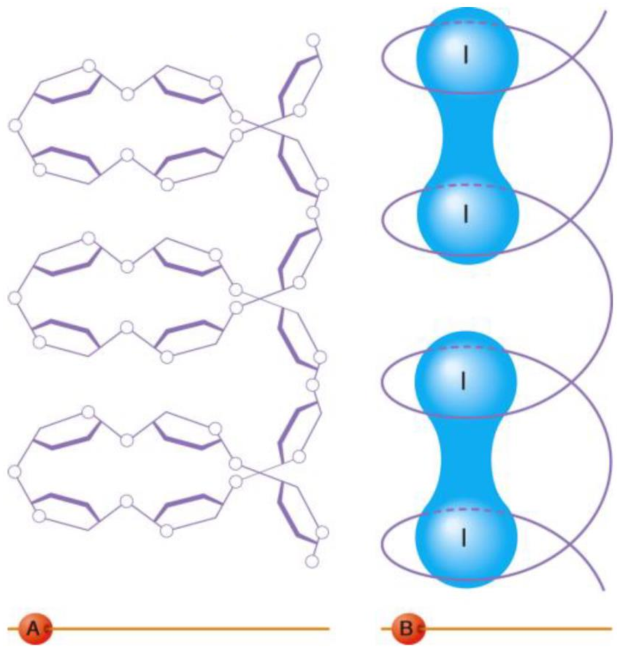


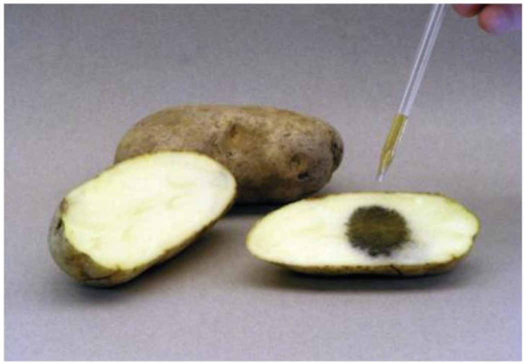
Amylose

10%-20% of the starch in plants is in this form.

Helix shape of amylose

The amylose chain is flexible enough to allow the molecules to twist into the shape of a helix. Because it packs more tightly, it is slower to digest than other starches.

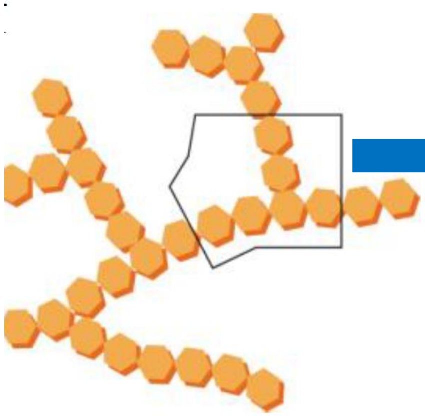


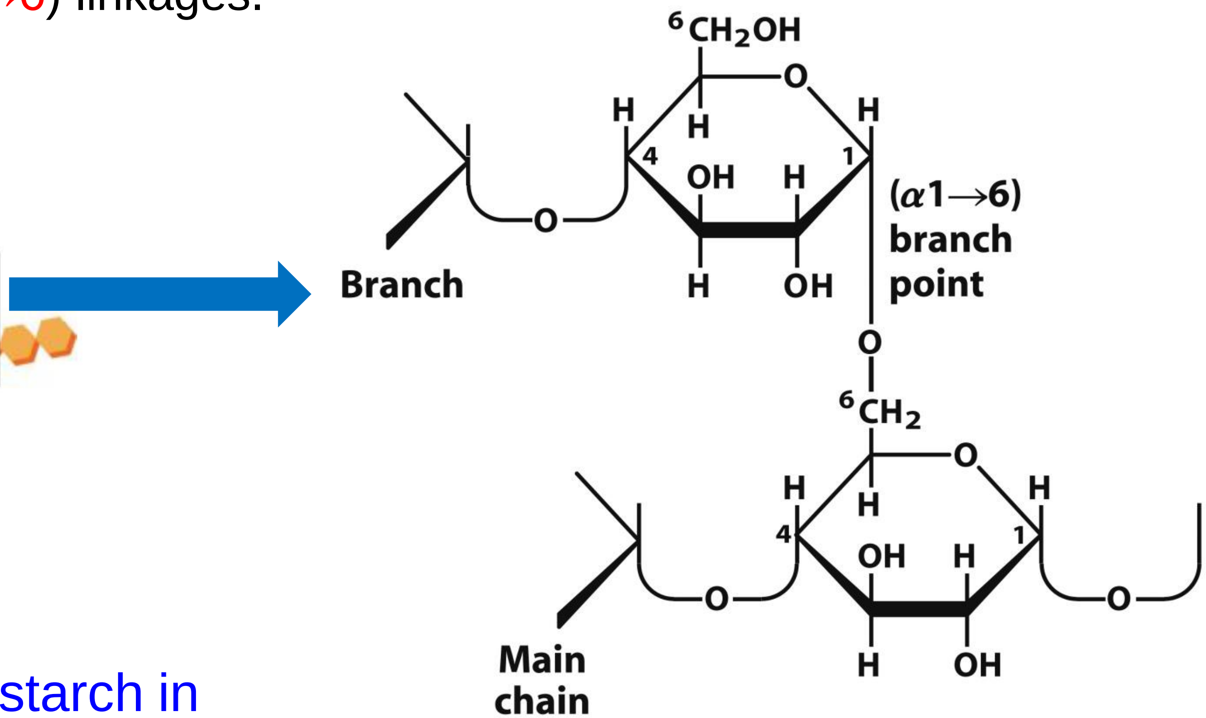


Amylose helices can trap molecules of iodine, forming a characteristic deep blue-purple color. Iodine is therefore often used as a test for the presence of starch.

- **Starch--Amylopectin**

Amylopectin has a high molecular weight (up to 200 million) and is highly branched. The glycosidic linkages joining successive glucose residues in amylopectin chains are (α1→4); the branch points (occurring every 24 to 30 residues) are (α1→6) linkages.





80%-90% of the starch in plants is in this form.

Figure 7-13b
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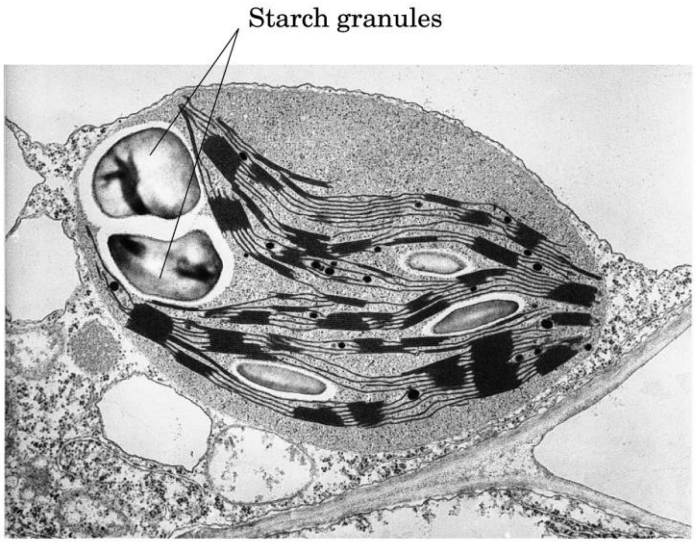
Amylose and amylopectin in starch granules



Chloroplast

Large starch granules in a single chloroplast. Starch is made in the chloroplast from D-glucose formed *photosynthetically.*

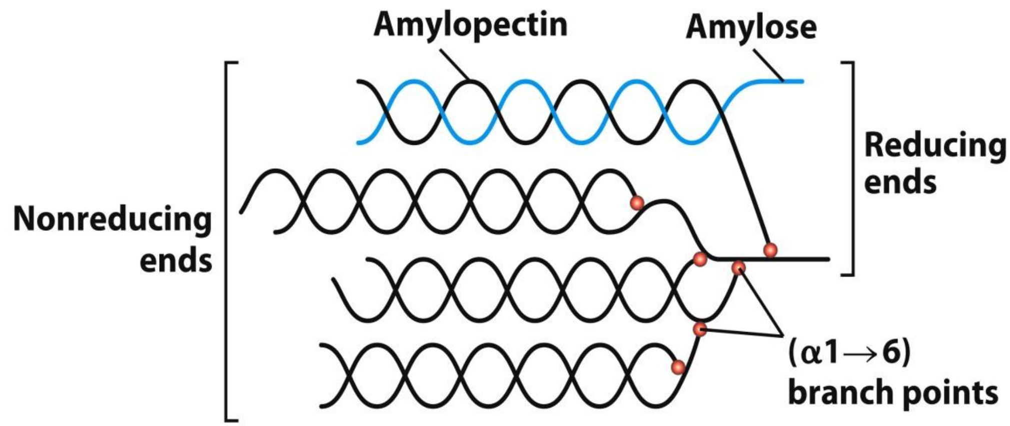


Figure 7-13c
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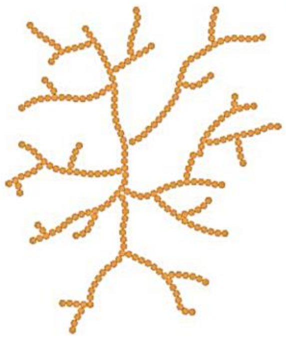
Strands of amylopectin (black) form double-helical structures with each other or with amylose strands (blue). Amylopectin has frequent (α1→6) branch points (red). Glucose residues at the nonreducing ends of the outer branches are removed enzymatically during the mobilization of starch for energy production.

- **Glycogen**



Glycogen, the main storage polysaccharide of animal cells, is structurally similar to amylopectin, containing both (α1→4) glycosidic linkages and (α1→6) branch points, but is more extensively branched (on average, every 8 to 12 residues) and more compact than starch.

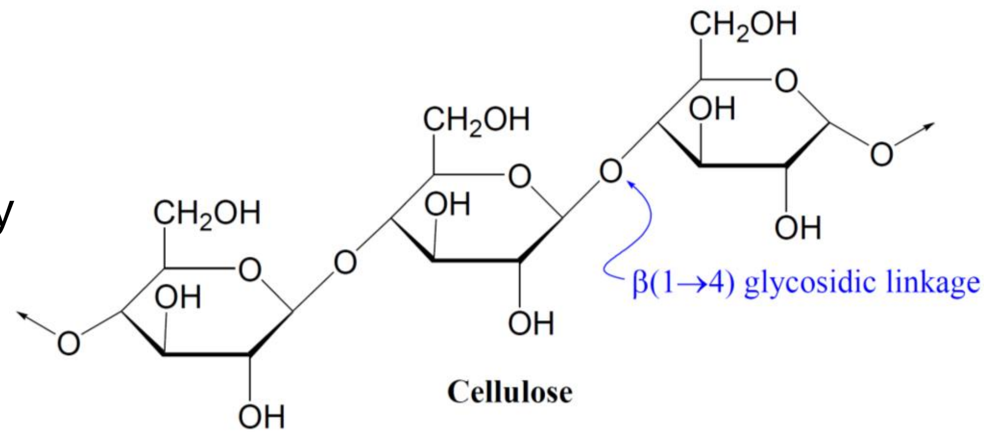
Because each branch in glycogen ends with a nonreducing sugar unit, a glycogen molecule with ***n*** branches has ***n* + 1** nonreducing ends, but **only one** reducing end.

Glycogen is abundant in the liver and muscles; on hydrolysis it forms D-glucose, which maintains normal blood sugar level and provides energy. When glycogen is used as an energy source, glucose units are removed one at a time from the nonreducing ends. Degradative enzymes that act only at nonreducing ends can work simultaneously on the many branches, speeding the conversion of the polymer to monosaccharides.

➢ Homopolysaccharides serve structural roles

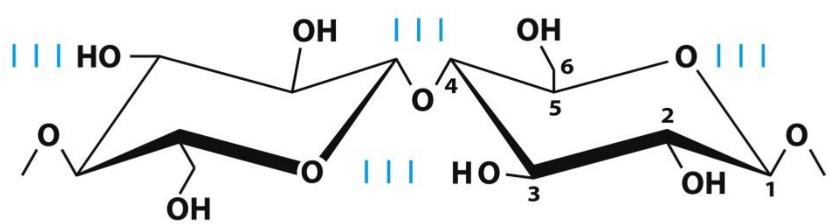
- **Cellulose**

Cellulose is a polymer consisting of long, *unbranched* chains of (**β1→4**) glycosidic linkages; it may contain from 10,000 to 15,000 D-glucose units.



Cellulose

Because of the (β1→4)-linkages, cellulose has a different overall shape from amylose, forming extended straight chains which hydrogen bond to each other, resulting in a very rigid structure.



(β1→4)-linked D-glucose units

Figure 7-14
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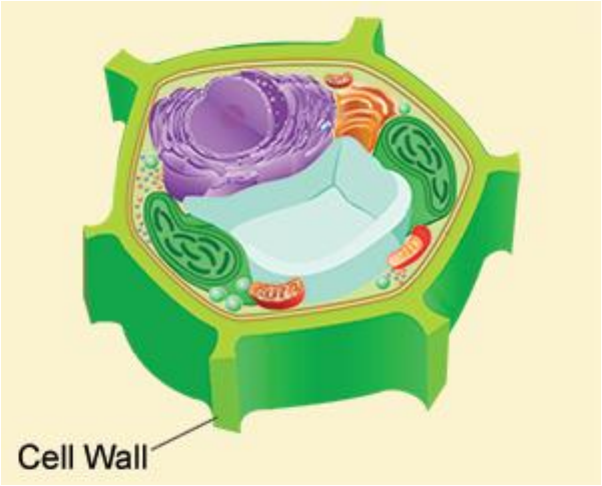
Cellulose is a fibrous, tough, water-insoluble substance, is found in the cell walls of plants, particularly in stalks, stems, trunks, and all the woody portions of the plant body. Cellulose constitutes much of the mass of wood, and cotton is almost pure cellulose.

Cellulose is the most important ***structural*** polysaccharide, and is the single most abundant organic compound on earth. It is the material in plant cell walls that provides strength and rigidity; wood is 50% cellulose.



Breakdown of cellulose

Glycogen and starch ingested in the diet are hydrolyzed by α-amylases and glycosidases, enzymes in saliva and the intestine that break (α1→4) glycosidic bonds between glucose units.

Termites readily digest cellulose only because their intestinal tract harbors a symbiotic microorganism, ***Trichonympha***, that secretes cellulase, which hydrolyzes the (β1→4) linkages.

A wide range of invertebrate animals, including arthropods and nematodes, have genes encoding cellulose-degrading.

Trichonympha

Most vertebrate animals **cannot** use cellulose as a fuel source, because they lack an enzyme to hydrolyze the (β1→4) linkages.

Exception: ruminant animals such as cattle, sheep, and goats harbor symbiotic microorganisms in the rumen (the first of their four stomach compartments) that can hydrolyze cellulose, allowing the animal to degrade dietary cellulose from soft grasses, but not from woody plants.

Dietary Fiber

- ✓ Dietary fiber consists of complex carbohydrates, such as cellulose, and other substances that make up the cell walls and structural parts of plants.

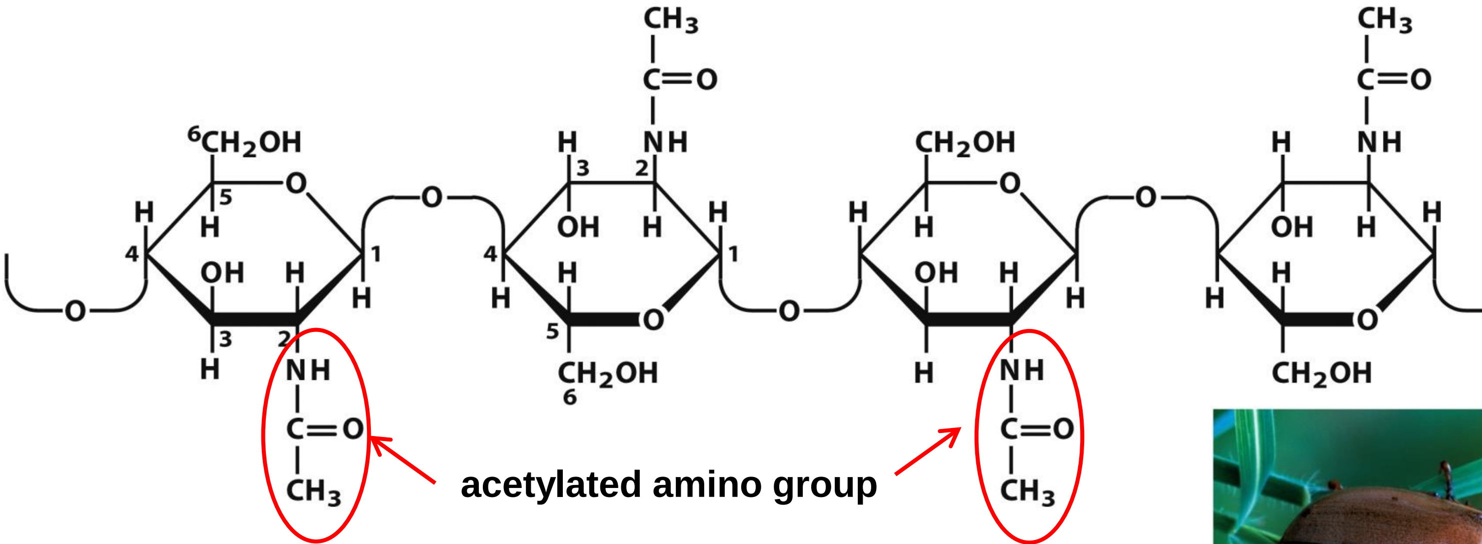
- ✓ Good sources of dietary fiber include cereal grains, oatmeal, fresh fruits and vegetables, and grain products.

- ✓ Soluble fiber, such as pectin, has a lower molecular weight, and is more water soluble. Soluble fiber traps carbohydrates and slows their digestion and absorption, thereby leveling out blood sugar levels during the day. Soluble fiber also helps to lower cholesterol levels by binding dietary cholesterol.

- ✓ Insoluble fiber, such as cellulose, provides bulk to the stool, which helps the body to eliminate solid wastes.

- **Chitin（几丁质）**

Chitin is a linear homopolysaccharide composed of ***N*****-acetylglucosamine** residues in (β1→4) linkage. The only chemical difference from cellulose is the replacement of the hydroxyl group at C-2 with an acetylated amino group. The polymer is extremely strong because of the increased hydrogen bonding provided by the amide groups.



Chitin is the principal component of the hard exoskeletons of nearly a million species of arthropods—insects, lobsters, and crabs, for example—and is probably the second most abundant polysaccharide.

Figure 7-16b
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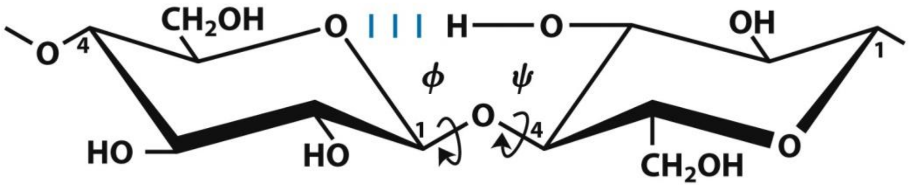
➢ Steric factors and hydrogen bonding influence homopolysaccharide folding

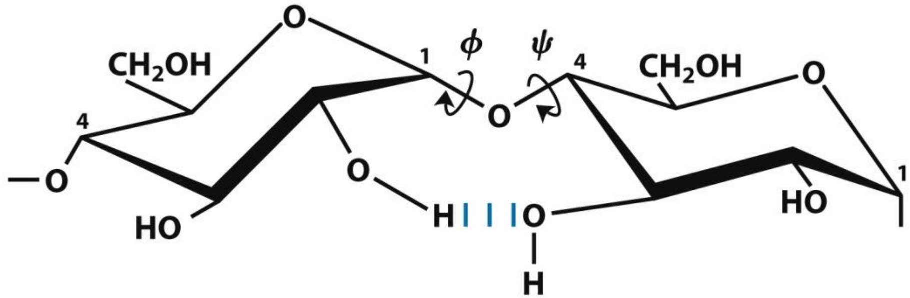
A series of rigid pyranose rings connected by an oxygen atom (the glycosidic bond).

In principle, free rotation about both C–O bonds linking the residues, rotation about each bond is limited by steric hindrance by substituents.

The three-dimensional structures of these molecules can be described in terms of the dihedral angles about the glycosidic bond, ϕ and ψ.



Cellulose
(β1→4)Glc repeats

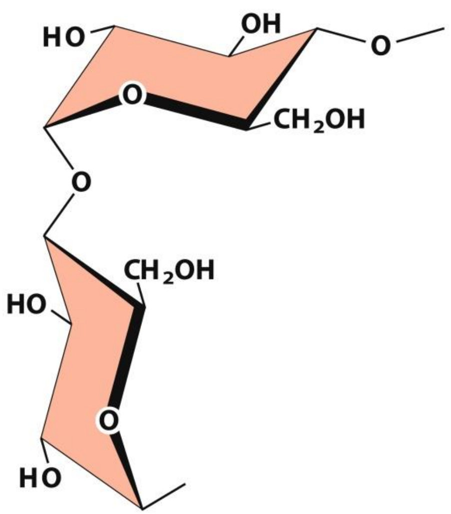


Amylose
(α1→4)Glc repeats

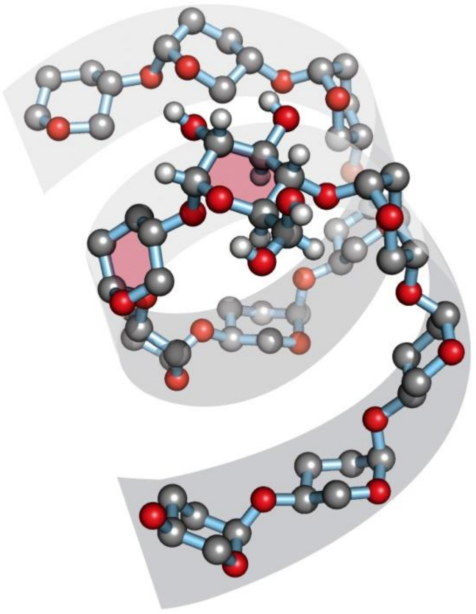
Hydrogen bonding has an especially important influence on their structure because polysaccharides have so many hydroxyl groups,.

Helical structure of starch (amylose)

The most stable three-dimensional structure for the (α1→4)-linked chains of starch and glycogen is a tightly coiled helix. The average plane of each residue along the amylose chain forms a **60°** angle with the average plane of the preceding residue, so the helical structure has **6** residues per turn.



(α1→4)-linked
D-glucose units



For amylose, the core of the helix is of precisely the right dimensions to accommodate iodine as complex.

The structure of cellulose

For cellulose, the most stable conformation is that in which each chair is turned 180° relative to its neighbors, yielding a straight, extended chain.

All —OH groups are available for hydrogen bonding with neighboring chains. With several chains lying side by side, a stabilizing network of ***interchain*** and ***intrachain*** hydrogen bonds produces straight, stable supramolecular fibers of great tensile strength.

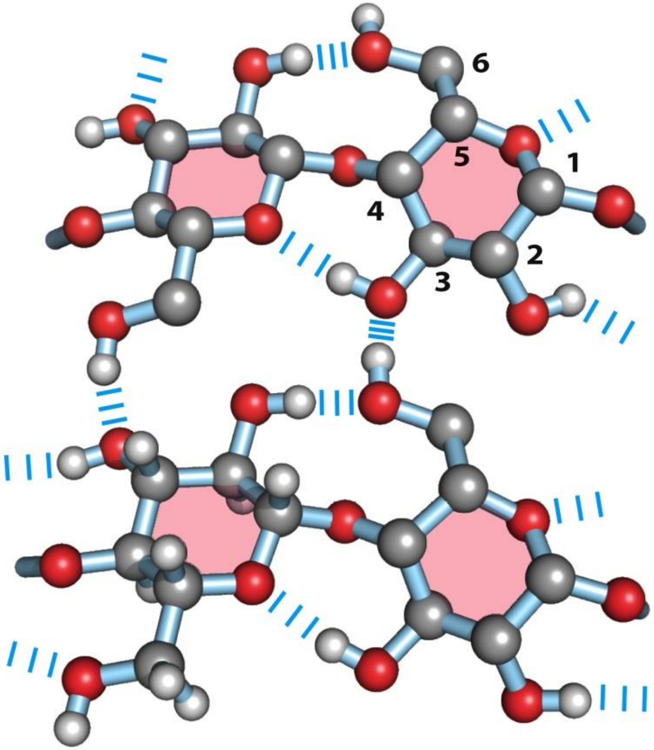


Figure 7-20
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Many manufactured products, including, paper, cardboard, rayon, insulating tiles, and a variety of other useful materials, are derived from cellulose. The water content of these materials is low because extensive interchain hydrogen bonding between cellulose molecules satisfies their capacity for hydrogen-bond formation.

TABLE 7–2 Structures and Roles of Some Polysaccharides

Polymer	Type*	Repeating unit†	Size (number of monosaccharide units)	Roles/significance
Starch				Energy storage: in plants
Amylose	Homo-	(α1→4)Glc, linear	50–5,000	
Amylopectin	Homo-	(α1→4)Glc, with (α1→6)Glc branches every 24–30 residues	Up to 10^6	
Glycogen	Homo-	(α1→4)Glc, with (α1→6)Glc branches every 8–12 residues	Up to 50,000	Energy storage: in bacteria and animal cells
Cellulose	Homo-	(β1→4)Glc	Up to 15,000	Structural: in plants, gives rigidity and strength to cell walls
Chitin	Homo-	(β1→4)GlcNAc	Very large	Structural: in insects, spiders, crustaceans, gives rigidity and strength to exoskeletons
Dextran	Homo-	(α1→6)Glc, with (α1→3) branches	Wide range	Structural: in bacteria, extracellular adhesive
Peptidoglycan	Hetero-; peptides attached	4)Mur2Ac(β1→4) GlcNAc(β1	Very large	Structural: in bacteria, gives rigidity and strength to cell envelope
Agarose	Hetero-	3)D-Gal(β1→4)3,6-anhydro-L-Gal(α1	1,000	Structural: in algae, cell wall material
Hyaluronan (a glycosamino-glycan)	Hetero-; acidic	4)GlcA(β1→3) GlcNAc(β1	Up to 100,000	Structural: in vertebrates, extracellular matrix of skin and connective tissue; viscosity and lubrication in joints

7.3 Glycoconjugates

Glycoconjugates are proteins or lipids covalently linked with carbohydrates, forming biologically active molecules.

Biological functions of glycoconjugates:

In addition to their important roles as stored fuels and as structural materials, polysaccharides and oligosaccharides are ***information carriers***.

- ✓ Providing communication between cells and their extracellular surroundings;
- ✓ Labeling proteins for transport to and localization in specific organelles, or for destruction when the protein is malformed or superfluous;
- ✓ Serving as recognition sites for extracellular signal molecules (growth factors, for example) or extracellular parasites (bacteria or viruses).

On almost every eukaryotic cell, specific oligosaccharide chains attached to components of the plasma membrane form a carbohydrate layer (the glycocalyx), serving as an information-rich surface that the cell shows to its surroundings. These oligosaccharides are central players in cell-cell recognition and adhesion, cell migration during development, blood clotting, the immune response, wound healing, and other cellular processes.

- **Three major types of glycoconjugates**

Proteoglycans（蛋白聚糖） are macromolecules of the cell surface or extracellular matrix in which one or more sulfated glycosaminoglycan chains are joined covalently to a membrane protein or a secreted protein.

Glycoproteins（糖蛋白） have one or several oligosaccharides of varying complexity joined covalently to a protein.

Glycolipids （糖脂） are membrane lipids in which the hydrophilic head groups are oligosaccharides.

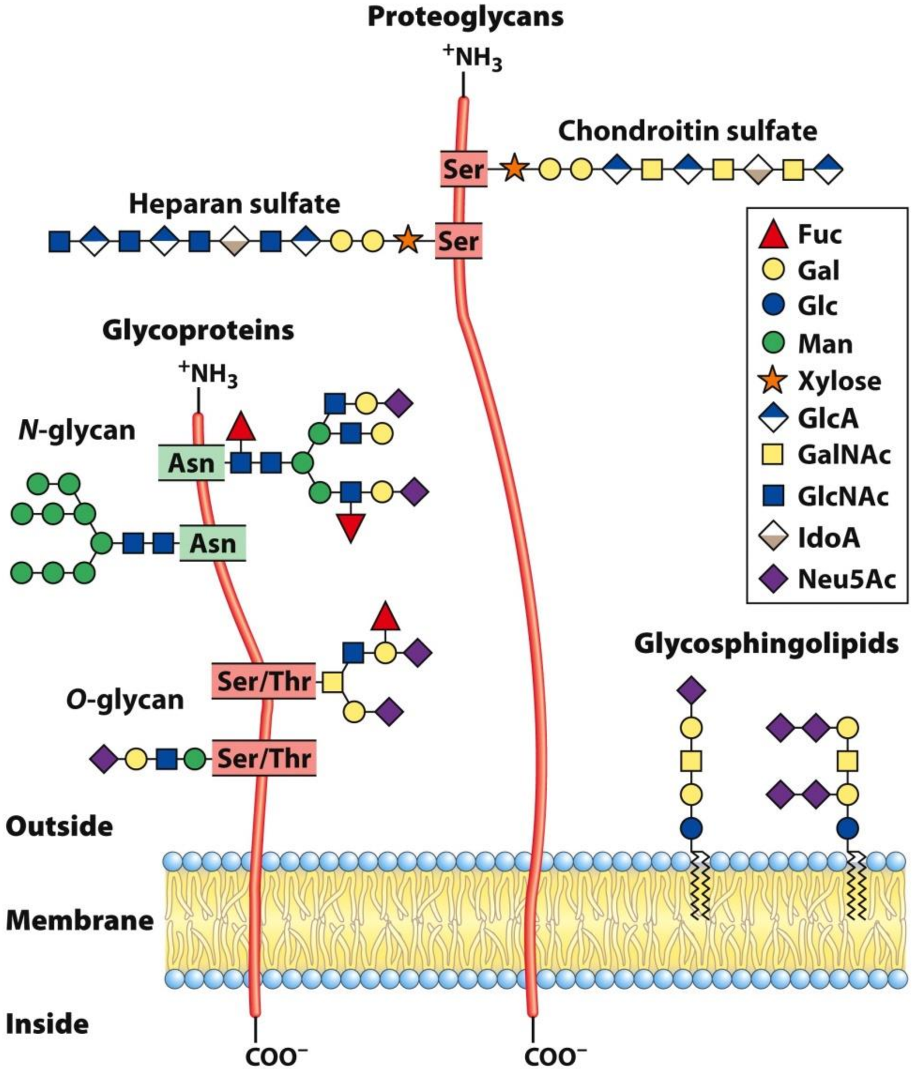

Figure 7-24
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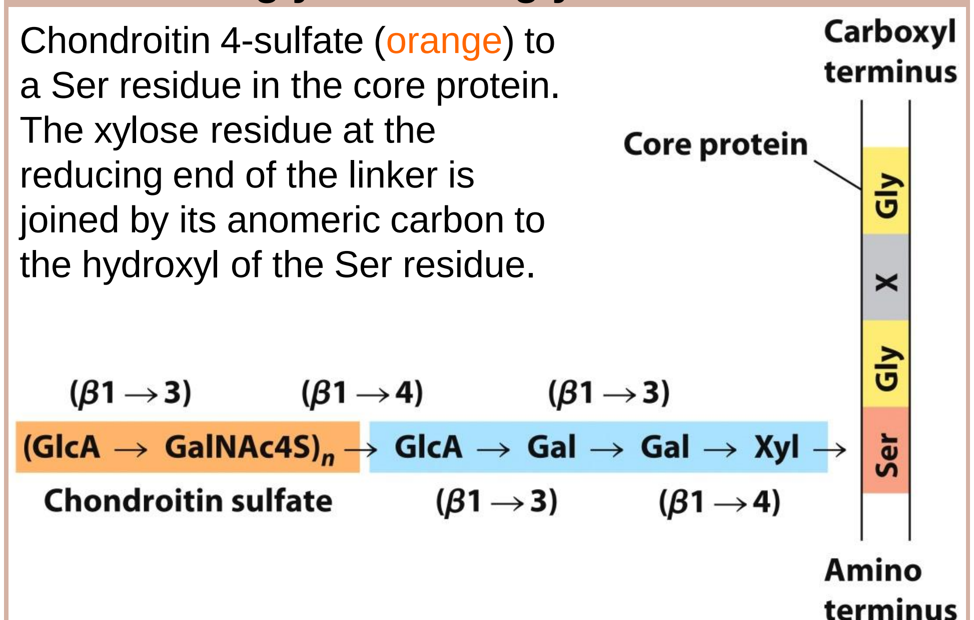
- **Proteoglycans（蛋白聚糖）** are glycosaminoglycan-containing macromolecules of the cell surface and extracellular matrix
  - Mammalian cells can produce about 40 types of proteoglycans.
  - These molecules act as tissue organizers, and they influence various cellular activities, such as growth factor activation and adhesion.
  - The basic proteoglycan unit consists of a "core protein" with covalently attached glycosaminoglycan(s). The point of attachment is a Ser residue, to which the glycosaminoglycan is joined through a tetrasaccharide bridge. The Ser residue is generally in the sequence **–Ser–Gly–X–Gly–** (where X is any amino acid residue).

A typical tetrasaccharide linker (blue) connects a glycosaminoglycan

Chondroitin 4-sulfate (orange) to a Ser residue in the core protein. The xylose residue at the reducing end of the linker is joined by its anomeric carbon to the hydroxyl of the Ser residue.



■ Membrane proteoglycans

Syndecans and **Glypicans** are two major families of membrane heparan sulfate proteoglycans.

- **Syndecan** core protein has a single transmembrane domain and an extracellular domain bearing three to five chains of heparan sulfate and in some cases chondroitin sulfate.
- **Glypican** is attached to the membrane by a lipid anchor, a derivative of the membrane lipid phosphatidylinositol.

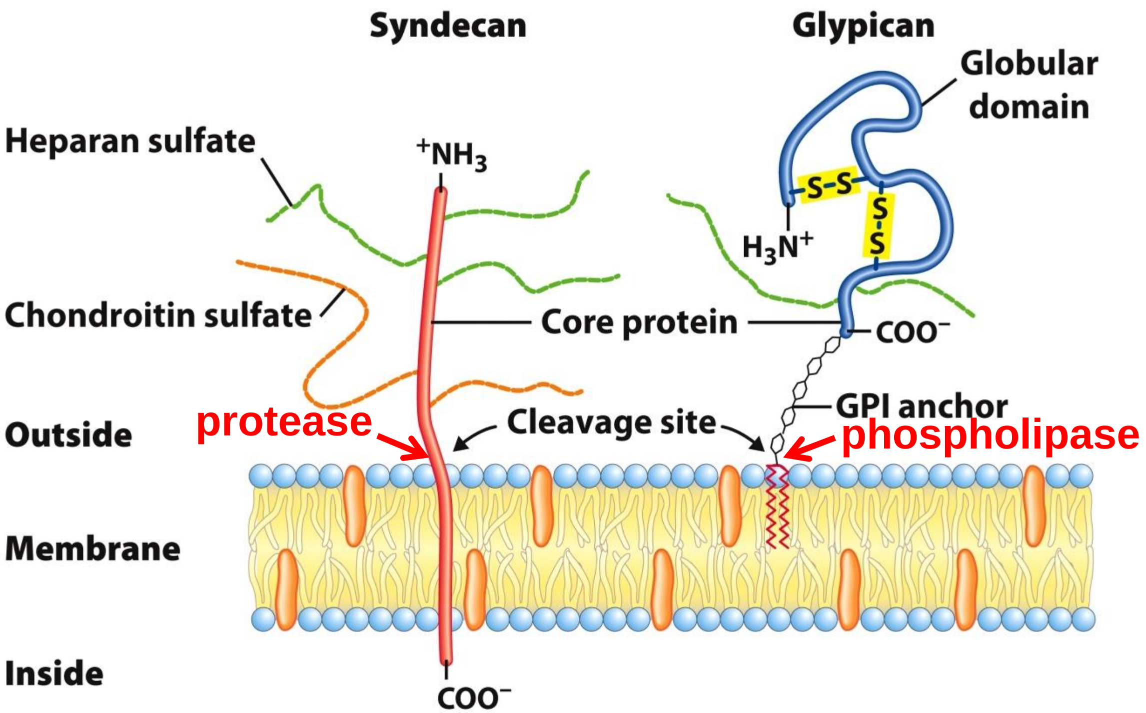


Figure 7-26a
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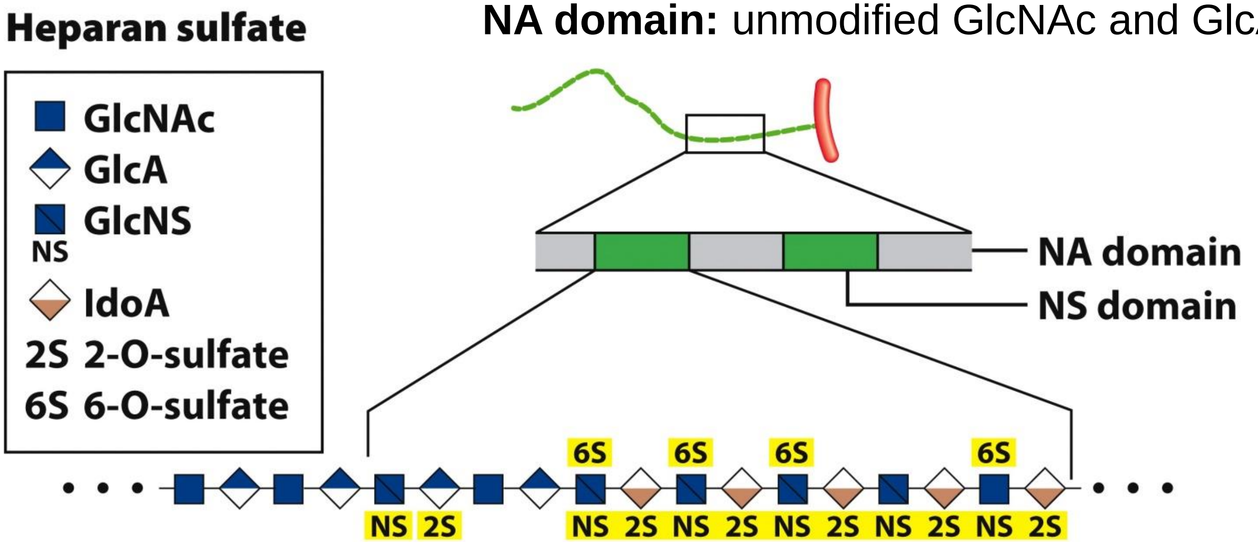
Chondroitin　软骨素
Heparan　　乙酰肝素

Heparan sulfate

NS domain: highly sulfated domain
NA domain: unmodified GlcNAc and GlcA residues



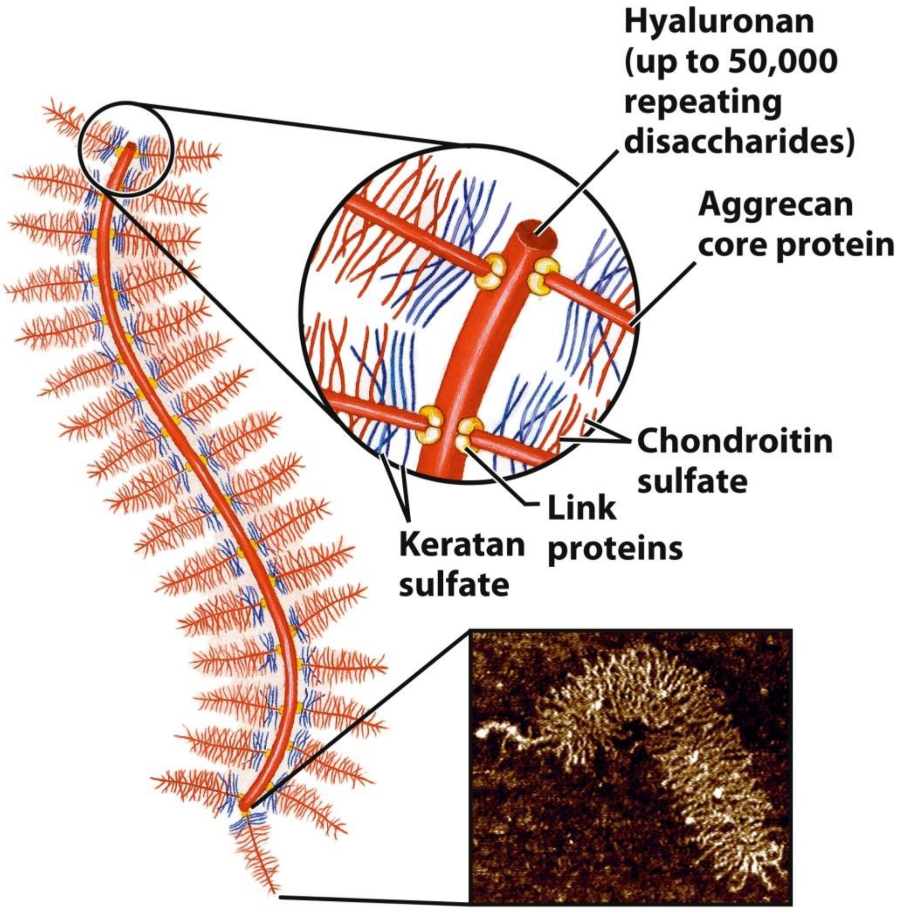
- The exact pattern of sulfation in the NS domain differs among proteoglycans.
- Heparan sulfate molecules with precisely organized NS domains bind specifically to extracellular proteins and signaling molecules to alter their activities.

■ Proteoglycan aggregates

Proteoglycan aggregates, many core proteins all bound to a single molecule of hyaluronan.

Aggrecan core protein has multiple chains of chondroitin sulfate and keratan sulfate, joined to Ser residues in the core protein, to give an aggrecan monomer of *M*r ~$2X10^6$.

When a hundred or more of these “decorated” core proteins bind a single, extended molecule of hyaluronate, the resulting proteoglycan aggregate (*M*r >$2X10^8$) and its associated water of hydration occupy a volume about equal to that of a bacterial cell!



Proteoglycan aggregate of the extracellular matrix

Chondroitin 软骨素
Keratan 硫酸角质素

- **Interactions between cells and the extracellular matrix**

The extracellular proteoglycans interact strongly with fibrous matrix proteins such as collagen, elastin, and fibronectin, forming a cross-linked meshwork that gives the whole extracellular matrix strength and resilience.

These interactions serve not merely to anchor cells to the extracellular matrix, but also to provide paths that direct the migration of cells in developing tissue and to convey information in both directions across the plasma membrane.

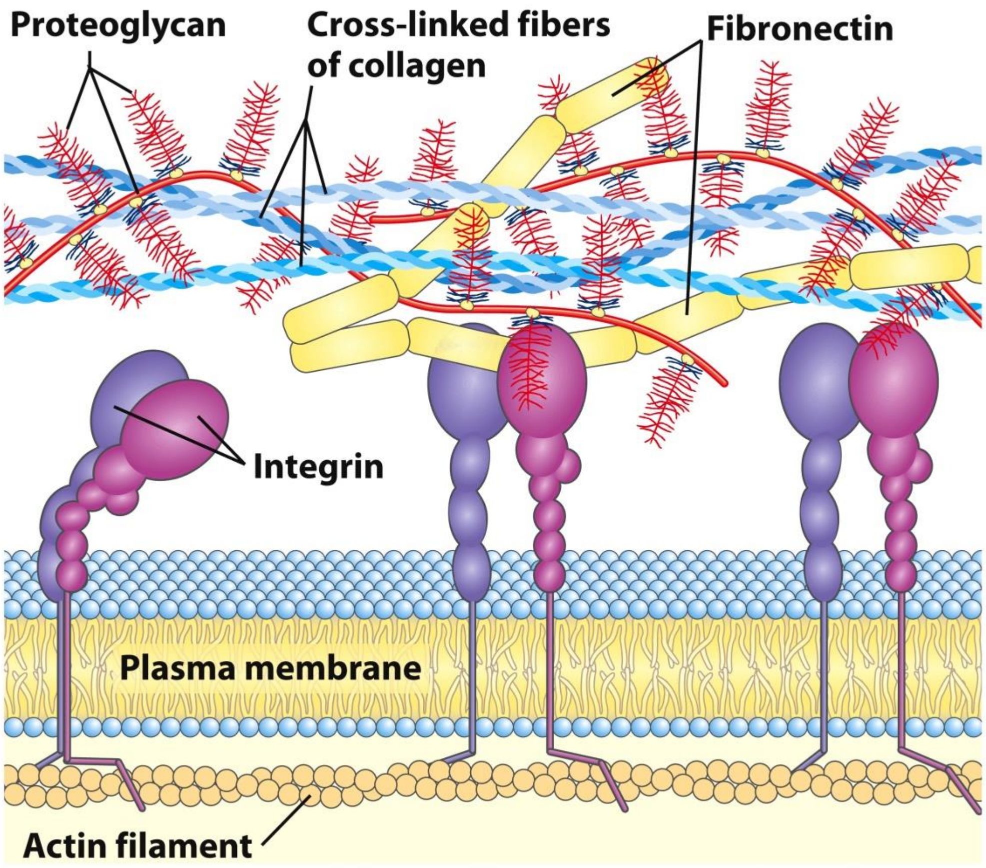


Figure 7-29
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➢ Glycoproteins（糖蛋白）

- Glycoproteins are carbohydrate-protein conjugates in which the glycans are **smaller** and more **structurally diverse** than the huge glycosaminoglycans of proteoglycans.

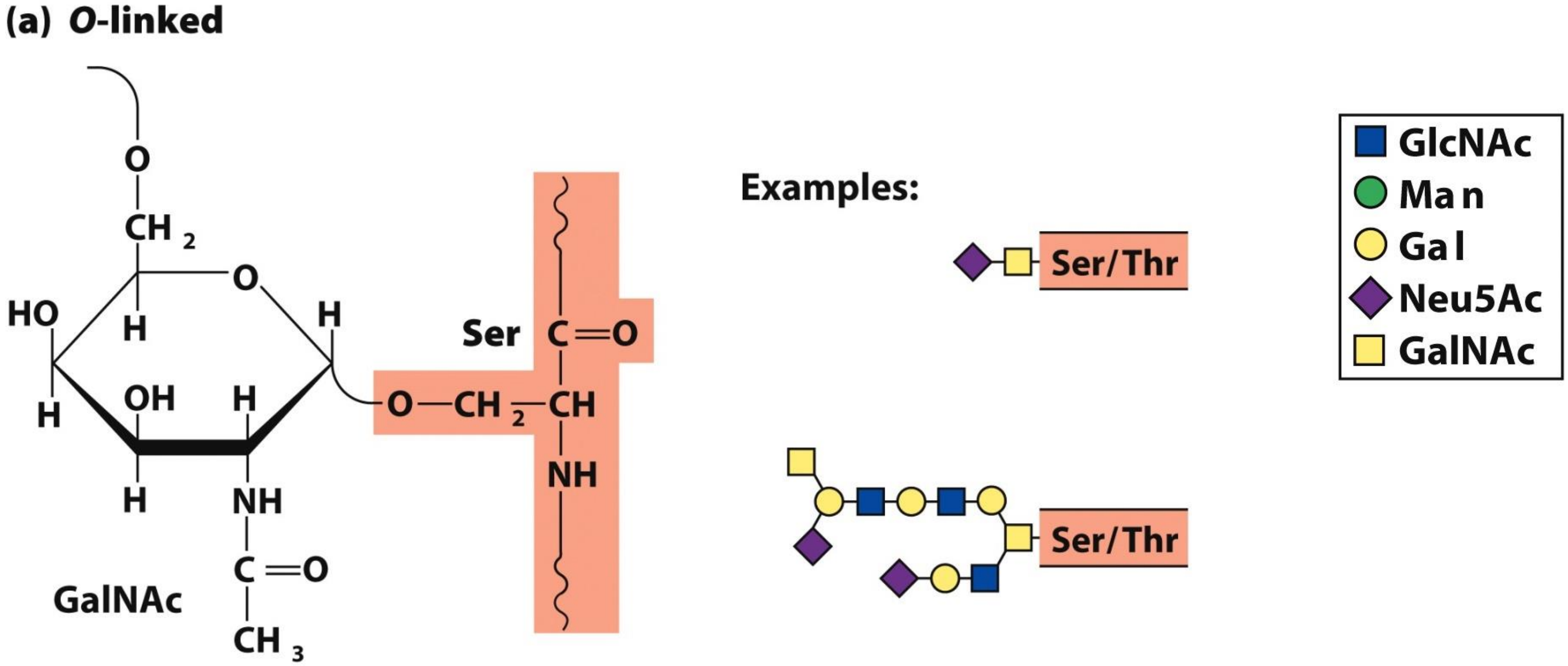
- They are found on the **outer face** of the *plasma membrane*, in the *extracellular matrix*, and *in the blood*. **Inside cells** they are found in specific organelles such as *Golgi* complexes, *secretory* granules, and *lysosomes*.

- The oligosaccharide portions of glycoproteins are less **monotonous** than the glycosaminoglycan chains of proteoglycans; they are **rich in information**, forming highly specific sites for recognition and high-affinity binding by other proteins.

■ Oligosaccharide linkages in glycoproteins.

- ***O*-linked glycosylation:**

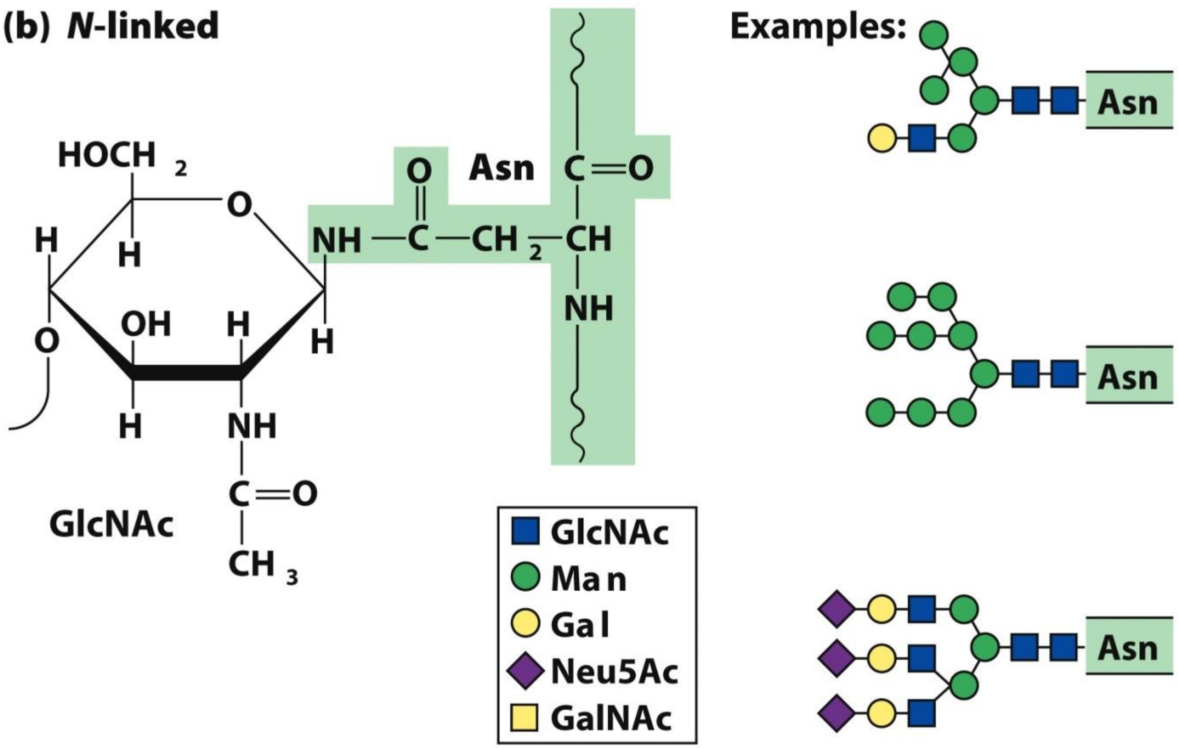
  Attached to the hydroxy oxygen of **serine** or **threonine** of proteins



There appears to be no specific consensus sequence for *O*-linked oligosaccharides, although regions bearing *O*-linked chains tend to be rich in Gly, Val, and Pro residues.

- ***N*-linked glycosylation:**

  Attached to the hydroxy oxygen of **asparagine** of proteins



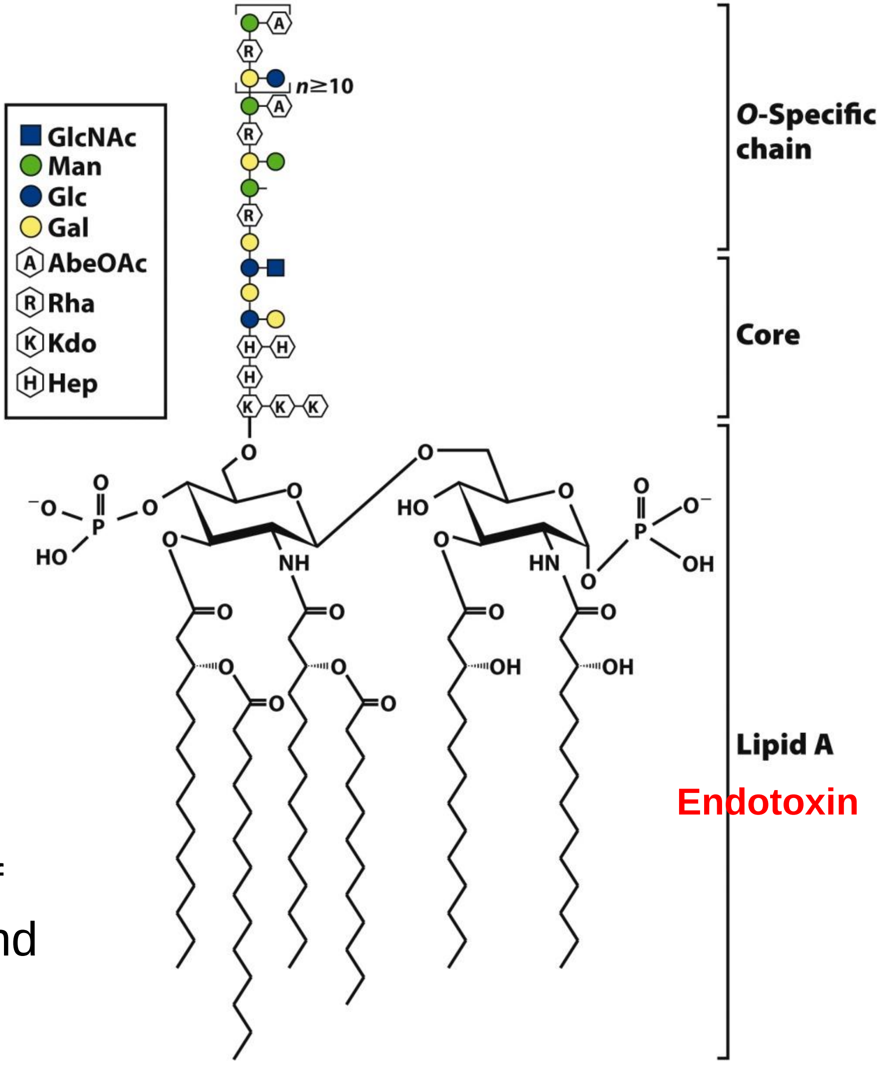
The attachment of a sugar molecule to a nitrogen atom in an **asparagine** (Asn, N) residue occurring in the tripeptide sequence **Asn-X-Se**r or **Asn-X-Thr** where X could be any amino acid except Pro, in a protein.

➢ Glycolipids（糖脂）

Glycolipids are membrane lipids in which the hydrophilic head groups are oligosaccharides, which, as in glycoproteins, act as specific sites for recognition by carbohydrate-binding proteins.

Glycolipids and Lipopolysaccharides are membrane components.

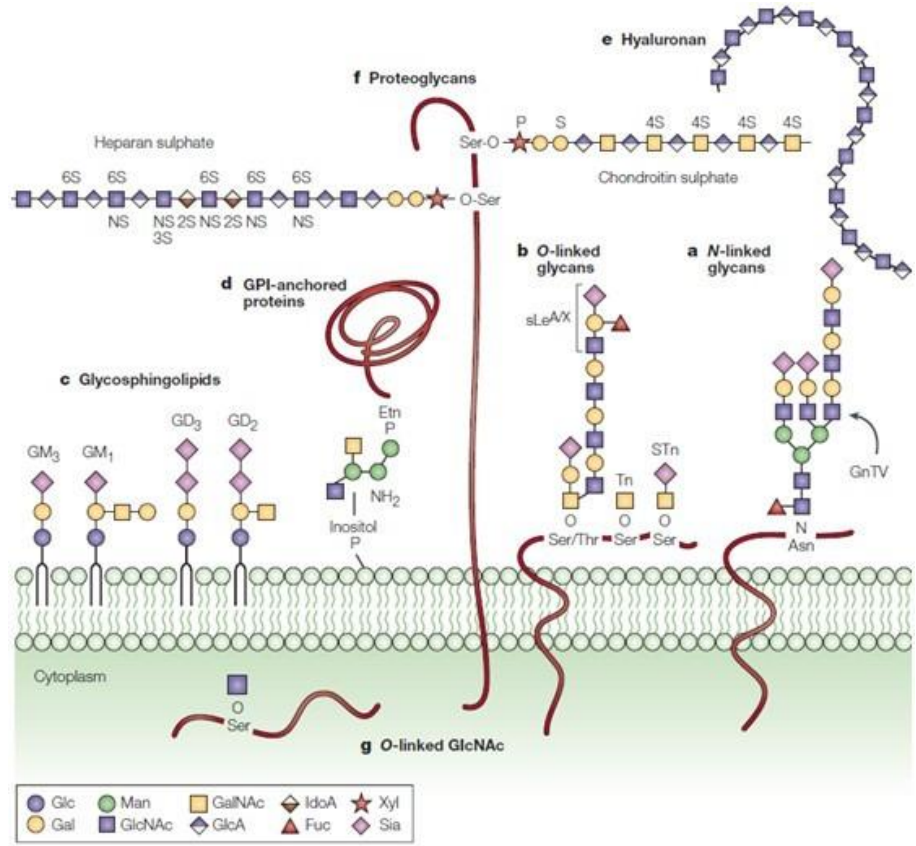
Lipopolysaccharides are the dominant surface feature of the outer membrane of gram-negative bacteria such as *E. coli* and *S. typhimurium*.

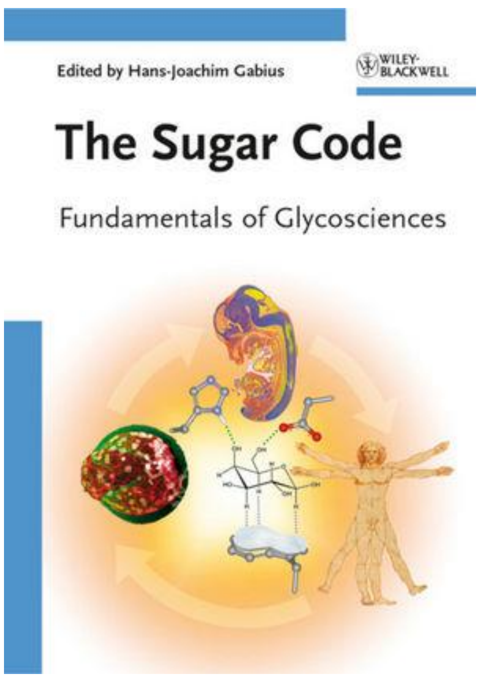


Bacterial lipopolysaccharides

7.4 Carbohydrates as Informational Molecules: The Sugar Code

- Cells use specific oligosaccharides to encode important information about intracellular targeting of proteins, cell-cell interactions, cell differentiation and tissue development, and extracellular signals.



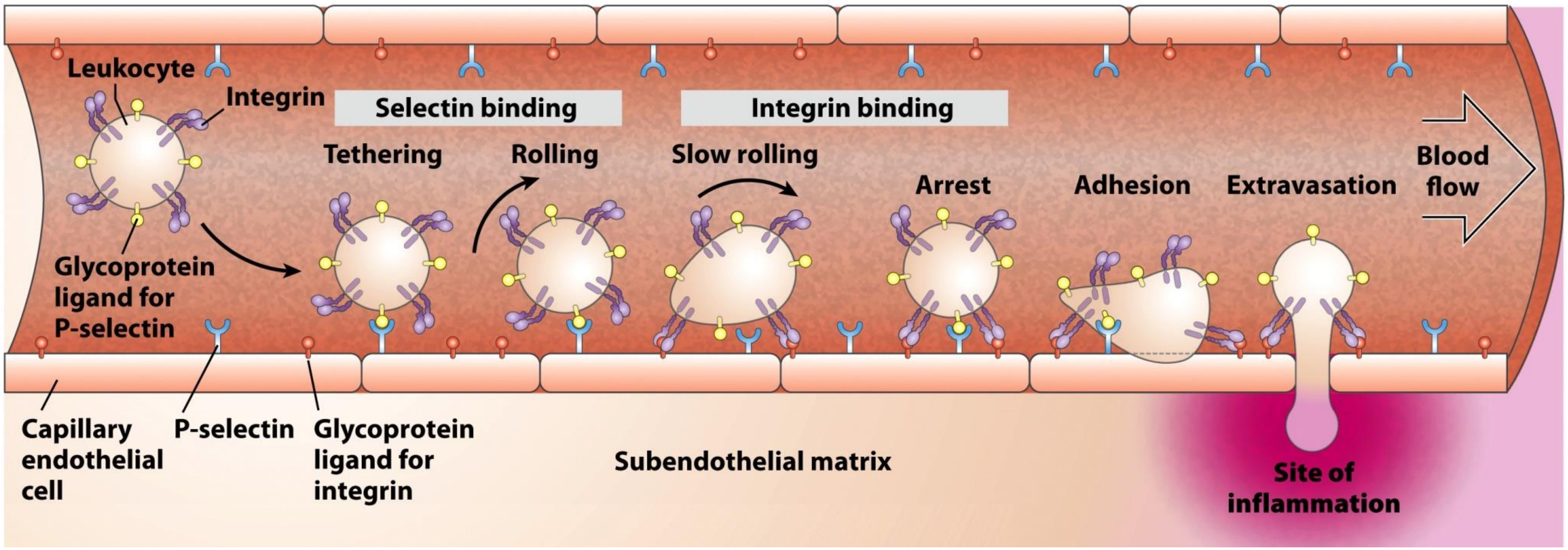


Glycobiology

The study of the structure and function of glycoconjugates, is one of the most active and exciting areas of biochemistry and cell biology.

➢ Lectins (凝血素): the sugar code readers

Lectins are a superfamily of proteins that bind carbohydrates with high specificity and with moderate to high affinity. Lectins are found in all organisms, serve in a wide variety of cell-cell recognition, signaling, and adhesion processes and in intracellular targeting of newly synthesized proteins.



Selectins: a subfamily of lectins

Integrin: a receptor for glycoproteins and are extremely important for cell adhesion

➢ Roles of oligosaccharides in recognition and adhesion

(a) Oligosaccharides are components of glycoproteins or glycolipids on plasma membranes, which can be bound by extracellular lectins.

(b) Viruses bind to surface glycoproteins as the first step in infection.

(c) Bacterial toxins bind to a surface glycolipid before entering a cell.

(d) Some bacteria adhere to and then colonize or infect animal cells.

(e) Selectins (lectins) in the plasma membrane of certain cells mediate cell-cell interactions.

(f) The mannose 6-phosphate receptor/ lectin of trans Golgi complex binds to the oligosaccharide of lysosomal enzymes, targeting them to the lysosome.

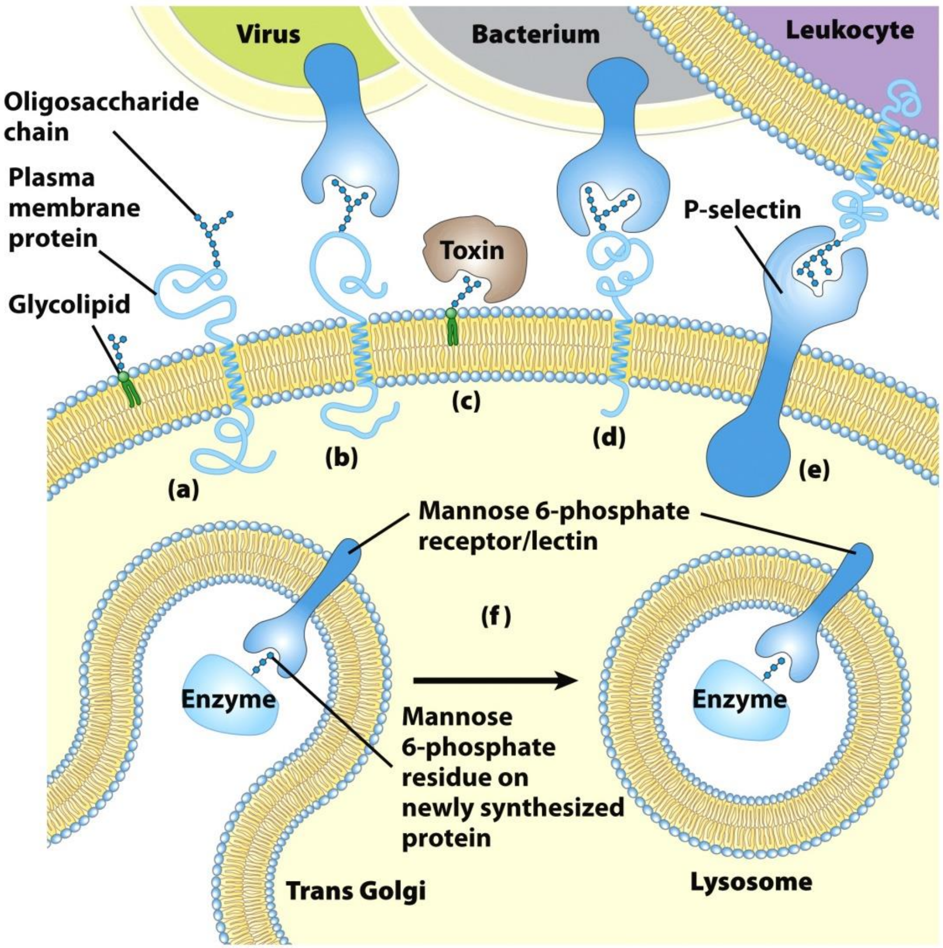


Figure 7-37
Lehninger Principles of Biochemistry, Sixth Edition

7.5 Working with Carbohydrates

➢ Methods of carbohydrate analysis

Mass spectrometry and NMR spectroscopy are powerful tool for oligosaccharide.

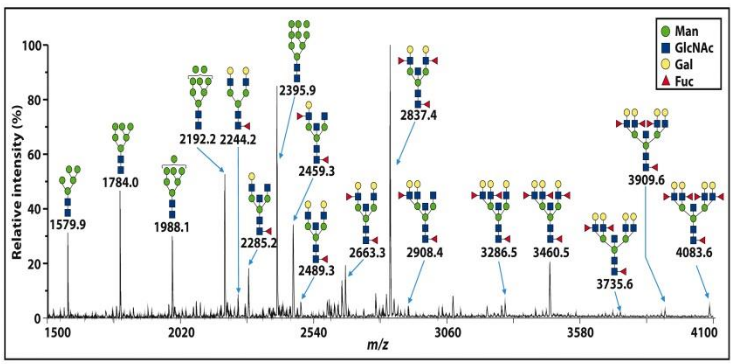
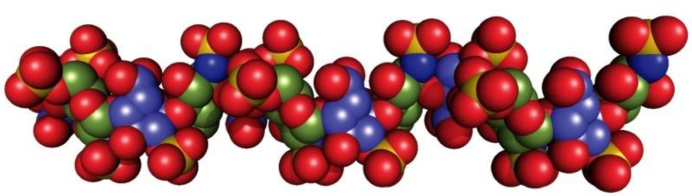


Figure 7-39
Lehninger Principles of Biochemistry, Sixth Edition



Heparin segment

Figure 7-22 part 2
Lehninger Principles of Biochemistry, Sixth Edition

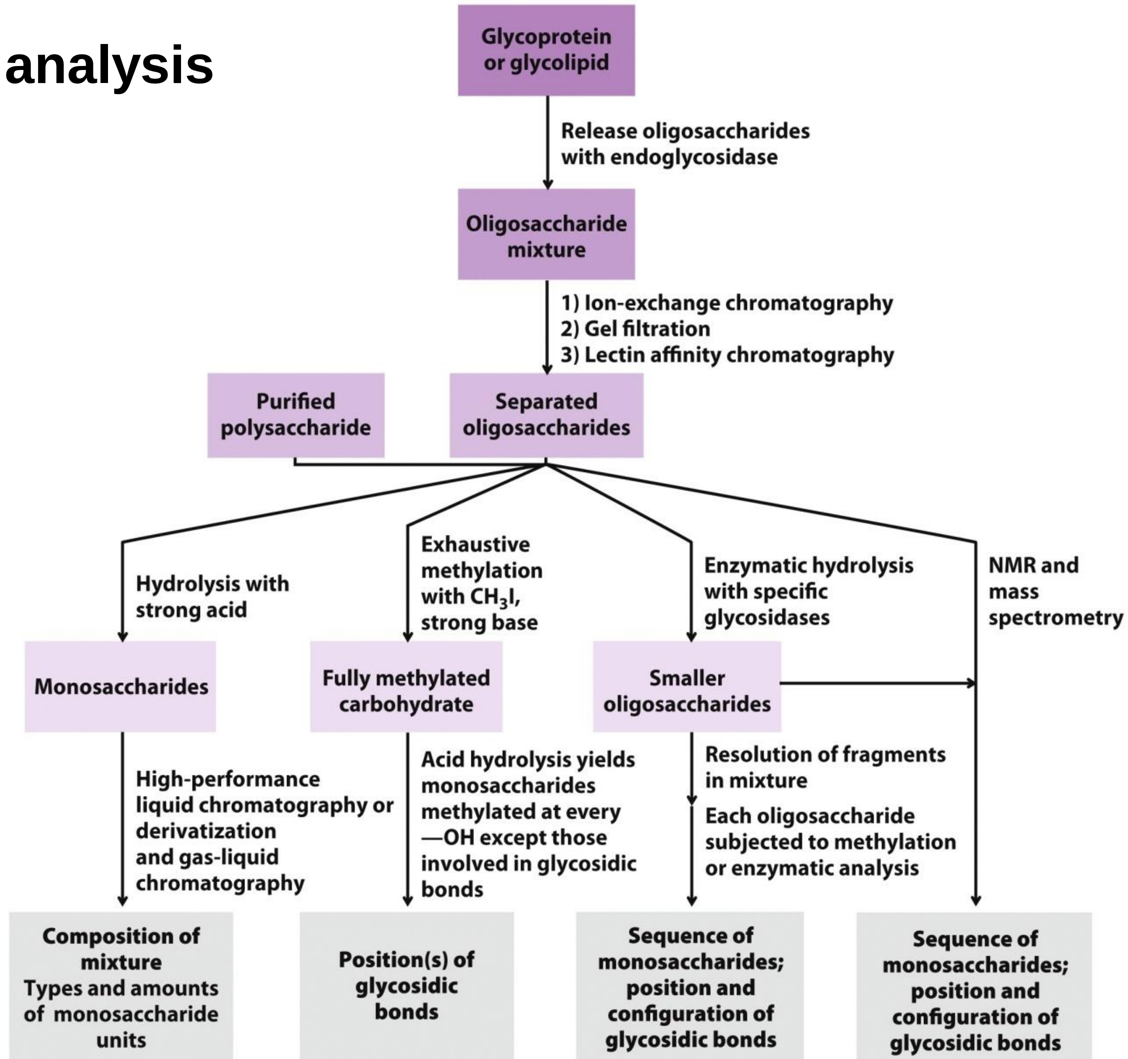
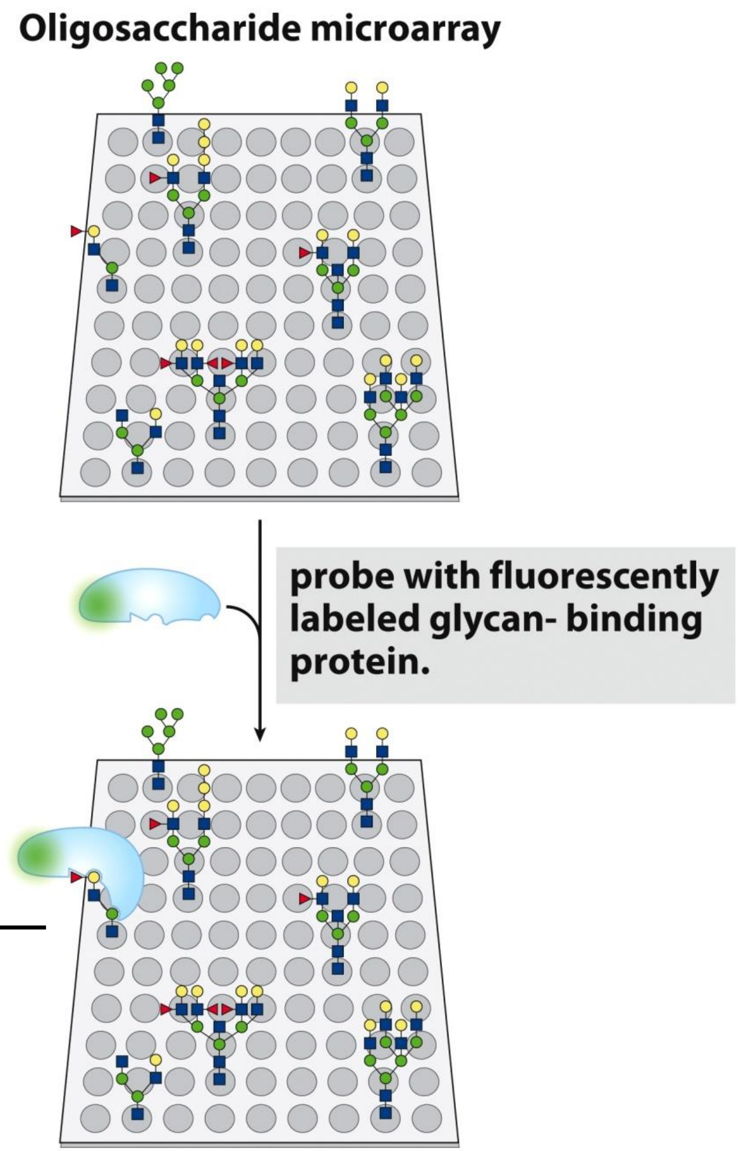
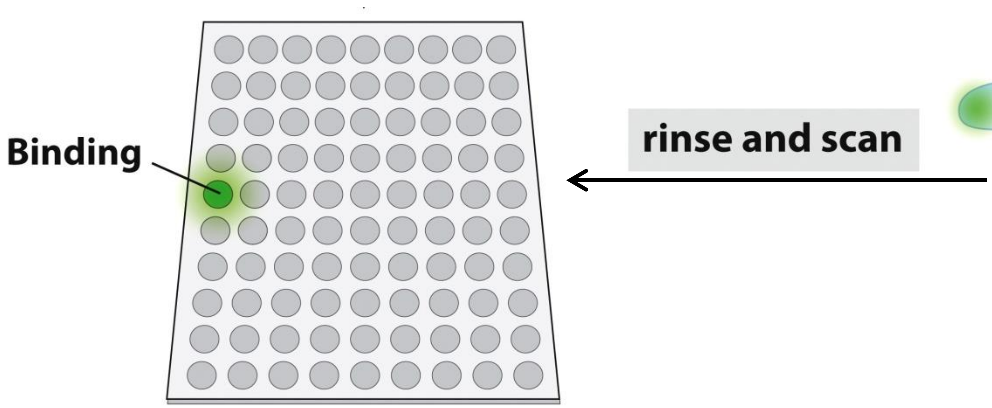


Figure 7-38
Lehninger Principles of Biochemistry, Sixth Edition

- **Oligosaccharide microarrays identify proteins with specific affinity for particular oligosaccharides**

Microarrays of pure oligosaccharides are useful in determining the specificity and affinity of lectin binding to specific oligosaccharides.





Take home messages …

- ✓ **Polysaccharides**
  Starch, glycogen, cellulose, chitin

- ✓ **Glycoconjugates:**
  Proteoglycans, Glycoproteins, Glycolipids

- ✓ **Carbohydrates as Informational Molecules: The Sugar Code**
  Roles of oligosaccharides