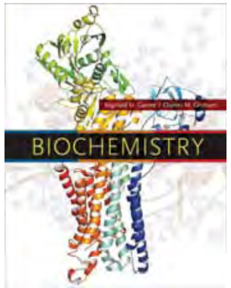
Chapter 7



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Carbohydrates and the Glycoconjugates of Cell Surfaces

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Before the class

- Ask your self…
  - Do you know what is stereochemistry?
  - What is R, S isomer? What is D, L isomer?
  - How do you define a sugar?
  - How sugars are linked into polymer?
  - What are functions of sugars?

Outline

- Part 1 Basic properties of carbohydrates
  - How are carbohydrates named?
  - What is the structure and chemistry of monosaccharides?
- Part 2 Oligo- and poly-saccharides
- Part 3 Glycoproteins and proteoglycans

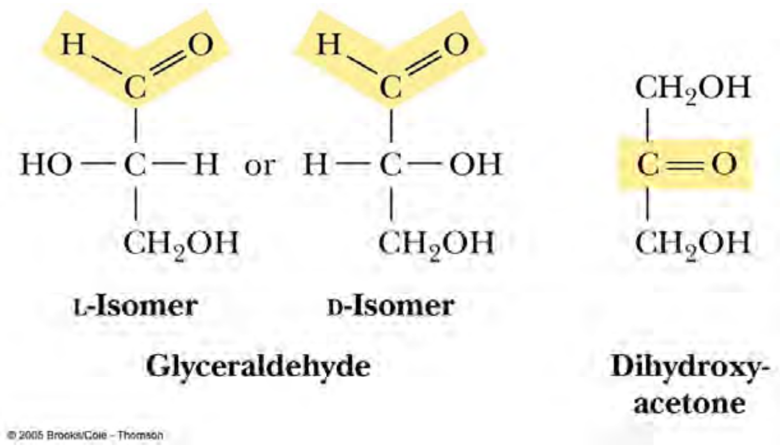
Classification of carbohydrates

- *Carbohydrates are hydrates of carbon*
- __醣：Monosaccharides (simple sugars) cannot be broken down into simpler sugars under mild conditions
- __醣：Oligo = "a few" - usually 2 to 10
- __醣：Polysaccharides are polymers of the simple sugars

Classification by organic chemistry point of view

- Carbohydrate: at least $C_3(H_2O)_3$
  - Each carbon has a functional group of –OH, except one.
- Aldoses and ketoses
  - contain aldehyde and ketone functions, respectively
- Triose, tetrose, etc.
  - denotes number of carbons

Triose, the simplest carbohydrate



有掌型對稱中心 **(chiral center)**

要 **tetrose** 才有掌型對稱

Stereochemistry Review

- Aldoses with 3C or more and ketoses with 4C or more are chiral
- Review Fischer projections and D,L system
  - D,L designation refers to the configuration of the highest-numbered asymmetric center
  - D,L only refers the stereocenter of interest
- D,L do not specify the sign of rotation of plane-polarized light (*levorotatory/dextrorotatory or -/+*), neither R/S isomer!
- D-form of monosaccharides predominate in nature
  - How about amino acids?

More Stereochemistry

- Enantiomers
  - Stereoisomers that are mirror images of each other
- Diastereomers
  - Pairs of isomers that have opposite configurations at one or more chiral centers but are NOT mirror images are
- Epimers
  - Two sugars that differ in configuration at only one chiral center are
  - Is epimer = enantiomer?

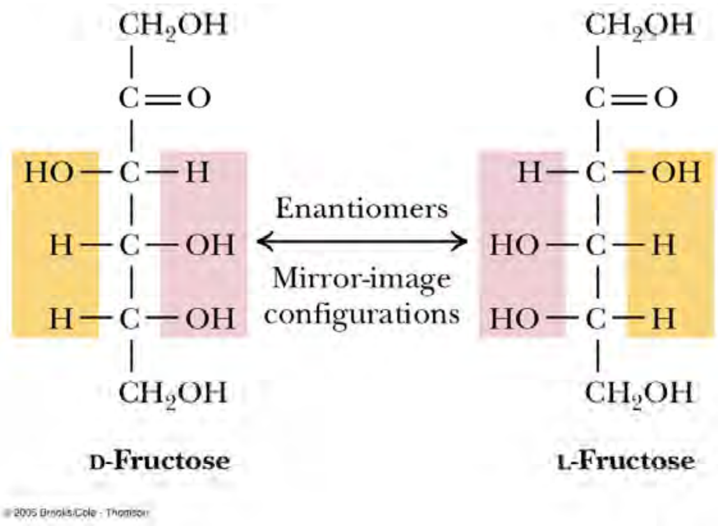


Figure 7.4 D-Fructose and L-fructose, an enantiomeric pair. Note that changing the configuration only at C5 would change D-fructose to L-sorbose.

D-aldoses

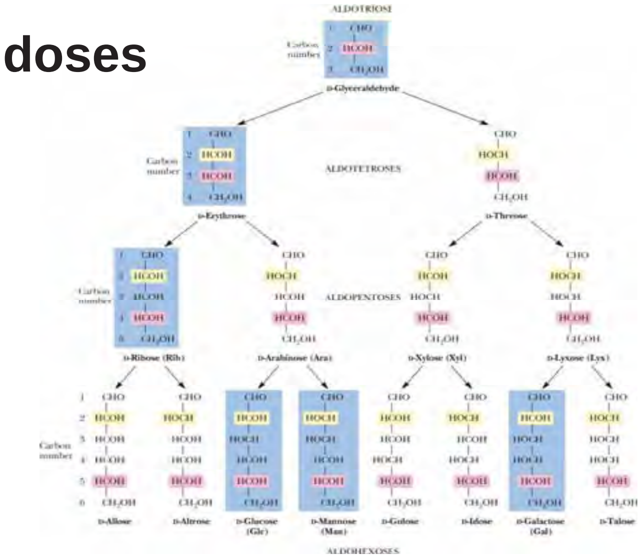


Figure 7.2 The structure and stereochemical relationships of D-aldoses having three to six carbons. The configuration in each case is determined by the highest numbered asymmetric carbon (shown in pink). In each row, the "new" asymmetric carbon is shown in yellow.

D-ketoses

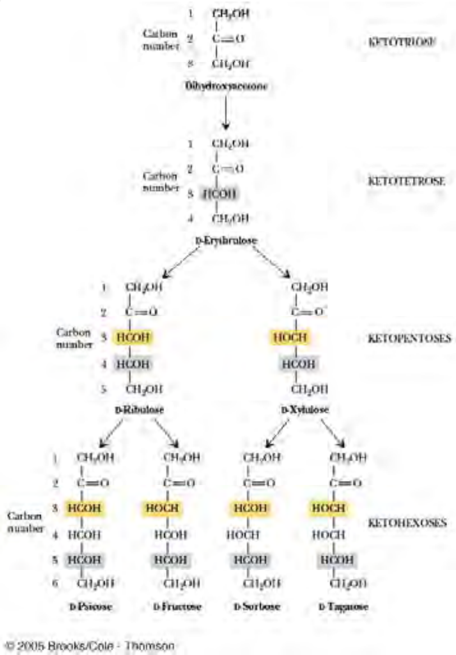
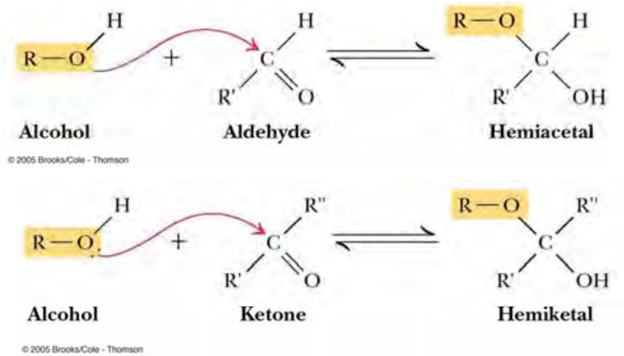


Figure 7.3 The structure and stereochemical relationships of D-ketoses having three to six carbons. The configuration in each case is determined by the highest numbered asymmetric carbon (shown in gray). In each row, the "new" asymmetric carbon is shown in yellow.

Cyclic monsaccharide structures

- Alcohol is very easy to react an aldehyde or ketone

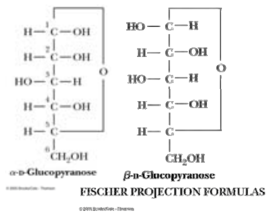


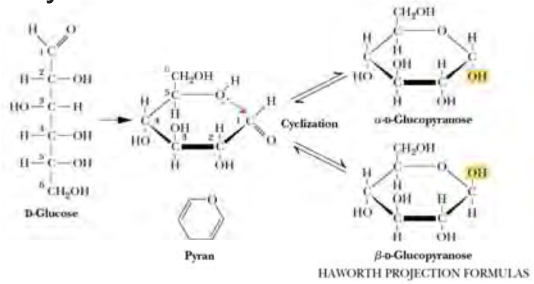
Properties of cyclic monosaccharide

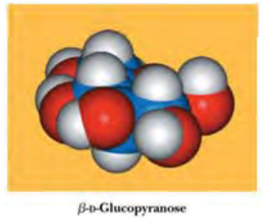
- Glucose (an aldose) can cyclize to form a cyclic hemiacetal (半縮醛)
- Fructose (a ketose) can cyclize to form a cyclic hemiketal (半縮酮)
- Cyclic forms possess anomeric carbons
  - For D-sugars, alpha has OH down, beta up
  - For L-sugars, the reverse is true
- Linear form sugar < 1% in solution!
- Reversible between linear and cyclic
  - Mutarotation

Cyclic hexoaldose

- Fisher projection vs. Haworth projection
- Note which –OH attacks C1
- New chiral center!
- Pyranose

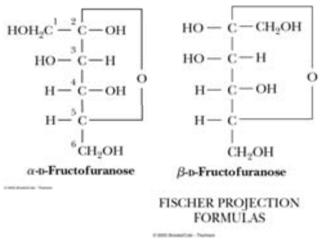


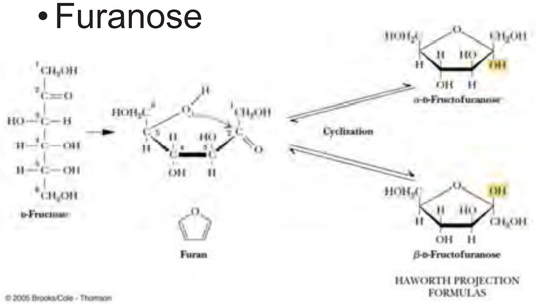


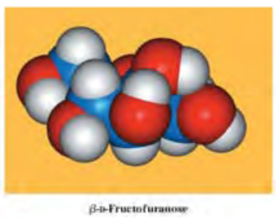


Cyclic hexoketose

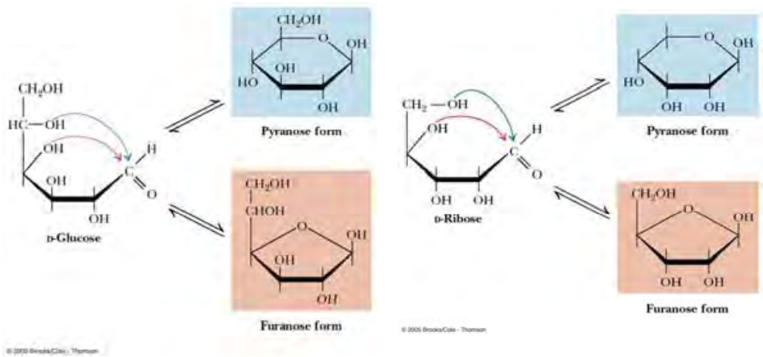
- Fisher projection vs. Haworth projection
- Note which –OH attacks C2
- New chiral center!
- Furanose





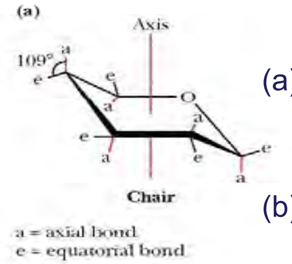


Furanose and Pyranose



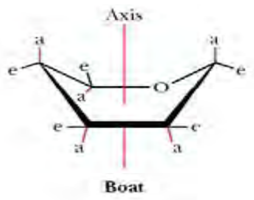
- D-Glucose can cyclize in two ways, forming either furanose or pyranose structures.
- D-Ribose and other five-carbon saccharides can form either furanose or pyranose structures.

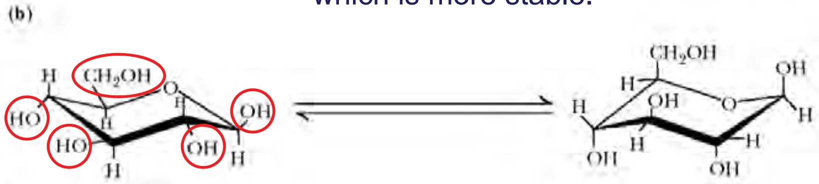
Cyclic monosaccharide ring is not in a plain



(a) A pyranose sugar has 2 conformations of chair and boat .

(b) β-D-glucose has 2 chair conformations, which is more stable.





All bulky groups are at equatorial bonds

Monosaccharide Derivatives

- Sugar acids
- Sugar alcohols: mild reduction of sugars
- Deoxy sugars: constituents of DNA, etc.
- Sugar esters: phosphate esters like ATP are important
- Amino sugars contain an amino group in place of a hydroxyl group
- Acetals, ketals and glycosides: basis for oligo- and poly-saccharides

Sugar acids

- Reducing power of sugars:
  - sugars with free anomeric carbons
  - will reduce oxidizing agents, such as peroxide, ferricyanide and some metals (Cu and Ag)
- These redox reactions convert the sugar to a sugar acid
- Glucose is a reducing sugar - so these reactions are the basis for diagnostic tests for blood sugar (Fehling test and Silver mirror test)

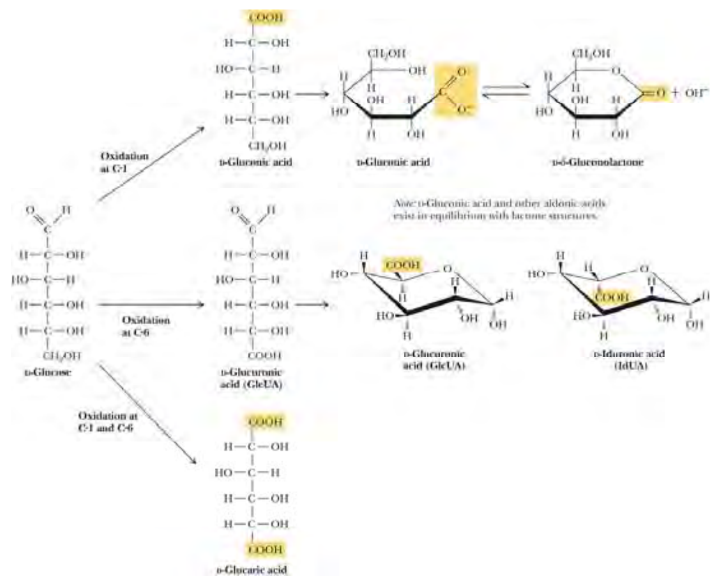
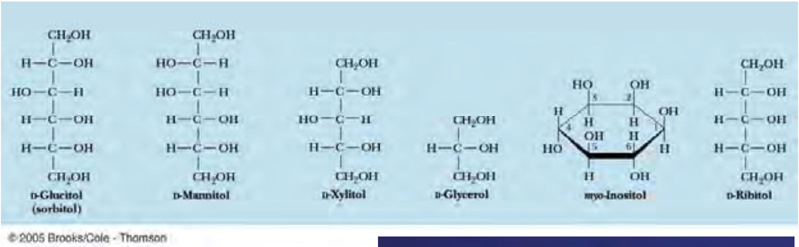


Figure 7.9

Sugar alcohol



Deoxy sugars

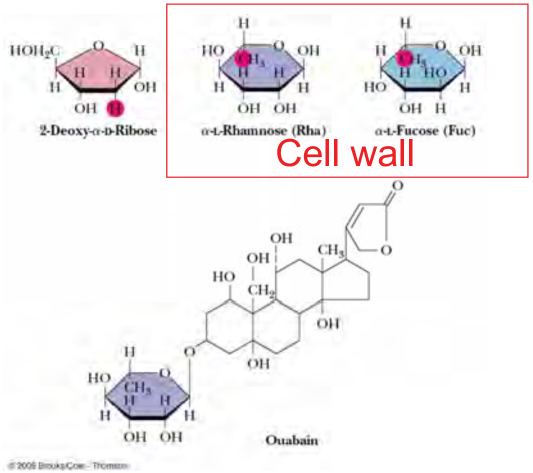


Figure 7.12 Several deoxy sugars and ouabain, which contains α-L-rhamnose (Rha). Hydrogen atoms highlighted in red are "deoxy" positions.

Sugar esters

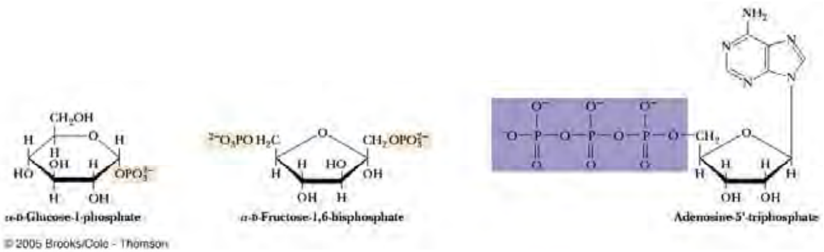
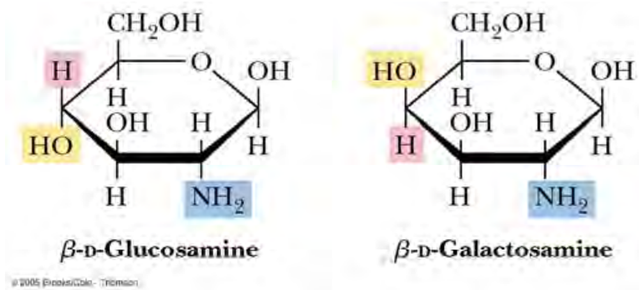


Figure 7.13 Several sugar esters important in metabolism.

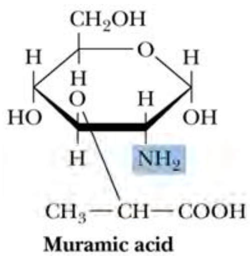
Amino sugars



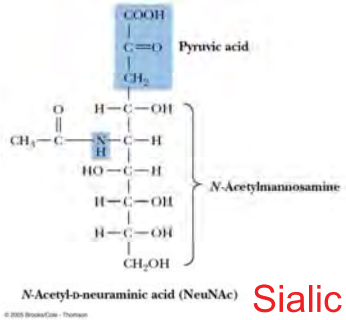
Chitin 的組成成份

Figure 7.14 Structures of D-glucosamine and D-galactosamine.

Amino sugars

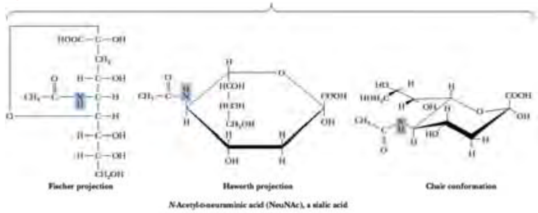


Cell wall

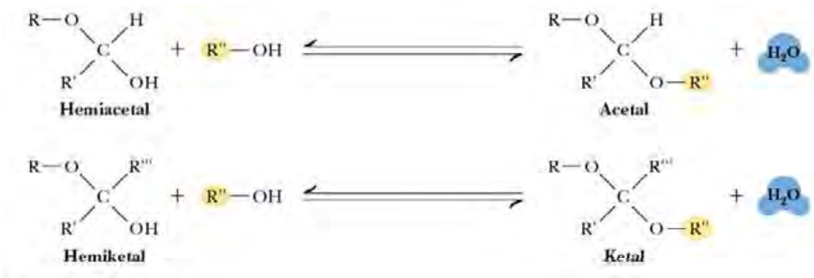


Sialic acid

gure 7.15 Structures
muramic acid and
euraminic acid and
everal depictions of
alic acid.



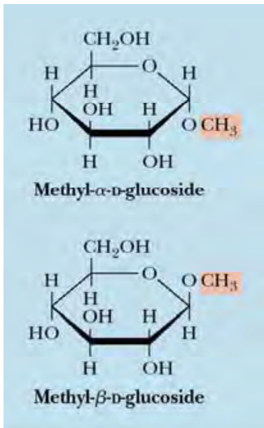
Formation of Glycosides



- Acetals and ketals can be formed from hemiacetals and hemiketals, respectively.

Glycosidic Bond

- The anomeric carbon of pyranose and furanose retention of the α- or β-configuration at the C-1 carbon.
- The new compound is called **glycoside.**
  - Ex: methyl-D-glucoside
- The new bond formed is called a **glycosidic bond**.



End of Part I

- Ask yourself……
  - How to classify monosaccharides?
  - Can you tell the differences of D-, L- form in stereochemistry?
  - Can you draw a glucose in Fischer projection and Haworth projection?
  - What is anomeric carbon?
  - What is glycoside?

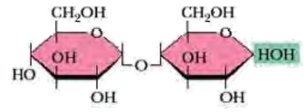
7.3 – What is the Structure and Chemistry of Oligosaccharides?

It's not important to memorize structures, but you should know the important features

- Be able to identify anomeric carbons and reducing and nonreducing ends
- Sucrose is NOT a reducing sugar
- Browse the structures in Figure 7.18 and Figure 7.19
- Note carefully the nomenclature of links! Be able to recognize alpha(1,4), beta(1,4), etc

Oligosaccharides

- A few monosaccharides are linked through glycosidic bonds
- Note how to name oligosaccharides
- Reducing or non-reducing oligosaccharides
  - Ex:



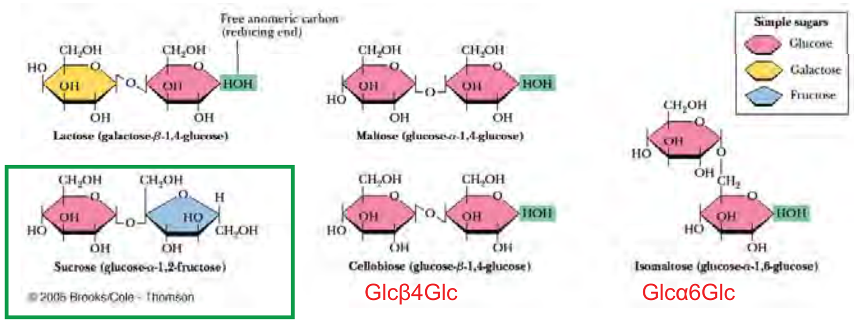
Maltose (glucose-α-1,4-glucose)

Maltose (reducing)

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

or Glcα4Glc

Disaccharides are the simplest oligosaccharides



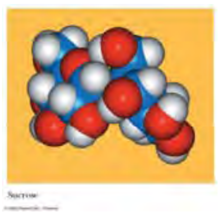
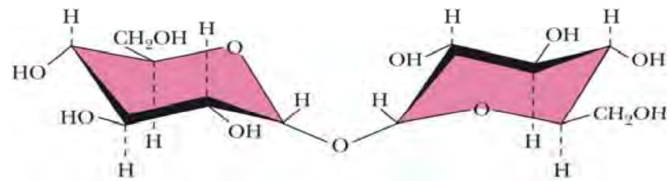


Figure 7.18 The structures of several important disaccharides. Note that the notation -HOH means that the configuration can be either α or β. If the -OH group is above the ring, the configuration is termed b. The configuration is α if the -OH group is below the ring as shown. Also note that sucrose has no free anomeric carbon atoms.

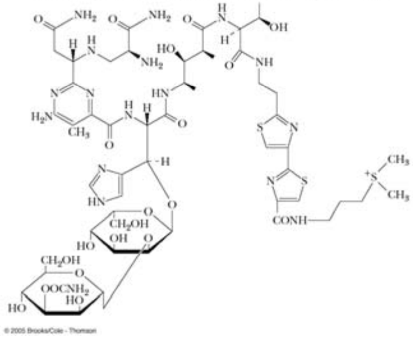
Trehalose

- In "hemolymph" (insect blood).
- The "blood sugar" is **trehalose**
  - nonreducing disaccharide.
- Trehalose may act as a natural cryoprotectant, protecting the insect from damage due to freezing temperatures.
- How to write the structure of trehalose?

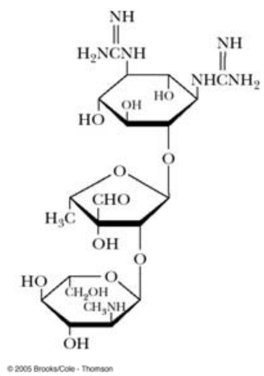


Some antibiotics are oligosaccharides or contain oligosaccharide groups.

Bleomycin A_2 (an antitumor agent used clinically against specific tumors)



Streptomycin (a broad-spectrum antibiotic)



Polysaccharides

- Nomenclature:
  - homopolysaccharide vs. heteropolysaccharide
- Functions of oligosaccharides
  - Storage molecules: Starch and glycogen
  - Structural molecules: Chitin and cellulose
  - Recognition molecules: Cell surface polysaccharides

Starch

A plant storage polysaccharide

- Two forms: amylose and amylopectin
- Most starch is 10-30% amylose and 70-90% amylopectin
- Branches in amylopectin every 12-30 residues
- Amylose has alpha(1,4) links, one reducing end

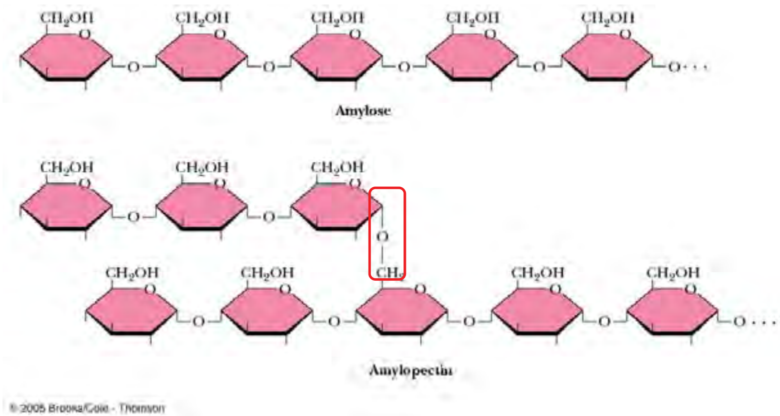
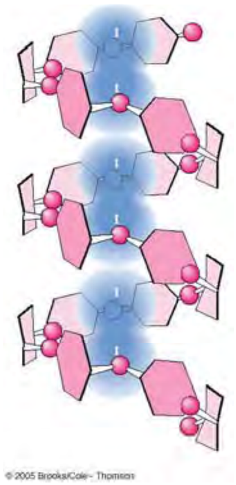


Figure 7.20 Amylose and amylopectin are the two forms of starch. Note that the linear linkages are $\alpha(1 \rightarrow 4)$, but the branches in amylopectin are $\alpha(1 \rightarrow 6)$. Branches in polysaccharides can involve any of the hydroxyl groups on the monosaccharide components. Amylopectin is a highly branched structure, with branches occurring every 12 to 30 residues.

Amylose

- Amylose is poorly soluble in water, but forms micellar suspensions
- In these suspensions, amylose is helical
  - iodine fits into the helices to produce a blue color



Hanjia's Biochemistry Lecture

Break down of starch

- Enzymes
  - α-amylase (endoamylase)
    - Salivary gland secretion, pancreatic juice
    - Low efficient with raw starch
  - β-amylase
    - Plant and microorganism
  - α(1-6)glucosidase
    - Limit dextrin
    - De-branch
  - Starch phosphorylase
    - Metabolism usage in plant

Hanjia's Biochemistry Lecture

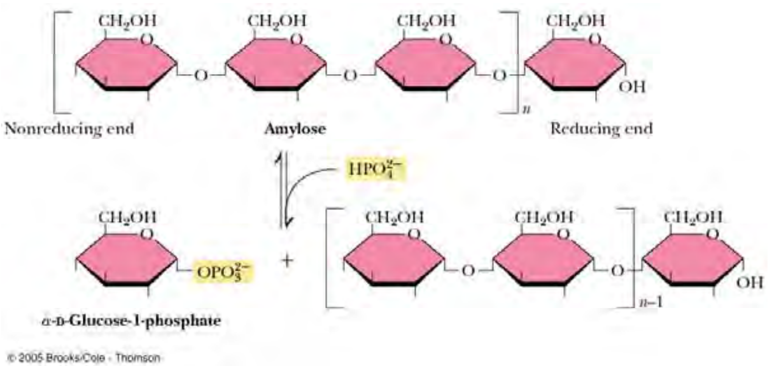


Figure 7.22 The starch phosphorylase reaction cleaves glucose residues from amylose, producing a-D-glucose-L-phosphate.

Hanjia's Biochemistry Lecture

Why Branching in Starch?

Consider the phosphorylase reaction...

- Phosphorylase releases glucose-1-P products from the amylose or amylopectin chains
- The more branches, the more sites for phosphorylase attack
- Branches provide a mechanism for quickly releasing (or storing) glucose units for (or from) metabolism

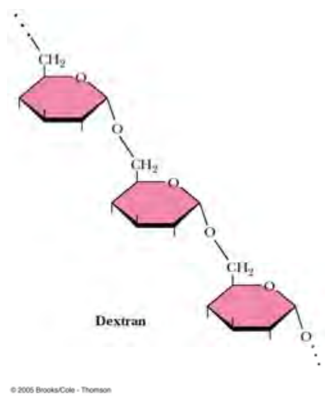
Glycogen

The glucose storage device in animals

- Glycogen constitutes up to 10% of liver mass and 1-2% of muscle mass
- Glycogen is stored energy for the organism
- Only difference from starch: number of branches
- Alpha(1,6) branches every 8-12 residues
- Like amylopectin, glycogen gives a red-violet color with iodine

Dextrans

- A small but significant difference from starch and glycogen
  - Alpha(1,6) linkage
  - Branches can be (1,2), (1,3), or (1,4)
  - Dental plaque
  - Isomaltose
- Application
  - Cross-linked dextrans are used as "Sephadex" gels in column chromatography
  - These gels are up to 98% water!



Structural Polysaccharides

- Composition similar to storage polysaccharides, but small structural differences greatly influence properties
- Beta(1,4) linkages make all the difference!
- Strands of cellulose form extended ribbons

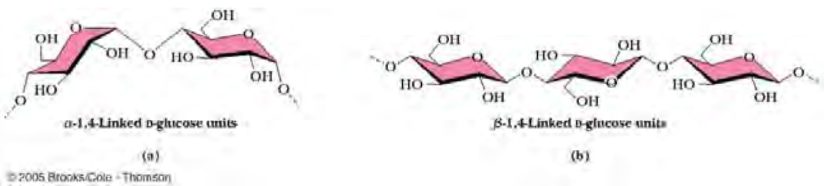


Figure 7.23 (a) Amylose, composed exclusively of the relatively bent α(1→4) linkages, prefers to adopt a helical conformation, whereas (b) cellulose, with β(1→4)-glycosidic linkages, can adopt a fully extended conformation with alternating 180° flips of the glucose units. The hydrogen bonding inherent in such extended structures is responsible for the great strength of tree trunks and other cellulose-based materials.

Cellulose

- Cellulose is the most abundant natural polymer on earth
- Cellulose is the principal strength and support of trees and plants
- Cellulose can also be soft and fuzzy - in cotton

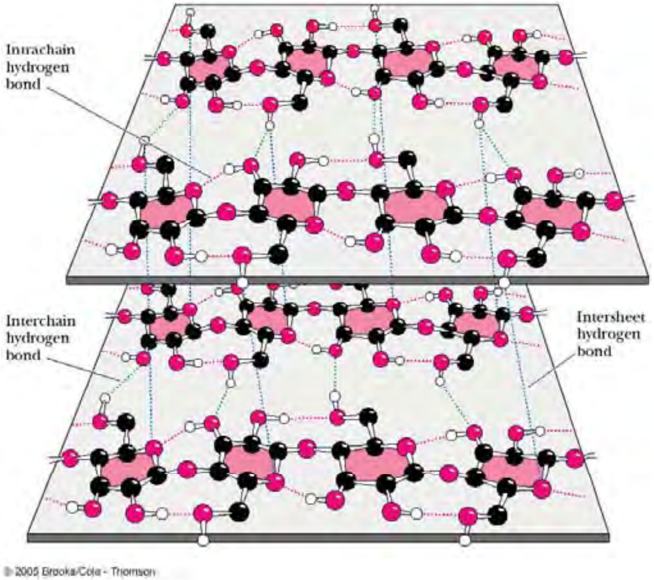
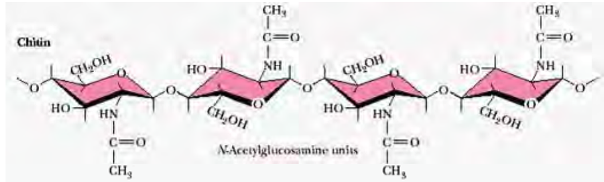


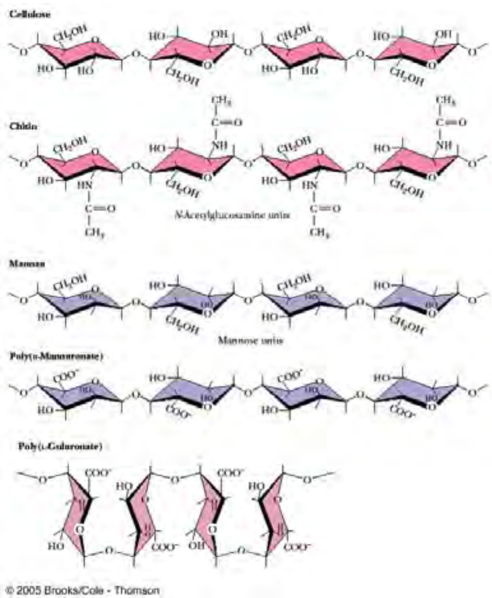
Figure 7.24
The structure of cellulose, showing the hydrogen bonds (blue) between the sheets, which strengthen the structure. Intrachain hydrogen bonds are in red and interchain hydrogen bonds are in green.

Chitin

- Exoskeletons of crustaceans, insects and spiders, and cell walls of fungi
  - similar to cellulose, but C-2s are N-acetyl
  - cellulose strands are parallel, chitins can be parallel or antiparallel

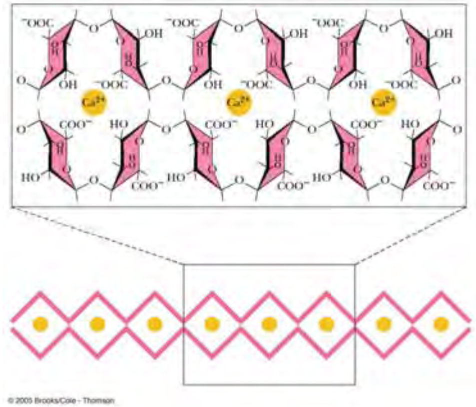


Like cellulose, chitin, mannan, and poly (D-mannuronate) form extended ribbons and pack together efficiently, taking advantage of multiple hydrogen bonds.



Alginates

- Alginates – Ca^{2+}-binding polymers in algae - Poly(α-L-guluronate)



- Agarose and agaropectin - galactose polymers
- Glycosaminoglycans - repeating disaccharides with amino sugars and negative charges

Agarose and agaropectin

- Galactose polymers
- D-galactose β3,6-anhydro-L-galactose
- The favored conformation of agarose in water is a double helix with a threefold screw axis.
- 高含水量
- 寒天

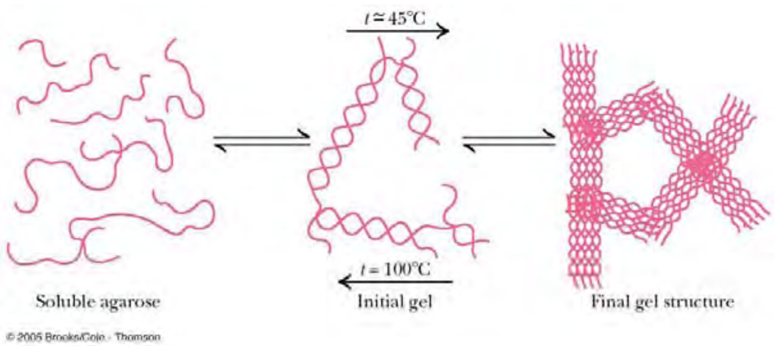


Figure 7.32 The ability of agarose to assemble in complex bundles to form gels in aqueous solution makes it useful in numerous chromatographic procedures, including gel exclusion chromatography and electrophoresis. Cells grown in culture can be embedded in stable agarose gel "threads" so that their metabolic and physiological properties can be studied.

Glycosaminoglycans (GAG)

- Heparin, with a very high negative charge, is a natural anticoagulant.
- Hyaluronates (consisting of up to 25,000 disaccharide units) are components of the vitreous humor of the eye and of synovial fluid, the lubricant fluid of the body's joints
- Chondroitins and keratan sulfate are found in tendons, cartilage, and other connective tissue
- Dermatan sulfate is a component of the extracellular matrix of skin
- Glycosaminoglycans are constituents of proteoglycans (discussed later in this chapter)

Structure of GAG

定義：
有 **amino sugar**且
單醣裡至少一個
酸性官能基

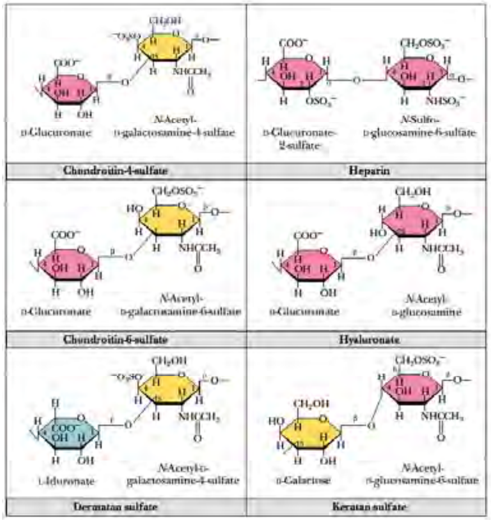


Figure 7.33 Glycosaminoglycans are formed from repeating disaccharide arrays. Glycosaminoglycans are components of the proteoglycans.

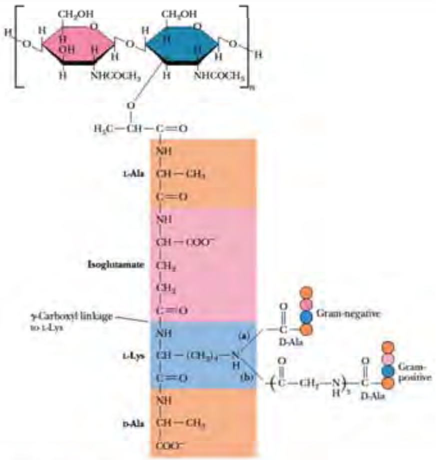
Bacterial Cell Walls

Composed of 1 or 2 bilayers and peptidoglycan shell

- Gram-positive: One bilayer and thick peptidoglycan outer shell
- Gram-negative: Two bilayers with thin peptidoglycan shell in between
- Gram-positive: pentaglycine bridge connects tetrapeptides
- Gram-negative: direct amide bond between tetrapeptides

Structure of peptidoglycan

- The tetrapeptides linking adjacent backbone chains contain an unusual γ-carboxyl linkage.
- There are 4 unusual things in **Tetrapeptide!**

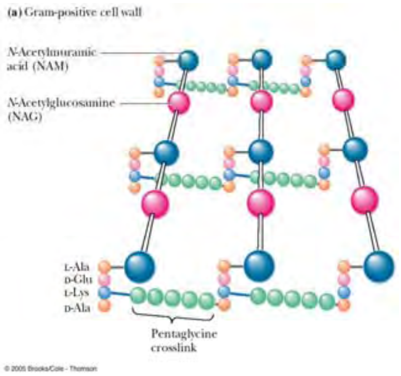


More Notes on Cell Walls

- Note the gamma-carboxy linkage of isoglutamate in the tetrapeptide
- Peptidoglycan is called murein - from Latin "murus", for wall
- Gram-negative cells
  - Are hairy with the lipopolysaccharide "hair"
  - Hydrophobic proteins:
    - C terminal Lys links to peptidoglycan **(10% diaminopimelic acid)**
    - N terminal Ser links to lipid

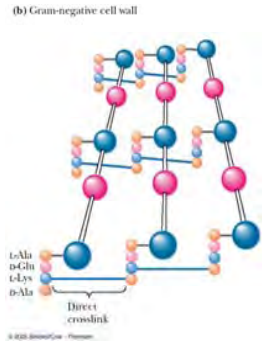
Gram positive bacteria

- The cross-link in Gram-positive cell walls is a pentaglycine bridge



Gram negative bacteria

- The linkage between the tetrapeptides of adjacent carbohydrate chains in peptidoglycan involves a direct amide bond



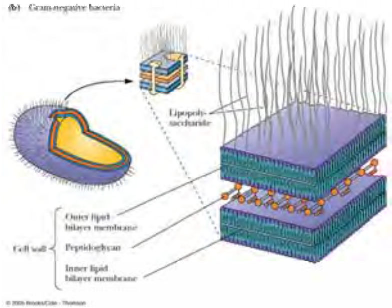
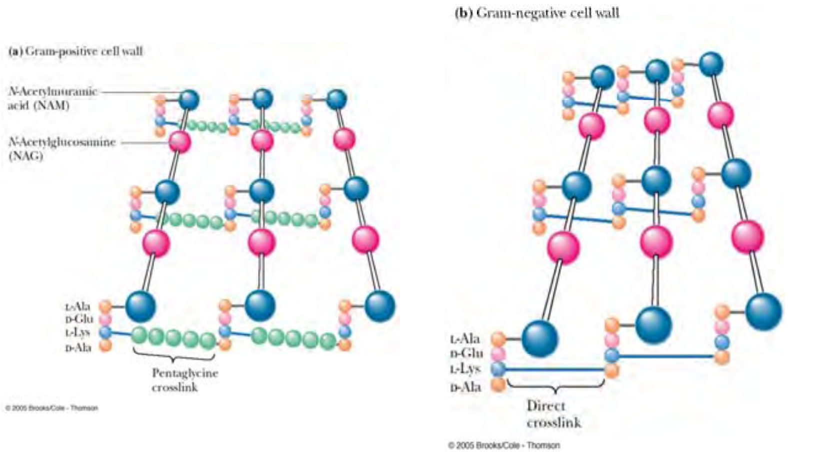
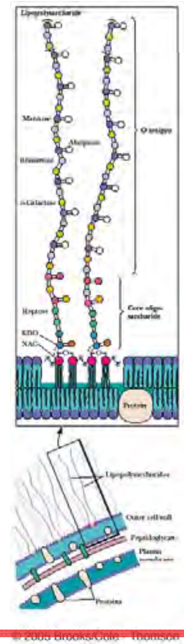


Figure 7.29
(a) The cross-link in Gram-positive cell walls is a pentaglycine bridge. **(b)** In Gram-negative cell walls, the linkage between the tetrapeptides of adjacent carbohydrate chains in peptidoglycan involves a direct amide bond between the lysine side chain of one tetrapeptide and D-alanine of the other.



LPS

- Lipopolysaccharide (LPS) coats the outer membrane of Gram-negative bacteria.
- The lipid portion of the LPS is embedded in the outer membrane and is linked to a complex polysaccharide.



Cell Surface Polysaccharides

- Animal cell surfaces contain an incredible diversity of glycoproteins and proteoglycans
- These polysaccharide structures regulate cell-cell recognition and interaction
  - Heart myocytes: synchrony when they make contact
  - Kidney cells contact with kidney cells but not liver cells
- Extracellular matrix (ECM):
  - The uniqueness of the "information" in these structures is determined by the enzymes that synthesize these polysaccharides

End of Part 2

- Ask yourself…
  - How to distinguish a disaccharide reducing sugar or not?
  - How to describe the linkage of polysaccharide?
  - What are the differences between storage and structural polysaccharides?
  - What is GAG?
  - What is the structure of bacteria cell wall?

Protein Covalently Linked with Oligosaccharide and Polysaccharide

- Structural protein, enzymes, membrane receptors, transport proteins, immunoglobin….

Many structures and functions!

- May be N-linked or O-linked
- N-linked saccharides are attached *via* the amide nitrogens of asparagine residues
- O-linked saccharides are attached to hydroxyl groups of serine, threonine or hydroxylysine

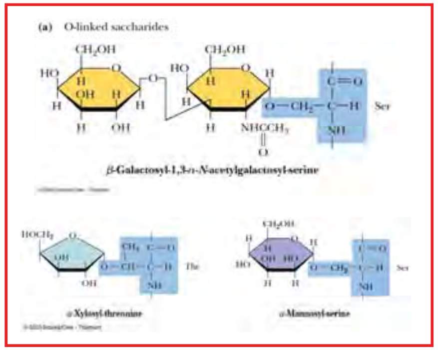
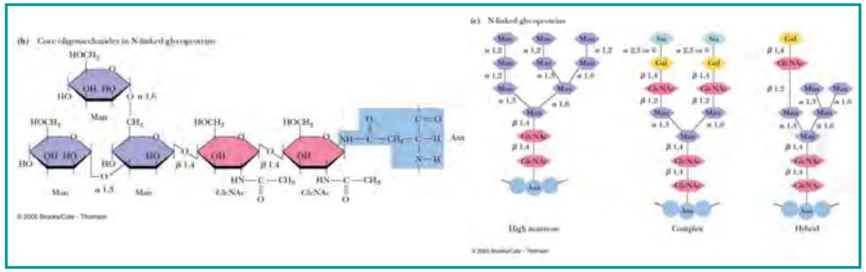


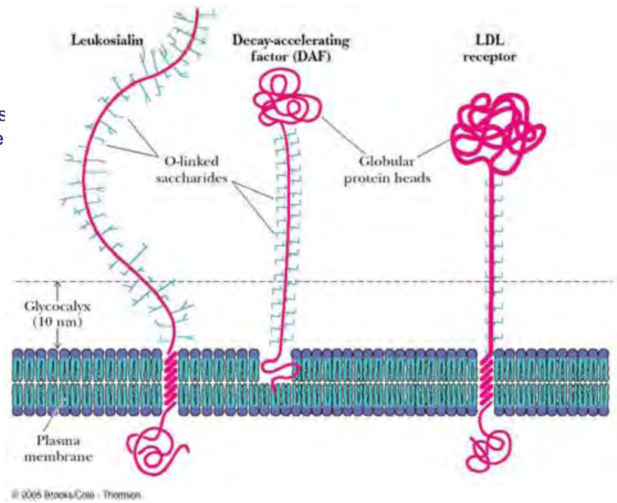
Figure 7.32
The carbohydrate moieties of glycoproteins may be linked to the protein via **(a)** serine or threonine residues (in the **O-linked saccharides**) or **(b)** asparagine residues (in the **N-linked saccharides**). **(c)** N-Linked glycoproteins are of three types: high mannose, complex, and hybrid, the latter of which combines structures found in the high mannose and complex saccharides.



O-linked Saccharides of Glycoproteins

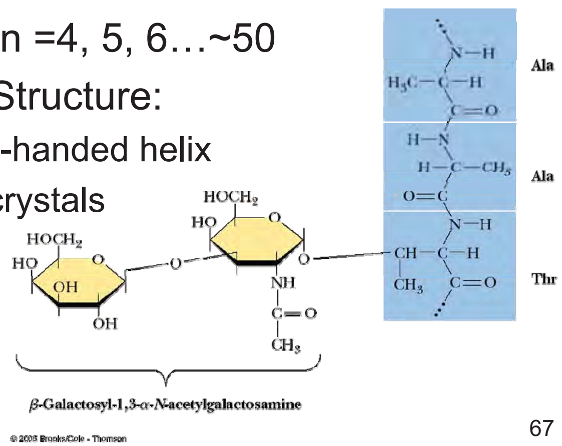
- Usually N-acetylgalactose, but mannose, galactose and xylose are also found.
- In certain viral glycoproteins and cell surface glycoproteins
- Function in many cases is to adopt an extended conformation.Ex. Mucin in mucous membrane
- Bristle brush structure extends functional domains up out of the glycocalyx

Figure 7.33 The O-linked saccharides of glycoproteins appear in many cases to adopt extended conformations that serve to extend the functional domains of these proteins above the membrane surface. *(Adapted from Jentoft, N., 1990,* Trends in Biochemical Sciences ***15:**291–294.)*



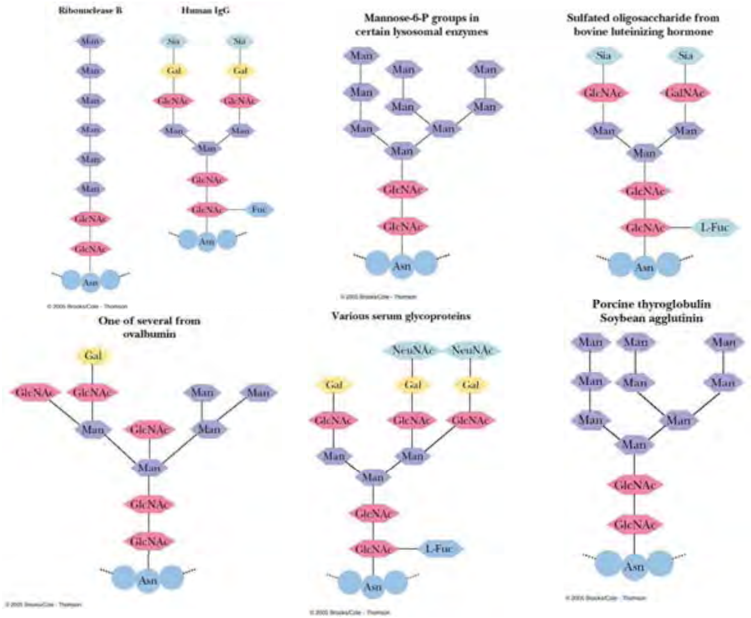
Antifreeze Glycoproteins

- AFGPs: found in the blood of Arctic and Antarctic fish.
- $[A\text{-}A\text{-}T]_n\text{-}A\text{-}A$: n =4, 5, 6…~50
- Flexible Rod Structure:
  - Threefold left-handed helix
  - Binds to ice crystals



N-linked Oligosaccharides

- Core structure: two N-acetylglucosamine and a branched mannose triad
- Found in many different proteins: immunoglobulin, ribonuclease B, ovalbumin, peptide hormones…
- Functions:
  - Alter the chemical and physical properties of proteins (solubility, mass, charge…)
  - Stabilize protein conformations and/or protect against proteolysis
  - Promote folding
  - Cleavage of monosaccharide units from N-linked glycoproteins in blood targets them for degradation in the liver



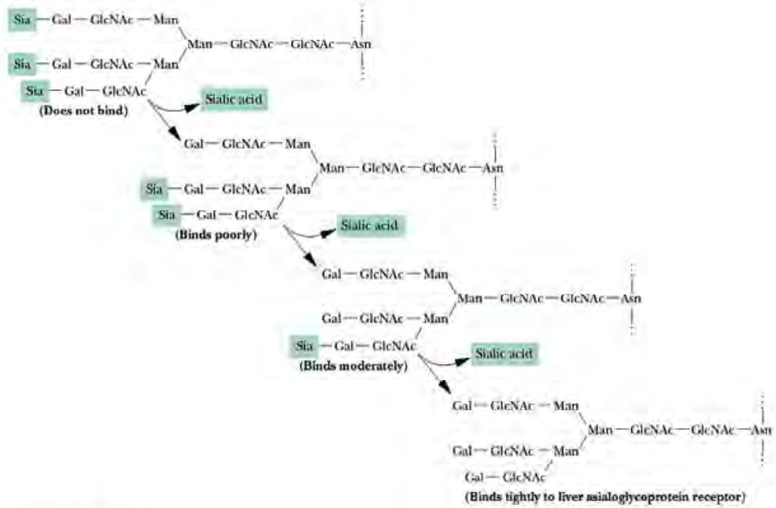


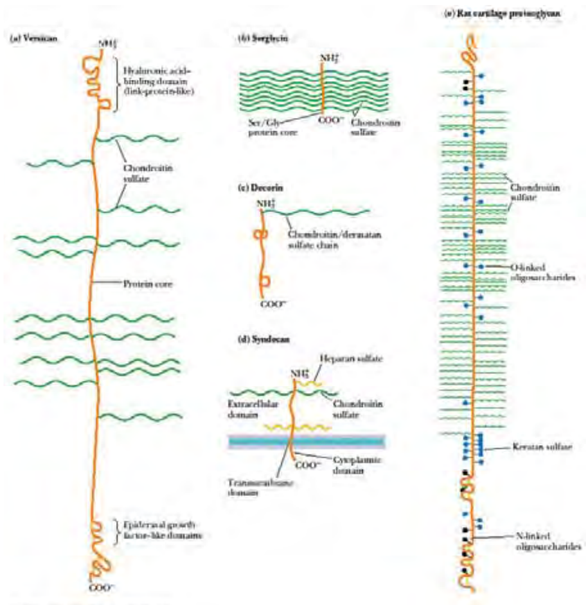
Figure 7.35

Progressive cleavage of sialic acid residues exposes galactose residues. Binding to the **asialoglycoprotein receptor** in the liver becomes progressively more likely as more Gal residues are exposed.

Proteoglycans

Glycoproteins whose carbohydrates are mostly glycosaminoglycans (GAG)

- Components of the cell membrane and glycocalyx
- Consist of proteins with one or two types of glycosaminoglycan
- Glycosamino-glycans O-linked to serine residues.
- Proteoglycans include both soluble proteins and integral transmembrane proteins.



Soluble:
Versican, Serglycin, Secorin, Cartilage

Transmembrane:
Syndecan

Protein Part of Proteoglycan

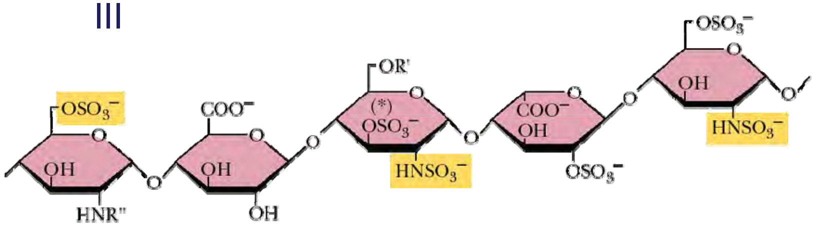
- Diversity
  - Size: 104 a.a. (Serglycin) ~ 2409 a.a. (Versican)
  - GAG linkage:
    - O-linked to Ser-Gly repeat
    - One or Two kinds of GAG
    - Serglycin: 49 a.a. are Ser-Gly repeat
    - Cartilage: 117 Ser-Gly pairs
    - Decorin: secreted by fibroblasts; 3 Ser-Gly pairs and only one of them linked with GAG

Proteoglycan Function 1

- Binding with other proteins
  - Example: syndecan - transmembrane protein - inside domain interacts with cytoskeleton, outside domain interacts with fibronectin
- Glue for ECM
- Mediating the binding of growth factors...
  - GAG binding sites: (BBXB and BBBXXBB)
    - Non-specific binding: through charge
    - Specific binding: Ex. Heparin and antithrombin III

Heparin

- Heparin, a carbohydrate having anticoagulant properties.
  - used by blood banks and also by physicians
  - This sulfated pentasaccharide sequence in heparin binds with high affinity to antithrombin III

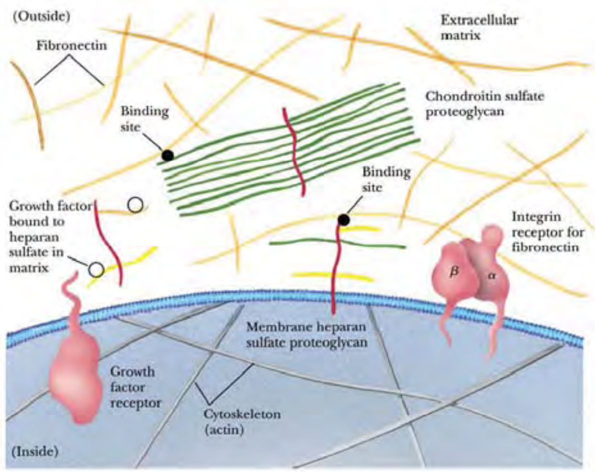


Proteoglycan Function 2

- Modulation of cell growth processes
  - Binding of growth factor proteins by proteoglycans in the glycocalyx provides a reservoir of growth factors at the cell surface
  - Heparin: inhibit cell proliferation (internalization)
  - Fibroblast growth factor: binds to heparin to prevent degradation and enchance activity
  - Transforming growth factor β: sitmulate the synthesis of proteoglycan
  - Growth hormone like? versican

ECM is a reservoir of growth factors

Figure 7.37 Proteoglycans serve a variety of functions on the cytoplasmic and extracellular surfaces of the plasma membrane. Many of these functions appear to involve the binding of specific proteins to the glycosaminoglycan groups.

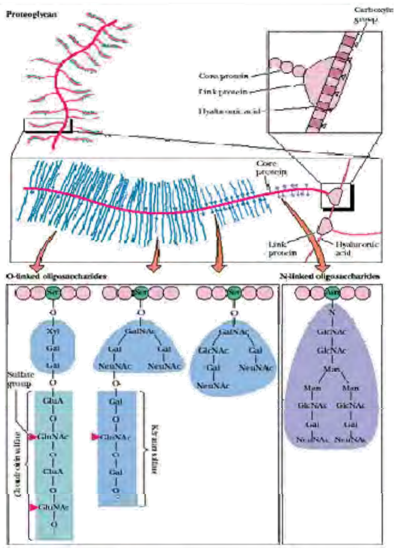


Proteoglycan Function 3

- Cushioning in joints
  - Cartilage matrix proteoglycans absorb large amounts of water. During joint movement, cartilage is compressed, expelling water!
  - In cartilage, these highly hydrated proteoglycan structures are enmeshed in a network of collagen fibers.

Hyaluronate

- Hyaluronate is the backbone of proteoglycan structures, such as in cartilage (MW > 2M).
  - The proteoglycan subunits consist of a core protein containing numerous O-linked and N-linked glycosaminoglycans.



Sugar Code and Lectins

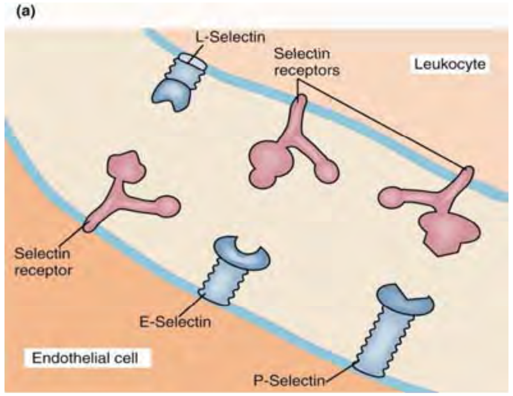
TABLE 7.1 Specificities and Functions of Some Animal Lectins

Lectin Family	Carbohydrate Specificity	Function
Calnexins	Glucose	Ligand-selective molecular chaperones in ER
C-type lectins	Variable	Cell-type specific endocytosis and other functions
ERGIC-53	Mannose	Intracellular routing of glycoproteins and vesicles
Galectins	Galactose/lactose	Cellular growth regulation and cell–matrix interactions
Pentraxins	Variable	Anti-inflammatory action
Selectins	Variable	Cell migration and routing

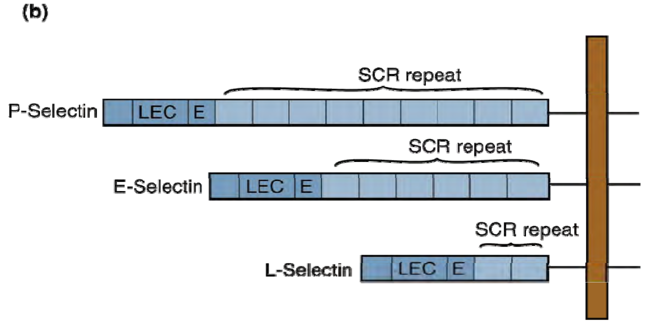
- Sugars are the "letters" of the **sugar code**. Lectins are the translators of the sugar code.
- Many processes, such as cell migration, cell-cell interactions, immune responses, and blood clotting, depend on information transfer using this code.

Selectin proteins modulate the inflammatory response

- Selectin-carbohydrate interactions modulate the rolling of leukocytes along the vascular wall, so that leukocytes can migrate efficiently to the sites of inflammation.
- P-selectin is storaged and released by induction of Histamine….



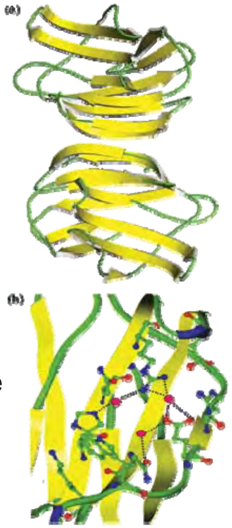
The primary structure of the selectin family of adhesion proteins



- LEC is the lectin domain.
- E is the epidermal growth factor domain.
- SCRs are "short consensus repeat" domains.

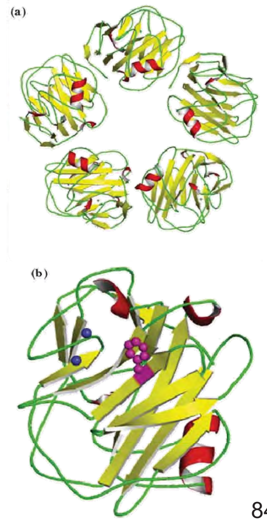
Galectins are Mediators of Inflammation, Immunity, and Cancer

- Galectins are a family of proteins with "carbohydrate recognition domains (CRDs) of about 135 residues
  - Human galectin-1 is a dimer of antiparallel β-sandwich subunits
  - Lactose binds at opposite ends of the dimer
  - H-bond networks maintain the structure of the galactose-binding sites.
- Galectins bind β-galactosides specifically



C-Reactive Protein – a Lectin That Limits Inflammation Damage

- Pentraxins are lectins that form planar, 5-membered rings
- C-reactive protein (CRP) is a pentraxin
  - limit tissue damage, acute inflammation, and autoimmune reactions
  - by binding to phosphocholine (PC) moieties on damaged membranes
  - a diagnostic marker of inflammation, an indicator of heart disease risk



End of Part 3

- Ask yourself…
  - What is glycoprotein? What are their functions?
  - What is proteoglycan? What are their functions?
  - What are the differences between proteoglycans and glycoproteins?
  - What is sugar code? How to read it?

End of the class

- After this class, you should have learned…
  - Stereochemistry of sugar
  - Cyclic of sugar
  - Glycosidic bond
  - Reducing sugar
  - Functions of sugar
  - Proteoglycan and glycoprotein
  - Sugar code

Exercise

9. A 0.2-g sample of amylopectin was analyzed to determine the fraction of the total glucose residues that are branch points in the structure. The sample was exhaustively methylated and then digested, yielding 50 umole of 2,3-dimethylglucose and 0.4 umole of 1,2,3,6-tetramethylglucose

a. what fraction of the total residues are branch points?

b. how many reducing ends does this amylopectin have?