CARBOHYDRATES

Course: Biochemistry I (BIOC 230)
Instructor: Dr. M. A. Srour
Textbook:
Principles of Biochemistry, 5th Ed., by L. A. Moran and others. 2014, Pearson. . Chapter 8

Carbohydrates

- Most abundant class of biological molecules on Earth
- Originally produced through CO_2 fixation during photosynthesis

Roles of Carbohydrates

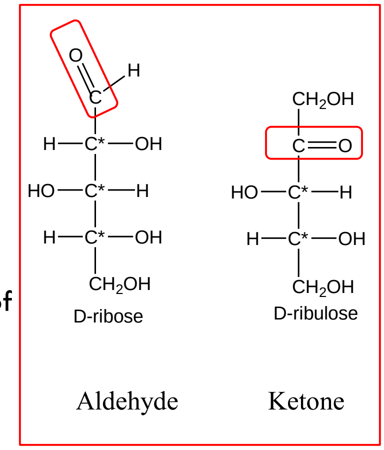
- Energy storage (glycogen,starch)
- Structural components (cellulose, chitin)
- Cellular recognition
- Carbohydrate derivatives include DNA, RNA, co-factors, glycoproteins, glycolipids

Three major size classes of Carbo.

- Monosaccharides (simple sugars) cannot be broken down into simpler sugars under mild conditions
- Oligosaccharides = "a few" - usually 2 to 10; Units or residues joined by glycosidic bonds. Most abundant is the disacch like sucrose
- Polysaccharides are polymers of the simple sugars; sugar polymers with more than 20 or so monosacch; like cellulose and glycogen

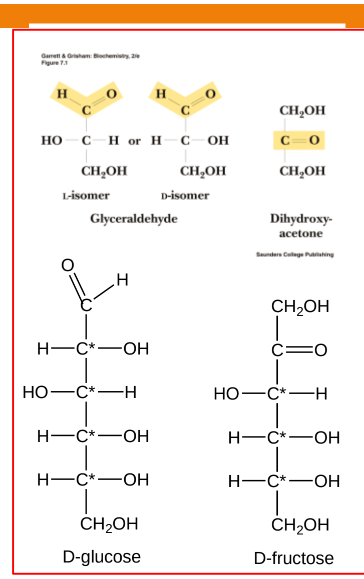
Monosaccharides

- Polyhydroxy ketones (ketoses) and aldehydes (aldoses)
- Aldoses and ketoses contain aldehyde and ketone functions, respectively
- Ketose named for "equivalent aldose" + "ul" inserted
- Triose, tetrose, etc. denotes number of carbons
- Empirical formula = $(CH_2O)_n$
- All common mono and disacch have names ending the suffix "ose"

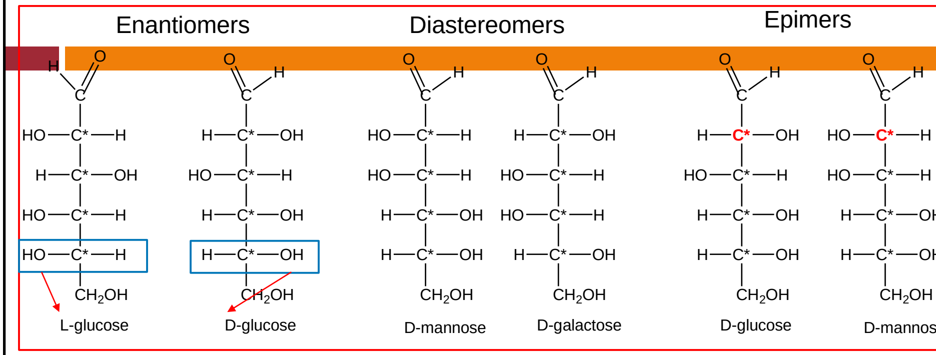


Monosaccharides are chiral

- Aldoses with 3C or more and ketoses with 4C or more are chiral
- The number of chiral carbons* present in a ketose is always one less than the number found in the same length aldose
- Number of possible stereoisomers = 2^n (n = the number of chiral carbons)



Stereochemistry



•<u>Enantiomers</u> = mirror images,

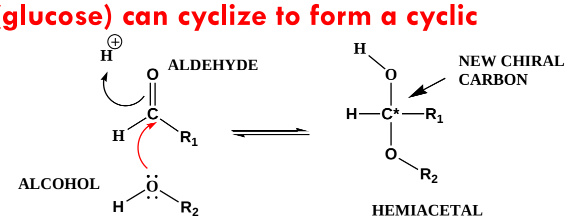
•Pairs of isomers that have opposite configurations at one or more chiral centers but are NOT mirror images are <u>diastereomers</u>

•Diastereomers that differ at ONLY ONE chiral center are called Epimers (e.g. D-Mannose and D-Galactose are epimers of D-Glucose at C2 and C4, respectively)

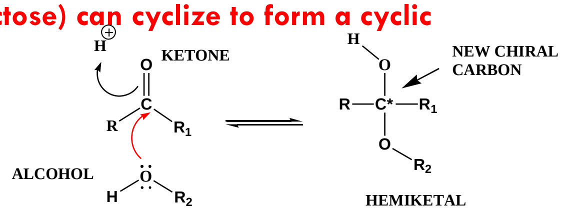
Cyclization of aldose and ketoses introduces additional chiral center

Review only!

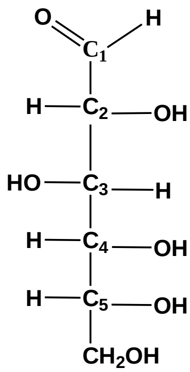
- **Aldose sugars (glucose) can cyclize to form a cyclic hemiacetal**



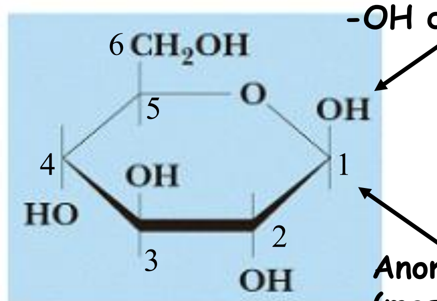
- **Ketose sugars (fructose) can cyclize to form a cyclic hemiketal**



Haworth (Ring form) and Fischer (Linear form) Projections of D-Glucose



-OH up = beta
-OH down = alpha

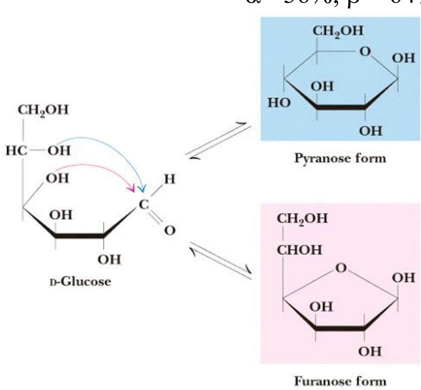


Anomeric carbon (most oxidized)

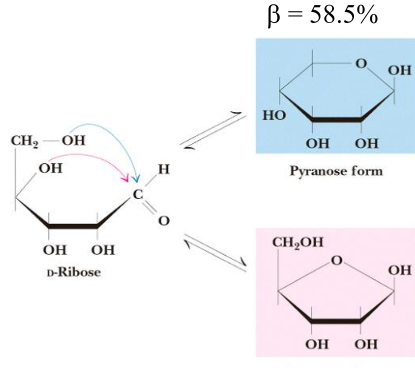
For all non-anomeric carbons, -OH groups point down in Haworth projections if pointing right in Fischer projections.
α-D-Glucose and β-D-Glucose are Anomers.

Monosaccharides can cyclize to form Pyranose / Furanose forms

α= 36%; β = 64%



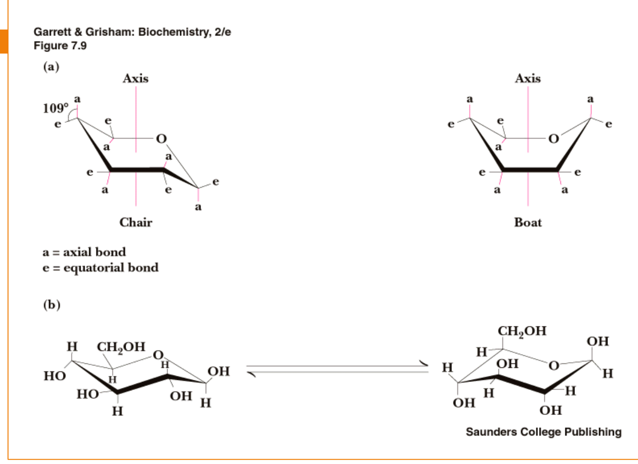
α = 21.5%
β = 58.5%



α = 13.5%
β = 6.5%

Pyranose: six-membered ring
Furanose: five-membered ring

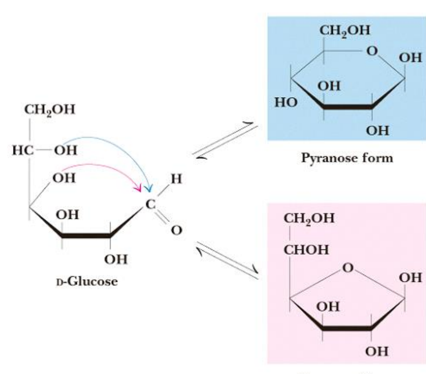
Conformation of Monosaccharides



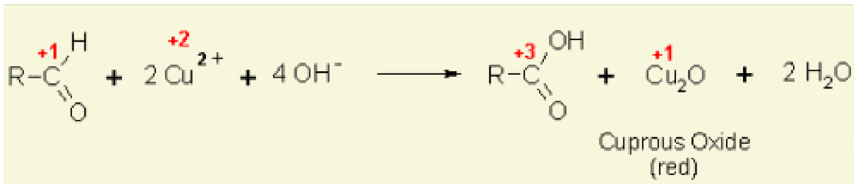
Pyranose sugars not planar molecules, prefer to be in either of the two chair conformations.

Reducing Sugars

- When in the uncyclized form, monosaccharides act as reducing agents.
- Free carbonyl group from aldoses or ketoses can reduce Cu^{2+} and Ag^{+} ions to insoluble products
- The carbonyl group itself is oxidized to a carboxyl group



Sugars as reducing agents



Blood glucose measurement in the diagnosis and treatment of diabetes

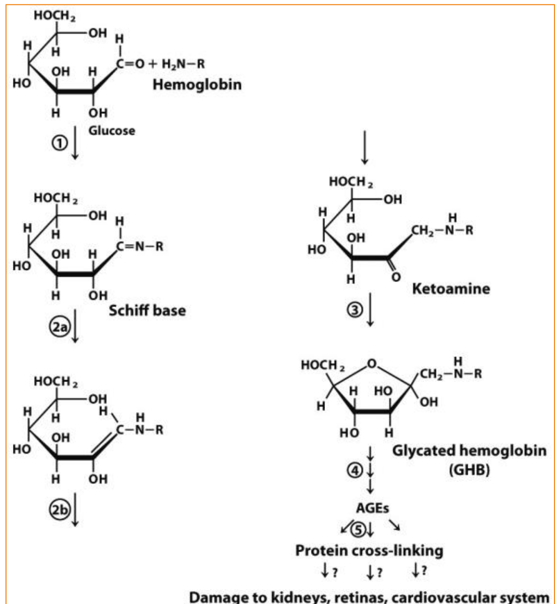
- Normal plasma glucose level is 70-100 mg/dL
- In type 1 DM, hyperglycemia is due to absence of insulin that normally serves to reduce blood glucose level
- Glucose in blood and urine can be determined by a simple assay using Glucose Oxidase method:
  - First rxn: β-D-glucose $+O_2 \rightarrow$ D-Glucono-δ-lactone + H2O2
  - Coupled rxn: H2O2 + chromogen or dye $\rightarrow$ change in color
- Glucose levels change overtime, & a single measurement may not reflect the average level over hours or days!!
- How we can determine the average glucose level over a period of 3-4 months??

Glycated Hemoglobin or HbA1c:

The nonenzymatic reaction of glucose with a primary amino group in hemoglobin

Reference range: Diabetic ≥6.4%

Diabetic patients are recommended to keep HbA1c <7%



Box 7-1 figure 2
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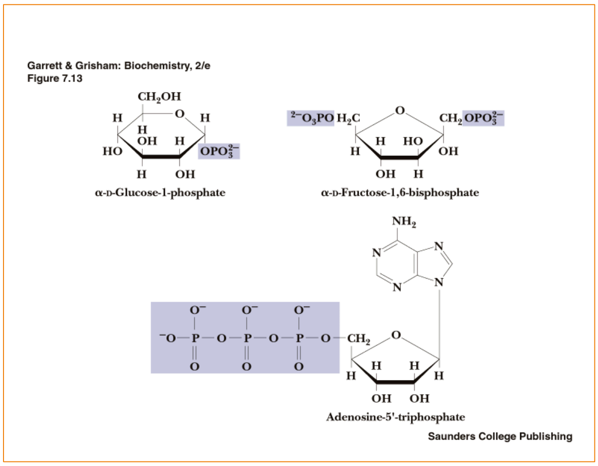
Derivatives of Monosaccharides

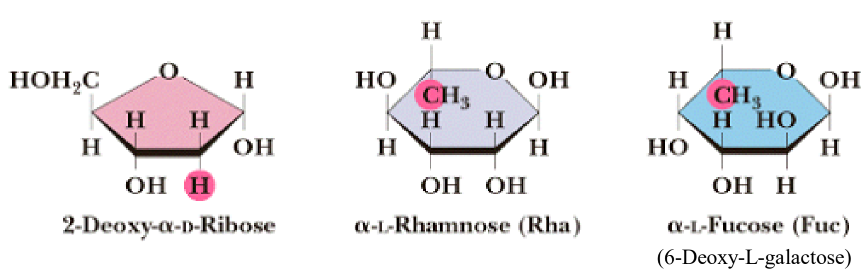
Sugar Phosphates

Monosacch. in metabolic pathways are often converted to phosphate esters



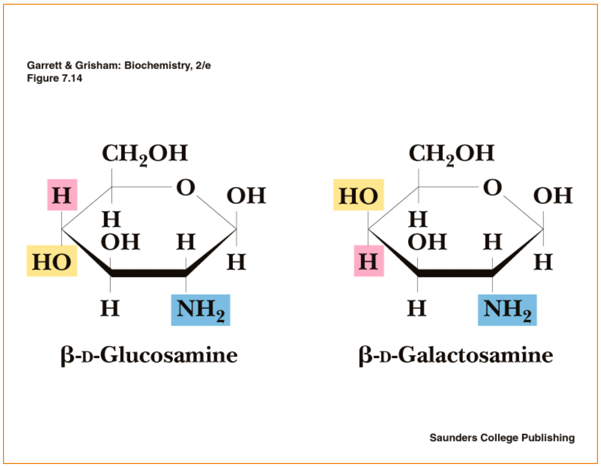
Deoxy Acids

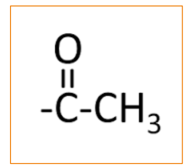
In Deoxy sygars, a hydrogen atom replaces one of the hydroxyl groups in the parent monosacch. E.g., 2-Deoxy-D-ribose is an important building block of DNA



Amino Sugars

An amino group replaces one of the hydroxyl groups in the parent monosacch. Sometimes the amino group is acetylated



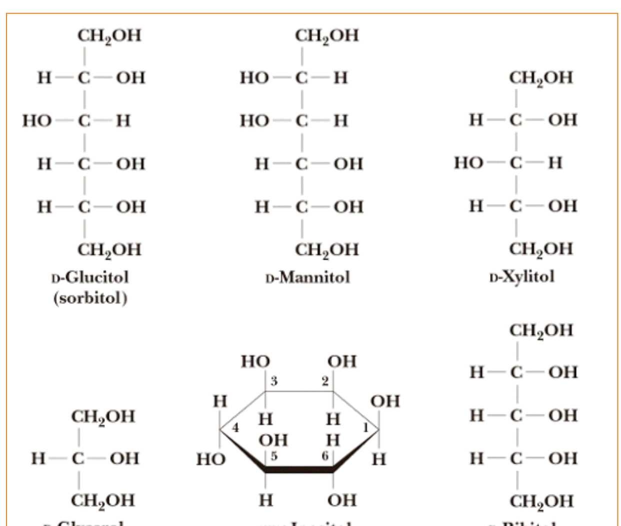


Acetyl group

Sugar alcohols

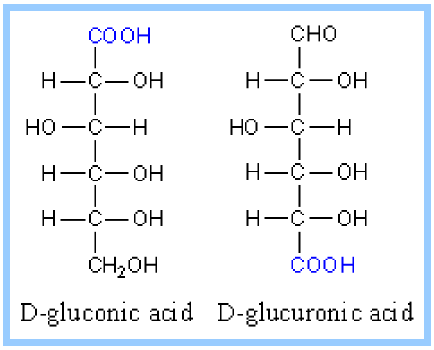
Carbonyl oxygen of parent monosacch. is reduced producing a polyhydroxyl alcohol.

Named by replacing the suffix "ose" with –itol.



Sugar acids

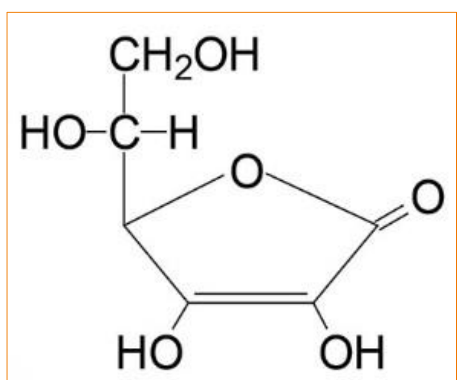
Sugar acids are carboxylic acids derived from aldoses, either by oxidation of C-1 to yield aldonic acid or by oxidation of the highest numbered carbon (the carbon bearing the primary alcohol) to yield alduronic acid.



Ascorbic acid

L-Ascorbic acid is derived from D-glucuronic acid.

Primates cannot convert glucuronate to ascorbic acid and thus must obtain it from diet.



Monosaccharide structures you need to know

- Glucose
- Fructose
- Ribulose
- Glyceraldehyde
- Dihydroxyacetone

Oligo- and polysaccharides

Disaccharides contain a glycosidic bond

- Disaccharides consist of two monosaccharides joined covalently by an O-glycosidic bond
- In disacch or polysacch, the chain with a free anomeric carbon is called reducing end
- Common disacch.:
  - Sucrose: table sugar and most abundant diascch found in nature,
  - Lactose: major carbo in milk
  - Maltose: present in malt; released during hydrolysis of starch

Formation of maltose

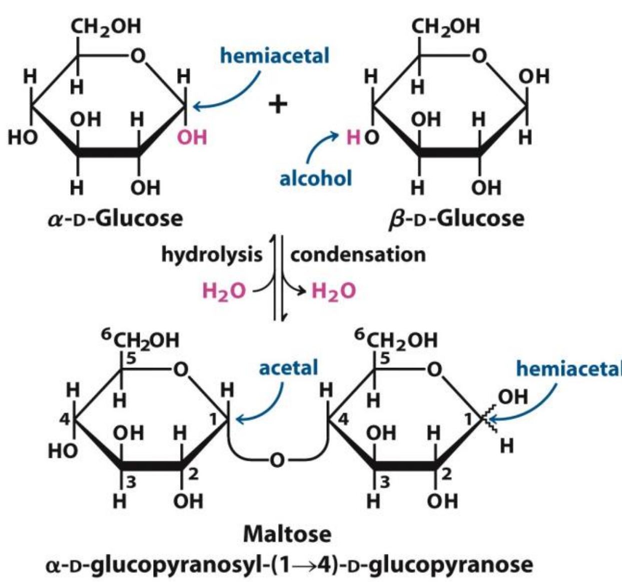


Figure 7-11
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Some common disaccharides

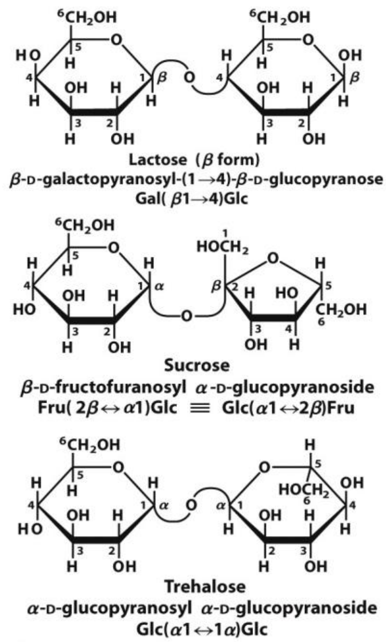

Figure 7-12
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TABLE 7–1 Symbols and Abbreviations for Common Monosaccharides and Some of Their Derivatives

Abequose	Abe	Glucuronic acid	GlcA
Arabinose	Ara	Galactosamine	GalN
Fructose	Fru	Glucosamine	GlcN
Fucose	Fuc	*N*-Acetylgalactosamine	GalNAc
Galactose	Gal	*N*-Acetylglucosamine	GlcNAc
Glucose	Glc	Iduronic acid	IdoA
Mannose	Man	Muramic acid	Mur
Rhamnose	Rha	*N*-Acetylmuramic acid	Mur2Ac
Ribose	Rib	*N*-Acetylneuraminic acid (a sialic acid)	Neu5Ac
Xylose	Xyl		

Note: In a commonly used convention, hexoses are represented as circles, *N*-acetylhexosamines as squares, and hexosamines as squares divided diagonally. All sugars with the "gluco" configuration are blue, those with the "galacto" configuration are yellow, and "manno" sugars are green. Other substituents can be added as needed: sulfate (S), phosphate (P), O-acetyl (OAc), or O-methyl (Ome).

Table 7-1
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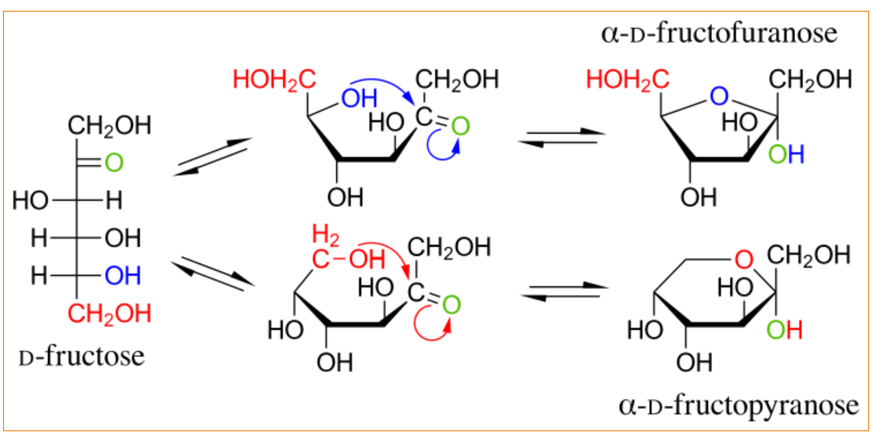
Class activity!

- Honey is an emulsion of microcrystalline D-fructose and D-glucose. Although D-fructose in polysaccharides exists mainly in the furanose form, a solution of crystalline D-fructose in honey, is a mixture of several forms with β-D-fructopyranose (67%) and β-D-fructofuranose (25%) predominating. Draw the Fischer projection for D-fructose and show how it can cyclize to form both the cyclized forms above.



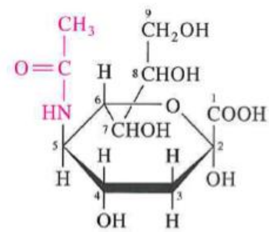
Comparison of sugar content of different fruits & honey for fructose and glucose

Food	**Fructose** g/100g (cals)	**Glucose** g/100 (cals)
Sucrose	50 (200)	50 (200)
Apples	5.9 (23.6)	2.4 (9.6)
Pears	6.2 (24.8)	2.8 (11.2)
Fruit juice	5-7 (20-28)	2-3 (8-12)
Raisins	29.8 (119.2)	27.8 (111.2)
Honey	40.9 (163.6)	35.7 (142.8)
High fructose corn syrup	55-90 (220-360)	45-10 (180-40)

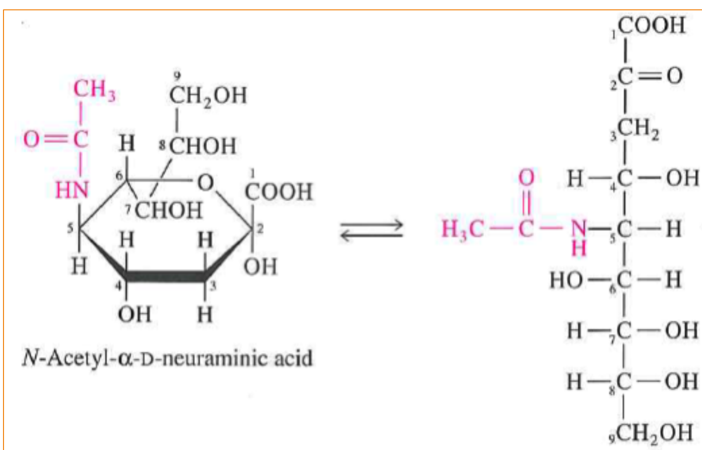
Class activity!

- Sialic acid (N-acetyl-α-D-neuraminic acid) is often found in N-linked oligosaccharides that are involved in cell-cell interaction. Cancer cells synthesize much greater amounts of it and it has been proposed as anticancer agent to block cell-surface interactions between normal and cancer cells.
- Study the structure of SA and answer the following questions:

- Is it an α or β anomeric form?
- Will SA mutorotate between α or β anomeric form?
- Is this a deoxy sugar?
- Will the open chain of SA be an aldehyde or a ketone?
- How many chiral enters are there in the sugar ring?



N-Acetyl-α-D-neuraminic acid



N-Acetyl-α-D-neuraminic acid

N-Acetyl-α-D-neuraminic acid (open-chain form)

Class activity!

- In the procedure for testing blood glucose, Glucose Oxidase is used per the following reaction:
- B-D-Glucose + O2 → D-Glucono-δ-lactone + H2O2
- Since Glucose oxidase is specific for –anomeric form of D-glucose, then why Total blood glucose is measured?

Polysaccharides

Polysaccharides

- Most carbo found in nature occur as polysaccharides
- Polysacch are called Glycans
- Polysacch differ from each other in the identity of their recurring monosacch units, in the length of their chains, in the type of bonds linking the units and in the degree of branching
- Most polysacch are classified according to their biological roles; as storage (starch and glycogen) or structural polysacch (cellulose and chitin)

Polysaccharides

- Polysacch are broadly divided into two broad classes:
- Homoglycans or Homopolysaccharides: contains only a single type of monomer. Function either as storage of monosacch or structural elements
- Heteroglycans or Heteropolysaccharides: contain two or more different kinds. Provide extracellular support for organisms

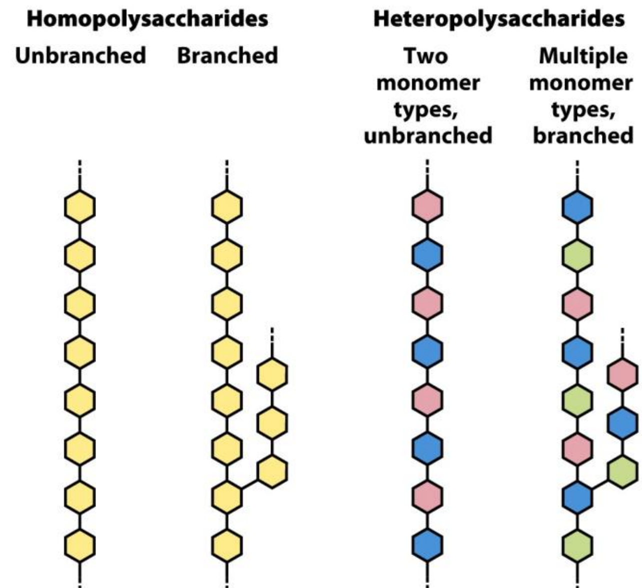


Figure 7-13
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An (α1→6) branch point of glycogen or amylopectin

Some Homopolysacch are stored forms of fuel

- Starch : contains two types of glucose polymers,
  - Amylose (unbranched chains of glucose residues connected by (α1->4) linkages
  - amylopectin (highly branched), with α1->6 linked branches

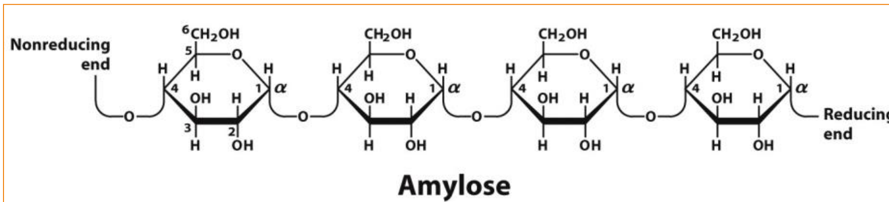

Figure 7-14a
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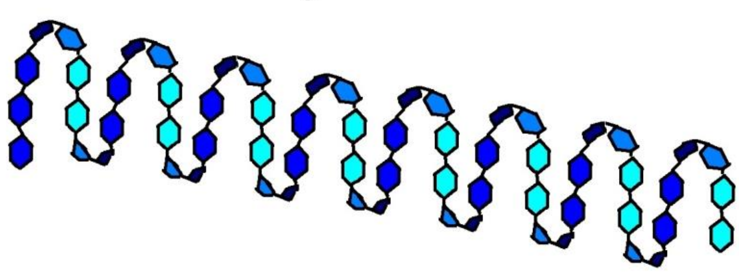
Amylose:
A linear polymer of D-glucose residues in (α1→4) linkage

A polymer of 100-1000 D-glucose residues

It is not truly soluble in water, forms micelles in water, and assume a helical structure

Amylose: left-handed helix

Amylose Helix



On average, 8 glucose residues per turn

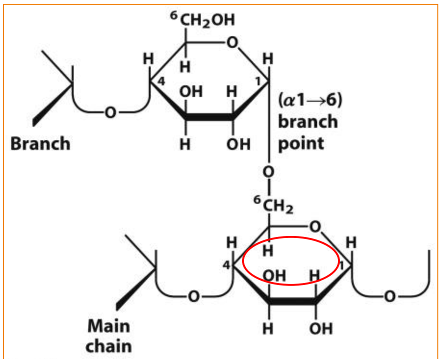


Figure 7-14b
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Amylopectin:
Branched version of amylose
Branches are linked via a (α1→6) glucosidic bond
Branching occurs on average every 25 residues
It contains 300-6000 glucose residues

Starch in human diet

- An adult human consumes ~300 grams of carbohydrates daily, much of which in the form of starch
- Raw starch granules resist enzymatic hydrolysis, but cooking causes them to absorb water and swell, the swollen starch is a substrate of two glycosidases
- Dietary starch is degraded in GI tract by the actions of α–amylase and de-branching enzyme
- α-amylase is an endoglycosidase that catalyzes random hydrolysis of α(1>4) glucosidic bonds of amylose and amylopectin

Some Homopolysacch are stored forms of fuel // cont'd

- Glycogen: main storage polysacch in animal cells. More extensively branched than amylopectin and more compact than starch, with α1->6 linked branches

- Dextrans : bacterial and yeast polysacch made up of a1->6 linked poly-D-glucose; all have a1->3 branches and some also have a1->2 or a1->4 branches.

Glycogen

- Branched polymer of glucose
- It contains the same types of linkages found in amylopectin, but branches are smaller and more frequent, occurring every 8-12 residues
- In general, glycogen molecules are larger than starch molecules containing up to ~50,000 glucose residues
- In mammals, glycogen accounts for up to 10% of the mass of liver and 2% of the mass of muscle

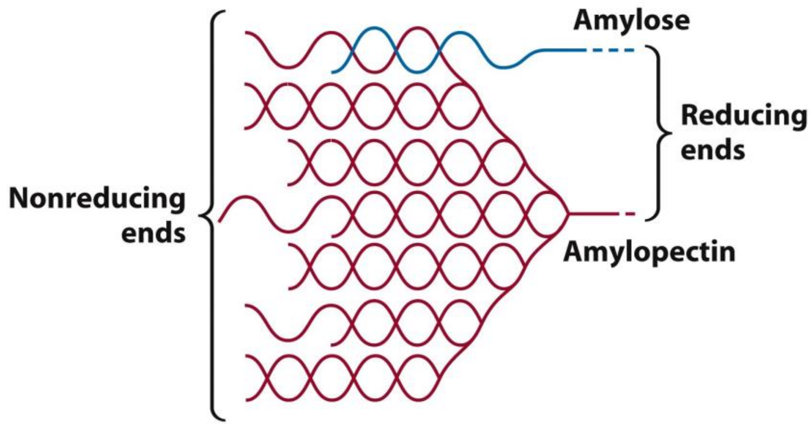

Figure 7-14c
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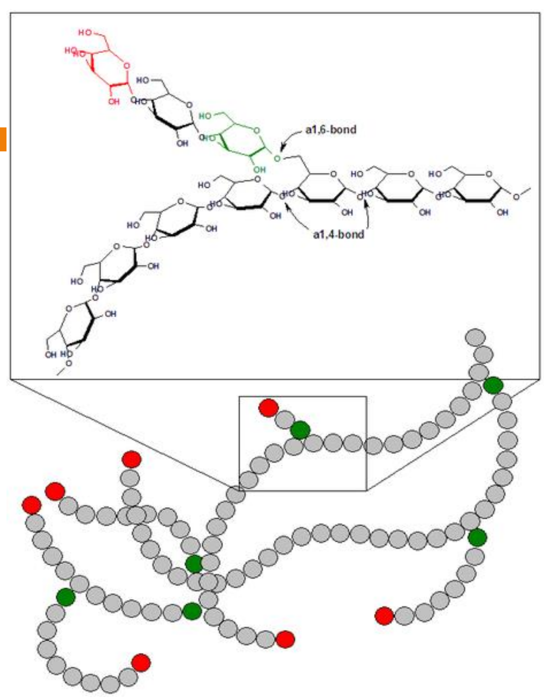
A cluster of amylose and amylopectin like that believed to occur in starch granules

Glycogen



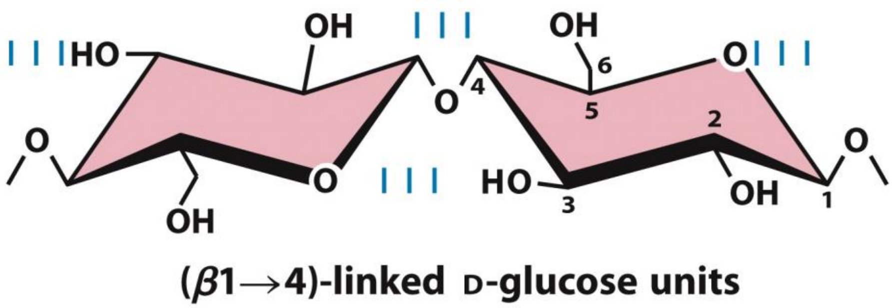
Class activity!

- Why not store glucose in its monomeric form??

Some Homopolysacch serve structural roles

- Cellulose: a fibrous tough water insoluble substance found in plant cell walls .
- Starch and glycogen are hydrolysed by a-amylase in saliva and intestine
- Most animals cannot hydrolyze cellulose
- Termites readily hydrolyze cellulose because they harbor microorganims *Trichonympha* that produce ***cellulase***

Cellulose. (a) Two units of a cellulose chain; the D-glucose residues are in (β1→4) linkage. The rigid chair structures can rotate relative to one another.



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Cellulose. (b) Scale drawing of segments of two parallel cellulose chains, showing the conformation of the D-glucose residues and the hydrogen-bond cross-links

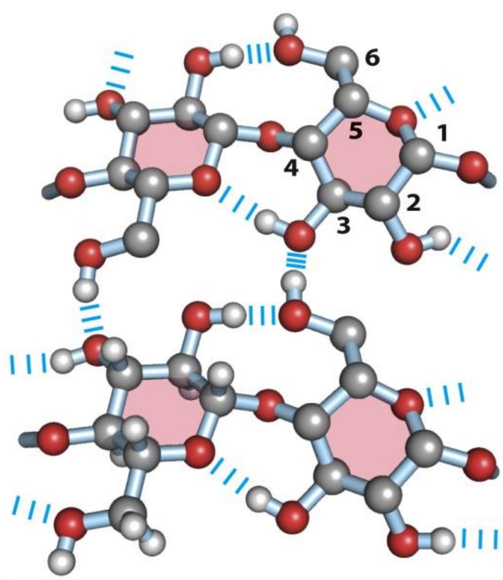

Figure 7-15b
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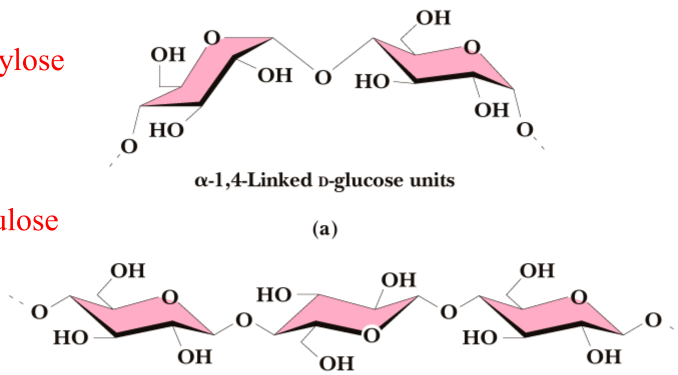
Cellulose vs Amylose

Garrett & Grisham: Biochemistry, 2/e
Figure 7.26

amylose

cellulose



α-1,4-Linked D-glucose units

(a)

β-1,4-Linked D-glucose units

(b)

Glucose units rotated 180º relative to next residue

Cellulose

- Consists of β-D-glucose
- Glucose molecules vary greatly in size, ranging from 300-15,000 D-glucose units
- Extensive hydrogen bonding within and between cellulose chains leads to formation of bundles and firbils
- Cellulose fibrils are water insoluble and strong and rigid
- Cotton fibers are almost entirely cellulose

Cellulose

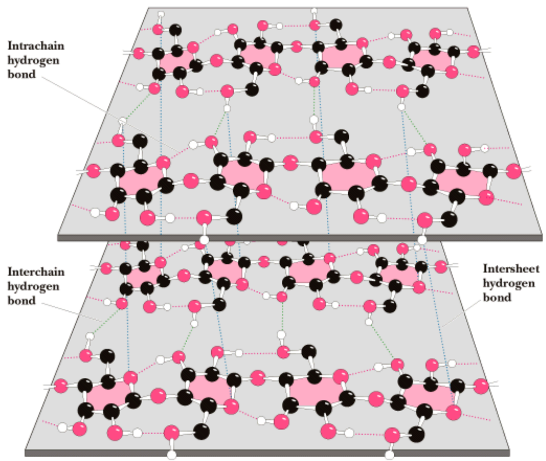
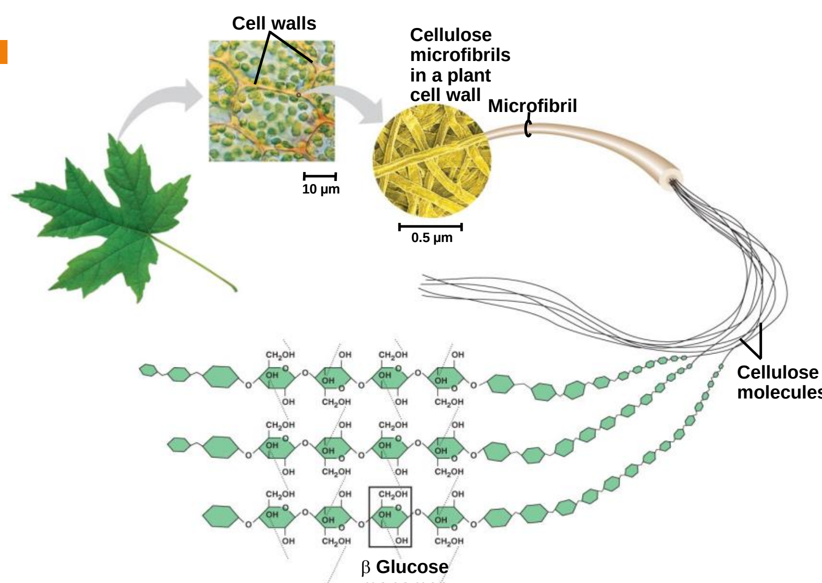


Fig. 5-8

The arrangement of cellulose in plant cell walls



Cellulose

Cellulose breakdown by wood fungi. All wood fungi have the enzyme cellulase, which breaks the (β1→4) glycosidic bonds in cellulose

Figure 7-16
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Some Homopolysacch serve structural roles

- Chitin: a linear polymer of N-acetylglucoseamine in (β 1->4) -linkage
- Differs from cellulose, in that the OH group at C2 is replaced by acetylated amino group

Chitin. (a) A short segment of chitin, a homopolymer of *N*-acetyl-D-glucosamine units in (*β*1→4) linkage.

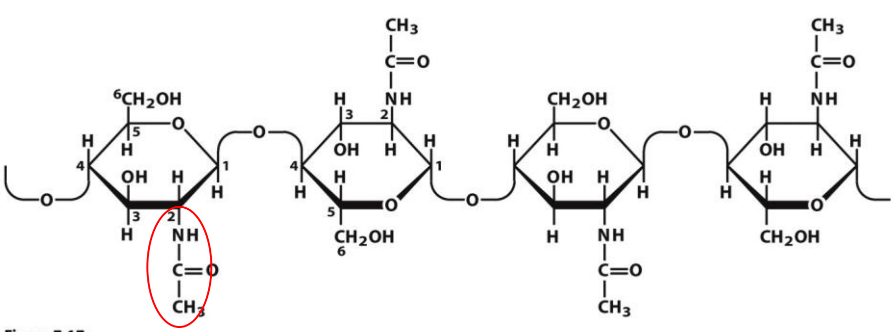

Figure 7-17a
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A spotted June beetle (*Pelidnota punctata*), showing its surface armor (exoskeleton) of chitin

Figure 7-17b
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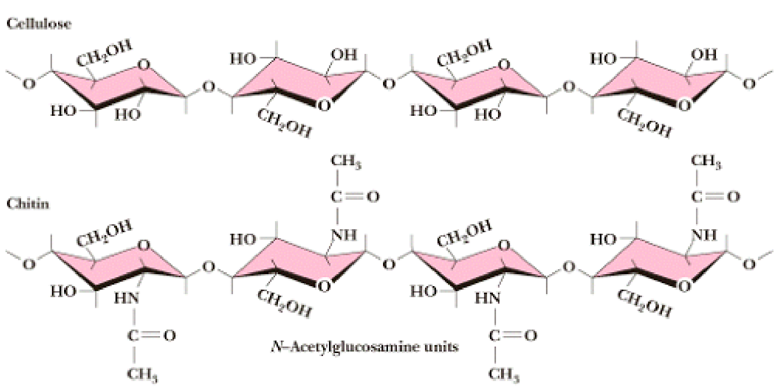
Chitin vs Cellulose



Glycoconjugates

Glycoconjugates

- Glycoconjugates consist of polysacch linked (conjugated with) proteins or peptides
- In most cases polysacch are heteroglycans
- Three types of glycoconjugates:
  - Proteoglycans
  - Peptidoglycans
  - Glycoproteins

Proteoglycans

- Proteoglycans: are complexes of proteins and a class of polysacch called glycosaminoglycans
- Proteoglycans are predominately found in the extracellular matrix (connective tissue) of multicellular animals
- Glycosaminoglycans: are a family of linear polymers composed of repeating disaccharide units.
- One of the two monosacch is always either D-glucosamine or D-galactosamine; and the amino group of the amino sugar can be acetylated forming GalNAc and GlcNAc
- The other monosacch is in most cases is usually an alduronic acid

Proteoglycans (cont'd)

- Specific hydroxyl and amino groups of many proteoglycans are sulfated
- The sulfate groups and caboxylate groups of alduronic acids makes these molecules polyanionic
- Up to 100 glycosaminoglycan chains can be attached to the protein of a proteoglycan
- Examples: Hyaluronic acid, chondroitin -4-sulfate, Keratan sulfate, heparin

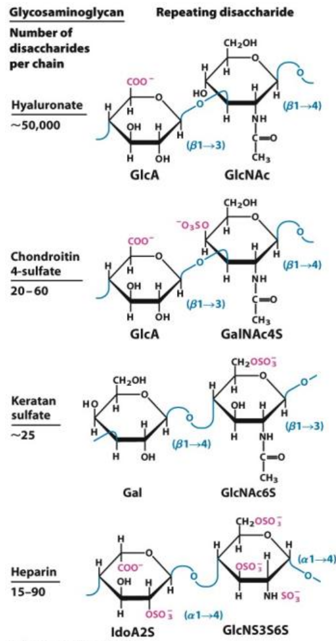


Figure 7-22 part 1
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See next slide for larger view!

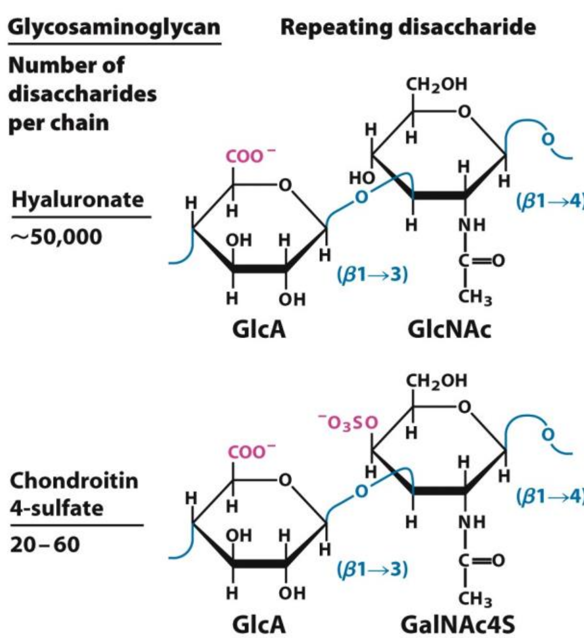

Figure 7-22 part 1a
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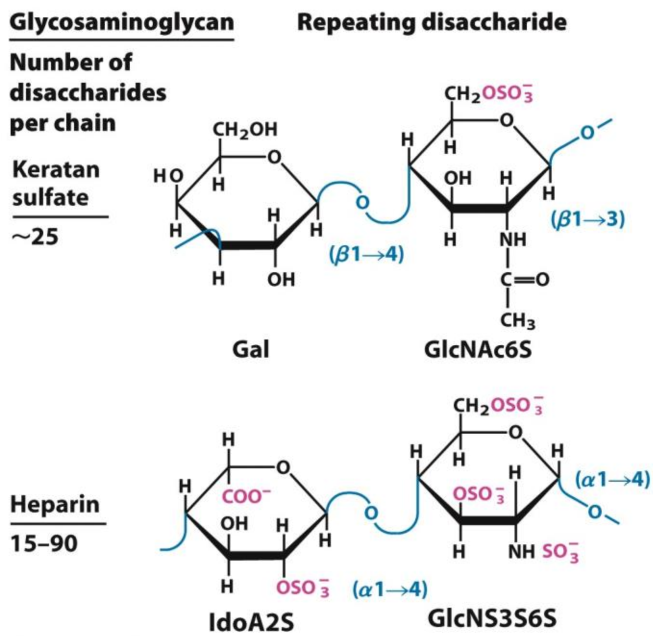

Figure 7-22 part 1b
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Hyaluranon of hyaluronic acid

- Repeating units: D-glucuronic acid and N-Acetylglucosamine
- Up to 50,000 disacch units
- Forms clear highly viscous solutions that serve as lubricants in synovial fluid of joints and give the vitreous humor of eye,
- Hyaluronan is also a component of ECM of cartilage and tendons to which it gives a tensile strength and elasticity

Other glycosaminoglycans

- Chondroitin sulfate: contributes to tensile strength of cartilage, tendons, ligaments and walls of aorta
- Dermatan sulfate contributes to pliability of skin, blood vessels and heart valves. Here GlcA is replaced by its 5-epimer IdoA
- Keratan sulfates have no Uronic acid and their sulfate content is variable.
- Present in cornea, cartilage, bone and a variety of horny structures like hair, horn, nails and claws

Heparan sulfate

- Heparan sulfate is produced by all animal cells and contains variable arrangements of sulfated and nonsulfated sugars
- Sulfated component allow it to interact with proteins including growth factors and ECM
- Heparin is a fractionated form of heparan sulfate derived mostly from mast cells
- Heparin is an inhibitor of coagulation that exert its effect by binding to and inhibition of antithrombin

Structure of proteoglycans

- Basic unit consist of a "core protein" with covalently attached glycosaminoglycans. Point of attachment is a Ser residue
- Ser residue is usually in the sequence –Ser-Gly-X-Gly; where X is any amino acid
- Up to 100 glycosaminoglycan chains can be attached to the protein of a proteoglycan
- Heteroglycan chains are usually covalently bound by a glycosidic linkage to the hydroxyl oxygen of serine residue: called O-linked glycans
- Not all glycans are covalently linked to protein
- Glycosaminoglycans can account up to 95% of the mass of proteoglycan

Structure of proteoglycans

- Cartilage is a mesh of collagen fibers interspersed with large proteoglycn aggregates
- The major proteoglycan of cartilage is called Aggercan. Its core proteins carries: ~30 moleculaes of keratans sulfate, ~100 molecules of chondroitin sulfate
- Aggrecan is a member of a small family of Hyalectans, proteoglycans that bind to hyaluronic acid
- Each aggregate resembles a brush
- These aggregates have hyaluronic acid and several other proteoglycans as well as a core protein and link protein

Proteoglycan structure, showing the tetrasaccharide bridge. A typical **tetrasaccharide** linker (**blue**) connects a glycosaminoglycan—in this case **chondroitin 4-sulfate (orange)**–to a **Ser residue (pink)** in the core protein.

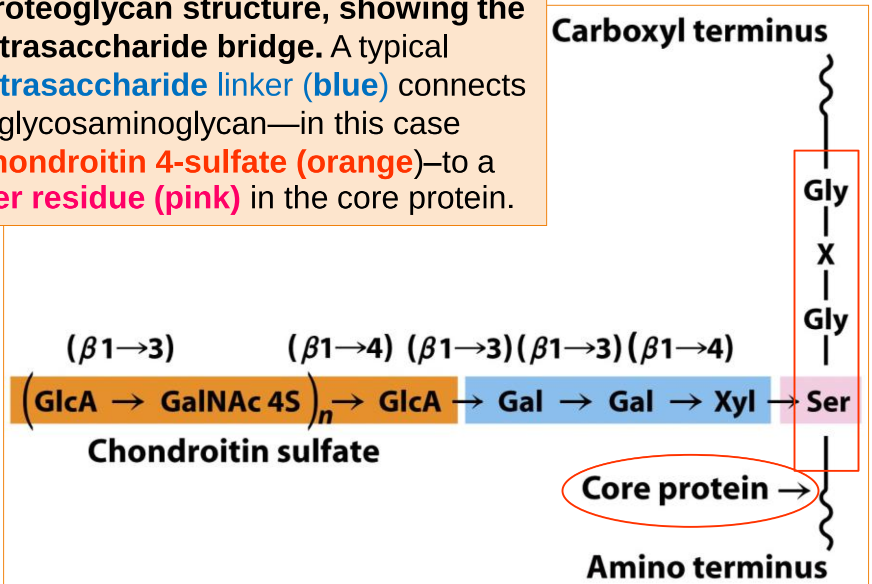

Figure 7-24
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Two families of membrane proteoglycans: Syndecan and Glycans

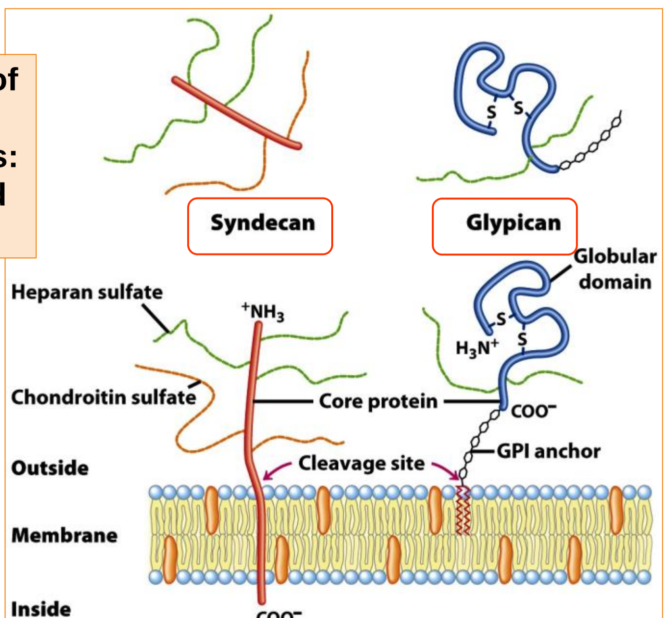

Figure 7-25a
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Proteoglycan aggregates

- Some proteoglycans can form aggregates, enormous supramolecular assemblies of hyaluronan
- In ECM, proteoglycan aggregates interact with other ECM components like collagen, elastin, fibronectin and form a cross-linked meshwork that gives the ECM strength and resilience
- The ECM meshwork functions to anchor cells to ECM and provide paths that direct migration of cells in developing tissue and to convey information in both directions across plasma membrane

Proteoglycan aggregate of the extracellular matrix

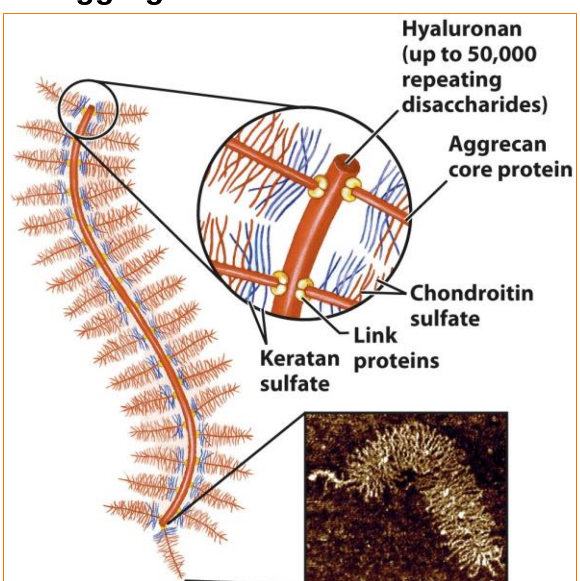

Figure 7-27
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Interactions between cells and the extracellular matrix

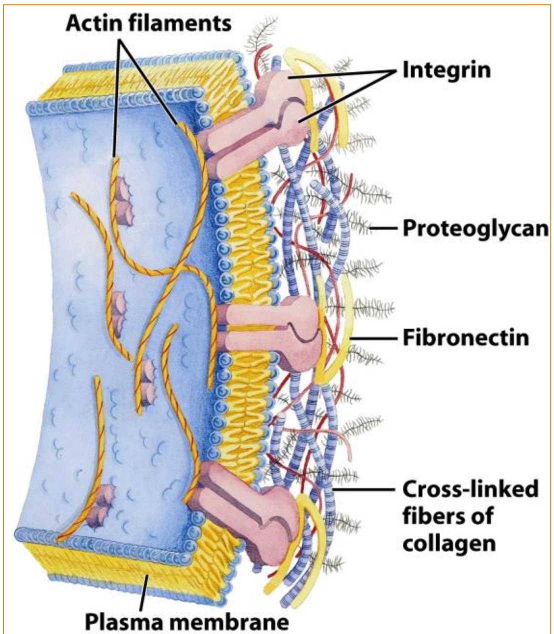

Figure 7-28
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Peptidoglycans

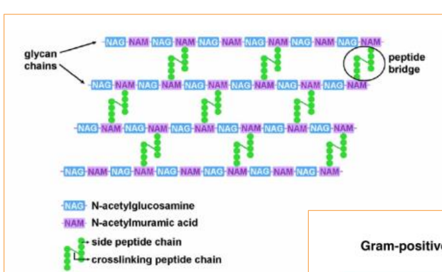
- Peptidoglycans are polysaccharides linked to small peptides
- Cell wall of many bacteria contain a special class of peptidoglycan with a heteroglycan component attached to a four- or five-residue peptide
- Heteroglycn is composed of alternating residues of GlcNAc and N-acetylMuramic acid (MurNAc)
- The antibacterial action of Lysozyme results from it ability to hydrolyse polysacch chain of peptidoglycans

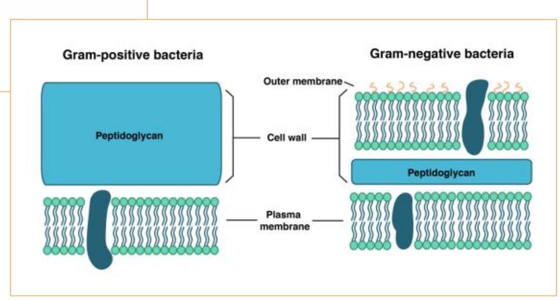
Peptidoglycans

- The Peptide component of petidoglycan varies among bacteria. In S. aureus, it is a tetrapeptide with alternating L and D amino acids: **L-Ala-D-Isoglu-L-Lys-D-Ala**
- The tetrapeptide is cross linked to another chain with a pentaglycine
- In Gram-negative bacteria there is a thin peptidoglycan layer between cell membrane and outer membrane
- In Gram-positive bacteria, there is a thick peptidoglycan layer that retains the stain in Gram stain

Class activity!

- Peptidoglycans in Gram positive & negative bacteria





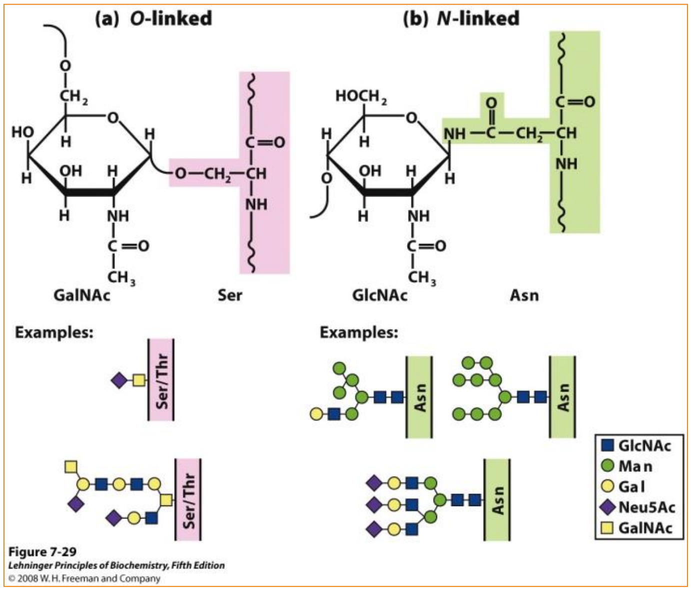
Class activity!

- What antibiotics inhibit synthesis of peptidoglycan in bacteria?
- Vancomycin interrupts cell wall synthesis by forming a complex with the C-terminal D-alanine residues of peptidoglycan precursors. Complex formation at the outer surface of the cytoplasmic membrane prevents the transfer of the precursors from a lipid carrier to the growing peptidoglycan wall by transglycosidases

Glycoproteins

- Glycoproteins are proteins that contain covalently bound oligosacch (i.e., proteins that are glycosylated, thus proteoglycans are a type of glycoproteins)
- In glycoproteins, glycans are smaller, branched and more structurally diverse than glycosaminoglycans
- Carbohydrate is attached at its anomeric carbon to –OH of a Ser or Thr residue (O-linked) or through an N-glycosyl link to the amide nitrogen of an Asn residue (N-linked)
- Carbo chain ranges from 1 to more than 30 residues
- Carbo may constitute ~80% of the total mass of glycoprotein

Oligosaccharide linkages in glycoproteins



Glycoproteins

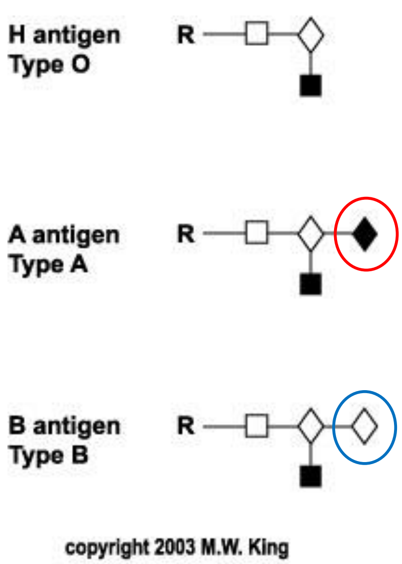
- A diverse group of proteins that includes enzymes, hormones, structural proteins and transport proteins
- Composition of oligosacch chain exhibit great variability and may vary among molecules of same protein

Class activity!

ABO blood groups

It is determined by a single gene on chromosome 9 with 3 alleles.

Phenotype	Genotype	ABO enzyme	Immunodominat sugar	Antigen
A	AA, AO	A enzyme	Fucose + GalNAC	A
B	BB, BO	B enzyme	Fucose + Gal	B
AB	AB	A enzyme & B enzyme	Fucose + GalNAC Fucose + Gal	A, B
OO	OO		Fucose	H



Blood ABO Antigens

Structure of the ABO blood group carbohydrates,
R represents the linkage to protein in the secreted forms, sphingolipid in the cell-surface bound form
open square = GlcNAc, **open diamond = galactose**, **filled square = fucose**, filled diamond = GalNAc,