Complex Carbohydrates & Glycolipids

Date:  Friday, Aug. 19, 2005*  Time:  10:30-11:50 AM*  
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*Be sure to consult the online schedule for this course for the definitive date and time for this lecture.

This material is closely related to Dr. Burridge’s topics on membrane biogenesis and exocytosis (August 19 and 22, respectively*), and all are relevant to the Lysosomal Storage Disorders case conference on August 23*. All the material presented (other than that in italics) is considered important to your understanding of the subject, but pay extra attention to any material in bold and/or red. Pretty much everything you will need to know for assessment purposes (exams) is in the notes below and/or in the associated .ppt slide set on the Molecules to Cells homepage, but there’s no substitute for coming to class and paying attention.  Students interested in additional details on these topics are directed to Lippincott’s Illustrated Reviews: Biochemistry (3rd ed), pp 155-170 and 205-210, and/or Voet, Voet, and Pratt, Fundamentals of Biochemistry, pp. 203-217 and 226-227.

Important Overall Concepts:
1. Complex carbohydrates are covalent complexes of proteins and sugars (glycoproteins and proteoglycans), or of lipids and sugars (glycolipids).
2. The carbohydrate groups direct glycoproteins to their correct location within the cell, or target them for export and function outside the cell.
3. Many of the interactions between cells are mediated by glycoproteins or glycolipids.
4. Genetic diseases of complex carbohydrate and glycolipid metabolism are generally due to defects in the catabolism of these molecules.

Objectives for complex carbohydrates: By the end of this lecture, you should:
a. understand the difference between glycoproteins and proteoglycans, and know the general roles of each.
b. know the role of carbohydrate moieties in determining blood type (A, B, or O).
c. understand the role of the carbohydrate moiety in targeting glycoproteins to their correct cellular or extracellular location.
d. understand how simple defects in processing of carbohydrate groups on glycoproteins can lead to serious diseases, such as I-cell disease.
Definitions and Characteristics of Complex Carbohydrates

A. Oligosaccharides and polysaccharides consist of monosaccharides (simple sugars) linked together by glycosidic bonds. For our purposes we will define oligosaccharides as containing \( \leq 15 \) sugar residues and polysaccharides as anything larger.

B. Glycosaminoglycans (aka mucopolysaccharides) consist of repeating disaccharides arranged in a linear polymer containing 50 to 25,000 disaccharide units. These molecules are highly negatively charged. Glycosaminoglycans (GAGs) are classified according to the disaccharide repeats and include hyaluronates, dermatan sulfates, chondroitin sulfates, keratan sulfates, heparan sulfates and heparin (heparin and heparan sulfates are distinguished primarily by the number of sulfate residues per residue).

For a figure showing the disaccharide composition of the glycosaminoglycans, go to [http://web.indstate.edu/thcme/mwking/glycans.html](http://web.indstate.edu/thcme/mwking/glycans.html)

It is not necessary to know the structures of the glycosaminoglycans at this time, but this terminology will come up again in the musculoskeletal, dermatology, pharmacology, and hematology portions of your organ system blocks in the second year.

1. General characteristics of the Glycosaminoglycans:
   - have slimy, mucous-like consistency
   - have high viscosity and low compressibility
   - are primarily located on cell surface and the extracellular matrix
   - are particularly abundant in extracellular spaces surrounding connective tissues such as cartilage, tendon, skin, and blood vessel walls
   - help lubricate cell joints
   - provide structural integrity to cells

2. Specific characteristics of selected Glycosaminoglycans:
   - Hyaluronic acid
     - very large (250-25,000 repeating disaccharides. Most other glycosaminoglycans contain 50-1000 repeating disaccharides)
     - forms mostly noncovalent aggregates with protein (most of other glycosaminoglycans and complex carbohydrates are covalently linked to protein)
   - Heparin
     - found primarily in intracellular granules of mast cells. Inhibits blood clotting (thus, its role is distinct from the other GAGs)

C. Proteoglycans are found on the cell surface and extracellular matrix. They are proteins to which linear GAG chains (up to 100 monosaccharide units) are covalently
attached through O-glycosidic bonds to Ser and Thr. These proteoglycan monomers generally are non-covalently associated with a hyaluronic acid core. The GAG chains remain separated from each other because of charge repulsion, giving them a "bottle brush" structure. Proteoglycans may also contain some N-linked oligosaccharides typical of those seen with glycoproteins near the end of the protein that is complexed with the hyaluronic acid core. Proteoglycans are often >90% carbohydrate by weight. The structure of a typical proteoglycan is shown at right.

For more information on glycosaminoglycans and proteoglycans, go to: http://web.indstate.edu/thcme/mwking/glycans.html

The clinical role of glycosaminoglycans and proteoglycans will be covered in the organ system blocks in the second year.

D. Glycoproteins are proteins which contain one or more oligosaccharide complexes covalently attached to the protein through either N-glycosidic linkages to Asn or O-glycosidic linkages to Ser or Thr. The oligosaccharide complexes attached to the glycoprotein are very different from the GAGs attached to the proteoglycans (see below). Glycoproteins also differ from proteoglycans in that they are generally <10% carbohydrate by weight.

Synthesis and Processing of Glycoproteins

The carbohydrate residues on glycoproteins are generally added to proteins in the endoplasmic reticulum (ER) and/or the Golgi. The proteins to which these oligosaccharides are attached have an N-terminal signal sequence that directs them to the ER. This mechanism is described in Dr. Burridge’s lecture on Membrane Biogenesis and is summarized in the figure on the right (the details of this process are not important for you to know). Click here for animation
1. Roles of Glycoproteins

- **Labeling of proteins to determine their cellular location** (see below)
- **Labeling of proteins to determine their half-life in the blood.** Most circulating glycoproteins have a terminal sialic acid (also called N-acetyl-neuraminic acid). Once the sialic acid residue has been removed by a cell surface neuraminidase, the glycoproteins are rapidly cleared from the blood by the liver.
- **Recognition of self vs foreign.** The role of surface glycoproteins in determining immune response will be discussed in much more detail when Immunology is covered in Block 4.
- **Cell adhesion.** The role of glycoproteins and proteoglycans in cell adhesion and cell-cell interactions will be discussed in more detail in Dr. Burridge’s lectures on “Cell-cell interactions” and “Extracellular matrix and integrins.” These molecules are involved in the phenomena of contact inhibition, whereby normal cells stop growing when they touch each other. The uncontrolled growth of cancer cells reflects their loss of contact inhibition.
- **Host-parasite interactions.** Viruses, bacteria, and parasites are identifiable in part because of their surface glycoproteins. However, they also often use surface glycoproteins on our cells as a site of attachment and entry. These issues will be discussed in Immunology, Virology, and Microbiology topics are covered in later blocks.
- **Blood group antigens** (see below)

2. **O-linked Glycoproteins:** The synthesis of O-linked glycoproteins occurs in stepwise addition of sugars in the Golgi. As an example, the ABO blood group type is determined by the oligosaccharide units attached to both secreted O-linked glycoproteins and to glycolipids on the surface of cells (see below). People with the O type blood group lack the enzymes that add either a terminal N-acetylgalactosamine (Type A) or a terminal galactose (Type B) to the oligosaccharide that is attached to their glycolipids and proteins. (more details in the glycolipids portion of this lecture)

For a figure showing the carbohydrate moieties corresponding to the ABO blood types go to [http://web.indstate.edu/thcme/mwking/protein-modifications.html](http://web.indstate.edu/thcme/mwking/protein-modifications.html)

3. **N-linked glycoproteins:** The synthesis of N-linked glycoproteins occurs via the addition of a single 14-residue oligosaccharide in the ER followed by stepwise processing of the oligosaccharide moiety as it passes through the Golgi. There are two major fates of the N-linked glycoproteins: if a phosphate group is added to terminal mannose residues in the cis-Golgi, no further processing occurs and the oligosaccharide is of the “high mannose” type. If no phosphate is added to the terminal mannose residues in the cis-Golgi, further processing occurs in the medial
and trans-Golgi and the resulting oligosaccharide is of the “complex” type. The overall process is shown below.

The addition of phosphate to the terminal mannose residues is a 2-step process, as shown below. You are not responsible for the enzyme name.

Glycoproteins that terminate in mannose 6-phosphate (e.g., have “high mannose” oligosaccharide moieties) bind to mannose 6-phosphate receptors on clathrin-coated vesicles that bud off from the Golgi and transport their contents to the lysosomes. This step is required for the delivery of lysosomal enzymes to the lysosomes (see figure on next page). Glycoproteins containing “complex” oligosaccharide moieties are generally destined to be either membrane-bound or secreted into the extracellular space. Genetic defects in enzymes involved in phosphorylating terminal mannose moieties result in abnormal targeting of newly synthesized glycoproteins; this rare disorder is known as I-cell disease (aka Mucolipidosis II). For additional details on this disorder, go to: http://genetics.accessmedicine.com/server-java/Arknoid/amed/mmbid/co_chapters/ch138/ch138_p01.html. For your information, this link is to the online version of “Metabolic and Molecular Bases of Inherited Disease,” an encyclopedic source for virtually all genetic disorders. It is accessible from the Health Sciences Library website (www.hsl.unc.edu) under “Books and Audiovisuals” – Find Electronic Books. Although more detailed than necessary for most of your needs this semester, it is probably the best source of information for this rare disease (there is no useful information on this disorder on UpToDate). Additional information is available.
Targeting of high-mannose glycoproteins to lysosomes via the mannose-P/mannose-P receptor pathway is described in more detail in Dr. Burridge’s “Membrane Biogenesis” lecture. It is summarized in the figure at right.

Key points on complex carbohydrates, glycosaminoglycans, and glycoproteins:

**Definitions:**
- **Glycosaminoglycans (mucopolysaccharides)** - carbohydrate groups attached to proteins in proteoglycans. They consist of long polymers of negatively charged disaccharides. This makes them slimy and viscous (relevant to their functions)
- **Proteoglycan** - mostly carbohydrate, with a little protein
- **Glycoprotein** - mostly protein with a little carbohydrate

**Functions of proteoglycans:**
- Compressibility (lubricants, shock-absorbers)
- Adhesion
- Structure
- Anticoagulant (heparin)
Functions of glycoproteins - carbohydrate moieties on glycoproteins involved in cellular localization (targeting)
- 1/2 in plasma
- cell surface phenomena
  - adhesion and other cell-cell interactions
  - immune recognition
  - binding sites for viruses and parasites
  - blood group antigens (glycolipids too)

Targeting of proteins to the endoplasmic reticulum
- role of SRP and signal sequence on nascent protein chain
- SRP receptor on ER; translation through pore in ER
- removal of SRP; removal of signal sequence

O-linked glycoproteins - stepwise addition of sugars in Golgi; blood group antigens are example

N-linked glycoproteins - synthesized in Golgi/ER system
- ER - core oligosaccharide added, some processing
- Golgi - additional processing
  - For lysosomal enzymes -
    - Pi added to terminal mannose
      - binds to mannose-P receptors and transported to lysosome
      - dissociation from receptors; removal of P
  - For secretory or cell-surface glycoproteins -
    - complex carbohydrate chain constructed in Golgi - NO mannose phosphorylation
      - (N-acetylglucosamine, mannose, galactose, and sialic acid units added)

I-cell disease - defective mannose phosphorylation results in mis-targeting of glycoproteins normally destined for lysosomes
Objectives for glycolipids: By the end of this lecture, you should:

a. know the general outline of the pathway for synthesis of the sphingolipids (recognize structures of sphingosine and ceramide and know which sugars characterize galactosylceramide and glucosylceramide (easy) and gangliosides

b. understand the similarities that define the group of inborn errors of metabolism classified as sphingolipidoses

c. know how localization of a sphingolipid, rate of turnover, and extent of a lysosomal enzyme defect interact to modulate the type and severity of disease

d. know the specific metabolic lesion associated with each disease covered (sadly, this is a favorite subject for MSLE Step 1 questions)

A. General points: Sphingolipids are a group of compound lipids; although not as quantitatively prominent as phospholipids, they are vital membrane components in many tissues. All cells contain some sphingolipid - sphingomyelin is a component of most plasma membranes, and also the source of free ceramide and sphingosine, components of important signaling cascades. Other sphingolipids are defined by the composition and sequence of sugars attached to the terminal alcohol group of ceramide (hence the term glycosphingolipids). Note that the common names for sphingolipids (e.g., cerebroside, sphingomyelin) actually define not a pure lipid, but a lipid class - heterogeneous with respect to the chain-length of attached fatty acids. Most sphingolipids are differentially distributed - they are present at different amounts, and are presumed to have different functions, in different cell types.

Just like proteins and other membrane components, all sphingolipids undergo metabolic turnover - they are synthesized, transported and deposited at their desired location, fulfill their function for some period of time, and then undergo metabolic turnover. This latter process involves internalization of membrane by endocytosis, transfer to lysosomes, and degradation by hydrolytic enzymes in lysosomes.

Sphingolipidoses: There are a number of “inborn errors of metabolism” in which the activity of an enzyme involved in degradation of a sphingolipid is greatly reduced or absent. The lipid just ahead of the metabolic block in the degradative pathway accumulates. Accumulation of this product in the cell may directly cause cell death, as well as the accumulation of phagocytic cells. These phagocytes in turn engulf...
and then try to digest the contents of the damaged cell; sometimes they themselves then “choke” on the accumulated product.

The rate and amount of accumulation of the metabolite prior to the metabolic block depends on how much the original glycosphingolipid is turning over. This in turn, depends on how much of the lipid is in the cell and how quickly it is being catabolized. A possible complication in elucidating the pathophysiology of such a disease includes the existence of minor pathways involved in degradation (a product of the minor pathway can be toxic). Sometimes the metabolic defect involves not in the enzyme itself, but rather a needed activating-factor protein. Individually, the inborn errors of sphingolipid metabolism are rare, yet collectively they are a clinical consideration. One reason is that there are, after all, many infants that fail to develop normally - and often a large number of possible disorders need to be considered and excluded. For more about symptoms, incidence, treatment, &c of these often largely neurological diseases, visit http://www.ninds.nih.gov/health_and_medical/disorders/ All sphingolipidoses are autosomal recessive, except for X-linked Farby’s Disease.

B. Biosynthesis of sphingolipids. Sphingolipids contain sphingosine. You are NOT responsible for the structure of these lipids. The backbone of sphingosine contains the 16 carbons of palmitic acid, as well as the amine group and 2 of the 3 carbons of the amino acid serine (the CO₂ of serine is displaced during the condensation). The basis of sphingolipid structure is actually ceramide, which is the N-acyl derivative of sphingosine. Addition of various sugars, which have been activated as nucleoside diphosphate sugars, results in formation of the various sphingolipids. Except for synthesis of galactosyl- and glucosyl-ceramides (cerebrosides) and sulfatides, details of the biosynthetic pathways are beyond the scope of this course.

C. Origin and structure of sphingosine and ceramide: The structure of sphingosine is shown above. Ceramide is also shown. In this example the fatty acid in the N-acyl position defines it as stearoyl-sphingosine. Other fatty acids could also be present (the fatty acid composition differs in different sphingolipids and in different cell types.)
D. **Ceramide distributes to different biosynthetic pathways:**
This depends on the cell type and lipid being synthesized. Important examples are illustrated - in outline in this figure and in more detail in later figures.

![Ceramide Distribution Diagram](image)

E. **Sphingolipids in myelinating cells.** In myelin-producing oligodendroglia (CNS) and Schwann cells (PNS), ceramide is made and rapidly converted to galactosylceramide (often trivially called cerebroside) by an enzyme, ceramide galactosyl-transferase; UDP-galactose donates the sugar. As mentioned earlier, galactosyceramide accounts for about a quarter of the lipid of myelin and therefore about 6% of brain weight. There is also present sulfatide (formed by sulfation of cerebroside at the 3'-OH of galactose), which accounts for some 5% of brain lipid. The presence of these galactolipids may account for some of the unusual structural features of the myelin membrane. Problems arise if the β-galactosidase involved in degradation of cerebroside is deficient. This disorder is called **Krabbe's disease.** It is also referenced as **globoid cell leukosystrophy** - in acknowledgment of the phagocytic cells that accumulate in white matter and attempt to digest the cerebroside. The pathophysiology involves not only accumulation of cerebroside but also production of an abnormal and highly toxic metabolite, galactosylphosphingosine (psychosine) that results when fatty acid is removed from cerebroside. This disorder is primarily of an infantile form.
Another disorder primarily involving myelinating cells is **metachromatic leukodystrophy** - a lack of **sulfatase** activity necessary for removal of sulfate from sulfatide. The accumulation of sulfatide leads, with certain dyes, to a colorful staining of autopsy histology sections - hence the name. This is a less severe disorder with a representation as juvenile or even adult forms. Although most cases are due to a defect in the sulfatase, some cases are due to a lesion in a required sulfatidase activator protein.

**F. Sphingolipids enriched in neurons:** Most glycosphingolipids actually have glucose (rather than galactose) linked directly to ceramide (different enzymes for synthesis). There is little or no free glucosylceramide as such; it is almost invariably present as part of more complex glycosphingolipids. In neurons these are usually gangliosides that are synthesized by stepwise addition of sugars from activated UDP-sugar donors. **Gangliosides** are defined as sphingolipids containing N-acetylneuraminic acid (NANA, aka sialic acid). The adjacent figure illustrates $G_{M1}$, a common ganglioside in brain, and inborn errors due to failure to degrade this lipid during normal metabolic turnover. There is a $G_{M1}$ gangliosidosis due to a defect in $\beta$-galactosidase (obviously not the same one causing Krabbe's disease), which is rare enough to probably not show up on any exam. Most people have heard of the next disease in line, **Tay-Sachs disease**, a defect in $\beta$-hexosaminidase (the enzyme removing NANA). There is accumulation of $G_{M2}$. Most cases are infantile. Patients with Tay-Sachs have a "cherry-red" spot in the back of their eyes. Again, a variant due to a defect in an activator protein is known. **Note that the above is a considerable oversimplification of the basics of Tay-Sachs disease, which is confounded by the presence of defects in different subunits of hexosaminidases, various activator proteins, &c.)** Look at [http://www.ntsad.org/index.htm](http://www.ntsad.org/index.htm) for more info on Tay-Sachs and related diseases like Fabry's and Gaucher's Ds (click on "The NTSAD Diseases Family" bar).

There are other disorders connected with degradation of gangliosides; these will not be discussed. Note, however, that far down the degradation pathway one arrives at glucosylceramide. A defect in $\beta$-glucosidase (**Gaucher's disease**) involves pathology in different organs; in some variants of the disease, neurological manifestations are due to pile up of glucosylceramide in neurons (see also later figure).
G. Sphingolipids and blood elements: All cells carry many antigens – and most cells have those corresponding to the ABO classification. These are particularly well-expressed in red blood cells, and so are an important consideration in transfusion (do you know your blood type? – you should). Anyway, these antigenic groupings correspond to minor variations in sugar composition of a sphingolipid on the surface of cells – see figure above. These antigens may also be expressed as the carbohydrate chain of a glycoprotein, which may be secreted into body fluids-determining secretor or non-secretor status). The red blood cells have a defined half-life and are then plucked out of the circulation and degraded. In Gaucher’s disease, this helps account for the pile-up of glucosylceramide in spleen, liver, bone marrow, and other locations.

H. Sphingolipids in other cells: Globoside, or more complicated lipids based on globoside, are widely distributed in many cell types. An inborn error of globoside catabolism (Fabry’s disease), due to a defect in an α-galactosidase, results in accumulation of a trihexoside (diagram) that leads to skin and kidney problems. As mentioned earlier, sphingomyelin is found in almost all cells, and is enriched in brain as a structural lipid. The biosynthesis of sphingomyelin follows an unusual pathway – the phosphocholine moiety is donated from phosphatidylcholine. As a side issue, note that although sphingomyelin and phosphatidylcholine seem very different lipids, they actually have a steric resemblance – they can both be drawn with a 3 carbon backbone and two long hydrophobic acyl residues buried in a membrane bilayer, with a polar phosphocholine group sticking out into the aqueous phase. Niemann-Pick disease is a serious infantile (type A) or adult (type B, less severe and with fewer neurological features) disorder involving a defect in sphingomyelinase activity and consequent accumulation of sphingomyelin.
As a confusing aside, in addition to use of the term Niemann-Pick in the context of glycosphingolipidoses, there is also a Niemann-Pick type C disease. This is a completely different disorder, a lesion in a protein that handles the intracellular processing of cholesterol as part of the process of LDL-receptor mediated endocytosis (lots more on this soon). Defective trafficking of intracellular cholesterol results in lysosomal accumulation of unesterified cholesterol, gangliosides, and other lipids. The prevalence of this childhood-onset neurodegenerative disease, characterized clinically by relentless neurological and intellectual deterioration, is about 1:150,000. Death inevitably occurs in early adolescence.

Mucopolysaccharidoses: These comprise another class of lysosomal storage diseases, characterized by defects in catabolism of carbohydrate chains on mucopolysaccharides (glycosaminoglycans). The general underlying pathophysiology is similar to that of the glycosphingolipidoses, and involves the abnormal accumulation of undegraded material in lysosomes. There are a number of diseases in this class, many characterized clinically by structural abnormalities and often severe mental retardation. This is relevant to the upcoming case conference on lysosomal storage diseases, and is only briefly mentioned here.
Finally, a little bit about **adrenoleukodystrophy**, so named because it is a progressive **demyelinating disease** associated with adrenal insufficiency. It is an X-linked recessive disease (Moms carry it and give it to half their sons) characterized by **accumulations of very long chain fatty acids (>22C) in brain** – cerebrosides, gangliosides, and sphingomyelin all have lots of these, accounting for the localization of pathology. Unlike most fatty acids, which are β-oxidized inside mitochondria, these VLCFA are normally degraded in peroxisomes, vastly underappreciated oxidative organelles. Accumulation of VLCFA is due to a defect in VLCFA~CoA activation, but the mutation is in an ABC transporter protein. The exact link between the ABC transporter protein and VLCFA~CoA synthase activation is unknown. Lorenzo Odone is the most famous ALD patient, and his parents are largely responsible for the movie “Lorenzo’s Oil” (which leaves a bad taste in many neurochemist’s mouths). If you wish to see Nick Nolte explain fatty acid biosynthesis to Susan Sarandon, using a chain of paper clips, just visit your local video store.

**Key Points for glycolipid metabolism**

**Sphingosine**, the backbone of sphingolipids (and an important component of signal transduction pathways) is synthesized from 16C **palmitic acid** and the amino acid, **serine**. The base molecule of sphingolipids is **ceramide**, the N-acyl derivative of sphingosine (fatty acid in an amide link with sphingosine). Addition of **sugars** to ceramide yields many different glycosphingolipids.

**Sphingolipidoses** result from defects in catabolism of **sphingolipids** (either the enzyme itself or required activator proteins); accumulation of undegraded sphingolipids, often in brain, leads to all kinds of problems. Most are autosomal recessive, except for X-linked Fabry’s Ds.

**Cerebroside** (galactosyl-ceramide) and **sulfatide** (sulfated cerebroside) are "myelin-specific" lipids. Defects in catabolism of cerebroside (**Krabbe’s Ds**: β-galactosidase) or sulfatide (**metachromatic leukodystrophy**: arylsulfatase A) result in their accumulation and destroy myelin.

**Gangliosides** contain glucosyl-ceramide and also all have N-acetyleneuraminic acid (NANA; aka sialic acid). Some are concentrated in neurons, and defective catabolism causes **Tay-Sachs Ds** (defect in β-hexosaminidase, the enzyme that removes NANA). **Gaucher’s Ds** results from a defect in β-glucosidase, the enzyme that takes the glucose off of glc-ceramide.

Glycosphingolipids are also important as **blood group antigens** on erythrocytes (**A**, **B**, & **O** blood types). They contain glc-ceramide and contribute to accumulation of this lipid in **Gaucher’s Ds**, especially in spleen.

**Fabry’s Ds** is a defect in degradation of **globoside**, a widely distributed glycosphingolipid. Accumulated undegraded globoside leads to kidney and skin problems.

**Sphingomyelin**, choline-P-ceramide, is enriched in **myelin**. Defects in sphingomyelin catabolism (sphingomyelinase; chops choline-P from sphingomyelin) results in **Niemann-Pick Disease**. Niemann-Pick **C Disease** involves a defect in intracellular trafficking of cholesterol.
Hunter’s and Hurler’s Syndromes are caused by defects in degradation of mucopolysaccharides such as heparin. Mucopolysaccharides accumulate in lysosomes and often lead to severe mental retardation and major deformities.