Review of Projections

1. What monosaccharide is this?
2. Which chiral center defines the stereochemistry?
3. How are left and right in Fischer projections related to up/down in Haworth?
4. How are up/down in Haworth related to axial/equitorial in stereochemical projections?

1. Where is the aglycone?
2. Where is the “reducing” end?
3. How many β-glycosidic linkages?
4. How many α-glycosidic linkages?
5. How many 3-substituted residues?
6. How many 6-substituted residues?
1. How many non-reducing termini?
2. How many glycosidic bonds?
3. Other than the NANA (N-acetylleuraminic acid, a type of sialic acid) residues, how many are α-glycosides?

**The "Primary" Sialic Acids (Salivic)**

N-acetyl-neuraminic acid (2-keto-5-acetamido-3,5-dideoxy-D-glycero-D-galacto-nonulosonic acid)

"Neu5Ac" “NANA”, “NeuAc”

KDN (2-keto-3-deoxy-D-glycero-D-galacto-nonulosonic acid)

KDO = an octulosonic acid,
KDN lacking C9 of side-chain (lipid A of LPS), not in animal cells

After Varki, A
Phylogenetic relationships of enzymes involved in the metabolism of sialic acids

HOMOLOGY

Angata and Varki
Chemical Reviews

After Varki, A

THE SIALIC ACIDS

-α-linkage to:
-α-lactamized or lactamized

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After Varki, A

Two Major Kinds of Sialic Acids in Mammalian Cells

N-ACETYLNEURAMINIC ACID (Neu5Ac)

N-GLYCOLYNEURAMINIC ACID (Neu5Gc)

After Varki, A
Glycans linked to lipids and lipid precursors

• Large O-linked Glycosaminoglycans and poly-lactosamine structures
• Glycoprotein N-linked and O-linked oligosaccharides
• Glycolipid oligosaccharides
Glycan synthesis in a cellular context

Overview
From ER through Trans-Golgi and points inbetween
On and into the ER

Dolichol-P-X
Glycosyl phosphatidylinositol (GPI)
Glycosphingolipids (GSL)

ER glycolipid synthesis

Dolichol-P-X
Glycosyl phosphatidylinositol (GPI)
Glycosphingolipids (GSL)
Minimal Defining Structure of a Glycosphingolipid

Glycan-O-Ceramide

Biosynthesis of Ceramide and Glucosylceramide
Major Classes of Glycosphingolipids

<table>
<thead>
<tr>
<th>Series</th>
<th>Designation</th>
<th>Core Structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lacto</td>
<td>(LcOSe4)</td>
<td>Galβ3GlcNAcβ3Galβ4Glcβ1Ceramide</td>
</tr>
<tr>
<td>Lactoneo</td>
<td>(LcnOSe4)</td>
<td>Galβ4GlcNAcβ3Galβ4Glcβ1Ceramide</td>
</tr>
<tr>
<td>Globo</td>
<td>(GbOSe4)</td>
<td>GalNAcβ3Galβ4Glcβ1Ceramide</td>
</tr>
<tr>
<td>Isoglobo</td>
<td>(GbiOSe4)</td>
<td>GalNAcβ3Galβ4Glcβ1Ceramide</td>
</tr>
<tr>
<td>Ganglio</td>
<td>(GgOSe4)</td>
<td>Galβ3GalNAcβ4Galβ4Glcβ1Ceramide</td>
</tr>
<tr>
<td>Muco</td>
<td>(MucOSe4)</td>
<td>Galβ3Galβ3Galβ4Glcβ1Ceramide</td>
</tr>
<tr>
<td>Gala</td>
<td>(GalOSe2)</td>
<td>Galβ4Galβ1Ceramide</td>
</tr>
<tr>
<td>Sulfatides</td>
<td></td>
<td>3-0-Sulfo-Galβ1Ceramide</td>
</tr>
</tbody>
</table>

Different Core structures generate unique shapes and are expressed in a cell-type specific manner

After Varki, A

Nomenclature Issues

Glycosphingolipid (GSL) = Glycan + Sphingolipid  
(named after the Egyptian Sphinx)

Glycosphingolipids often just referred to as “Glycolipids.”  
“Ganglioside”: a GSL with one or more sialic acid residues

Example of nomenclature:  
Galβ3GalNAcβ4(Neu5Acα3)Galβ4Glcβ1Cer = GM1 in the Svennerholm nomenclature  
OR  
II³Neu5Ac-GgOSe₂-Cer  
in the official IUPAC-IUB designation

After Varki, A
Neutral GSLs
- CMS
- CDS
- CTS
- Globoside
- asialo-GM1

Acidic GSLs (Gangliosides)
- GM1
- GD1a
- GD1b
- GT1

GSLs
- Asialo-GM1
- GM1
- GD1a
- GD1b

Gangliosides
- hiC3
- iPSCs
- hiC3 NC
- Neural crest cells
Pathways for Gangli-series Glycosphingolipid biosynthesis

• Ceramide is utilized for Ganglioside synthesis and for GalCer synthesis
• GM3 is a branching substrate for production of essentially all complex gangliosides
• Biosynthesis exhibits branch exclusivity (sialyltransferases cannot take a-series to b-series or b-series to c-series)
• Extension with neutral residues utilizes the same transferases regardless of series

Turnover and Degradation of Glycosphingolipids

• Internalized from plasma membrane via endocytosis
• Pass through endosomes (some remodelling possible?)
• Terminal degradation in lysosomes - stepwise reactions by specific enzymes.
• Some final steps involve cleavages close to the cell membrane, and require facilitation by specific sphingolipid activator proteins (SAPs, also known as “liftases”).
• Individual components, available for re-utilization in various pathways.
• At least some of glucosylceramide may remain intact and be recycled
• Human diseases in which specific enzymes or SAPs are genetically deficient (storage diseases)
Biological Roles of Glycosphingolipids

- Thought to be critical components of the epidermal (skin) permeability barrier (Glc-Cer delivers Cer to stratum corneum)
- Organizing role in cell membrane. Thought to associate with GPI anchors in the trans-Golgi, forming “rafts” which target to apical domains of polarized epithelial cells
- May also be in glycosphingolipid enriched domains (“GEMs”) which are associated with cytosolic oncogenes and signalling molecules
- Physical protection against hostile environments
- Binding sites for the adhesion of symbiont bacteria.
- Highly specific receptor targets for a variety of bacteria, toxins and viruses.

Biological Roles of Glycosphingolipids

- Specific association of certain glycosphingolipids with certain membrane receptors.
- Can mediate low-affinity but high specificity carbohydrate-carbohydrate interactions between different cell types.
- Targets for autoimmune antibodies in Guillian-Barre and Miller-Fisher syndromes following Campylobacter infections and in some patients with human myeloma
- Shed in large amounts by certain cancers - these are found to have a strong immunosuppressive effects, via as yet unknown mechanisms
Embryonic Lethal. Embryogenesis proceeded into gastrulation with differentiation into primitive germ layers and embryo patterning but abruptly halted by a major apoptotic process. Deficient embryonic stem cells able to form endodermal, mesodermal, and ectodermal derivatives but were strikingly deficient in ability to form well differentiated tissues. However, hematopoietic and neuronal differentiation could be induced in culture.

Consequences of Glucosylceramide Synthase gene disruption

Consequences of Lactosylceramide Synthase gene disruption

Early embryonic lethal, associated primarily with extra-embryonic tissues (trophoblast, extraembryonic membranes).
Enhanced sensitivity to insulin. Enhanced insulin receptor phosphorylation in skeletal muscle. Protection from high-fat diet-induced insulin resistance. Is GM3 ganglioside a negative regulator of insulin signaling?

Salt-and-Pepper (S&P) Syndrome

- Identified in 1 African-American family in the southeastern US at the Greenwood Genetic Center, Greenwood, SC
- Profound intellectual disability
- Failure to thrive
- Seizure disorder
- Neural crest defects: Midface hypoplasia, scattered dermal hyper- and hypopigmentation

Charles Schwartz, Greenwood Genetic Center
Allelic to a disorder described in Ohio and Pennsylvania Amish communities (caucasian)

Salt and Pepper Syndrome

Old Amish Infantile Epilepsy Syndrome

S&P Syndrome: missense mutation (E332K) in ST3GalV (GM3 Synthase), middle of S-motif
Amish Syndrome: truncation (R232X) in ST3GalV, between L- and S-motif

Pennsylvania Amish

Viable, fertile, normal life span. Sensory deficits especially related to pain sensation.

Consequences of SialylTransferase II (GD3 synthase) gene disruption

A Pennsylvania clinic working with Amish and Mennonite communities could be a model for personalized medicine.
Male Sterility. Late Onset Peripheral Nerve Demyelination possibly related to loss of ligands for Myelin Associated Glycoprotein (Siglec-4). Reduction in neural conduction velocity in some nerves. Compensatory increase in GM3 and GD3 in the brain.

Sheikh, KA, et al. (1999) PNAS 96, 7532
Sudden death phenotype. Extremely susceptible to induction of lethal seizures by loud sounds. Further characterization in progress.

Double KO: Mice Expressing only GM3 in the Brain

Back to the ER

Glycosyl phosphatidylinositol (GPI)
Examples of GPI-Anchored Proteins

**Cell surface hydrolases**
- alkaline phosphatase
- acetylcholinesterase
- 5’ nucleotidase

**Adhesion molecules**
- neural cell adhesion molecule
- heparan sulfate proteoglycan

**Protozoal antigens**
- trypanosome VSG
- leishmanial protease
- plasmodium antigens

**Mammalian antigens**
- carcinoembryonic antigen
- Thy-1

**Others**
- scrapie prion protein
- folate receptor
- decay accelerating factor

Structure of the Basic GPI Anchor

After Hart, G.
Structural Analysis of the GPI Anchor

Enzymatic and chemical cleavage sites are useful in identifying GPI anchored membrane proteins

GPI-biosynthetic pathway in mammalian cells

- Flip from cytoplasmic face to ER lumen after addition of GlcN to PI (equilibrium)
- Fixed on lumenal face by further acylation
- Sequential addition and modifications
- Transamidation reaction for linkage to protein prior to deacylation (different from T. brucei)
After Freeze, H

GPI anchors synthesized by enzyme complexes

Catalyzed transamidation reaction: Ethanolamine attacks peptide bond, C-term peptide is leaving group

After Freeze, H
Examples of C-Terminal Sequences Signaling the Addition of GPI-Anchors

<table>
<thead>
<tr>
<th>Protein</th>
<th>GPI signal sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetylcholinesterase (Torpedo)</td>
<td>NQFLPKLLNATA C DGELESSTSSSKGIIIFYVLSILYFY</td>
</tr>
<tr>
<td>Alkaline phosphatase (placenta)</td>
<td>TACDLAPPAGTT D AAHPGRSWPALLPLLAGTLLLETATAP</td>
</tr>
<tr>
<td>Decay accelerating factor</td>
<td>HETTPNKSGGTT S GTRRLSHTCGFTLTGLGLTVLMTT</td>
</tr>
<tr>
<td>PARP (T. Brucei)</td>
<td>EPEPEPEPEPEPEP G AATLKSVALPAIAAALVAAF</td>
</tr>
<tr>
<td>Prion protein (hamster)</td>
<td>QKESQAYYDGRR S SAVLFSSPPVILLISFLIFMLVG</td>
</tr>
<tr>
<td>Thy-1 (rat)</td>
<td>KTINVRDKLVK C GSISLILVQNTSWLLLLLSSLFLQATDFISI</td>
</tr>
<tr>
<td>Variant surface glycoprotein (T. Brucei)</td>
<td>ESNCKWENNACK D SSILVTKKFALTVVSAAFVALLF</td>
</tr>
</tbody>
</table>

Boldfaced amino acid is the site of attachment of the GPI. Sequence to the right of the space is cleaved from the protein by the transpeptidase upon anchor addition.
After Freeze, H

Paroxysmal Nocturnal Hemoglobinuria

- A hematopoietic stem cell disorder characterized by intravascular hemolytic anemia (sudden onset, intermittent/episodic). Abnormal blood cells lack GPI-anchored proteins due to a mutation in the PIG-A gene.
- Lack of GPI-anchored complement regulatory proteins, such as decay-accelerating factor (DAF) and CD59, results in complement-mediated hemolysis and hemoglobinuria.
Nocturnal hemoglobinuria, not clear why morning urine is enriched in hemoglobin breakdown products (variable course)

Morning Report, Toronto General Hospital; http://morningreporttgh.blogspot.com/2010/05/

GPI-linked proteins protect RBCs from complement-mediated lysis

Accumulation of hemoglobin breakdown products in kidney tubule eventually leads to tubule pathology


Back again to the ER

Dolichol-P-X
Topological model for the enzymatic reactions leading to Dol-P biosynthesis de novo on the cytoplasmic face of the ER

GlcNAc

Biosynthesis of N-Glycans:
Production of GlcNAc-P-P-Dolichol


Biosynthesis of the N-Glycan Precursor on the Cytosolic Leaflet of the Endoplasmic Reticulum (ER)


Biosynthesis of the N-Glycan Precursor on Lumenal Leaflet of ER

Completion of Biosynthesis of N-Glycan Precursor on Lumenal Leaflet of ER - and Transfer to Protein


Topological model for lipid intermediate synthesis, translocation and the role for Dol-P-P/Dol-P phosphatases in the recycling of Dol-P-P/Dol-P in the ER

**General Principles Regarding Lipid-Linked Glycans**

– Glycan synthesis is compartmentalized within cells
– Precursors begin as lipid-linked species on the cytoplasmic face of the ER, requiring that substrates be flipped for further processing
– Donor substrates contribute to more than one class of glycoconjugate
– Nucleotide sugar donors are used for cytoplasmic face extension and for Golgi extension; Dolichol-linked donors are used for ER extension of N-linked glycan precursors
– The assembly-line model for glycan extension in the Golgi apparatus may not be as applicable to glycolipid synthesis as it is to glycoprotein glycosylation
– Some precursors and intermediates in glycolipid synthesis influence signaling pathways
– Glycosphingolipids and GPI-anchored proteins associate in membrane microdomains (rafts, GEMs)