

Principle of Carbohydrate Analysis

BCMB 8020

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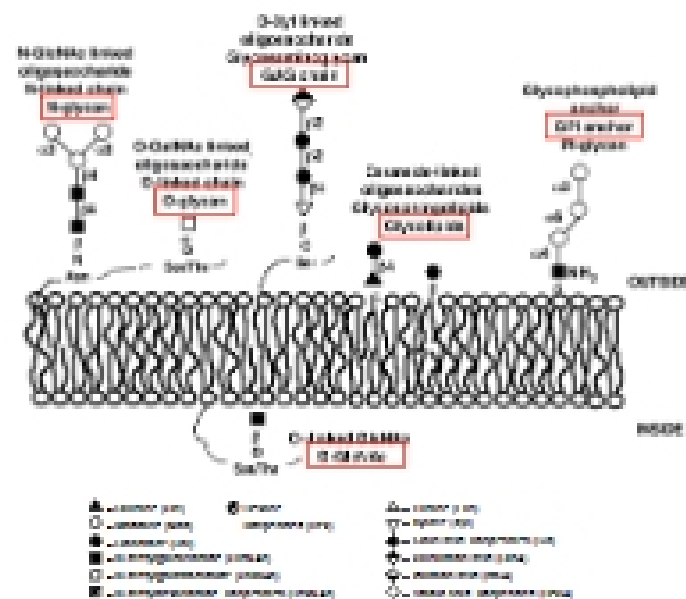
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Outline

- I. Class of carbohydrate (types of glycosylation)
- II. Why study carbohydrate
- III. Approaches for carbohydrate analysis
- IV. Sample paper

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I. Species of Carbohydrate Moieties



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II. Roles of Glycoprotein-associated Carbohydrates

1. Quality Control/folding.
(deglycosylation/reglycosylation)
- glycosyltransferase
2. Solubility
- Peroxidase
3. Circulating half-life
- Lutropin (LH), follicle-stimulating hormone (FSH)
4. Cell-cell interactions
- lymphocyte homing, cell growth, tumor metastasis

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Significance

Fully understanding the biology of glycoconjugates requires in-depth knowledge of the carbohydrate chains

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III. Experimental approaches for carbohydrate analysis

Table 1. Primary structural features of a complex carbohydrate

General description	Specific examples
Qualitative and quantitative composition, i.e., nature and number of constituent monosaccharides including absolute configuration (D- or L-) and ring size (pyranose [6] or furanose [5])	Gal, GlcNAc, Man, Fuc in ratio 3:4:3:1 D-Gal, D-GlcNAc, D-Man, L-Fuc Mang, GalF
Positions of glycosidic linkages	α , β
Sequence of monosaccharides, including occurrence of branchpoints (double or triple substitution of a monosaccharide)	Man (1-6)Man (1-3)Man (1-4)GlcNAc
Nature, number, and location of appended noncarbohydrate groups: phosphate, sulfate, acetate (peptide, lipid)	Ido3S6O3, Neu5Ac9OAc

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III. Experimental approaches for carbohydrate analysis

Table 2. Secondary structural features of a complex carbohydrate

Conformational aspect	Example
Precise ring conformation of each monosaccharide	Complete set of H-C-C-H dihedral angles
Orientation of monosaccharides with respect to each other	Torsional angles ϕ , ψ around glycosidic bonds and/or interatomic distances
Flexibility of the spatial structure	Dynamics parameters (rotational correlation times, order parameters)

- For most carbohydrates, the secondary and higher-order structures in solution are not readily defined, due to their inherent flexibility.
- The secondary and higher-order structure analysis of carbohydrates is not discussed in further detail in this lecture. Instead, the analytical methods used to determine composition and sequence of carbohydrates are discussed

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Carbohydrate Analysis Offers Unique Challenges

- Branched
- Synthesis is not "template driven"
- Alternative linkage positions are possible
- Alternative anomeric configurations are possible
- Cell-type specific glycosylation
- Influence of environmental conditions
[Glucose] [NH₂] pH
- Site-specific glycosylation
- Microheterogeneity

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III. General Considerations for Analyzing the Primary Structure of a Carbohydrate

- Due to enormous structural diversity of naturally occurring glycans, their structural analysis requires a flexible approach
- The choice of methodology and the final result expected are dictated by the amount and purity of carbohydrate material available

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III. General Considerations for Analyzing the Primary Structure of a Carbohydrate

Information is expected to obtain through serial carbohydrate analysis

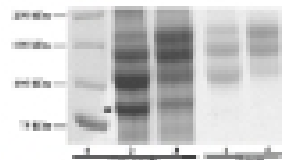
- Presence of carbohydrate moiety ?
- Class of carbohydrate ?
- Quantity and monosaccharide composition ?
- More detailed analysis: linkage and sequence ?

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IIIA. Presence of Carbohydrates ?

Chemical Reaction

Periodic acid-Schiff (PAS) reaction: Based on the susceptibility of sugars to periodate oxidation. Sensitivity: 5-10 ng of glycoprotein



PAS-PAGE analysis of the 3-layer glycoprotein of *C. albicans* (molecular weight 100,000 Da). Lane 1, molecular mass standards; lanes 2-4, 3-layer glycoprotein from continuous culture; lanes 5 and 6, 3-layer glycoprotein from batch culture; lanes 7 and 8, *C. albicans* plus staining; lanes 9 and 10, periodic acid-Schiff staining. Reimer, G. *et al.*, *J. Microbiol.* 2008

Radioactive labeling

- Proteins metabolically labeled with radioactive sugar precursors. After purification, the presence of carbohydrate moiety can be detected by radioactivity. Sometimes, the ability to label proteins with specific radioactive precursors may suggest the presence of specific types of glycoprotein or carbohydrate structures.
- Label endogenous glycan by radioactive sugar nucleotides by glycosyltransferases

Lectin binding

Lectins are carbohydrate-binding proteins. Sensitivity: 5-10 ng. Based on the specificity, the lectin binding assay may also facilitate to determine the type of carbohydrate

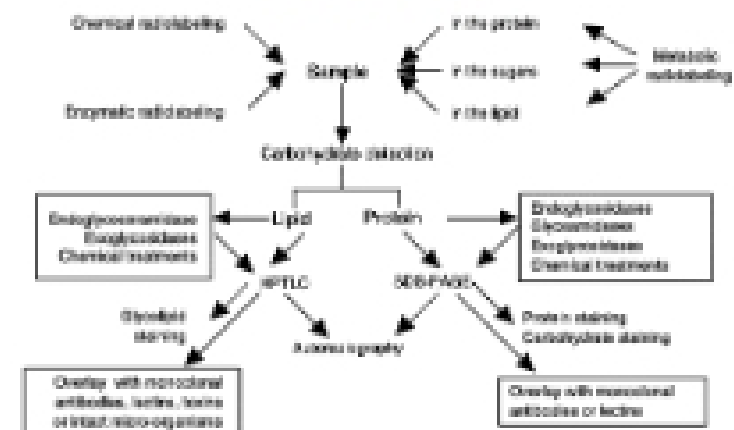
Enzyme digestion

Carbohydrate degrading enzymes

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IIIA. Presence of Carbohydrates ?

Basic Strategies for the detection of carbohydrates in glycoconjugates



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