



Glycomics

How are the structures of carbohydrates investigated?

Carbohydrate structure has been investigated for over a century. Yet by the mid-twentieth century, despite prodigious efforts, investigations of glycans lagged far behind those devoted to nucleic acids and proteins. Why? First, life scientists believed that carbohydrates act solely as structural materials or energy sources. Second, the technology required to analyze their structural complexities was yet to be developed. During the past 25 years, however, there has been a growing recognition of the informational role of carbohydrates in biological processes. Combined with the development of powerful chemical and physical techniques, this recognition has led to significant progress in carbohydrate structure determination.

Glycomics is the investigation of the structural and functional properties of all glycans in a cell type or organism. Their structural characterization begins with the release of oligosaccharides from purified glycoproteins or glycolipids. The oligosaccharides attached to proteins can be released by enzymes called glycosidases or by chemical methods such as hydrazinolysis and β -elimination. In hydrazinolysis, hydrazine (NH_2NH_2) is used to cleave glycosyl-amine linkages between a sugar residue and an asparagine residue. β -Elimination is the base-catalyzed nonhydrolytic cleavage of an *O*-glycan covalently attached to the hydroxyl group of a serine or threonine residue of a protein. Glycolipids are treated with enzymes called lipases that catalyze the removal of a lipid component. Once oligosaccharides are released from a glycoconjugate, they are separated by ion exchange, gel, or affinity chromatography (p. 173) and/or various electrophoresis methods. The monosaccharide composition of a purified oligosaccharide is then determined by acid hydrolysis, followed by the formation of volatile monosaccharide derivatives. These in turn are analyzed by high-temperature separation techniques known as gas-liquid chromatography (GLC) alone or GLC combined with mass spectrometry (GLC-MS). In GLC the volatile components of a mixture are separated by their relative tendencies to dissolve in a liquid impregnated stationary phase through which a mobile gas phase flows.

Determination of the original positions and the precise linkages of the monosaccharide components in an oligosaccharide

begins with a chemical technique called exhaustive methylation. In this method a methylating agent such as methyl iodide converts all of the free OH groups of the oligosaccharide to OCH_3 groups. After acid hydrolysis cleaves the glycosidic bonds, but not the methyl ether bonds, the methylated products are analyzed by GLC. Analysis reveals the position of the free OH group on each molecule, which marks the position of a glycosidic bond. Treatment of oligosaccharides with an exoglycosidase reveals the identity of terminal sugar residues from the nonreducing end.

Other techniques used to analyze oligosaccharide structure include MALDI MS (p. 176) and nuclear magnetic resonance (NMR). NMR is a type of spectroscopy in which atomic nuclei such as ^1H and ^{13}C that have magnetic properties are aligned with a strong magnetic field. Skilled biochemists determine the three-dimensional structures of carbohydrates and other biomolecules using short pulses of electromagnetic radiation. Analyzing its absorption reveals the identity of nuclei and their chemical environments.

In recent years *sugar chips*, also referred to as glycochips, have been developed to analyze the specific binding properties of glycan-binding proteins. A sugar chip is a type of microarray, a glass, plastic, or silicon chip to which hundreds or thousands of different oligosaccharides have been robotically printed. (Microarrays were originally developed to analyze gene expression profiles, see p. 680.) Each spot on the sugar chip contains one specific glycan. Sugar chips are used to characterize the specificity of glycan binding proteins (lectins from the surface of a particular cell type or a virus). After the addition of the sample glycan binding protein to each of the glycan spots on the chip, gentle rinsing can remove unbound sample proteins. The chip is then analyzed to determine which glycan binding proteins are still present. In one commonly used protocol, the sample proteins are initially linked to an antibody. After the rinsing step, a second antibody linked to a fluorescent molecule that binds to the first antibody is applied to the chip. The pattern of carbohydrate binding is determined by computer-assisted analysis. In this screening process, the presence and intensity of fluorescence or its absence indicate which glycans are ligands for the binding protein.

SUMMARY: Thanks to such technologies as GLC, MALDI MS, NMR, and glycochips, investigators are determining the structural and functional properties of the vast number of carbohydrates that occur in living organisms.

