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Review Article

The Third Alphabet of Life: Carbohydrate-Protein Interactions

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Abstract: The structural diversity of oligosaccharides found in glycoconjugates is enormous. This is due to the number of different ways in which sugar monomers may be linked to each other regarding linkage position, anomeric configuration, pyranosidic or furanosidic ring form and chain branching. It has been proposed that these factors contribute to the exquisite potential of oligosaccharides to establish a code system of biological information. The information contained in these structures is decoded by complementary sites present on carbohydrate binding proteins (lectins).

Key Words: Biological code, carbohydrate, lectin, differentiation, development

Hayatın Üçüncü Alfabesi: Karbonhidrat-Protein Etkileşimleri

Özet: Glikokonjugatların yapısında bulunan oligosakkaritler büyük yapısal farklılıklar gösterir. Bu farklılık şeker monomerlerinin aralarında oluşturdukları bağların çok değişken olmasından, anomerik konfirigasyon, piran ve furan halka yapıları ve dallanmalardan, kaynaklanır. Bu farklı yapı sayesinde oligosakkaritlerin biyolojik enformasyon içeren bir kod sistemi oluşturduğu kabul edilmektedir. Bu sistemde şifrelenmiş olan biyolojik bilgi, şekerlere spesifik proteinler (lektinler) tarafından tanınmaktadır.

Anahtar Sözcükler: Biyolojik kod, karbonhidrat, lektin, farklılaşma, gelişme

Introduction

For the first half of the 20th century proteins and carbohydrates were considered separate classes of natural products, and most chemists and biochemists had little doubt about which of the 2 deserved their attention. Such an attitude was based largely on the widely held belief that carbohydrates are dull compounds and that they serve only as structural or protective materials (e.g., cellulose in plants and chitin in insects) and as an energy source (glycogen in animals), but lack any biological specificity (1). However, there is increasing evidence, formulated over 20 years ago, that carbohydrates act as recognition determinants in a variety of physiological and pathological processes (2-11). This concept evolved with the realisation that carbohydrates have enormous potential for encoding biological information (12,13). The messages encoded in the structures of complex carbohydrates are deciphered through interactions with complementary sites on carbohydrate-binding proteins, chiefly lectins (14). Processes in which the participation of carbohydrate-lectin interactions was clearly demonstrated include intracellular trafficking of enzymes, clearance of glycoproteins from circular systems and a wide range of cell-cell interactions (15-19).

Oligosaccharides occurring in biological systems are often linked to lipids or proteins. The resultant glycoconjugates encompass glycoproteins (including proteoglycans), glycopeptides, peptidoglycans and glycolipids as well as lipopolysaccharides. Glycoconjugates are found inside the cells, both in the cytoplasm and subcellular organelles, and within cell membranes as well as in extracellular fluids and matrices (20). Furthermore, oligosaccharides are integral parts of many important classes of macromolecules, such as structural proteins, enzymes, transport proteins, hormones, immunoglobulins, cell adhesion molecules, toxins and lectins (20). The biological functions of these sugar chains may include merely structural effects by influencing and controlling the conformation and stability of a protein, but also the modulation of the functional activity of a protein, exposure of target structures for toxins, antibodies and micro-organisms, masking of such epitopes and the provision of ligands for specific binding events that mediate cell-cell or cell-matrix interactions, thereby ¨intervening in the social life of cells" (20-22).

Based on the nature of the linkage between their polypeptide chains and their oligosaccharide chains glycoproteins can be divided into 3 major classes: (1) O-glycans, involving the hydroxyl side chain of serine or threonine and a sugar such as GalNAc, (2) N-glycans, involving the amide nitrogen of asparagine and GlcNAc, and (3) GPI anchor (GPI-anchored or GPI-linked). Those linked to the carboxyterminal amino acid of a protein via a phosphorylethanolamine moiety are joined to an oligosaccharide, which in turn is linked via glucosamine to phosphotidylinositol (23-25). There are considerable differences in the number distribution of glycan chains along the polypeptide backbone according to the glycoprotein type. Generally N-linked glycoproteins contain a few oligosaccharide chains (1 to 5), which are separated by several amino acid residues, whereas O-linked glycoproteins contain a high number of chains in close proximity that form clusters (26). In addition, some glycoproteins contain both N-and O-linked oligosaccharides.

Similar to glycoproteins, glycolipids also represent a structural heterogeneous group of glycoconjugates found in all living organisms ranging from bacteria to humans. In animals and humans, glycosphingolipids prevail, in which ceramide provides the lipid anchor (27). The carbohydrate chains, usually containing glucose at their reducing termini, are linked via an O-glycosidic bond.

The sugar code

Proteins, nucleic acids and glycoconjugates (glycopoteins and glycolipids) are large molecules essential to all living cells. While nucleic acids and proteins are linear molecules in which the building blocks are joined together by identical bonds (phosphodiester and amide bonds, respectively), the glycan moieties of glycoconjugates are usually branched and the monosaccharide building blocks may be joined to one another in many different linkages. This has important implications for biosynthesis. In the case of proteins and nucleic acids every new molecule is copied from a pre-existing template molecule acting as a mould. Glycans cannot be copied from a mould and are manufactured on an assembly line in which individual components are incorporated sequentially. The potential number of all possible linear and branched isomers of small oligosaccharides has recently been calculated to be much larger than previous estimations. Seven structural elements lead to a large number of isomers, including multiple ring sites as points of glycosidic attachment, α/β anomerity, pyranose/furanose configuration and branching structure. For example, 3 different hexoses may be linked to each other to form over 1000 different trisaccharides (23,28-30).

Carbohydrate-protein interactions in development

Embryonic development is a carefully coordinated and complex process with a genetically determined time schedule in which considerable changes occur in cell differentiation-related properties such as growth, cell alignment, cell mobility and cell overlapping (2). The mechanism by which embryo cells migrate and orient themselves in a highly organised manner is among the crucial problems of the biology of development. Understanding the biological roles of the diverse oligosaccharides of glycoproteins, proteoglycans and glycolipids has been a major challenge in cell biology. Work with monoclonal and polyclonal antibodies has shown that there are great changes in the display of oligosaccharides at the surface of cells during embryonal development and differentiation (31-36). This suggested that such oligosaccharides may be important as ligands in macromolecular interactions that determine the way cells migrate or respond to various microenvironments. The recently gained access to transgenic animals with defined defects in N-linked glycosylation has provided experimental proof that complex-type oligosaccharides are indeed required for viability in embryogenesis (17).

In a number of studies (37-41) investigators have used specific oligosaccharide/lectin staining patterns in the definition of the dynamics of tissue development in various embryonic models. In all these studies, the oligosaccharide is viewed as a surface ligand that signals subsequent specific changes in cellular differentiation (i.e. the development of tissue/organ-specific function) through interaction with its specific lectin receptor.

Using a model of neurite outgrowth in the nasal cavity, Puche et al. (42) showed that galectin-1 was essential for appropriate topologically specific projections in the olfactory buds. This is evidence that this galactose-specific endogenous lectin orchestrates the spatial organisation of specific cellular subsets. The investigation by Shuler (43) illustrates the need for spatial and temporal integration of growth/differentiation functions in craniofacial development.

Carbohydrates in cancer

O- and N-glycan chains of glycoproteins, as well as the level and the spectrum of glycoprotein expression, may be altered in tumour cells. In general, N-glycans are often highly branched and sialylated in cancer, while O-glycans are often truncated and sialylated (44-46). Many of the tissue and blood group antigens that can be detected with antibodies have been found to change in cancer (47-50). The increased number of branches in tumour cells provides attachment sites for additional poly N-actetyllactosamine chains, sialic acid residues and other determinants that may function in cell adhesion or other biological processes and in the protection of the cancer cell surface, promoting survival in the blood stream and invasion of tissues (46,47).

Human colorectal polyps that tend to develop into carcinomas and apparently benign polyps may exhibit changed carbohydrate activities (51,52). For example, there may be an increase in sialyl-Le^x as well as an appearance of extended Le^y and Le^x antigens during the development to cancer and this may be correlated with the malignant potential of polyps (53,54). Colon cancer tissue produces glycoproteins and mucins with abnormal glycosylation patterns that may resemble foetal patterns (55,56). In addition, the genes encoding the peptide moieties of glycoproteins and mucins may undergo alterations of expression in colon cancer. These events may be related to malignant potential and development of the tumour; the mechanism inducing these changes is still not understood (47).

Recent studies have indicated that overexpression of galectin genes may occur in neoplastic cells and may be correlated with tumor progression (57,58). The expression of galectins on vascular endothelial cells suggests that they are involved in the adhesion and invasion of tumour cells. In vitro, antibodies against galectin-1 inhibit the adhesion of lymphoma cells to human microvascular endothelial cells (59).

Carbohydrate-mediated drug targeting

Targeted drug delivery involves the design and synthesis of carriers displaying ligands that mediate the binding of a drug/carrier complex to a receptor (60). Subsequent internalisation of the carrier/drug complex leads to accumulation of the drug in the target cells and exclusion from non-target cells that lack the requisite receptor (61,62). Site-specific drug or gene delivery to diseased cells may be accomplished utilising oligosaccharides that are recognised by membrane lectins (62-65). Examples include liver parenchyma asialo-receptors that recognise terminal galactose residues, Kuppfer and liver endothelial cells that recognise 4-sulphated GlcNAc residues and a variety of cells that express mannose-6-phosphate receptors. The drug carriers may be glycoproteins such as asialofetuin or lactosaminated albumin. It is presumed that the ligand-receptor complex will be actively endocytosed and the drug made available at cellular target sites (47). Targeted drug delivery needs to be a built-in mechanism that causes intracellular liberation of the drug from the carrier. To achieve this, drugs have been linked to carriers via acid-label linkers that are cleaved following endocytosis (66,67). Linkers that are stable in the circulation but are degraded by proteases present in lysosomes have also been developed (68-71).

Conclusion

Research on biological roles of glycoconjugates has made impressive progress in recent years. This has left footprints in virtually all fields of biology and medicine. Technical advances in oligosaccharide synthesis, purification and structural analysis, together with the steadily growing knowledge about endogenous binding partners such as lectins, have enabled research activities in the different areas of glycosciences to exert an obvious influence on various disciplines in basic and applied sciences. In view of ample documentation of carbohydrate expression in animal tissues it is no longer appropriate to consider trying to find out the biological roles of carbohydrates as a bashful search for any tenuous thread of evidence for a physiological relevance.

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