Review

Glycoproteins: Biologically Vital Macromolecules

[1]NASIR-UD-DIN, [2]EVELYNE WALKER-NASIR,
[1]M.A.K.MALGHANI AND [1]S.ALTAF HUSSAIN
[1]*Department of Chemistry, University of Baluchistan, Quetta, Pakistan*
[2]*Biogen, S.A. Geneva, Switzerland.*

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Amongst the glycoconjugates, the group of glycoproteins represent biologically abundant and clinically vital molecules. From immunoglobulines to hormones, membrane constituents of normal and transformed cells, as well as in the processes of reporduction, glycoproteins play a significant role.

Quite recently, it has been recognised that living organisms form a large number of biologically important polymers [1-6], in which proteins and carbohydrates are covalently linked and in which the sugar moiety has a significant function. In fact not long ago a concerted effort was applied to remove carbohydrates, utilizing even the most drastic alkaline conditions in order to obtain pure proteins. However, now it seems that most proteins are glycoproteins as are many polysaccharides, inlcuding starch and glycogen. Furthermore, there are sufficient suggestions that the sugar chains of glycoproteins contribute to important biological functions. These include biological recognition, such as specification of blood types [5], regulation of the half-life of glycoproteins in the circulatory system [7-14], stabilization protein conformation [6], protection of exposed tissue, bronchial, gastrointestinal and cervical [1], as well as glycoprotein uptake by cell [15]. The glycoprotein uptake by the cells [15] and the presence in the circulatory system of these macromolecules has opened possibilities for directing chemotherapy to specific sites in the body and for enzyme replacement therapy for the treatment of genetic disorders. Of particularly significance is the possibility that the carbohydrates of cell membrane glycoproteins play a dominant role in differentiation [16], growth [17] and intercellular recognition [18,19] and in many pathological processes including malignancy [20]. Glycoprotein may also function (Tamm Horsfall) to regulate circulatory activity of cytokines [21]. Carbohydrate moiety in this glycoprotein, (Uromodulin) is responsible for its biological activity [22]. Currently the progress rate in the biochemistry, molecular biology and biological aspects of glycoproteins is unfolding, and there is every suggestion that implications of these macro-molecules will gain in importance.

Glycoproteins exist in cells both in soluble and membrane bound forms in addition to being present in the intercellular matrix and in extracellular s ecretory fluids. Human serum is a rich source of glycoproteins where nearly all the proteins except two, albumin and proalbumin, contain sugars. The monosaccharides of common occurence in glycoproteins are hexoses, N-acetylhexosamines, uronic acids, a deoxyhexose and two pentoses. In addition, a complex monosaccharide derived from a nine carbon straight-chain sugar known as neuraminic acid is a common component of mammalian glycoproteins [1]. Neuraminic acid has been known to exist in man and primates as N-acetylneuraminic acid and in other animal species as N-glycolyneuraminic acid [23]. The hydroxyl groups of sugars in glycoproteins are substituted by other groups, particularly in secretory fluids, such as bronchial, intestinal and cervical mucuses, and in viral glycoproteins by sulfate groups [24], or in lysosomal hydrolases and yeast mannans, by phosphate groups [25,26].

A singificant feature of glycoproteins is the carbohydrates-protein linkage. Two distinct types of linkage dominate: an N-glycosidic linkage between the anomeric carbon of N-acetylglucosamine and nitrogen of the amide group asparagine and the other involving the O-glycosidic linkage between N-acetylgalactosamine, galactose and xylose of the carbohydrate chains to the hydroxyl groups serine, threonine, hydroxylysine, and hydroxyproline of the protein moeity (Fig.1). Of rare occurrence are the O-glycosidic linkage such as that between mannose and serine or between L-fucose and threonine. However, N-acetylglucosamine-asparagine contains the only known N-glycosidic linkage in the glycoproteins. A single glycoprotein such as immunoglobulin and glycoprotein hormones may contain more than one type

	N-Glycosyl Linkage			Source
	N-Acetylglucosamine		Asparagine	Serum glycoproteins, glycoprotein hormones, enzymes and cell-membrane glycoproteins
	O-Glycosyl Linkage			
1.	N-Acetylgalactosamine	⟶	Serine or Threonine	Mucins, Fetuin, Epiglycanin, Antifreeze glycoprotein
2.	Xylose	⟶	Serine or Threonine	Proteoglycans
3.	Fucose	⟶	Serine or Threonine	Normal human urine and rat tissue
4.	Galactose	⟶	Serine or Threonine	Cuticle collagen
5.	Mannose	⟶	Serine or Threonine	Yeast and Fungal glycoproteins
6.	Galactose	⟶	Hydroxylysine	Glomerular basement membrane and tropocollagen
7.	Galactose	⟶	Hydroxyproline	Plant cells
8.	Arabinose	⟶	Hydroxyproline	Potato lectin

Fig.1: Attachment of oligosaccharide to proteins.

of carbohydrate-protein linkage. More recently it has been recognized that O-glycosidically linked oligosaccharide present complexity by providing diversity of sugar linkages at the ultimate sugar residue linked to protein.

Monosaccharides are multi-functional organic compounds, usually with three or more free hydroxyl groups of rather similar chemical reactivity, in addition to a primary hydroxyl group of different chemical reactivities. Furthermore, there is the problem of anomery, since glycosidic linkages may be either α or β i.e., the oxygen atom on carbon number one can assume different planes of the sugar ring. Diverse reactivity of functional groups in a single sugar unit requires a meticulous design and the explicit execution of a complex series of reactions to introduce a carbohydrate chain in the glycoprotein. The synthesis of oligosaccharides in vivo required therefore, considerable enzyme specificity; in vitro it is a complex task, and there are few reports on such syntheses. However, a consequence of this structural complexity and variability is the capability of the sugars to serve as carriers of biological information. In contrast to peptides and oligonucleoitides, in which the information is based entirely on the number of different monomeric units, in oligosaccharides the information rests also in the position and anomeric configuration of the glycosidic unit and in the branching points. As a consequence sugar macromolecules can store substantially more information per unit weight than proteins and nucleic acids.

Protein moiety

The protein backbone of glycoproteins may have different peptide sequences although the carbohydrate

chains may show some similarity in structures. The biosynthesis of the protein moiety of the glycoproteins is analogous to that of nonglycosylated proteins, so that their structure is under regulation of the genetic code. As a consequence, all molecules of a given protein are identical. Glycosylation of proteins is not primarily regulated by genes, and the biosynthesis of glycoproteins is controlled by the transferases in the absence of template. Furthermore, the sugars may be added to peptide chains in vivo, nonenzymatically. The best example of this type of glycosylation is in haemoglobin A_{1c}, present in minute amounts in the red blood cells of humans.

Haemoglobin A_{1c} is formed by nonenzymic attachment of glucose to the αamino groups of the aminoterminal valine of the β-chain in haemoglobin A. Higher levels of haemoglobin A_{1c} and glycosylated serum albumin are present in diabetic patients. There is a strong suggestion that quantitation of these glycoproteins may be a better test than glucose analysis for evaluating carbohydrate metabolism in normal and diabetic individuals.

Glycoprotein structure

Recent advances in the knowledge of the structure and metabolism of glycoproteins and insight into the biosynthesis and function would be impossible without the development of new and sophisticated techniques for their isolation, purification and structural characterization. Essentially, the purification of glycoproteins is performed by methods commonly used in protein and polysaccharide chemistry such as gel filtration, ion-exchange chromatography, agarose and sodium dodecyl sulfate polyacrylamide gel electrophoresis and isoelectric focusing. Among the newer techniques now more commonly utilized is the purification of glycoproteins by the use of lectins i.e. affinity chromatography. Lectins are a group of sugar-specific proteins of nonimmune origin that are common in plant but are also found in microorganisms and animals. These proteins bind to specific sugars, essentially with specific structure, and hence can be effectively used to purify a mixture of glycoproteins. In addition, this binding property imparts an important functional behaviour of lectins, i.e., agglutination of cells which have glycoproteins and glycolipids on the cell surface. Amongst the commonest lectins are concanavalin A (specific for αmannose and α-glucose), peanut lectin (specific for galactose), wheat germ agglutinin (specific for β-N-acetylglucosamine and N-acetylneuraminic acid), and soybean agglutinin (specific for N-acetylgalactosamine). Because the glycoproteins bind non-covalently to either solubilized or immobilized lectins, the complexes thus formed can be dissociated by adding the sugar for which the lectin is specific. Due to this behaviour a pure glycoprotein can be isolated from a crude biological extract, or a mixture of glycoproteins that differ in sugar composition or in the structure of the carbohydrate moiety can be resolved.

Largely glycoproteins contain several monosaccharides and different sugar chains. Because of microheterogeneity there are variations in the number and type of carbohydrate units attached to the protein backbone of glycoproteins. Due to the multifunctional character of the carbohydrate chains as well as protein moiety, the macromolecules assume a distinct chemical behaviour. In addition, the capability of these macromolecules to form aggregated structures due to intra- and intermolecular activity and the physical diversity in behaviour makes their handling and the study of their physical properties difficult. Therefore, due to the complexity of these organic

Table-1:

Few natural glycoproteins and their origin

Glycoprotein	Source	Size (Molecular weight)	Carbohydrate (Content percent)
Serum glycoproteins			
IgG immunoglobulin	Human	150,000	10
Thyroglobulin	Calf	670,000	8
Prothrombin	Human	72,000	8
Hormones			
Human chorionic gonadotropin	Urine	38,000	31
Erythropoietin	Urine	34,000	29
Membrane constituents			
Human glycophorin	Erythrocytes	31,000	61
Bovine rhodopsin	Retina	40,000	7
Enzymes			
Alkaline phosphatase	Mouse liver	130,000	18
Bromelain	Pineapple	33,000	36
Other			
Human interferon	Leucocytes	26,000	26

molecules the characterization of glycoproteins , is a difficult task. The nature of the carbohydrate units and the microheterogeneity present in the glycoproteins permit, with great difficulty, structural elucidation on an intact molecule. Traditionally, the glycopeptides isolated from proteolytic digests of a glycoprotein or β-eliminatedborohydride reduced olgiosaccharides are the starting materials for structural studied of carbohydrates. For the study of the protein moiety various reactions are used depending upon the amount of carbohydrate in the glycoprotein.

There is, however, still continuous progress being made in developing microanalytical techniques, since most glycoproteins of value are available in homogeneous form in small quantities. However, refinement of carbohydrate and amino acid methodology and the development of new techniques have almost made it possible to do a reasonably complete structural study in a short time on as little as 0.5 mg of material. The easier structural feature in the glycoproteins to indentify is the carbohydrate peptide linkage, since the various linkages have different stabilities towards acid and alkali. The N-glycosidic linkage is relatively stable to mild acid but is hydrolyzed under stronger conditions. The O-glycosidic linkages vary markedly in their sensitivity to alkali. The galactosylhydroxylysine linkage is highly stable whereas the O-glycosidic linkage to serine and threonine is readily cleaved by alkali. The common procedure in the structural investigation of glycoproteins is the elimination of carbohydrate chains from the proteins by mild alkaline treatment in the presence of sodium borohydride. Under these conditions the glycosidic

bonds are cleaved, and serine and threonine residues are converted into alanine and α-aminobutyric acid respectively, whereas the sugars involved in the glycosidic linkage are converted into the corresponding sugar alcohols. The sugar commonly involved in such type of linkage in the secretory glycoproteins is N-acetyl-galactosaminitol. Quantitative assay in the loss of serine and theornine, increase in the amounts of alanine and α-aminobutyric acid and indentification of the sugar alcohol formed provide information as to the nature of the carbohydrate-peptide linkage. In addition it also suggests an approximate number of O-glycosidically linked residue in the glycoprotein.

For the structural analysis of carbohydrates the information of importance pertains to the sequence and linkage of sugar residues. Methylation analysis [27,28], which involves the substitution of free hydroxyl groups by methoxyl groups, followed by acid hydrolysis [29,30], is the most important and frequently used method in the structural investigations of complex carbohydrates. The partially methylated sugar, after reduction with sodium borohydride and acetylation with acetic anhydride, are analyzed by gas-liquid chromatography - mass spectrometry [31-36]. In order to avoid possible mass symmetries in the alditol derivatives, it is useful to use borodeuteride instead of borohydride [37] for the reduction. Also oximes [37] and aldonitriles [38-40] have been used to avoid mass symmetries. The position of O-methyl and O-acetyl groups in these derivatives can be ascertained from the specific fragmentation observed in the highly specific electron impact mass spectra. The sequence of sugars in the oligomers and their anomeric configuration is best analyzed by the use of glycosidases. Glycosidases are now available in highly purified form, and they display strict stereochemical and in some cases linkage specificity [41,42]. Furthermore, information on the anomeric linkages as well as on the sequence of sugars and their linkage by high-resolution protein nuclear magnetic resonance (NMR) spectroscopy has been in recent years a subject of extensive studies. Beyond doubt NMR spectroscopy of carbohydrate chains in the glycoproteins, secretory as well as of serum origin, has added a new dimension in the study of structure-function relationship with the combined application of high performance liquid chromatography and 500 MHz NMR spectroscopy, a number of oligosaccharide structures have been characterized which otherwise could not be identified [43-47].

The protein moiety of glycoprotein, as described earlier, is synthesized by the same mechanism that produces nonglycosylated proteins. Synthesis of the protein takes place on polyribosomes, although initiated on free ribosomes. Attachment of the free ribosome to the membrane of the endoplasmic reticulum is initiated by a signal sequence of the nascent peptide chain. Addition of sugar to proteins is a cotranslational event, involving alternation of amino acids already in existence in the polypeptide. Glycosyltransferases catalyze the stepwise synthesis of both O-linked and N-linked oligosaccharides. The enzymes that transfer sugars from their nucleotide and catalyze the transfer to sugars to the proteins [48] in the case of O-linked oligossachardes begins with the transfer of N-acetyl-galactosamine to the hydroxyl group of threonine or serine. Biosynthesis of the oligosaccharides sequence in the glycoproteins is regulated by the availability of transferases, and is, therefore, achieved without the involvement of RNA or DNA. The sequence is, however, indirectly regulated by the transferases, which are the primary gene products. In

Table-2: Lectin sources and some of their characteristics

No.	Lectin source	Molecular weight	Blood group specificity	Carbohydrate specificity
1.	Peanut	110,000	Neuraminidase-digested A, B, O or T antigen	β-D-Galp-(1→3)-D-GalNAcp
2.	Jack Bean (concanavalin A)	104,000	Non specific	α-D-Manp>α-D-Glcp>α-D-GlcNAcp
3.	Horse Gram A	113,000	A >>Az	α-D-GalNAcp
	B	109,000		
4.	Soybean	120,000 - 122,000	A >>0 B	α-D-GalNAcp, β-D-GalNAcp
5.	Lentil	42,000 - 63,000	Non specific	α-D-Manp>α -D-Glcp, α-D-GlcNacp
6.	Asparagus pea A	120,000	0 A	α-L-Fucp, 2-O-Me-D-Fucp
	B	58,000		
	C	117,000		
7.	Red Kidney bean	126,000 - 136,000	Non specific	
8.	Pea	49,000 - 53,000	Non specific	α-D-Manp>α-D-Glcp α-D-GlcNac
9.	Castor bean	120,000	Non specific	β-D-Galp>α-D-Galp
10.	Potato	100,00	Non specific	β-D-GalNacp-(1→4)-[β-D-GlcNAcp-(1→4)] β-D-GlcNAc
11.	Wheat gum	36,000	Non specific	β-D-GleNAcp(1→4)β-D-GlcNAcp-(1→4)-β-D-GlcNAcp>β-D-GlcNAcp-(1→4)-β-D-GlcNAc; NANA

serum type glycoproteins containing an N-glycosyl linkage between asparagine and N-acetylglucosamine lipid linked oligosaccharide precursors are formed in animals [49], yeast and plants for the enbloc transfer to the amide group of an asparaginyl residue that is part of the sequence Asn-X-Ser/Thr in a growing peptide. The transfer of the oligosaccharide from the lipid-linked oligosaccharide to the growing peptide probably takes place on the inner side of the endoplasmic reticulum, and the completion of the complex oligosaccharide units occurs in the Golgi apparatus, a sub- cellular organelle that consists of membrane sacs. A recent development of significant consequence was the discovery of glucose residues in N-glycosylated glycoproteins, since this sugar had not been encountered before. It is now established that the glucose residues in the glycoprotein are present as a means to introduce further sugar residues and not as final components of the macromolecule.

Glycoconjugates in pathogenesis

Since biologically important glycoproteins are very widely distributed in nature and all cell membranes are coated with sugar-containing molecules it is evident that the contirbution of the sugar must be significant. The readers are referred to reference t for a comprehensive review of functions of the sugar moiety in glyco-conjugates.

The surface of mammalian cells posesses a variety of antigentic molecules, that may characterize the cell with regard to individual [50,51], and tissue [52] of origin. Definite antigens at the cell surface may reflect the stage of embryonic development [53], and antigens may develop from envi-

ronmental factors i.e., exposure to viruses [54], chemicals [55], or radiation [56]. Neoplasia can result from these factors, and the cell surface of neoplastic cells possesses antigens which have arisen from these environmental factors [57-58] or they may possess suppressed embryonic or fetal antigens [59]. These molecules, antigens, known as tumor associated or tumor specific antigens, can not usually be identified at the surface of normal cells similar to those from which the neoplasm was derived.

In the past decade, attempt to correlate transformation properties with macromolecular structure at the cell surface have not been conclusive. Some researchers have reported a correlation between metastatic potential and low concentrations of cell surface glycoproteins in mammary tumor cells, whereas others have observed large quantities of high-molecular weight, endogenous O-glycosylated glycoproteins in metastatic carcinoma cells grown in vitro [60-62]. There have been different reports concerning cancer-related cell surface carbohydrates and invasiveness. An increased degree of branching and sialic acid density in surface carbohydrates from tumor cells is generally observed. Studies in human malignancies as well as in a wide variety of cell systems have revealed that the presence of cancer-related surface carbohydrates correlates with the manifestation of the malignant phenotype of cells rather than with the transformed mode of growth in vivo or in vitro [63-65]. As the intrinsic factor of malignant cells is to invade neighbouring tissue it is expected that alterations in the surface carbohydrates may be responsible for difficient tissue control leading to invasion. Recently a report using an organ culture model [66] has been recorded [67]. Oncogenes and oncoviruses are known, their influence on the alteration as well as transformation of cell-surface glycoproteins is yet to be clearly defined, however. Among other glycoconjugates, heparin, a polymer of 2-acetamido-2-deoxy-D-glucose, glucouronic acid and idouronic acid residues linked to a protein through a linkage region of $(Gal)_2$-xyl, a trisaccharide, linked to a serine residue to the protein presents a delicate case of interaction with protein. Heparin is synthesized and stored in mast cells in close vicinity of blood vessels and released from mast cells when desired. The blood anticogulant activity of heparin rests on its ability to interact with high affinity to antithrombin, a plasma protein that inhibits the proteases participating in the coagulation mechanism. The main antithrombin (AT) binding region in heparin is contained in the octasaccharide (Fig. 2). The vital and shortest oligosaccharide sequence in heparin, able to bind to antithrombin and to selectively reinforce its activity towards blood coagulation factor Xa, was at most of the size of a pentasacc-

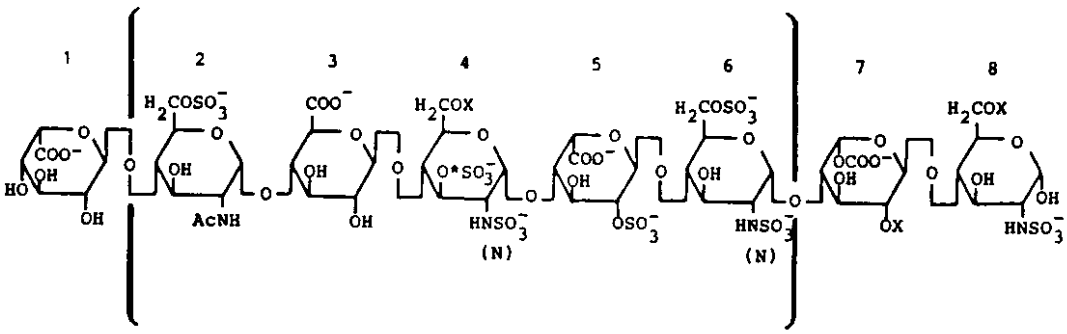

Fig.2: Structure of the AT-binding Octasaccharide.

haride [88,71], units 2-6 within brackets. There are structural variants within the pentasaccharide, the actual AT-binding unit, and these are indicated by X (H or SO_3, Fig. 2) or by the sugar unit, glucouronic acid or idouronic acid. The 3-O-sulfate group of sugar unit 4, marked by an asterisk, is unique to the AT-binding region of the heparin molecule. In addition, each of the sulfate group marked by (N) in Fig. 2 is essential to the high affinity binding of antithrombin. Thrombosis, i.e., the formation of clots inside the blood vessels, could occur as a result of transformed binding sites or disfunction of the heparin-antithrombin complex. Transformation or metastatic potential may be caused by a variety of mechanism, however. There exists scope of more intensive investigations in this area to define the exact mechanism of cell-transformation.

A variety of disorders are generated by disfunction of glyco-conjugates and particularly glycoproteins; only two i.e., neoplasia and thrombosis have been considered in this article. The precedence of these disorders over others is given in this review because of the preeminence of the pathology of these vitally significant disorders.

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