Glycoproteins in Disease

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ABSTRACT

The class of carbohydrate macromolecules known as glycoproteins exhibits great structural diversity. Their key role in the function of biological systems includes determination of cell membrane structure and function as well as antigenic determinants. In the recent decade, the detection of inborn errors of metabolism of these macromolecules as well as changes in their composition following mutagenesis and oncogenesis have highlighted the key role they play in the biology of man. The application of analytic techniques for their quantitation and structural characterization has resulted in their increasing utility in diagnostic medicine. They may be used not only as markers of active and inactive disease, but they also have become important in the diagnosis of congenital defects and tumors.

Introduction

Glycoproteins are heteropolysaccharides, that is, they are protein macromolecules with carbohydrate prosthetic groups. The chemical properties and functions of the carbohydrate moiety are intimately related to the protein structure. The carbohydrate component captures and entrains water in the vicinity of the glycoprotein and has functional significance in terms of its solubility and the movement of small molecules. The carbohydrate component contains galactose, mannose, glucose, N-acetylglucosamine, sialic acid, fucose and/or xylose and may constitute one to eight percent of their total weight.

By the application of techniques such as digestion with proteolytic enzymes, fractionation by gel filtration, ion exchange and thin layer chromatography, small glycopeptides have been isolated and their chemical structure established. The glycopeptide linkage regions consist of glycoside bonds; N-glycoside linkages of N-acetylglucosamine to asparagine; O-glycoside bond linkage of the β-hydroxyl of serine or threonine to the sugar. The O-glycoside bond is sensitive to mild alkaline hydrolysis and the liberated carbohydrate is reduced with sodium borohydride yielding a sugar alcohol. Compositional analysis involves definition of the carbohydrate sequence, identification of the enantiomeric configuration (alpha or beta linkage) and definition of the sugar hydroxyl groups involved in the glycosidic linkage.
Analytic techniques include (1) degradation of sugars from the non-reducing end of the oligosaccharide (exoglycosidases such as neuraminidase, β-galactosidase, β-N-acetylgalcosaminidase, α-mannosidase and others) (2) specific endoglycosidases and (3) acid hydrolysis, hydrazinolysis or acetolysis. Periodate oxidation and/or methylation has been employed for the chemical production of substituted hydroxyl groups on the sugars prior to fragmentation.

Glycopeptides linked by the β-hydroxyl group of serine or threonine to N-acetylgalactosamine include human IgA, the β-subunit of human chorionic gonadotropin, fetuin, human red blood cell membrane sialoglycoprotein as well as human gastric mucin and the core region of human blood group substances. The N-acetylgalactosaminoyl-asparagine linkage is found in two types of oligosaccharides: simple oligosaccharides containing mannose and N-acetylgalactosamine and complex oligosaccharides containing these two carbohydrates moieties and sialic acid, galactose and fucose. Single glycopeptides may contain both linkages as exemplified by thyroglobulin and human serum IgM. Additionally, glycoproteins may contain both complex N-glycoside and O-glycoside linked chains, i.e., human IgA and red blood cell membrane sialoglycoprotein.

One other structural feature of glycoproteins merits comment, e.g., the presence of sialic acid. Sialic acid is usually attached by its carboxyl end and is found at the terminal non-reducing end of the carbohydrate moiety. Three sialic acid derivatives are found in human glycoproteins. These are N-acetylenuraminic acid, N,O-diacytlenuraminic acid and N-glycolylneuraminic acid. The chemical characteristics of sialic acid and its location in the glycoprotein complex have suggested four potential functions.

First, with regard to blood group substances, submaxillary secretions, urinary mucoproteins, glycolipids, sialic acid appears to be vital for hemagglutination by influenza virus. Viral attachment to the erythrocyte is dependent upon the complimentary configuration in the virus to neuraminic acid (the virus contains neuraminidase). Second, sialic acid is chemically functional in determining viscosity. Third, it is necessary for the biological activity of several hormones (i.e., gonadotropins). Fourth, sialic acid is a structural requisite for the biological function of Castles intrinsic factor.

Brief mention should be made of the structural characteristics of the blood group substances type A, B and O. These glycoproteins exist in insoluble form on the erythrocyte surface, and in water soluble form in saliva, gastric juice, ovarian cyst fluid, semen and other secretions. Approximately 75 percent of the normal population are secretors for the A, B and O blood groups in biologic fluids. The remaining 25 percent of the population, non-secretors, secrete a glycoprotein known as the Le" glycoprotein. Blood group glycoproteins are not found in association with nervous tissue, epithelium, skin appendages, bone and cartilage.

**Plasma Glycoproteins**

The plasma glycoproteins normally exist in the precipitable plasma protein fraction. In 100 ml of serum there are approximately 120 mg of hexose, 80 mg of hexosamine, 60 mg of sialic acid and 90 mg of fucose. In general, the plasma glycoprotein fraction contains mannose and galactose in an approximate 1:1 relationship. Gamma globulins and, to a slightly lesser extent, beta and gamma globulins are particularly rich in carbohydrate. The monosaccharides in the various globulin fractions tend to be relatively similar; however, the gamma globulin fraction is enriched in fucose and contains a mannose to galactose ratio of three.
Ten percent of the total hexose in plasma is contained in the seromucoid fraction. The major component is the α-l-acid globulin which contains at least six fractions on cellulose chromatography. This glycoprotein has a higher water solubility and heat stability, a low isoelectric point, a molecular weight of approximately 40 to 45 × 10³ daltons and contains 40 percent carbohydrate. It contains five to eight polysaccharide units linked to the protein core with each saccharide unit consisting of approximately eight to 15 monosaccharide units. A common monosaccharide sequence found in the seromucoid fraction is the sialylgalactosyl-glucoaminoylmannose region. The carbohydrate-protein linkage is alkaline resistant indicative of an N-glycosidic bond between N-acetyl-glucosamine and the β-amide of asparagine.

Two metal precipitable glycoprotein fractions are present in plasma. They are the zinc α-2-glycoprotein and the barium α-2-glycoprotein. These have molecular weights of 41 and 49 × 10³ daltons, respectively, and contain approximately 16 to 18 percent total carbohydrate.

Another major plasma glycoprotein is haptoglobin,—the hemoglobin binding glycoprotein which migrates electrophoretically in the α-2-globulin region. This fraction has well defined genetic variants that are of significance in clinical medicine. The β-1-globulin fraction contains several well-defined glycoproteins including the β-1C and β-1A globulins which function in the serum complement complex. The major iron binding plasma glycoprotein, transferrin, resides in this fraction. This glycoprotein is synthesized in the liver and functions in the transport of iron from sites of absorption and hemolysis to sites of hemoglobin synthesis. It is also important in the secondary defense mechanism against infection. A variety of molecular genetic variants has been described.

The plasma α-1-macroglobulin consists of a mixture of glycoproteins containing about 10 percent of their molecular weight as carbohydrate and a galactose-mannose ratio of 1:2. The major defense system of humoral immunity, the immunoglobulins, are all glycoproteins and contain approximately 1.5 to 2 percent carbohydrate.

Two major components of the coagulation system, namely prothrombin and fibrinogen are glycoprotein macromolecules. In the conversion of prothrombin to thrombin, the major chemical change is the release of the carbohydrate moiety. In addition to these major plasma glycoproteins, a variety of circulating hormones are glycoproteins. These include hormones such as thyroglobulin, interstitial cell stimulating hormone, follicle stimulating hormone, human chorionic gonadotropin, thyroid stimulating hormone and erythropoietin.

This vast diversity of molecular species has resulted in the inability to define a unifying concept for the role of glycoprotein. Clearly, these macromolecules subserve functions such as enzymes, hormones, lubricants, metal transporters and clotting agents, to mention simply a few. Possibly a common function relates to the mechanisms involved in the cellular secretion of glycoproteins. Other roles of the carbohydrate fraction are the determination of molecular tertiary structure and the degree of hydration of the macromolecule. In layered membranes the carbohydrate fraction of glycoproteins clearly determines the density of packing and porosity of membranes as well as active transport of certain electrolytes. As mentioned previously, the sialic acid component of the saccharide residue appears to be functional in determining viscosity properties, antigenic specificity of blood groups, stability of the molecule and resistance to degradation.
In clinical situations, a rise in serum glycoproteins is observed in response to stress which is mediated by glucocorticoids. Administration of cortisol to the experimental animal or human results in an elevation of glycoproteins equivalent to that seen in stress reactions. The mechanisms of this response is unclear. Additionally, a rise in the glycoprotein level in plasma is associated with renal damage. This phenomenon is associated with enhancement of glucosamine incorporation into the glycoproteins, presumably as a result of failure of production of the normal renal inhibitor of glycoprotein synthesis.

In certain chronic diseases, elevated serum glycoproteins are also seen,—a rise in the α-1-acid glycoprotein fraction with decreased sialic acid content. Following surgical injury, elevations of α-1-acid glycoprotein, haptoglobin, fibrinogen, C-reactive protein and ceruloplasmin are seen. In the stress reaction to surgical injury, no elevation of the α-2 macroglobulin fraction is observed. This is in contrast to the elevated glycoprotein response seen following physical exercise. All plasma glycoprotein fractions are increased and elevated levels of α-2 macroglobulin, transferrin and α-1 antitrypsin are noted.

In association with either pregnancy or the use of contraceptive steroids, a rise in fibrinogen, ceruloplasmin, transferrin and some α-globulins occurs. A degree of specificity is observed since a decrease is seen in the α-1-acid glycoprotein fraction. This decrease is predictable since progesterone and other steroids normally bind this glycoprotein and reduce their biological activity.

Glycoproteins function as acute reactants manifesting quantitative plasma alterations in association with multiple stressful conditions. The most exciting and recent application of quantitative glycoprotein assessment is the application of radioimmunoassay and electrophoretic methods in three clinical areas involving the measurement of α-fetoprotein (AFP), α-1 antitrypsin (A,T), carcinoembryonic antigen (CEA) and tumor antigens.

**Alpha-Fetoprotein**

α-Fetoprotein is a fetal glycoprotein synthesized and secreted by the fetal liver, gastrointestinal tract and yolk sac. Peak maternal serum concentrations are observed during the first trimester and, subsequently, rapidly decline during later gestation. Newborn infant serum levels may be as high as 86 to 90 μg per ml and then decline over the first two to three months of post-natal life. The pioneering observations of Brock and Sutcliff of increased α-fetoprotein concentration in the amniotic fluid of pregnancies with children having anencephaly of spina bifida initiated the era of the application of the quantitative determination of this glycoprotein for prenatal diagnosis of congenital malformations. This observation served as the impetus for the development of sensitive techniques of radioimmunoassay to quantitate α-fetoprotein and led to the recognition that elevation of AFP occurred not only with malformations but also with neoplasms and liver disease.

Studies have indicated that over 90 percent of neural tube defects may be identified *in utero* by the antenatal α-fetoprotein determination. It is thought that this glycoprotein appears in amniotic fluid as a consequence of transudation of plasma and serum through open neural tube defects. The situations in which amniotic fluid α-fetoprotein determination yields false negative results occur in those fetuses with skin-covered defects of the neural tube. The general applicability of this technique was limited owing to the need to measure amniotic fluid levels and, therefore, was performed only in families with an increased risk. The clin-
ical usefulness was compromised since 90 percent of fetuses with neural tube defects are born to families in whom there is no known increased risk factor.

Subsequent investigations have indicated that α-fetoprotein levels in maternal serum are diagnostic for neural tube closure defects. In the past three years, this approach has been validated. Maternal α-fetoprotein levels increase progressively from the 11th to 13th gestational week until the 25th to 27th week. Thereafter the levels rise slowly, plateau and decline around parturition. The progressive increase of maternal serum α-fetoprotein levels from the 13th through 25th week of gestation necessitates precise definition of gestational age requiring sonography to verify gestational age. Factors which limit the application of this methodology are multiple births and fetal death. To avoid the confounding issue of either multiple gestation or fetal demise, diagnosis should include measurement of serum prolactin levels. Human placental lactogen increases with multiple births and decreases in association with fetal death.

A variety of anomalies are associated with increased amniotic α-fetoprotein levels. These are omphalocele, gastroschisis, sacrococcygeal teratoma, congenital nephrosis and atresia of the upper gastrointestinal tract. No studies have indicated whether or not these conditions are associated with increased maternal serum α-fetoprotein levels.

Since α-fetoprotein is synthesized by fetal liver, the association of increased levels of AFP with both neoplastic and non-neoplastic liver disease suggested themselves. Recent studies indicated that quantitative estimation of serum α-1-fetoprotein may be helpful in distinguishing neonatal hepatitis from biliary atresia. Studies have indicated whether or not these conditions are associated with increased maternal serum α-fetoprotein levels.

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Alpha-1 Antitrypsin

The major plasma antiprotease is the glycoprotein α-1 antitrypsin. This glycoprotein has a molecular weight of approximately 54,000 daltons and contains significant sialic acid residues. In the early 1960's, Laurell established the relationship of a deficiency of this antiprotease with chronic obstructive lung disease. The observation was based upon the recognition that a deficiency in the serum α-globulin region of plasma protein electrophoretograms was present in individuals with chronic lung disease. Since that time, two clinical phenotypes have been associated with a deficiency of α-1 antitrypsin.

The first is the presence of chronic obstructive lung disease and pulmonary emphysema in individuals homozygous for α-1 antitrypsin deficiency (ZZ-phenotype). The second is the association of obstructive hepatic disease (cholestatic) in children with the ZZ-phenotype on electrophoresis of α-1 antitrypsin. There are at least 11 alleles for the α-1 antitrypsin
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Glycoprotein and 22 combinations of different structural $\alpha$-1 antitrypsins have now been recognized. The commonest variant associated with disease is the Z-phenotype; additionally, hepatic disease has now also been associated with the S variant. These electrophoretic variants are identified by crossed immunodiffusion techniques (starch gel electrophoresis and second dimension agar electrophoresis) or by isoelectric focusing on polyacrylamide gel stabs. In heterozygotes for the Z-phenotype an increased incidence of chronic obstructive lung disease is suggested. It is probable that the production of disease in an $\alpha$-1 antitrypsin deficiency heterozygote occurs as a consequence of interplay of environment and genotype. One environmental factor in the production of emphysema in these individuals is cigarette smoking. Individuals heterozygous for the Z-allele have normal liver function, yet on hepatic biopsy show intracellular PAS-positive granules ($\alpha$-1 antitrypsin).

The ZZ-phenotype appears associated with at least three clinical pictures,—adult ZZ homozygous chronic obstructive lung disease, the childhood (ZZ-phenotype) cholestatic obstructive liver disease as well as children and young adults with both hepatic and pulmonary disease. The ZZ electrophoretic phenotype results from mutations in the protein portion of the glycoprotein, defects in the sialyl transferase enzyme or a defect in primary glycoprotein structure.

Glycoproteins as Markers of Tumors

The quantitation of serum glycoproteins serves as markers of neoplastic processes and is based on several observations (table I). Essential is the recognition that tumors may release or secrete products which differ from normal cells. These differences may reflect quantitative differences in the level of normally secreted substances or they may represent qualitative differences as evidenced by ectopic synthesis in cancerous tissue of hormones (production of erythropoietin, ACTH, parathormone or gonadotropins). One unique secreted tumor glycoprotein marker is the synthesis of monoclonal immunoglobulin by certain tumors. In the past three years, a new class of secretory products of neoplastic cells has been recognized. These are antigens found and produced during fetal life.

With normal differentiation, there is postulated to be synthesis of repressor substances, either associated with or causing the transition from a pluripotent cell to a differentiated cell. Thus, “primitive phase specific macromolecules” cease to be produced or are synthesized at a markedly reduced rate in mature tissues. Changes in the fetal markers (antigens) during maturation may fail to occur during oncogenesis and thus result in the persistence of these embryonic markers.

In model systems, the persistence of fetal glycoprotein antigens has been induced by malignant transformation with SV40 and avian myeloblastosis virus as well as following transformation with chemical mutagens. Thus, carcinogenesis may be associated with the presence or production of new cellular antigens and the depression of the host genome resulting in the appearance of fetal or pluripo-

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*Carinoembryonic antigen
†Fetal sulfoglycoprotein
§Beta-fetoprotein
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tent antigens. Implicit in these postulations was that fetal antigen persistence could occur in other diseases such as maturation arrest and malformations. This is indeed the case.

Chronic infections which are therapy resistant have resulted in the production of such glycoproteins implying that proper stimulation can lead to the production of embryonic markers by normal cells.

**Carcinoembryonic Antigen**

Most well studied is the glycoprotein fetal carcinoembryonic antigen (CEA) which occurs during normal development during the second through sixth gestational months and is present in cells of entodermal germ cell origin. The observation has been validated that neoplasms of the gastrointestinal tract are associated with markedly elevated plasma CEA levels and that the degree of elevation relates to tumor prognosis. Levels rise or are high with serosal penetration of the cancer, regional lymph node involvement or distant metastases.

High carcinoembryonic antigen levels have been found in association with adenocarcinoma of the pancreas. The serum CEA antigen determination has greater diagnostic value than any other laboratory finding for diagnosis of carcinoma of the pancreas. Elevation of CEA antigen occurs with pancreatitis; however, values of less than 10 ng per ml are found and there is virtually no overlap with malignant disease.

Elevations of CEA antigen may occur with all entodermally derived structures. It was predictable that elevated serum levels would occur in association with gastric carcinoma. Other glycoprotein fetal antigens are elevated in association with gastric tumors. Specifically, this includes elevated level of the fetal sulfoglycoprotein (FSA) which is found in 90 percent of patients with gastric carcinoma. Fetal sulfoglycoprotein detection methods have been employed in large populations (14,000 individuals) and only one percent had a positive FSA antigen. In this study, four individuals were asymptomatic and shown to have gastric carcinoma and the fifth had gastric polyposis. This antigen is structurally related to the blood group antigens and is present in the fetal stomach during early embryonic development.

CEA antigen is increased in serum and urine of patients with bladder carcinoma. The CEA antigen has diagnostic value for the detection of ovarian neoplasms.

α-Fetoprotein has diagnostic value for the detection of hepatocellular carcinomas; thus, levels are elevated in liver carcinoma, hepatitis, hepatoblastoma and cholangiomas. More recently, another fetogloboprotein has been clinically useful, β-fetoprotein (BFP). This antigen is synthesized in the fetal liver and elevated serum levels occur with hepatomas. The antigen appears to be immunologically indistinguishable from liver ferritin. In non-entodermally derived tumors, glycoprotein markers have diagnostic usefulness. Two specific situations are the detection of F and S antigens. Elevations of these antigens in serum and biologic fluids have diagnostic utility for leukemia and lymphomatous neoplasms. The F antigen appears to be a product of reactive lymphocytes whereas the S antigen appears to be a product synonymous with that found in fetal liver and neonatal thymus and is thought to be de-differentiation antigen.

**Conclusion**

Glycoproteins are ubiquitous macromolecules that function in the secretory processes, cell surfaces, and transport systems of cells. Early application of their measurement in clinical medicine as non-specific markers of stress and disease has been surpassed in recent times by the development of highly sensitive methods which allow their detection as markers of congenital malformations and neoplasms.

The study of the clinical utility of measurement of fetal embryonic glycoproteins
epitomizes what F. Hopkins wrote in 1927: “It is making, I know, a great claim, but I believe that it will be the ultimate privilege of advancing biochemistry to tempt all biologists—including the physician—always to picture mentally—as a habit of mind—the molecular events which underlie the changes of form and visible appearances which interest them, and, on the other hand, to demonstrate to chemists, that the molecular events they have studied so fully in systems more or less homogenous, gain enormously in interest, in spite of the complications involved, when they are organized and coordinated in systems involving changing form and elaborate structure.”

References


