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Review

Protein Glycosylation and Diseases: Blood and Urinary Oligosaccharides as Markers for Diagnosis and Therapeutic Monitoring

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Background: **N- and O-oligosaccharide variants on glycoproteins (glycoforms) can lead to alterations in protein activity or function that may manifest themselves as overt disease.**

Approach: **This review summarizes those diseases that are known to be the result of an inherited or acquired glycoprotein oligosaccharide structural alteration and that are diagnosed in blood or urine by chemical characterization of that oligosaccharide alteration.**

Content: **The biochemical synthesis steps and catabolic pathways important in determining glycoprotein function are outlined with emphasis on alterations that lead to modified function. Clinical and biochemical aspects of the diagnosis are described for inherited diseases such as I-cell disease, congenital disorders of glycosylation, leukocyte adhesion deficiency type II, hereditary erythroblastic multinuclearity with a positive acidified serum test, and Wiskott-Aldrich syndrome. We also review the laboratory use of measurements of glycoforms related to acquired diseases such as alcoholism and cancer.**

Conclusions: **Identification of glycoprotein glycoforms is becoming an increasingly important laboratory contribution to the diagnosis and management of human diseases as more diseases are found to result from glycan structural alterations.**

Most plasma membrane and secretory proteins are glycosylated. Oligosaccharides are integral part of these macromolecules and greatly affect their physical properties and biological functions. The glycosylation pathways occur in the cytosol, endoplasmic reticulum (ER),[4] and the Golgi complex and involve transport steps, processing glycosidases, and glycosyltransferases.

This review addresses the inherited and acquired diseases in which biological diagnosis is based on alterations in the oligosaccharide structure of glycoproteins secreted into blood and urine and confirmed by measuring the activity of cellular enzymes involved in the altered glycosylation pathway *(1)*.

O- and N-Oligosaccharide Structures of Glycoproteins

Oligosaccharides are covalently linked to proteins through nitrogen or oxygen, to Ser or Thr, or to Asn in O- or N-linked oligosaccharides, respectively *(2, 3)*.

In O-glycosylated proteins, the oligosaccharides range in size from 1 to >20 sugars, displaying considerable structural (and antigenic) diversity. Moreover, these oligosaccharides are not uniformly distributed along the peptide chain; they are clustered in heavily glycosylated domains. *N*-Acetylgalactosamine (GalNAc) is invariably linked to Ser or Thr (Fig. 1). Mannose residues are never detected in mature O-glycans.

N-Oligosaccharides have a common core structure of five sugars and differ in their outer branches. The first sugar residue, N-acetylglucosamine (GlcNAc) is bound to Asn included in a specific tripeptide sequence (Asn-X-Thr or Ser). N-Oligosaccharides are classified into three main categories: high mannose, complex, and hybrid (Fig. 1). High-mannose oligosaccharides have two to six additional mannoses linked to the pentasaccharide core and

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[4] Nonstandard abbreviations: ER, endoplasmic reticulum; GalNAc, *N*-acetylgalactosamine; GlcNAc, *N*-acetylglucosamine; SA, sialic acid; Con A, concanavalin A; AGP, α_1-acid glycoprotein; CDG, congenital disorders of glycosylation; TRF, transferrin; LAD, leukocyte adhesion deficiency; HEMPAS, hereditary erythroblastic multinuclearity with a positive acidified serum test; WAS, Wiskott-Aldrich syndrome; ALP, alkaline phosphatase; AFP, α-fetoprotein; and LCA, *Lens culinaris* agglutinin.

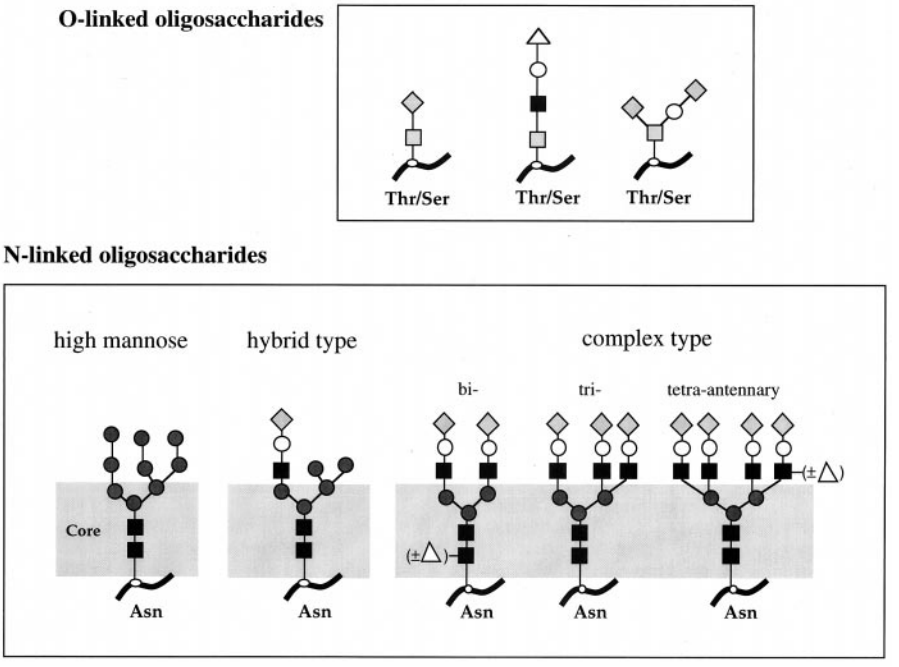

Fig. 1. O- and N-linked oligosaccharide structures.

(Top), examples of O-linked oligosaccharides. *(Bottom)*, different structures of N-linked oligosaccharides. ▨, GalNAc; ■, GlcNAc; ○, galactose; ●, mannose; △, fucose; ◆, SA; ∼, polypeptide chain.

forming the branches. Complex-type oligosaccharides have two or more branches, each containing at least one GlcNAc, one Gal, and eventually one sialic acid (SA); they can be bi-, tri-, or tetraantennary (Fig. 1). Hybrid oligosaccharides contain one branch that has the complex structure and one or more high-mannose branches. Glc residues are never detected in mature complex N-oligosaccharides. Serum glycoproteins mostly consist of complex type N-oligosaccharides. O- and N-oligosaccharide chains may occur on the same peptide core.

Biosynthesis Steps of O- and N-Oligosaccharides

O-Oligosaccharide biosynthesis begins in the cis Golgi with the transfer of the first sugar residue, GalNAc, from a nucleotide sugar by a specific polypeptide *O*-GalNAc transferase, to a complete polypeptide chain (Fig. 2). The glycan chain then grows by the addition of GlcNAc, Gal, and Fuc residues in the medial Golgi. Sialylation continues throughout the trans Golgi. There are several possible pathways to construct O-glycans, depending on the substrate specificity and intracellular arrangement of glycosyltransferases. However, it is far less complex than the processing of N-oligosaccharides *(4, 5)*.

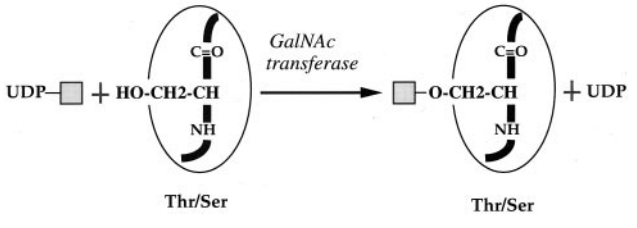

Fig. 2. Initiation of the biosynthesis of O-linked oligosaccharides.

UDP, uridine diphosphate; ▨, GalNAc; ∼, polypeptide chain.

The biosynthesis of N-oligosaccharides (Fig. 3) begins in the ER with a large precursor oligosaccharide that contains 14 sugar residues. The inner five residues constitute the core, which is conserved in the structure of all N-linked oligosaccharides. This precursor is linked to

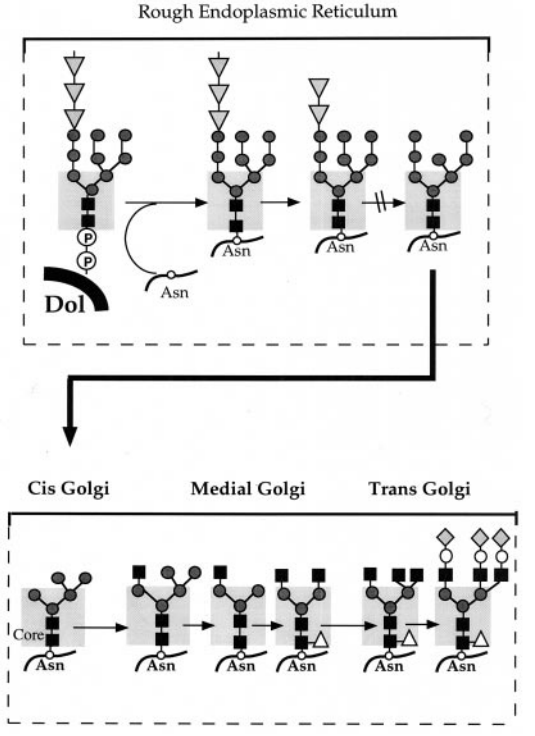

Fig. 3. Processing of N-linked complex oligosaccharides.

■, GlcNAc; ○, galactose; ●, mannose; ▼, glucose; △, fucose; ◆, SA; ∼, polypeptide chain. *Dol*, dolichol; *P*, phosphate.

dolichol pyrophosphate, which acts as a carrier for the oligosaccharide. This lipid-linked oligosaccharide is transferred "en bloc" to an Asn residue on the growing polypeptide chain *(6)*. While the nascent glycoprotein is still in the rough ER, all three Glc residues and one mannose residue are removed by specific glycosidases, producing an oligosaccharide with 10 residues instead of 14. The maturation of the N-oligosaccharides takes place in the Golgi complex. This pathway involves a coordinated and sequential set of enzymatic reactions, which remove and add specific sugar residues. The enzymes involved (glycosidases and glycosyltransferases) are located in the cis, medial, and trans Golgi *(7)*. The reaction product of one enzyme is the substrate for the next. When present, SA residues are always at the terminal nonreducing ends of oligosaccharides. The high-mannose and hybrid oligosaccharides appear as intermediates along the processing pathway. The complex type is the mature form of N-linked oligosaccharides.

Catabolic Pathways of N- and O-Oligosaccharides

Lysosomes, which contain the complete array of glycosidases and proteases, constitute the major catabolic organelle for glycoproteins. After complete hydrolysis, free products (amino acids and monosaccharides) exit the lysosomes and are either recycled or secreted in the extracellular fluids. Fig. 4 illustrates the degradation pathway of N-oligosaccharides, which begins with the elimination of Asn by *N*-aspartyl-β-glucosaminidase and is achieved by a β-mannosidase. In contrast to N-oligosaccharides, the catabolism of O-oligosaccharides is poorly documented and likely involves sequential and ordered events catalyzed by specific osidases, which remain to be characterized *(8, 9)*.

Catabolism of oligosaccharides also occurs in the cytosol. This concerns mostly the catabolism of oligomannosides, which originate either from the ER or from dolichol intermediates. There is intense intracellular trafficking of free mono- and oligosaccharides among the ER, cytosol, and lysosomes, which involves specific membrane carriers.

Microheterogeneity of Glycans

Wild-type glycoproteins consist of mixtures of glycosylated variants, known as glycoforms, in which the same peptide sequence is associated with the association of more than one oligosaccharide at the same glycosylation site. Indeed, a single oligosaccharide may have different structures depending on the folding of the peptide moiety and its recognition as acceptor by glycosyltransferases. Oligosaccharide structures also depend on the cell type and its enzymatic equipment, its developmental stage, and its nutritional or pathological state. The true structural diversity is enormous. This raises the question of using recombinant glycoproteins for therapeutic purposes, insofar as the oligosaccharide chains of the produced glycoproteins have to be structurally close to those

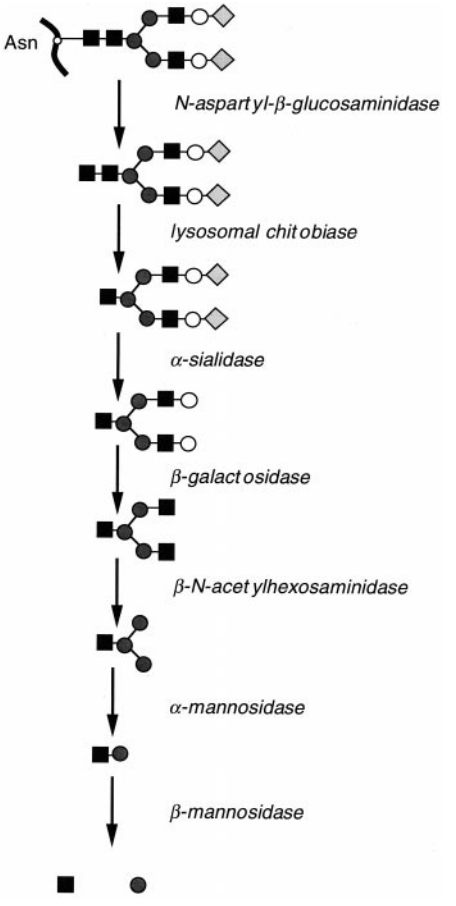

Fig. 4. Catabolic pathway for N-glycoproteins.
■, GlcNAc; ○, galactose; ●, mannose; ◇, SA; ∼, polypeptide chain.

of the wild-type glycoproteins and compatible with the immune system *(10, 11)*.

These glycoforms can be classified into restricted subsets of glycoproteins, using tools such as lectins *(12)*, which recognize specifically a sugar residue or a small oligosaccharide structure. Affinity chromatography and crossed immuno-affinoelectrophoresis currently are used for biological diagnosis *(13)*.

Using lectins greatly helps biologists to identify glycoforms and to detect variations in the relative proportions of glycoforms for a specific glycoprotein. For example, concanavalin A (Con A), the best-known plant lectin, specifically recognizes the trimannosidic structure of a N-glycoprotein if mannose residues are accessible *(14)*. Schematically, Con A interacts with biantennary oligosaccharides but not with tri- and tetraantennary structures *(15)*.

Fig. 5 illustrates how crossed immuno-affinoelectrophoresis in the presence of Con A, a simple and rapid method, can provide information about glycosylation. On the basis of the four Con A patterns of α_1-acid glycoprotein (AGP) shown in Fig. 5 (from a healthy adult, a pregnant woman, the umbilical cord blood from a healthy newborn, and from an infected newborn suffering from

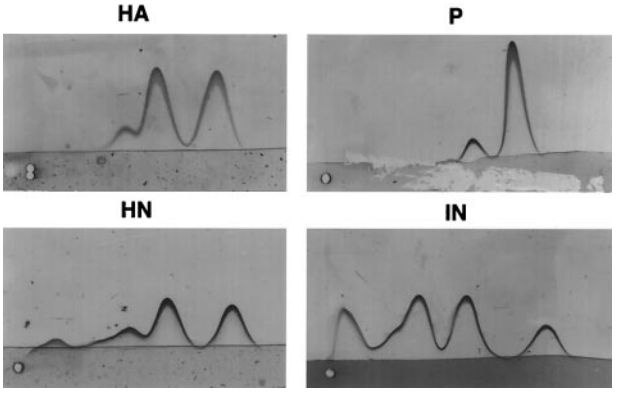

Fig. 5. Crossed immuno-affinoelectrophoresis in the presence of Con A of serum AGP from a healthy adult (*HA*), a pregnant woman (*P*), and from healthy (*HN*) and infected (*IN*) newborns.

listeriosis), it appears that (*a*) hormonal changes during pregnancy alter glycosylation of AGP with an increase of unretained forms of AGP; (*b*) AGP is not transferred from the mother to the fetus through the placental barrier; and (*c*) in response to infection, the fetus is able to produce some mediators that alter its AGP patterns without altering the AGP pattern of its mother.

Functions of Oligosaccharides in Glycoproteins

Oligosaccharides affect the overall conformation of a glycoprotein and therefore may affect any or all of its functions *(16, 17)*.

Few general rules that relate oligosaccharide structure to function have been established. Tridimensional conformation allows oligosaccharides to be recognized by receptors and to mediate events such as cell trafficking, cell adhesion, and biological half-life. These biological events involve biochemical interactions between oligosaccharides as ligands and proteins named lectins *(18)*. Ashwell and Morell *(19)*, when discovering the hepatic asialoglycoprotein receptor, identified the first mammalian lectin. It recognizes the ultimate Gal residue of desialylated serum glycoproteins and rapidly clears them from blood by endocytosis.

Cell and cell interactions involving a specific oligosaccharide structure are also well documented in the literature. The well-known recruitment of neutrophils to sites of inflammation as part of the cell's defense against bacterial infection is initiated by the interaction of a specific oligosaccharide present at the surface of a cell with its lectin counterpart, which is called a selectin *(20)*.

Numerous carbohydrate-binding specificities involved in infection and toxicity have been identified for a wide variety of microorganisms *(21)*.

Inherited Diseases

O- and N-glycan chain structures of glycoproteins may be altered in many diseases, inherited as well as acquired diseases.

I-CELL DISEASE, OR LEROY DISEASE

I-Cell disease is a severe and rare genetic disease, inherited in an autonomic recessive manner and caused by the lack of Golgi GlcNAc phosphotransferase *(9, 22–25)*.

Clinical features. Typical patients present with mental retardation, coarse facial features with gingival hyperplasia, and short-trunk dwarfism; they generally die in the first decade of life *(23)*.

Biochemical aspects. Whereas the oligosaccharides on secretory and membrane N-glycoproteins are processed to complex-type units, mannose residues on lysosomal enzymes become phosphorylated. The phosphorylation of mannose residues is a two-step procedure involving two separate enzymes in the cis Golgi: GlcNAc phosphotransferase and GlcNAc1 phosphodiester *N*-acetylglucosaminidase. GlcNAc phosphotransferase recognizes only lysosomal enzymes as substrates and catalyzes the addition of a GlcNAc phosphate residue to mannoses of the branches. *N*-Acetylglucosaminidase removes the GlcNAc group, leaving the phosphate attached to the carbon of the mannose (Fig. 6). Subsequently, the phosphorylated lysosomal enzymes bind tightly and specifically to a receptor, the mannose 6-phosphate receptor, a transmembrane protein of the trans Golgi, which directs the enzymes into vesicles coated with clathrin. After depolymerization, the

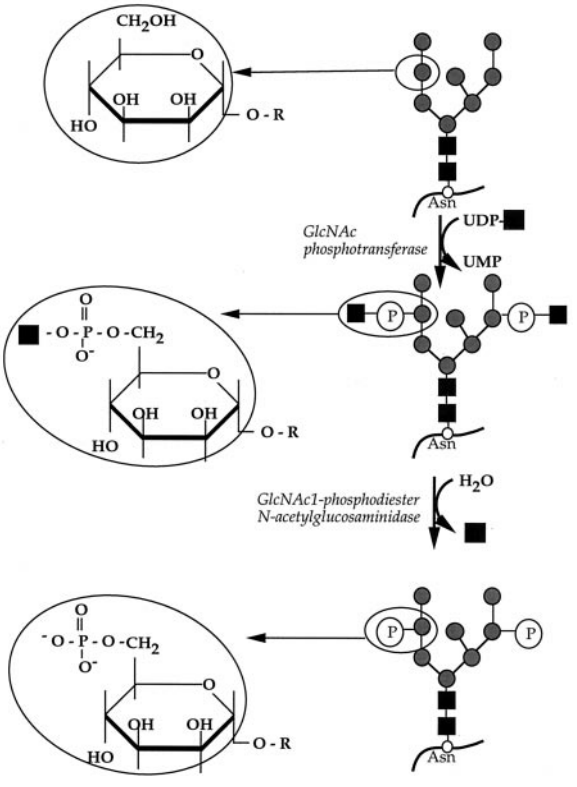

Fig. 6. The two steps of the phosphorylation of lysosomal enzymes.
●, mannose; ■, GlcNAC; ∼, polypeptide chain; *P*, phosphate; *UMP*, uridine monophosphate; *UDP*, uridine diphosphate; *R*, oligosaccharide group.

uncoated vesicles fuse with late endosomes in which the low pH allows the phosphorylated enzymes to dissociate from their receptors. Finally, transport vesicles containing the lysosomal enzymes fuse with lysosomes.

The lack of the first enzyme, GlcNAc phosphotransferase, causes I-cell disease, a lysosomal storage disease, which leads to a deficiency of multiple enzymes in fibroblasts and macrophages. Lysosomal enzymes with normal enzymatic activity are no longer directed to the lysosomes, but are secreted into the extracellular space. Alternative mechanisms must operate in other cell types to target lysosomal enzymes into lysosomes. Thus, hepatocytes, Kupffer cells, and leukocytes from patients with I-cell disease contain nearly normal concentrations of enzymes despite their deficiency in phosphotransferase activity.

Laboratory diagnosis. Biochemical diagnosis is based on strongly increased plasma lysosomal enzyme activities and is confirmed by the decrease of GlcNAc phosphotransferase activity in fibroblasts. The gene encoding GlcNAc phosphotransferase has not been cloned. No defects in GlcNAc1 phosphodiester *N*-acetylglucosaminidase or in the mannose 6-phosphate receptor have been reported.

CONGENITAL DISORDERS OF GLYCOSYLATION

Another newly delineated group of carbohydrate-related genetic diseases is termed congenital disorders of glycosylation (CDG; previously known as carbohydrate-deficient glycoprotein syndrome) *(26)*. Two types are described, based on the enzymatic defect: type I corresponds to the CDG that involve enzymatic steps up to the assembling of the glycoprotein; type II are the CDG in which processing is involved. The most common one is CDG Ia (>300 patients worldwide), which is attributable to a deficiency in cytosolic phosphomannomutase activity in the metabolism of mannose *(27)*.

Clinical features. CDG other than type Ib are multisystemic disorders involving neurological dysfunction. CDG Ia infants have a typical morphology with abnormal distribution of subcutaneous fat and inverted nipples.

Clinical symptoms of CDG Ib are limited to intestinal and hepatic disease.

Biochemical aspects. Phosphomannomutase catalyzes the transformation of mannose 6-phosphate to mannose 1-phosphate, which is then transformed to GDP mannose, a precursor of mannose for the biosynthesis of N-glycoproteins (Fig. 7). Phosphomannomutase deficiency (CDG Ia) leads to the synthesis of glycoproteins present in the serum as a mixture of various glycoforms: normally glycosylated, and partially or totally devoid of oligosaccharide chains. This is illustrated with transferrin (TRF) in Fig. 8. The gene encoding for the incriminated phosphomannomutase-2 is located on chromosome 16p13 *(28)*, and numerous mutations and one single-pair deletion have been identified recently *(29)*.

CDG Ib is caused by defective phosphomannose isomerase activity, which reversibly converts fructose 6-phosphate into mannose 6-phosphate. Mannose treatment has been used successfully to correct clinical and biochemical symptoms *(30)*.

The very recently described CDG Ic (or V) is caused by a defect in dolichyl-P-Glc:$Man_9GlcNAc_2$-PP dolichyl glucosyltransferase activity, which adds the first of three

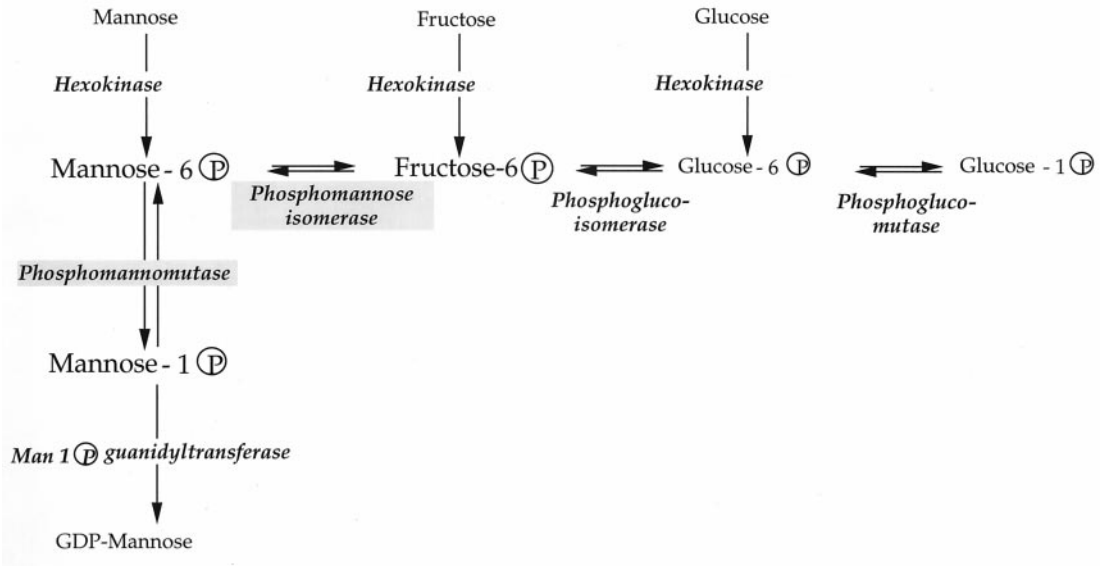

Fig. 7. Metabolism of mannose in relation to N-glycosylation.
Mannose, glucose, and fructose are precursors of GDP-mannose. *P*, phosphate.

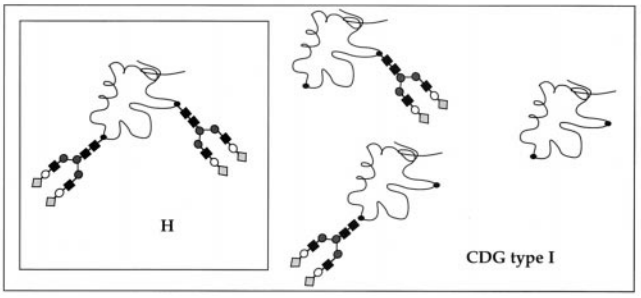

Fig. 8. Different glycoforms of serum TRF from a healthy subject (*H*) and from a *CDG type I* patient.

■, GlcNAc; ○, galactose; ●, mannose; ◊, SA; ∼, polypeptide chain.

glucosyl residues to the nascent N-glycoproteins in the ER *(31)*.

Serum glycoprotein glycosylation alterations observed in CDG Ib and Ic are similar to those in CDG Ia.

CDG type II (two described patients) is related to a deficiency in GlcNAc transferase II (cis Golgi), which leads to the synthesis of complex N-glycoproteins devoid of one branch *(32, 33)*.

Laboratory diagnosis. CDG are diagnosed by demonstrating the presence of abnormally glycosylated serum glycoproteins. Several electrophoretic methods may be used: (*a*) isoelectrofocusing, which detects the different glycoforms as a function of their SA content [Serum TRF, which was for the first time studied by Stibler et al. *(34)* in relation to alcoholism, is currently analyzed *(35)*.]; and (*b*) Western blotting, which detects the different glycoforms as a function of their molecular weights. Fig. 9 shows typical Western blot profiles of serum TRF, haptoglobin, AGP, and α_1-proteinase inhibitor from a healthy adult and a CDG Ia patient *(36)*.

The enzymatic deficiencies and the corresponding gene mutations can be demonstrated in leukocytes and in cultured skin fibroblasts from patients *(27, 29, 30)*.

CDG type II also is easily diagnosed by Western blot analysis of serum TRF and by demonstrating the GlcNAc transferase II deficiency and its related gene mutations *(33, 36)*.

LEUKOCYTE-ADHESION DEFICIENCY TYPE II

Leukocyte-adhesion deficiency type II (LAD II) is a rare severe immunological disease *(37)*.

Clinical features (38, 39). LAD II is clinically similar to the well-described LAD I, in that is also involves severe mental retardation, short stature, and dysmorphic features. The two syndromes differ in the molecular basis of their adhesion defects. LAD I is caused by a deficiency in the CD18 integrin-adhesion molecule, whereas LAD II

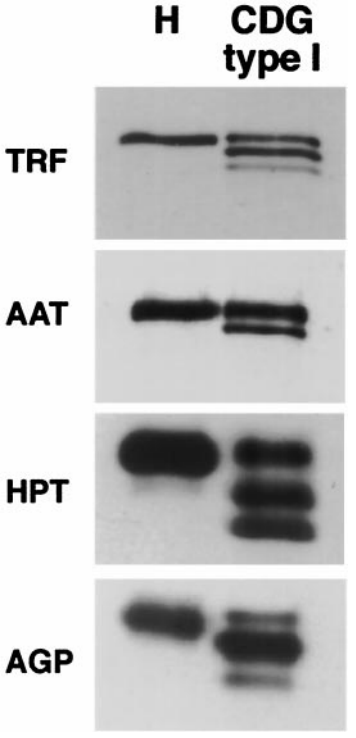

Fig. 9. Western blot of serum *TRF*, haptoglobin (*HPT*), *AGP*, and α_1-antitrypsin (*AAT*) from a healthy adult (*H*) and a *CDG type I* patient.

neutrophils are devoid of sialyl Lewis x, which is a ligand for the selectin family.

Biochemical aspects. The selectin family, including L-, E-, and P-selectins *(40)*, mediates rolling and tethering of leukocytes along the walls of the microvasculature before adhesion and extravasation. The selectins contain lectin-like domains that bind fucosylated oligosaccharides such as sialyl Lewis x [SA(α2,3),Galβ1,4(Fuc (α1,3))GlcNAc].

In addition to being sialyl Lewis x negative, LAD type II patients express oligosaccharide structures that are devoid of Fuc(α1,2)Gal, Fuc(α1,4)GlcNAc, and Fuc(α1,6)GlcNAc. These fucose residues are the products of at least four different fucosyltransferases, but it is unlikely that this genetic defect is a fucosyltransferase deficiency. The reported impaired fucosylation could be attributable to a defect either in the transport of fucose to the Golgi lumen or in any enzymes in the synthetic pathway of GDP-Fuc, which is the substrate for the fucosyltransferases. It appears to be caused by a defect in the conversion of GDP-Man to GDP-Fuc. Thus, the lack of sialyl Lewis x on the neutrophils would be secondary to a general fucose deficiency.

Laboratory diagnosis. Biochemical diagnosis could be established by evaluating the absence of the sialyl Lewis x epitope on neutrophil membranes using specific anti-sialyl Lewis x antibodies.

HEREDITARY ERYTHROBLASTIC MULTINUCLEARITY WITH A POSITIVE ACIDIFIED SERUM TEST

Hereditary erythroblastic multinuclearity with a positive acidified serum test (HEMPAS) *(41)*, also called congenital dyserythropoietic anemia type II *(42, 43)*, is a rare autosomal recessive disorder caused by membrane abnormality, with >300 known patients, and is a multifactorial disease.

Clinical features. Congenital dyserythropoietic anemia type II patients suffer from a long-life anemia, hepatosplenomegaly, liver hemosiderosis, and cirrhosis.

Biochemical aspects. HEMPAS erythrocyte band 3 shows a complete absence of poly *N*-acetyllactosamine. The disease might be heterogeneous because the primary defect is not totally clear. Indeed, some cases are related to GlcNAc transferase II deficiency *(44)*. Other patients had been identified with normal GlcNAc II activity but no detectable activity in α-mannosidase II. Both enzymes are necessary to convert complex immature N-glycans to complex mature N-glycans. In addition, the defect seems to be limited to hematopoietic cells, which does not totally correspond to the clinical features.

Studies on linkage analysis and allele segregation showed that there was no linkage between congenital dyserythropoietic anemia type II phenotype and the chromosomal regions containing the candidate genes that code for GlcNAc transferase II and α-mannosidase II. Ialoscin et al. *(45)* and Gasparini et al. *(46)* suggest that the disease is most likely attributable to a defect of a transcriptional factor regulating both enzymes.

Laboratory diagnosis. Biochemical diagnosis can be made by showing the presence of abnormally glycosylated band 3 in the electrophoretic pattern of erythrocyte membranes of patients, using sodium dodecyl sulfate-polyacrylamide gel electrophoresis.

WISKOTT-ALDRICH SYNDROME

Wiskott-Aldrich syndrome (WAS) is an X-linked, rare disease characterized by eczema, thrombocytopenia, and immunodeficiency *(1, 47)*.

Clinical features. The clinical features begin early in life and include recurrent infections, bleeding, and eczema. The prognosis is grave. Circulating platelets are decreased in number and size. IgM concentrations usually are below normal, IgG concentrations are normal, and IgA and IgE concentrations are high. Monoclonal gammopathy is common.

Biochemical aspects. WAS is caused by defects of the *WAS* gene *(48)*, which encodes the WAS protein, involved in proliferation and differentiation of the hematopoietic progenitors *(49)*. A specific defect in O-linked glycans in lymphocytes has been reported with the abnormal expression on WAS peripheral blood mononuclear cells of a highly O-glycosylated integral membrane, sialoglycoprotein, CD43. N-Linked glycans are normal. In addition, spectacular differences in the activities of two O-glycosyltransferases, core 2 GlcNAc transferase and α-2,6-sialyltransferase, are related to the abnormal O-glycan synthesis *(50, 51)*.

Other than the deficiency of galactosyltransferase I involved in the assembly of glycosaminoglycans *(52)*, WAS is the only genetic disease involving abnormal O-glycosylation identified at present.

Laboratory diagnosis. Biochemical diagnosis can be established by evaluating the O-glycosylation of CD43 in blood mononuclear cell membranes.

GLYCOPROTEINOSES

The common feature of these diseases is the genetic deficiency of any one of the lysosomal glycosidases involved in the catabolism pathway of glycoproteins (single-entity incidence between 1 in 100 000 and 1 in 250 000) *(53)*.

Clinical features. Most of these diseases are characterized by different clinical phenotypes, depending the age of the patient. The clinical feature in the infantile form is most often dominated by neurologic symptoms. Retinal abnormalities are frequent.

Biochemical aspects. The deficiency of one of the lysosomal enzymes involved in the catabolic pathway of glycoproteins leads to the accumulation of undegraded oligosaccharides in lysosomes and increased urinary excretion of carbohydrate material produced by the cytolysis of sick cells. An accumulation of glycolipids and mucopolysaccharides is also observed because the oligosaccharide moiety is degraded by the same set of enzymes.

Laboratory diagnosis. The biochemical diagnosis is based on chromatography of urinary oligosaccharides. Low cell-specific glycosidase activity confirms the results obtained by chromatography.

Mutations or deletions of some of the genes that code specific enzymes have been identified and can be demonstrated in some glycoproteinoses. Table 1 illustrates the main biochemical features of these diseases.

Acquired Diseases

Given that glycan synthesis involves enzymatic reactions, it is easily subjected to variations each time the enzymes or substrate concentrations vary. Therefore, every change or stimulation in the status of the producing cell is a potential source of glycosylation variations, even if protein synthesis remains unchanged. Study of the glycoforms of one or more glycoproteins will therefore give information about the cell function and the related disease.

Table 1. Glycoproteinoses.

Urinary oligosaccharides	Disease	Enzymatic defect	Gene
Sialyl-oligosaccharides	Sialidose I	Sialidase	Unknown
	Sialidose II	Sialidase	Unknown
	Galactosialidosis	Sialidase and galactosidase with a defect in a "protective" protein	Unknown
Galactosyl-oligosaccharides	GMI gangliosidosis	β-Galactosidase	Known (mutations)
	Morquio type B	β-Galactosidase	Known (mutations)
Glucosaminyl-oligosaccharides	Sandhorf disease	β-Hexosaminidase B	Known (deletion)
Fucosyl-oligosaccharides	Fucosidosis	α-Fucosidase	Known (mutations)
R-GlcNAc(β1-N)Asn[a]	Aspartylglucosaminuria	*N*-Aspartyl-β-glucosaminidase	Known (mutations)
α-Mannosyl-oligosaccharides with a GlcNAc residue at the reducing end	α-Mannosidosis	α-Mannosidase	Unknown
Man(β1-4)GlcNAc	β-Mannosidosis	β-Mannosidase	Unknown

[a] R, oligosaccharide group.

CARBOHYDRATE-DEFICIENT TRANSFERRIN AND CHRONIC ALCOHOL DRINKING

Biochemical aspects. Human TRF is a serum glycoprotein that exists in many different forms determined by genetic polymorphisms, iron saturation, and oligosaccharide composition. TRF contains two N-linked complex-type major biantennary oligosaccharides *(54)*. In normal plasma, the major glycoform is tetrasialylated (80%) with a small proportion of pentasialylated and trisialylated forms (15%). The hexa-, di-, mono-, and asialylated subtypes are present in very low concentrations.

In chronic alcoholics, the plasma concentration of tetrasialylated TRF decreases, whereas the concentration of disialylated TRF increases *(55)*. When alcoholism is associated with malnutrition and/or hepatocellular deficiency, the percentages of mono- and asialylated TRF also increase.

Laboratory diagnosis. Serum carbohydrate-deficient transferrin, which represents partially and totally desialylated TRF, currently is measured to detect and monitor chronic drinkers.

Isoelectric focusing was first used to separate serum TRF isoforms, but the current method uses anion-exchange chromatography to separate serum TRF isoforms, which are then measured by RIA or enzyme immunoassay *(56, 57)*. Commercial kits (CDTect EIA) are available.

ALKALINE PHOSPHATASE IN BONE AND HEPATOBILIARY DISEASES

Biochemical aspects. Human alkaline phosphatase (ALP) exists as several isoforms that may appear in plasma under different conditions of health and disease *(58)*. Four structural genes that encode ALP have been sequenced. The tissue-nonspecific *ALP* gene is expressed in osteoblasts, hepatocytes, kidney, early placenta, and other cells. The ALP isoforms from bone and hepatocytes, which are coded by the same gene, are differently glycosylated, depending the glycosyltransferases in both cell types. Healthy adults generally have approximately equal plasma activities of the liver and bone glycoforms. The source of an increase in serum ALP can be documented by identifying the ALP oligosaccharide moiety.

Laboratory diagnosis. Wheat germ agglutinin is a lectin that interacts preferentially with bone ALP. Its use in association with affinity techniques leads to an increase in the specificity and sensitivity of total serum ALP measurement *(59, 60)*. Clearly, serum bone ALP is a very reliable marker of bone formation. However, this method was used first some years ago and is being replaced by immunological assays that use a monoclonal antibody that recognizes the bone glycoform.

MALIGNANT DISEASES

Tumor-associated antigens often involve carbohydrates and are termed "TACAs" for tumor-associated carbohydrate antigens, which are found in both glycolipids and N- and O-linked oligosaccharides of glycoproteins *(61–63)*. These tumor-associated carbohydrate antigens seem to function mainly as adhesion molecules based on either carbohydrate interactions or carbohydrate selection interactions. Some of these antigens are found exclusively in mucin-type glycoproteins and are known as T, sialyl T, Tn, and sialyl Tn, each defined by a specific monoclonal antibody (Fig. 10). Their specific or high degrees of expression in certain types of cancer prompted researchers to evaluate their potential use as diagnostic and/or prognostic tools. It appeared that if their "diagnostic value" for cancer detection was low, they could have "prognostic value", particularly in the early stages of

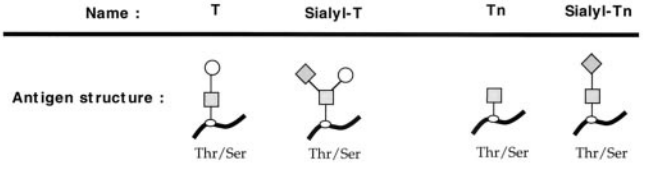

Fig. 10. Tumor-associated carbohydrate antigens.
○, galactose; ◆, SA; ▨, GalNAc; ∼, polypeptide chain.

tumor development. The clinical usefulness of their expression in tumors or in sera of cancer patients is under investigation in comparison studies with available tumor markers.

Oligosaccharide alterations are not restricted to surface antigens, but are also detected in serum glycoproteins. α-Fetoprotein (AFP), an oncofetal glycoprotein, in particular has been studied. Altered and different glycosylation of serum AFP has been found in serum from patients suffering from hepatocellular carcinoma and seminomatous germ-cell tumors *(64)*. Specific lectins, which recognize these altered oligosaccharides, are used in affinity electrophoresis, which is easy to perform routinely.

Biochemical aspects. AFP, one of the major plasma glycoproteins in early embryonic life, is synthesized in the yolk sac and then by the liver *(64)*. In an advanced stage of pregnancy, the concentration of liver-synthesized AFP in fetal serum decreases, whereas the albumin concentration increases. After birth, the serum AFP concentration becomes very low. In hepatocellular carcinomas and in germ-cell tumors, AFP is re-expressed, and its serum concentration greatly increases.

During pregnancy, AFP is also subjected to variations in the oligosaccharide moiety, giving different glycoforms, the proportions of which depend on the cell types secreting AFP. In adults, glycoforms of re-expressed AFP specific for hepatic regeneration *(65)* and yolk sac-derived tumors *(66)* are identified by the presence of Fuc on the first GlcNac of the core or an additional bisected GlcNAc on the first Man, respectively.

Laboratory diagnosis. The use of a combination of two lectins, Con A and *Lens culinaris* agglutinin (LCA) allows laboratorians to distinguish between benign liver disorders and hepatocellular carcinoma and between hepatic and germ-cell tumors. The presence of Fuc on the first GlcNAc residue of a biantennary oligosaccharide largely favors the interaction of LCA with such oligosaccharide structures, whereas the presence of a bisected GlcNAc residue at the trimannosidic core dramatically decreases the interaction of biantennary oligosaccharides with Con A. Briefly, serum AFP from hepatocellular carcinoma reacts with LCA and Con A, whereas AFP from yolk sac tumors binds LCA and does not react with Con A (Fig. 11). Several studies investigating diagnostic tools for hepatocellular carcinoma have shown that the percentage of LCA-reactive serum AFP is more sensitive than the serum AFP concentration itself. This is particularly interesting in the monitoring of patients with chronic liver diseases *(67)* because the incidence of hepatocellular carcinoma in association with cirrhosis is high.

The simultaneous determination of Con A- and LCA-reactive glycoforms in crossed immuno-affinoelectrophoresis for AFP is also particularly useful for differentiating seminomatous from nonseminomatous germ-cell tumors in neonates and infants in whom the serum AFP concentration is in the upper physiological range *(68)*.

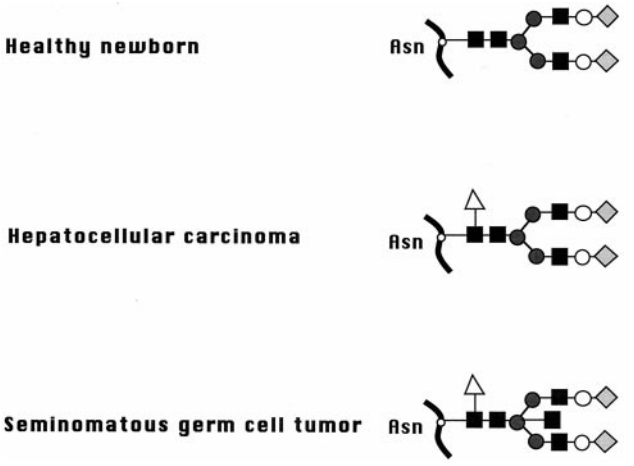

Fig. 11. Serum AFP glycan chain from a healthy newborn and from patients with hepatocellular carcinoma and with a seminomatous germ-cell tumor.

■, GlcNAc; ○, galactose; ●, mannose; △, fucose; ◆, SA; ∼, polypeptide chain.

OTHER DISEASES

Changes in the oligosaccharide moieties of glycoproteins are linked to many other disorders. The immune complexes from patients with rheumatoid arthritis are particularly rich in asialo-agalactosyl IgG, and these changes in IgG glycosylation are a consistent feature of patients with rheumatoid arthritis *(69)*.

The oligosaccharide profile of acute phase proteins is modified in inflammatory disorders *(70, 71)*, without any relation to the serum concentrations of these glycoproteins during acute inflammation. In several serum glycoproteins, such as AGP, TRF, and α_2-HS glycoprotein, during acute inflammation the number of branches is reduced, whereas during chronic inflammation, the number increases. These changes are easily demonstrated by crossed immuno-affinoelectrophoresis in the presence of Con A.

Conclusion

Sugar residues and oligosaccharide chains play diverse and crucial roles in several biological processes. Some inherited and nongenetic diseases are clearly the results of alterations in their structures. The techniques that have been developed have enabled biologists to detect variations in glycosylation and have thus become valuable diagnostic tools. Aberrant sugar chains may also alter the global structure of glycoproteins, particularly the peptide moiety. This raises the question of their interference in biochemical methods and particularly in immunological methods.

Glycobiology is a very active field of research that may lead to new approaches in the diagnosis and prognosis of human diseases.

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