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REVIEW

A retrospective and prospective view of glycopathology*

Akira Kobata‡

Tokyo Metropolitan Institute of Gerontology, 35-2 Sakaecho, Itabashi-ku, Tokyo 173, Japan

***Keywords*: Glycopathology, sugar chains, human chorionic gonadotropin, *N*-acetylglucosaminyltransferase**

***Abbreviations*: hCG, human chorionic gonadotropin; GnT, *N*-acetylglucosaminyltransferase; Py, polyoma; BHK, baby hamster kidney; DSA, *Datura stramonium* agglutinin; RSV; Rous sarcoma virus**

The dawning of glycopathology

Elucidation of the functional roles of nucleic acids as key materials for protein formation has completely changed the field of biological research. Establishment of molecular biology in a narrow sense, which deals with nucleic acids and proteins as information molecules, has certainly made and continues to make contributions to many aspects of life science. In the field of pathology, investigation of the abnormalities in nucleic acids is expected to elucidate the genetic background of various diseases. Hence, the human genome project, which plans to elucidate the structure of complete human genome, is being promoted on an international scale.

The information networks constructed by nucleic acids and proteins are, however, so rigid and essential for the maintenance of living organisms, that most of the serious defects induced in these molecules will lead to cell death. Most diseases should be brought about by abnormalities induced in the control mechanisms of cells, which are not essential for the maintenance of life itself, but are useful for the maintenance of social behaviour of differentiated cells in multi-cellular organisms.

Most of the secretory and membrane bound proteins, produced by the cells constructing multi-cellular organisms, contain covalently linked sugar chains and are called glycoproteins. Because of the difficulties associated with the structural study of the sugar chains of glycoproteins, the functional aspects of the sugar moieties of glycoproteins were previously ignored during the long history of protein research. However, recent development of various sensitive methods to elucidate the structures of the sugar chains has opened up a road to investigate these molecules, precisely.

In view of the recent finding that the sugar chains of glycoproteins play an important role in the control of cellular function and cellular recognition, investigation of the sugar chains formed under pathological states is expected to afford many important data for the understanding of various diseases. In this review article, I would like to introduce our work related to the pathology of glycoproteins to indicate the importance of this research field.

Alteration of the sugar chain structures of glycoproteins produced by tumours

It has been known since the early 1970s that altered glycosylation occurs in the glycoproteins produced by tumour cells. This information was obtained by using various indirect methods, such as monosaccharide analysis, comparison of the sizes of the glycopeptides obtained after exhaustive pronase digestion, and the study of the binding properties of glycoproteins with several lectins. This phenomenon, however, is considered very important for understanding tumours, because the sugar chains of glycoconjugates on the cell surface and in the inter-cellular matrix have been found to play important roles in a cell's interactions with its environment. Alteration in the carbohydrate structures of glycoproteins found in various tumours are, therefore, considered to be the basis of the abnormal social behaviour of tumour cells, such as invasion into the surrounding tissues and metastasis.

‡To whom correspondence should be addressed. E-mail: kobata@center.tmig.or.jp

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Establishment of hydrazinolysis [1], a chemical means to release quantitatively the N-linked sugar chains of glycoproteins as oligosaccharides, was effective in discovering the structural alterations induced in the sugar chains of glycoproteins produced by tumour cells, by comparing the sugar patterns of a glycoprotein produced by a malignant cell and its normal counterpart.

1) Pathology of the N-linked sugar chains of human chorionic gonadotropin

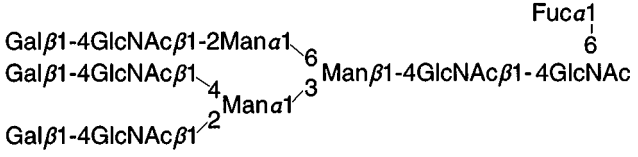
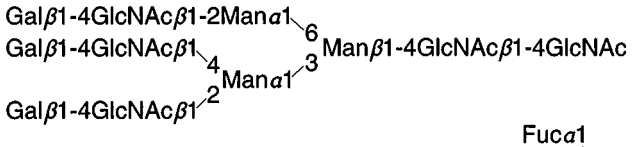
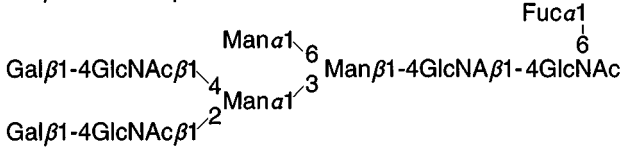
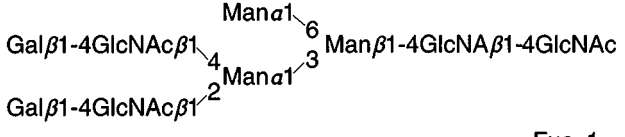
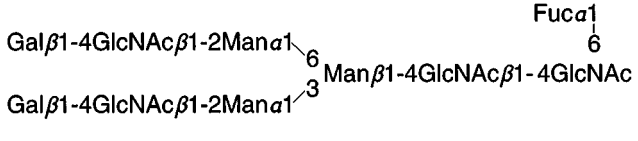
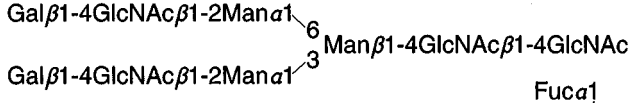
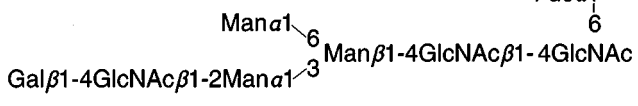
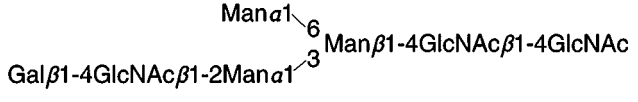
The first case of the application of hydrazinolysis in the study of altered glycosylation was successfully performed on human chorionic gonadotropin (hCG). HCG is a glycoprotein hormone produced by trophoblasts of placenta. High levels of hCG are also detected in the blood and the urine of patients with a variety of trophoblastic diseases. Therefore, urinary and serum hCG levels have been measured as useful markers for the diagnosis and prognosis of trophoblastic diseases as well as normal pregnancy. Many sensitive methods to determine the level of hCG in body fluids have been developed. Although these methods eliminate problems caused by interfering materials coexisting with hCG in serum and urine, none of them provided a way to discriminate hCGs from various trophoblastic diseases. Differential diagnosis of trophoblastic diseases is important because it is essential for the proper treatment of the diseases. Hydatidiform mole is considered to be essentially a benign lesion, although the rate of incidence of choriocarcinoma in patients with this disease is much higher than in normal pregnancy. Some hydatidiform moles show apparently more malignant characteristics than others, such as invasion into the surrounding tissues and metastasis, and are pathologically discriminated from typical moles by the name of invasive mole. Although prophylactic chemotherapy is effective in reducing the development of choriocarcinoma, use of chemotherapy at the time of mole removal is still controversial because of the drug toxicity. Therefore, any method to discriminate an invasive mole from hydatidiform mole would be useful to avoid indiscriminate prophylactic chemotherapy.

Comparative study of the oligosaccharides, released from hCGs purified from the urine of pregnant women and patients with trophoblastic diseases, revealed that interesting alterations were found in the sugar chains of hCGs purified from urine of patients with malignant trophoblastic diseases [2–5]. As summarized in Table 1, hCGs from pregnant women and patients with hydatidiform mole contain the sialylated forms of oligosaccharides E, F and H in approximately a 1 : 2 : 1 molar ratio. The hCGs from patients with invasive mole contain the sialylated forms of oligosaccharides A, B, E, F, G and H. The hCGs from patients with choriocarcinoma contain either sialylated or non-sialylated forms of all the eight oligosaccharides shown in Table 1. These results indicated that an abnormal expression of *N*-acetylglucosaminyltransferase (GnT) IV is the key that alters the glycosylation of hCG in choriocarcinoma. Since oligosaccharides A, B, C, and D were not detected in the hCGs from pregnant women and patients with hydatidiform mole, GnT IV, which catalyses the formation of the GlcNAcβ1-4Manα1-3 group, should not be expressed in the hCG producing cells. The presence of oligosaccharides A and B in hCG indicated that GnT IV is abnormally expressed in invasive mole. Expression of oligosaccharides C and D together with oligosaccharides A and B indicated that the abnormally expressed GnT IV in choriocarcinoma shows specificity for monoantennary sugar chains as well as biantennary sugar chains. It was reported by Schachter's group [6] that GnT IV solubilized from Golgi membrane can add a β-*N*-acetylglucosamine residue to the monoantennary sugar chains. However, oligosaccharides C and D have not been detected in the glycoproteins produced by various normal cells. Hence, we called them abnormal biantennary sugar chains, expecting them to become important tumour markers in the future. Therefore, a control mechanism to prevent formation of abnormal biantennary sugar chains must exist in the Golgi apparatus of normal cells. In connection with this problem, recent finding by Takeuchi's group [7] of two different GnT IV genes in human liver might be important.

The structural alterations, so far described, indicate that invasive mole should be considered as a premalignant stage, because ectopic expression of an enzyme was found. Therefore, discrimination of invasive mole from regular hydatidiform mole was considered most important for the accurate diagnosis of malignancy in trophoblastic diseases. This was successfully performed by using an immobilized *Datura stramonium* agglutinin (DSA) column [8]. Oligosaccharides with the Galβ1-4GlcNAcβ1-4(Galβ1-4GlcNAcβ1-2)Man group are retained on this column [9].

Urine samples from pregnant women and patients with trophoblastic diseases were passed through a small DSA-Sepharose column with or without sialidase pretreatment. The columns were washed with buffer, and then eluted with 0.1 N acetic acid. The amount of hCG in the washings and in the acetic acid eluates was determined by enzyme-immunoassay using an anti-hCG antibody, and the percentages of hCGs bound to the lectin column were calculated. As summarized in Figure 1A and B, urinary hCGs from pregnant women and patients with hydatidiform mole were not bound to the column, even after sialidase digestion. The values of invasive mole patients were also low without sialidase digestion. However, they became much higher after sialidase treatment (Figure 1C). The results in the case of choriocarcinoma hCGs showed more variation than others. Some of them gave results very similar to those obtained with invasive mole hCGs (Figure 1D). A significant portion of a choriocarcinoma hCG bound to the column, although the value became much higher after desialylation. One of the choriocarcinoma hCGs bound

Table 1. Desialylated sugar patterns of urinary hCGs obtained from normal pregnant women and various trophoblastic diseases.

	Structures	*Normal*		*Hydatidiform mole*			*Invasive mole*		*Choriocarcinoma*			
		1	*2*	*1*	*2*	*3*	*1*	*2*	*1*	*2*	*3*	*4*
A		–	–	–	–	–	% 5.9	6.7	20.7	34.5	31.2	20.0
B		–	–	–	–	–	6.1	2.3	2.3	10.8	11.8	8.0
C		–	–	–	–	–	–	–	12.2	12.9	8.8	14.1
D		–	–	–	–	–	–	–	9.6	11.4	12.6	15.5
E		24.0	25.2	25.5	25.7	26.2	58.5	29.6	7.2	5.3	6.4	6.5
F		48.7	49.1	49.0	51.2	50.3	9.5	21.4	4.0	5.2	6.9	5.0
G		–	–	–	–	–	1.6	9.2	8.8	5.0	4.3	5.3
H		27.3	25.7	25.5	23.1	23.5	18.4	30.8	35.2	14.9	18.0	25.6

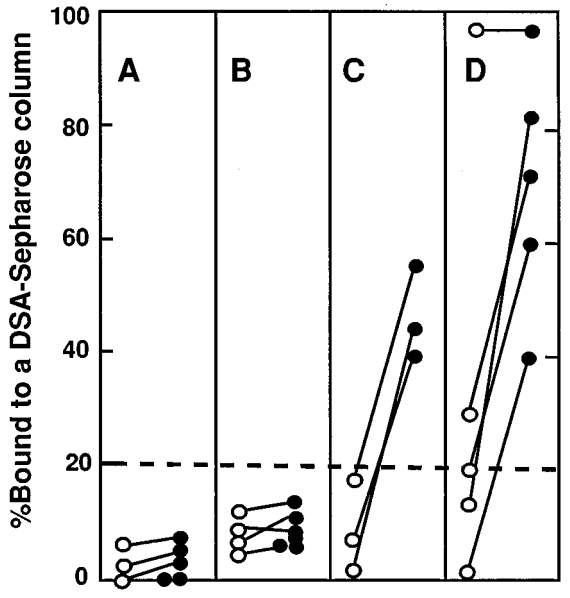


Figure 1. Percentage molar ratio of hCGs bound to a DSA-Sepharose column before (○) and after (●) sialidase treatment [7]. A, B, C and D represent the data obtained from urine samples from normal pregnant women, hydatidiform mole patients, invasive mole patients and choriocarcinoma patients, respectively.

completely to the column before sialidase digestion. Therefore, the affinity column chromatography using a DSA-Sepharose column can be effectively used to discriminate malignant hCGs from normal and hydatidiform mole hCGs in urine samples.

2) 'Warren-Glick' phenomenon

In 1969, Meezan *et al.* [10] reported an interesting phenomenon observed in the sugar chains of the plasma membrane glycoproteins of malignant cells. They cultured mouse 3T3 fibroblasts and their Simian virus (SV)-transformants in media containing [^{14}C]glucosamine and [^{3}H]glucosamine, respectively. After mixing the harvested cells, the plasma membrane fraction was isolated, and the glycoproteins in the membrane preparation were converted to glycopeptides by exhaustive pronase digestion. When the radioactive glycopeptide mixture was analysed by gel permeation chromatography, the ^{3}H-labelled glycopeptides from transformed cells were larger than the ^{14}C-labelled glycopeptides from non-transformed cells. The studies of many researchers were stimulated by this interesting observation and it was confirmed that the expression of the large sugar chains occurred in the malignant cells obtained by viral, chemical and spontaneous transformation [11–13]. Since the chick embryo cells, transformed by a *ts* mutant of Rous sarcoma virus (RSV), expressed the large sugar chains at the permissive temperature but not at the non-permissive temperature [14], the phenomenon was considered to be an expression of true transformed phenotype. Since Warren and Glick published many papers on this phenomenon, it has become known as the 'Warren-Glick' phenomenon.

Extensive analyses have been performed to elucidate the molecular basis of the Warren-Glick phenomenon. By analysing the behaviour of glycopeptides obtained from normal and malignant cells on a concanavalin A-Sepharose column, Ogata *et al.* [15] estimated that the molecular basis of the 'Warren-Glick' phenomenon is the increase in tri- and tetraantennary complex type N-linked sugar chains in malignant cells. This estimation was confirmed by the structural study of the radioactive oligosaccharides released by hydrazinolysis from the glycopeptide mixture [16]. As summarized in Table 2, oligosaccharides chemically released from glycopeptides from the polyoma (Py) transformant of baby hamster kidney (BHK) cells were more enriched in the complex type sugar chains containing the Galβ1-4GlcNAcβ1-6(Galβ1-4GlcNAcβ1-2)Man group as well as the elongated outer chains, than those from BHK cells.

The increase of complex type sugar chains with a 2,6-branched outer chain and of those with the elongated outer chains were also found to occur in RSV transformed BHK cells by Pierce and Arango [17]. Therefore, the increase of these sugar chains might well be considered as the molecular basis of the 'Warren-Glick' phenomenon.

The structural alteration of N-linked sugar chains shown in Table 2 could arise from the change in β-*N*-acetylglucosaminyltransferase (GnT) activities caused by the abnormal genetic control in malignant cells. It could also arise from changes in the membrane components in malignant cells, because transferases are embedded in the Golgi membrane. In order to confirm which of these possibilities are correct, activities of a series of GnTs in BHK cells and Py-BHK cells were investigated. So far, five different GnTs (as shown in Figure 2) have been found to add β-*N*-acetylglucosaminyl residues at different positions of the trimannosyl core. In addition, GnT VII, which adds an β-*N*-acetylglucosamine residue at the C-3 position of the galactose residue, should occur in both cells because the Galβ1-4GlcNAc repeats were detected in the outer chain moieties of some oligosaccharides shown in Table 2.

By comparative study of the six GnTs in the homogenates of BHK and Py-BHK cells [18], it was found that GnT V activity was increased approximately three-fold in Py-BHK cells (Table 3). In contrast, the same levels of GnTs I, II, IV and VII were detected in both cells. No GnT III activity was detected in the two cell types. These results agreed with the fact that no bisected N-linked sugar chain was detected in the membrane glycoproteins of both cell types (Table 2). Van den Eijnden and Schiphorst [19] reported that GnT VII works most favourably on the sugar chains containing the Galβ1-4GlcNAcβ1-6(Galβ1-4GlcNAc β1-2)Manα1-6 group. Therefore, a slight increase in the percentage of the sugar chains with elongated outer chains can also be explained by the increase of GnT V activity in Py-BHK cells.

Table 2. N-linked sugar chains of the membrane glycoproteins of BHK and Py-BHK cells.

Oligosaccharide structures	*BHK*	*Py-BHK*
Galβ1→4GlcNAcβ1→2Manα1↘6 Galβ1→4GlcNAcβ1→2Manα1↗3 Manβ1→4GlcNAcβ1→4GlcNAc	26%	22%
Galβ1→4GlcNAcβ1→2Manα1↘6 Galβ1→4GlcNAcβ1↘4 Manα1↗3 Manβ1→4GlcNAcβ1→4GlcNAc Galβ1→4GlcNAcβ1↗2	10%	> 4%
Galβ1→4GlcNAcβ1↘6 Manα1↘6 Galβ1→4GlcNAcβ1↗2 Manβ1→4GlcNAcβ1→4GlcNAc Galβ1→4GlcNAcβ1→2Manα1↗3	3%	< 6%
Galβ1→4GlcNAcβ1↘6 Manα1↘6 Galβ1→4GlcNAcβ1↗2 Manβ1→4GlcNAcβ1→4GlcNAc Galβ1→4GlcNAcβ1↘4 Manα1↗3 Galβ1→4GlcNAcβ1↗2	5%	< 15%
(Galβ1→4GlcNAcβ1→3)$_n$ { Galβ1→4GlcNAcβ1↘6 Manα1↘6 Galβ1→4GlcNAcβ1↗2 Manβ1→4GlcNAcβ1→4GlcNAc Galβ1→4GlcNAcβ1↘4 Manα1↗3 Galβ1→4GlcNAcβ1↗2	16%	< 25%

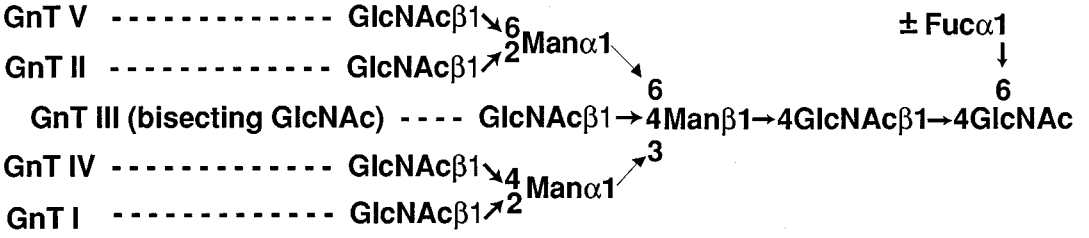


Figure 2. GnTs responsible for the formation of complex type sugar chains.

Although the 'Warren-Glick' phenomenon is widely observed in malignant cells, it is also true that not all malignant transformants show this phenomenon. For example, MT1 cells, an early SV-40 gene transformant obtained from mouse fibroblast NIH3T3 cells, does not show the 'Warren-Glick' phenomenon, although it has been considered malignant because it grows in soft agar. Occurrence of such exceptions led us to the idea that the 'Warren-Glick' phenomenon might be correlated to a higher order of malignant characters such as tumorigenesis and metastasis. Actually, studies of several transformants derived from NIH3T3 cells, and the transformants of 3Y1 cells, a fibroblast line established from Fisher rat embryo, obtained by transfection with the various fragments of human adenovirus type 12 DNA, revealed that expression of the 'Warren-Glick' phenomenon and tumorigenicity are highly correlated [20].

Complex type sugar chains with the Galβ1-4GlcNAcβ1-6(Galβ1-4GlcNAcβ1-2)Manα1-6 group bind to L_4-PHA, an isolectin of the red kidney bean which specifically agglutinates leukocytes. Accordingly, the amount of sugar chains

Table 3. Relative activities of various GnTs in the homogenates of BHK and PY-BHK cells.

Acceptors	*GnTs*	*BHK*	*Py-BHK*	*Py-BHK/ BHK*
M_2GN	I	100	100	1.00
M_5GN	I	1470	1436	0.98
M_3GN	I and II	1480	1635	1.10
GN_2M_3GN	IV	43	40	0.93
	V	13	44	3.38
GN_3M_3GN	V	24	69	2.82
$G_4GN_4M_3GN$	VII	45	48	1.07

containing the Galβ1-4GlcNAcβ1-6 (Galβ1-4GlcNAcβ1-2)Manα1-6 group on the surface of a cell can be roughly estimated by staining the cell with [^{125}I]L_4-PHA. Dennis *et al.* [21] investigated the binding of [^{125}I]L_4-PHA to a mouse mammary carcinoma SP-I, which shows no metastatic characteric and its metastatic transformants obtained by inserting various oncogenes. An important finding obtained by this study was that the pulmonary metastatic characters of the cells are positively correlated with the amount of [^{125}I]L_4-PHA bound to them. Similar results were obtained by comparative study of the oligosaccharide patterns released from mouse B16 melanoma cells and their variants with different metastatic characters by hydrazinolysis [22]. Recent investigation by Kawano *et al.* [23, 24] on the surface sugar chains of the F1 clone of B16 melanoma and two of its lectin mutants, which lost the pulmonary metastatic character, revealed that the decrease in the complex type sugar chains containing the Neu5Acα2-3Galβ1-4GlcNAcβ1-6(Neu5Acα2-3Galβ1-4GlcNAcβ1-2)Manα1-6 group in the surface glycoproteins is associated with the loss of metastatic properties in the two mutant cell lines.

3) Other important observations on the altered glycosylation in tumours

The glycoproteins produced by the normal liver of mammals do not contain bisected sugar chains, indicating that GnT III is not expressed in mammalian hepatocytes. Ectopic expression of bisected sugar chains in hepatoma was found by the comparative study of the N-linked sugar chains of γ-glutamyl transpeptidases (γ-GT) purified from rat liver and rat AH-66 hepatoma [25]. However, this interesting finding could not be applied to the diagnosis of human hepatoma, because the expression of bisected sugar chains was not prominent in the γ-GT produced by human hepatoma [26]. This is a good example indicating that some of the altered glycosylation of proteins produced by tumour cells are species-specific. The γ-GT purified from human hepatoma was found to be more enriched in the tri- and tetra-antennary sugar chains, indicating the expression of the 'Warren-Glick' phenomenon in this glycoprotein. Expression of the abnormal biantennary sugar chains, found in the choriocarcinoma hCG, was also observed in this glycoprotein.

Further studies of altered glycosylations in other glycoproteins produced by human hepatoma have been published by Yamashita's group [27–29]. Interested readers should consult for the cited papers.

Carcinoembryonic antigen (CEA) is a glycoprotein antigen, which was found by Gold and Freedman [30] in the glycocalyx of the epithelial cells of the fetal digestive tract and the adenocarcinoma cells derived from the adult digestive tract. The antigen has become one of the most widely used tumour markers, the serum level of which is used for long term monitoring of the prognosis of patients with colon, breast or lung adenocarcinoma after surgery. Glycoproteins cross-reacting with anti-CEA antibodies were found in normal human faeces, meconium, lung and spleen as well as haematopoietic cells including granulocytes and monocytes. The largest cross-reacting antigens in meconium and adult faeces were named non-specific cross-reacting antigen-2 (NCA-2) [31] and normal fecal antigen-2 (NFA-2) [32], respectively.

By successful cloning the cDNA of CEA, it was found that NFA-2 is the same gene product as CEA [33–35]. NCA-2 is also considered to have the same amino acid sequence. It has been known from an early stage in CEA research that CEA contains a large amount of sugars. Since NFA-2, NCA-2 and CEA are produced by colon epithelial cells of normal adults, those of fetus at their last differentiation stage, and those in malignant states, comparative study of their sugar chain structures was expected to give useful information related to the development and malignant transformational changes of the sugar chains of colon glycoproteins. It is also expected that the data may contribute to improvements in the diagnostic value of CEA, if any difference among the sugar chains of CEA and CEA-related antigens in normal tissues are found.

A comparative study of CEA, NFA-2 and NCA-2 [36–38] revealed that they have different sets of N-linked sugar chains reflecting the developmental and malignant transformational changes of the sugar chains of colon glycoproteins. High mannose type sugar chains and the abnormal biantennary sugar chains were detected only in CEA. The branching structures of the complex type sugar chains of the three antigens were almost the same. However, Type 1 chain (Galβ1-3GlcNAc) is detected in the outer chain moieties of NFA-2 and NCA-2, but not in those of CEA. The acidic sugar chains of NFA-2 and NCA-2 contain the Galβ1-3(SO_4^--6)GlcNAc and the Neu5Acα2-3Galβ1-3GlcNAc groups. In contrast, the Galβ1-4(SO_4^--6)GlcNAc and the Neu5Acα2-6Galβ1-4GlcNAc groups are detected in CEA. These interesting findings are expected to be useful for discriminating CEA from the related antigens produced by non-malignant tissues.

Function and pathology of the sugar chains of human immunoglobulin G (IgG)

IgG is composed of two types of polypeptide chains: called heavy (H) chain and light (L) chain with a stoichiometry of H_2L_2. Each H chain contains an N-linked sugar chain at the Asn 297 [39, 40]. The structural study of whole sugar chains from human IgG samples, purified from sera of healthy individuals, revealed that several unique characteristics are included in these sugar chains [41]. Only 25% of the sugar chains are sialylated. This is very unusual because the N-linked sugar chains of other serum glycoproteins are highly sialylated. Another characteristic feature of the sugar chains of IgG is the occurrence of extremely high microheterogeneity. In Figure 3, the largest neutral portion of the sugar chains of IgG is shown. The microheterogeneity is mainly produced by the presence or absence of the two galactoses, the bisecting GlcNAc and the fucose residue underlined in the structure in Figure 3. Therefore, at least sixteen different sugar chains are detected as the neutral portion of the sugar chains of IgG. The addition of sialic acid residues to these neutral sugar chains produces more than thirty different sugar chains.

Despite this extremely high heterogeneity, the molar ratios of each oligosaccharide present in the IgG samples obtained from sera of healthy individuals are quite constant [42]. The percentage molar ratio of the neutral, monosialyl and disialyl oligosaccharides released from IgG samples purified from the sera of healthy individuals were close to 76 : 18 : 6. The percentage molar ratio of the bisected sugar chains and those of fucosylated ones were 18 and 85–86, respectively. Myeloma IgGs, which are supposed to be the products of monoclonal B cells, also contain biantennary complex type sugar chains. However, the percentage molar ratio of each oligosaccharide released from 13 myeloma IgGs was not constant [42]. The neutral oligosaccharides ranged from 58 to 90%, monosialyl oligosaccharides from 9 to 42% and disialyl oligosaccharides from 0 to 9%. Oligosaccharides with bisecting GlcNAc were 6–58%. The structural variation of the sugar moieties of the monoclonal IgGs are not correlated with their subclasses. A possible explanation of these data is that B cells are a mixture of clones which are equipped with different sets and ratios of glycosyltransferases responsible for the maturation of the complex type N-linked sugar chains. The constancy in the ratio of oligosaccharides of serum IgG preparations from healthy individuals indicates that the ratio of B cells with different sets of glycosyltransferases is quite constant in individuals.

Interestingly, the sugar chains of IgG purified from sera of patients with rheumatoid arthritis are predominently devoid of galactose residues [43]. The galactose deficiency in rheumatoid arthritis is considered to be limited to the sugar chains of the IgG molecule, because the transferrin samples purified from the sera of patients with rheumatoid arthritis contain fully sialylated biantennary sugar chains as in the case of those from healthy individuals (Endo T., Kobata A. unpublished data). Therefore, the relative lack of galactose residues on rheumatoid IgGs could be induced by the abnormality of the galactosyltransferase in B cells rather than by the degradation by β-galactosidase during their circulation.

The comparative study of the homogenates of B cells obtained from patients with rheumatoid arthritis and healthy individuals revealed the following interesting findings [44]. Human B cells contain a galactosyltransferase that galactosylate quite specifically the N-linked sugar chains of IgG molecules. This enzyme in the B cells of patients with rheumatoid arthritis galactosylates IgG molecules less effectively than normal, because the affinity of the enzyme for UDP-Gal is lower than the enzyme found in healthy individuals. It was subsequently found that the degalactosylated IgG binds less effectively to the C1q and Fc-receptor. However, no decrease in binding to polyclonal rheumatoid factor and protein A was observed in the degalactosylated IgG [45]. These results indicate that the function of IgG molecules can be modified by the different degree of maturation of their sugar chains.

Recent development of an ELISA-based assay for the detection of agalacto-IgG in serum samples [46] will surely be useful for further clinical study of this interesting phenomenon.

The prospects for glycopathology

Various lysosomal exoglycosidase deficiencies have been known for a long time as congenital diseases related to the abnormal turnover of the sugar chains of glycoconjugates. However, the examples introduced in this review indicate that the number of diseases known to be caused by abnormalities in sugar chains has expanded tremendously in recent years. A distinguishing trait of these newly-described

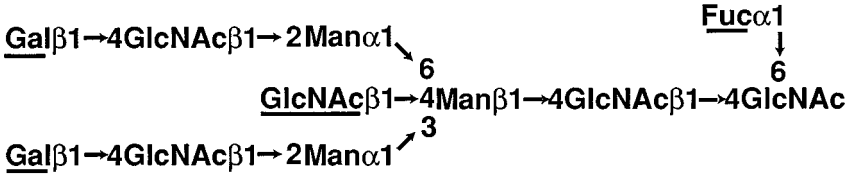


Figure 3. The largest desialylated N-linked sugar chain detected in human IgG.

diseases is that they are related to abnormalities in the biosynthesis of the sugar chains. Accumulation of our knowledge in the biosynthesis and the functional roles of the sugar chains of glycoproteins, with the advance in methods of studying the sugar chain structures, is contributing to this new genre, which might be called glycopathology.

As indicated in this review, comparative studies of the sugar chains of glycoproteins produced in various tumours and their normal counterparts indicate that the malignant alteration in sugar chains is quite variable. However, it is also true that a quite reproducible alteration is induced in a particular glycoprotein produced by a particular tumour. Therefore, it is possible to develop novel methods for the diagnosis and prognosis of various tumours by investigating the malignant alteration in the sugar chains of their glycoproteins. In relation to this, I would like to emphasize here that many tumour markers are glycoproteins. As exemplified by the study of hCG, searching for the tumour specific alteration in their sugar chains may unravel the evidence useful for increasing the values of these proteins as diagnostic tumour markers.

As in the case of the abnormal biantennary sugar chains, abnormal sugar chains produced by cancer cells can include those which have never been detected in normal human glycoproteins. Detection of the abnormal biantennary sugar chains in γ-GT from human hepatoma and CEA as well as hCG, suggest that some of such alterations may work as a new common antigen widely induced in many tumours. Therefore, elucidation of the mechanism that prevents formation of the abnormal biantennary sugar chains in normal cells and the mechanism of the override of this inhibition in tumour cells is expected to be an interesting target for future study.

Many preliminary studies, which will contribute to the development of glycopathology, have been reported. These include genetic disorders caused by a defect in N-linked sugar chain biosynthesis or processing, such as HEMPAS [47] and carbohydrate-deficiency glycoprotein syndrome [48]. Wiskott-Aldorich syndrome was found to be related to the abnormality of O-linked sugar chains [49]. Because of the complex nature of the biosynthetic mechanism of the sugar chains of glycoproteins, many other diseases with associated glycosylation abnormalities are expected to be found in the near future.

In contrast to proteins, sugar chains are not formed by the direct transfer of the information stored in genes, but are formed by the concerted action of glycosyltransferases coded by their structural genes. Therefore, the structures of the sugar chains of glycoproteins can be modified by a change in physiological condition of cells. The finding of organ-specific differences in the sugar chain structures of glycoproteins [50] indicates that the expression of glycosyltransferases are both quantitatively and qualitatively modified by the development and the differentiation of cells in multi-cellular organisms.

That the sugar chain structures of some glycoproteins could be modified by ageing was shown by the finding that the galactose content of human serum IgG decreases in aged persons [46, 51]. Hence the alteration induced in the sugar chains of IgG can partly explain the phenomenon of immunodeficiency observed in aged persons.

Dementia is one of the most important targets of ageing research because it severely lowers the quality of life of an aged person. By pathological study of the brains of patients with Alzheimer's disease, deposition of β-amyloid was observed as an event highly correlated with the disease. Structural study of β-amyloid [52] and subsequent cloning of the gene, producing its polypeptide portion, revealed that a protein called APP is a precursor of β-amyloid [53]. Since a large amount of APP is also produced in healthy brain, elucidation of the mechanism to induce an abnormal cleavage of APP producing β-amyloid is considered as a key step of this line of study. In view of the fact that APP is a glycoprotein, study of its sugar chains and age related alteration of their structures may be important.

Since the biosynthesis of sugar chains is not controlled by the interpolation of a template, the structures of sugar chains are much less rigidly pre-programmed than those of proteins and nucleic acids. Accordingly, age related alterations in the sugar chains of various glycoproteins is an important target to solve various pathological problems found in aged individuals.

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