Review

Exo- and endoglycosidases revisited

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Abstract: Many glycosidases, which work as useful reagents for the studies of structures and functions of free and conjugated oligosaccharides, have been found and thoroughly purified. These enzymes are classified into exo- and endoglycosidases by their glycon specificities. Their usefulness and limits as reagents are explained in detail in this review.

Endoglycosidases, which were originally found in the culture fluid of bacteria and in the extracts of plants, are now widely found in the mammals including humans. The physiological roles of these enzymes are discussed in relation to the oligosaccharides accumulated in the urine of patients with exoglycosidase deficiencies. Furthermore, PNGase is found to play important roles in the ER-associated degradation pathway of glycoproteins.

Recent studies of the glycosidases in *Bifidobacteria* have revealed that GNB/LNB pathway, which uniquely exist in this bacteria, works for the expression of Bifidus factor activity of human milk oligosaccharides, an important topic in the baby nutrition. This interesting field will be introduced in detail in one section of this article.

Keywords: aglycon specificity, Bifidus factor, glycon specificity, glycosidase-deficiencies, GNB/LNB pathway, human milk oligosaccharides

Enzymes, which hydrolyze sugar chains are called glycosidases. The topics related to glycosidases are very wide. For example, various glycosidases obtained from microorganisms, and recombinant glycosidases are used as important industrial reagents to produce monosaccharides from various homoglycans. Furthermore, applications of glycosidases as important industrial reagents in the fields of food, pharmaceutical, and paper manufacturing industries are well known.

In this review article, however, I would like to limit the subjects to the glycosidases, which have been used as useful reagents for the structural studies and functional studies of sugar chains. In addition, the physiological roles of these enzymes in the producing cells will also be discussed.

1. Glycosidases used as reagents for the structural studies of sugar chains

1-1. Exoglycosidases. Exoglycosidases are the enzymes, which release particular monosaccharides from non-reducing termini of oligosaccharides and the sugar chains of glycoproteins and glycolipids. The general structure of α-L-fucopyranoside is shown in Fig. 1. Exoglycosidases, which hydrolyze this compound into L-fucose and ROH, are called α-L-fucosidases. In this article, discrimination of D- and L- of monosaccharides are ignored, and the enzymes will be simply named α-fucosidases.

α-Fucosidases work on α-fucopyranosides, but not on its anomeric isomers: β-fucopyranosides, and on the other glycosides having different monosaccharides.

As described so far, exoglycosidases usually show strong substrate specificities to both monosaccharide moiety and its anomeric structure. Therefore, they are named by the monosaccharide and its anomeric

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Abbreviations: β-HexNAc'ase: β-*N*-acetylhexosaminidase; DNS: 5-dimethylaminonaphthalene-1-sulfonyl; ERAD: ER associated degradation; Fuc: L-fucose; Gal: D-galactose; GalNAc: *N*-acetyl D-galactosamine; GL-BP: galacto-*N*-biose/lacto-*N*-biose I-binding protein; Glc: D-glucose; GlcNAc: *N*-acetyl D-glucosamine; $GlcNAc_{OT}$: tritium-labelled *N*-acetyl D-glucosaminitol; LN*n*T: lacto-*N*-*neo*tetraose; LNT: lacto-*N*-tetraose; Man: D-mannose; Neu5Ac: *N*-acetylneuraminic acid; PNGase: peptide:*N*-glycanase.

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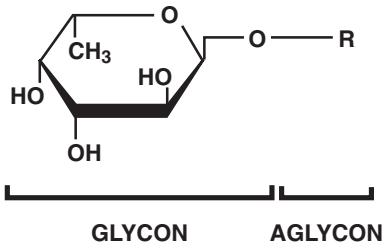


Fig. 1. General structure of α-L-fucopyranoside.

structure, which they hydrolyze, like α-fucosidase, or β-galactosidase etc. This substrate specificity is called **glycon specificity**. By using the substrate specificities of exoglycosidases, we can easily determine the monosaccharide located at the non-reducing termini of a sugar chain including its anomeric configuration.

Furthermore, monosaccharide sequence of a sugar chain can be easily determined, by sequential exoglycosidases digestion, since exoglycosidases release monosaccharides only from the non-reducing termini of a sugar chain.

For example, the sugar chains, which have the tetrasaccharide group: Galβ1-4GlcNAcβ1-2Manα1-3Manβ1—at their non-reducing termini, can be degraded to the Manβ1—group, only when they are sequentially digested with β-galactosidase, β-*N*-acetylglucosaminidase and α-mannosidase, by releasing one mole each of galactose, *N*-acetylglucosamine and mannose.

How many monosaccharides are released at each step of exoglycosidase digestion can easily be determined by using Bio-Gel P-4 column chromatography.[1)]

As an example, analytical data of a biantennary complex-type sugar chain obtained from human serum transferrin by hydrazinolysis followed by NaB^3H_4 reduction are shown in Fig. 2.

1-2. Glycon and aglycon specificities of exoglycosidases. Glycon specificities of some exoglycosidases are not so strict as others. For example, the β-glucosidase, purified from Almond emulsin, hydrolyzes not only β-glucopyranosyl linkage but also β-galactopyranosyl linkage.[2)] Many β-*N*-acetylglucosaminidases hydrolyze β-*N*-acetylgalactosaminyl linkages also, and are called β-*N*-acetylhexosaminidases. Namely, these enzymes do not recognize the epimeric difference at the C-4 position of the substrates. However, enzymes, which show such wide glycon specificities, are rather rare, and most exoglycosidases hydrolyze only one monosaccharide linkage. Now, I would like to describe about the **aglycon specificity** of glycosidases. This specificity is related to the structure of R in Fig. 1.

Many α-fucosidases were found from various sources. Among them, the enzymes purified from the hepatopancreas of marine gastropods can hydrolyze all α-fucopyranosyl derivatives having various R groups.[3)] In contrast, the α-fucosidase purified from the culture fluid of *Bacillus fulminans* hydrolyzes the Fucα1-2Gal group but no other disaccharides having Fucα1- residue at their non-reducing termini.[4)] Two α-fucosidases were purified from Almond emulsion.[5)] Among them, α-fucosidase I hydrolyzes the α-fucosyl residues of the Galβ1-3(Fucα1-4)GlcNAc group and the Galβ1-4(Fucα1-3)GlcNAc group, but cannot hydrolyze other α-fucosyl linkages.[6)] Accordingly, not only the number of α-fucosyl residues at the non-reducing termini of a sugar chain, but their binding sites in the sugar chain can be determined by using the three α-fucosidases. Quite recently, Sakurama *et al.*[7)] elucidated the structural basis of differences in the aglycon specificities of two α-fucosidases purified from *Bacteroides thetaiotaomicron*. Hopefully, this will open the door to elucidate the structural basis of differences of aglycon specificities of other α-fucosidases in the future.

Similar aglycon specificities were also found in other exoglycosidases, and effectively used for the structural analysis of sugar chains. For example, many β-galactosidases hydrolyze all disaccharides containing Galβ1- residue at their non-reducing termini, but cannot remove the galactose residue of the Galβ1-3(Fucα1-4)GlcNAc group and the Galβ1-4(Fucα1-3)GlcNAc group. β-Galactosidase purified from the culture fluid of *Diplococcal pneumoniae* can hydrolyze the Galβ1-4GlcNAc group but not the Galβ1-2GlcNAc, the Galβ1-3GlcNAc and the Galβ1-6GlcNAc groups.[8)]

A more complicated result was obtained by the study of β-galactosidase purified from Jack bean. The enzyme hydrolyzes the Galβ1-4GlcNAc group 50 times faster than the Galβ1-3GlcNAc group at low enzyme concentration. However, at high enzyme concentration, the enzyme hydrolyzes both groups at almost the same speed.[9)]

Many α-mannosidases hydrolyze all α-mannosyl linkages. However, the enzyme purified from *Aspergillus saitoi* hydrolyzes the Manα1-2Man group only, and cannot hydrolyze the Manα1-3Man and the Manα1-6Man groups.[10)] Accordingly, this enzyme has been effectively used to characterize the high mannose-type sugar chains, which contain large amount of the Manα1-2Man groups. It was also

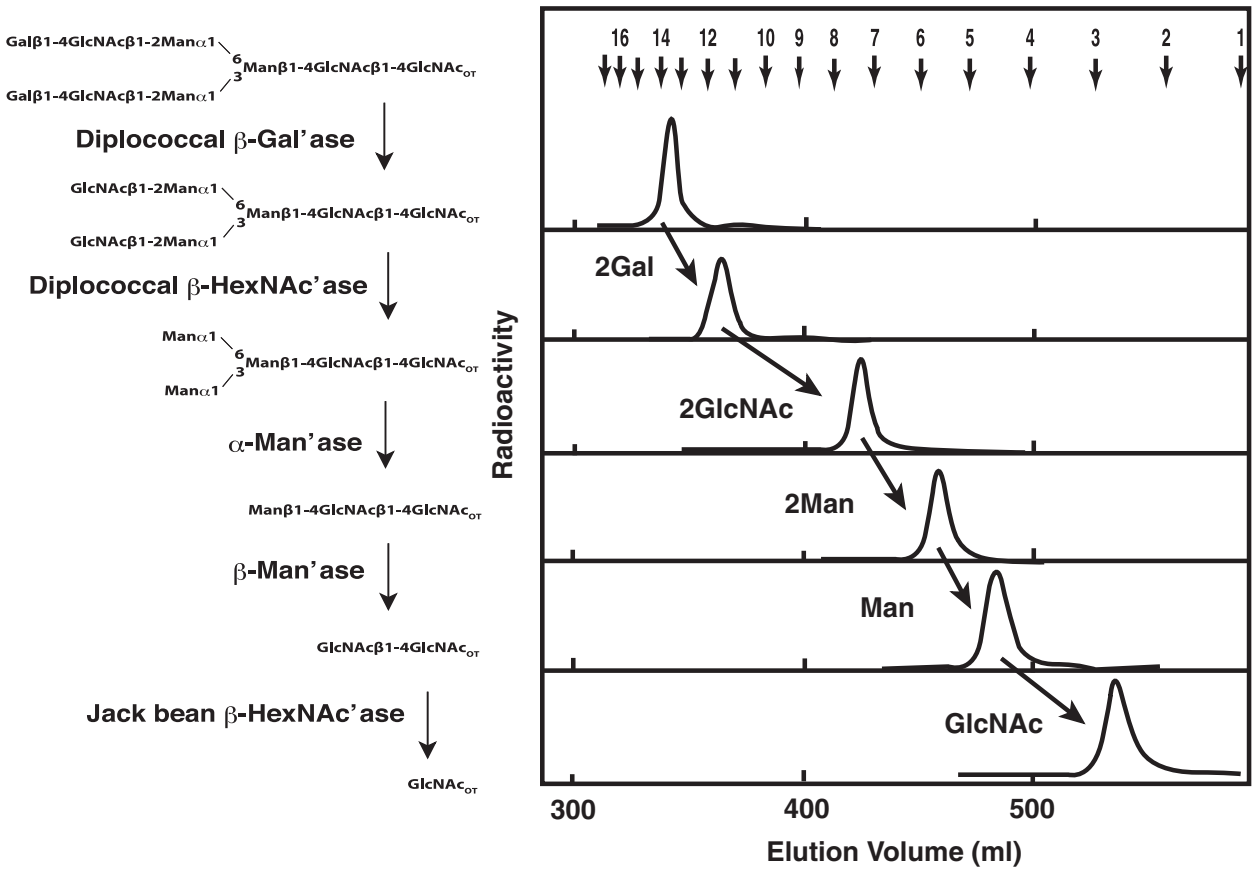


Fig. 2. Sequential exoglycosidases digestion analysis of the tritium labeled biantennary nonasaccharide obtained from human serum transferrin by hydrazinolysis. In the right figure, radioactive oligosaccharide at each step of exoglycosidase digestion was analyzed by Bio-Gel P-4 column chromatography. Arrows at the top of the figure are the elution positions of glucose oligomers (numbers indicate those of glucose units). β-Gal'ase, β-galactosidase; β-HexNAc'ase, β-*N*-acetylhexosaminidase; α-Man'ase, α-mannosidase; β-Man'ase, β-mannosidase.

known that the α-mannosidase purified from Jack bean hydrolyzes the Manα1-6Man group and the Manα1-2Man group at almost the same rate, but hydrolyzes the Manα1-3Man group at only 1/15 speed.

Different aglycon specificities were also found in the sialidases purified from various sources. The enzymes purified from *Clostridium perfringens* and from *Arthrobacter ureafaciens* hydrolyzes the Neu5Acα2-3Gal, the Neu5Acα2-6Gal and the Neu5Acα2-6GalNAc groups at almost the same rates. In contrast, the enzyme purified from *Vibrio cholera* hydrolyzes these three groups at the rates of 11:4:1, and the enzyme from influenza virus cleaves only the Neu5Acα2-3Gal group (Takasaki, S. and Kobata, A. unpublished data).

The most prominent aglycon specificity was found by the study of β-*N*-acetylhexosaminidase purified from *Diplococcus pneumoniae*,[11] and used for the structural studies of complex-type and hybrid-type sugar chains. This enzyme hydrolytically cleaves the GlcNAcβ1-2Man, the GlcNAcβ1-3Man and the GlcNAcβ1-6Gal groups, but cannot hydrolyse the GlcNAcβ1- residues shown in bold letters in Fig. 3. Namely, bisecting GlcNAc is resistant to the enzymatic digestion, and also inhibits the hydrolysis of the GlcNAcβ1-2 residue linked to the Manα1-6 arm. Furthermore, the GlcNAcβ1-6Man group is resistant to the enzyme digestion and also inhibits the hydrolysis of the GlcNAcβ1-2Man group on the same mannose residue. As described already, more delicate structural study of the sugar chains can be performed by using the aglycon specificities of exoglycosidases.

As an example of such study, structural study of a mixture of two octasaccharides[12] obtained from human milk will be introduced.

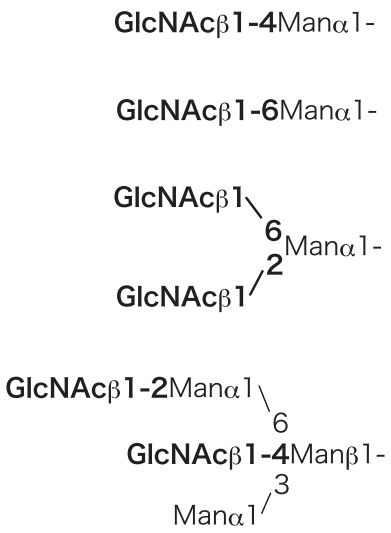


Fig. 3. The GlcNAcβ1- residues (bold letters), which are resistant to diplococcal β-*N*-acetyl hexosaminidase digestion.

By combining the sequential exoglycosidase digestion and methylation analysis, this octasaccharides fraction was found to be a mixture of oligosaccharides shown in Fig. 4A. When this oligosaccharides mixture was incubated with Almond α-fucosidase I, only the fucose residues close to the non-reducing termini were removed and the Galβ1-3(4)GlcNAcβ1- portions were exposed. When the mixture of these heptasaccharides was digested with a mixture of diplococcal β-galactosidase and Jack bean β-*N*-acetylhexosaminidase, approximately 30% of the oligosaccharides were converted to pentasaccharides and remaining 70% stayed as heptasaccharides. When the mixture of heptasaccharides was digested with a mixture of Jack bean β-galactosidase and Jack bean β-*N*-acetylhexosaminidase, all oligosaccharides were converted to a mixture of pentasaccharides. When this pentasaccharides mixture was digested with Almond α-fucosidase, it was converted to tetrasaccharide. This tetrasaccharide was completely converted to GlcNAcβ1-3Galβ1-4Glc by digestion with diplococcal β-galactosidase. Because diplococcal β-galactosidase cleaves the Galβ1-4GlcNAc group but not the Galβ1-3GlcNAc group, the tetrasaccharides fraction is concluded to be a pure lacto-*N*-*neo*tetraose. Based on this information, the original octasaccharides fraction was found to be a mixture of the octasaccharides constructed by addition of the Galβ1-3(Fucα1-4)GlcNAcβ1- group and the Galβ1-4(Fucα1-3)GlcNAcβ1- group at the C-3 position of the non-reducing terminal galactose of lacto-*N*-fucopentaose III: Galβ1-4(Fucα1-3)GlcNAcβ1-3Galβ1-4Glc in 7 to 3 molar ratio.

Usually, exoglycosidase activities were measured by using *p*-nitrophenyl glycosides as substrates. However, some of the enzymes purified by using the artificial substrates are not useful for the structural studies of the sugar chains because they do not work well on the natural sugar chains. Furthermore, many exoglycosidases, which have high aglycon specificities and very useful for the structural study of sugar chains, cannot be detected because they do not hydrolyze *p*-nitrophenylglycosides. Therefore, one must be careful on such evidences in searching for a new useful exoglycosidases.

1-3. Endoglycosidases. As described already, exoglycosidases hydrolytically cleave the monosaccharide residues located at the non-reducing termini of sugar chains. Many enzymes, which hydrolyze proteoglycans and various homoglycans, were known to hydrolyze the inner part of the sugar chains. Namely the glycon specificities of these enzymes are not directed to monosaccharides but to larger parts of sugar chains.

Since early 1970th, such group of enzymes, which work on the N-linked and O-linked sugar chains of glycoproteins, have been found and effectively used for the structural studies of sugar chains of complex carbohydrates. Since these enzymes cleave the inner part of sugar chains, they are generically called endoglycosidases. In Table 1, endoglycosidases that work on N-linked sugar chains and O-linked sugar chains are summarized. In order to use these enzymes as the effective reagents for the structural studies of the sugar chains of glycoproteins, their exact substrate specificities must be elucidated.

The detailed substrate specificities of Endo D, Endo H, Endo C_{II}, diplococcal endo-α-*N*-acetylgalactosaminidase, and four endo-β-galactosidases from different origins have been elucidated as summarized in Table 2 and Table 3. Namely, these enzymes have glycon specificities recognizing from disaccharide to pentasaccharide. It was also found that Endo C_I has the same substrate specificity as Endo D.[16]

1-3-1. Endo-β-N-acetylglucosaminidases and their application to structural study of glycopeptides. Because of its unique substrate specificity, Endo H could release all high mannose-type sugar chains and hybrid-type sugar chains, and used as a very effective reagent for the structural studies of these sugar chains. Since none of the four endo-β-*N*-acetylglucosaminidases could act on the complex-type sugar chains, which are the largest population of the N-linked

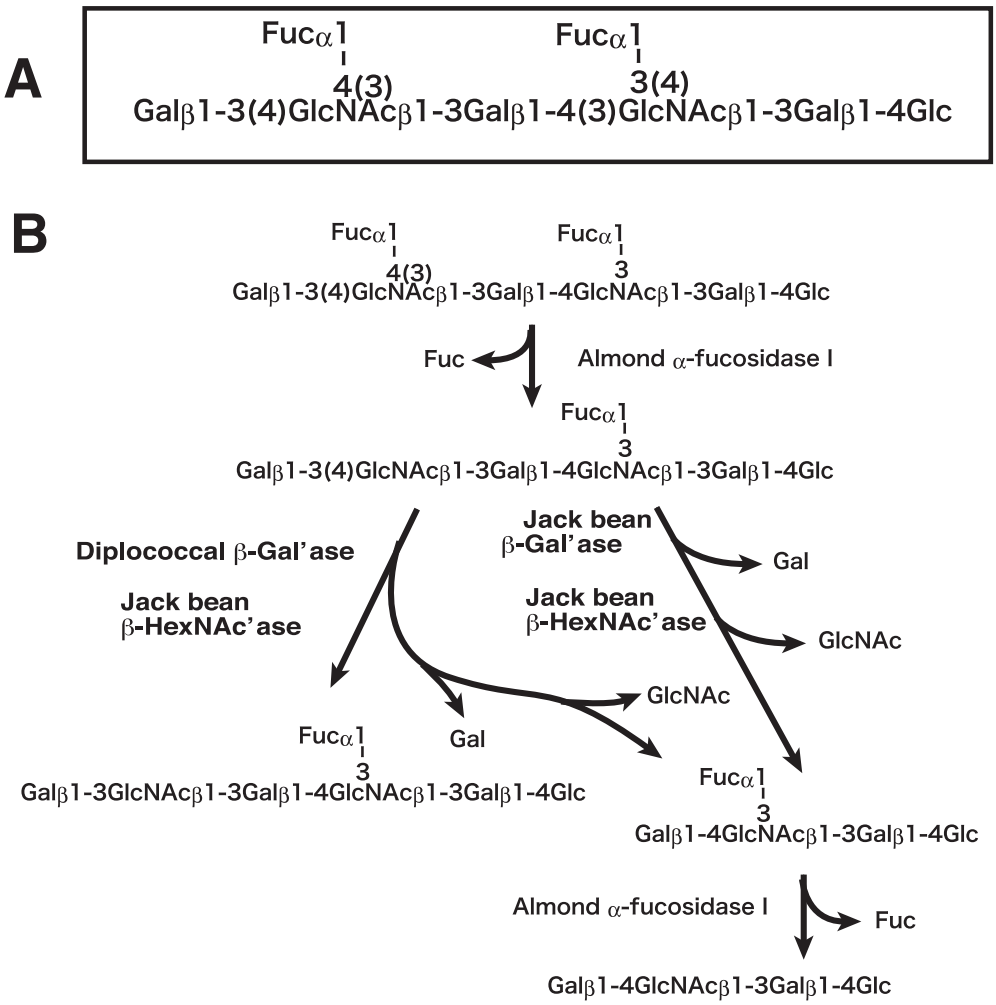


Fig. 4. Structural elucidation of an octa-saccharides mixture obtained from human milk. *β*-Gal'ase, *β*-galactosidase; *β*-HexNAc'ase, *β*-*N*-acetylhexosaminidase.

sugar chains, structural studies of these sugar chains were mainly performed by using hydrazinolysis[30] at the early stages of N-linked sugar chains research.

Many endo-*β*-*N*-acetylglucosaminidases were found from various sources later.[31),32] However, they all have similar substrate specificities, and none of them acts on the complex-type sugar chains. An interesting enzyme called Endo A was purified from *Arthrobacter protophormiae*.[26] This enzyme, which is induced by adding glycopeptides containing high mannose-type sugar chains in the culture medium, shows the same substrate specificity as Endo C_{II}, and shows strong transglycosylation activity together with hydrolytic activity.[33] The unique character of this enzyme will open the gate of production of neoglycoproteins as will be discussed at the end of this review article.

In 1982, Elder and Alexander found a new endo-*β*-*N*-acetylglucosaminidase in the culture fluid of *Flavobacterium meningosepticum*, and named it Endo F.[24] Interesting evidence is that this enzyme cleaves the complex-type sugar chains together with the high mannose-type sugar chains. However, the usefulness of the enzyme was not established because detailed substrate specificity was not investigated.

In 1990, Kadowaki *et al.* found an endo-*β*-*N*-acetylglucosaminidase, which acts on the complex-type sugar chains, in *Mucor hiemalis* and named it Endo M.[25] They then comparatively investigated in detail the substrate specificities of Endo M and Endo F.[34] By using DNS (5-dimethylaminonaphthalene-1-sulfonyl) derivatives of Asn-oligosaccharides as substrates, they found that both enzymes showed the same activities to Asn-oligosaccharides obtained from

Table 1. Endoglycosidases acting on the sugar chains of complex carbohydrates

Names	Sources	References
Endo-β-*N*-acetylglucosaminidase		
D (Endo D)	*Diplococcus pneumoniae*	13
H (Endo H)	*Streptomyces griseus*	14
L (Endo L)	*Streptomyces plicatus*	15
C_I and C_{II} (Endo C_I and C_{II})	*Clostridium perfringens*	16
F (Endo F)	*Flavobacterium meningosepticum*	24
M (Endo M)	*Mucor hiemalis*	25
A (Endo A)	*Arthrobacter protophormiae*	26
	Fig	17, 18
	Mammalian organs	19
	Hen oviduct	20
Endo-α-*N*-acetylgalactosaminidase		
	Diplococcus pneumoniae	21, 22
Endo-β-galactosidase		
	Diplococcus pneumoniae	23
	Escherichia freundii	27
C	*Clostridium perfringens*	28
Endo-β-Gal_{GnGa}	*Clostridium perfringens*	29
Peptide:*N*-glycanase (PNGase)		
	Almond emulsin	50
	Flavobacterium meningosepticum	51
	Animal tissues	56, 57

ovalbumin, indicating that both enzymes act on the high mannose-type and the hybrid-type sugar chains almost at the same rates. In contrast, Endo M acts on the biantennary complex-type sugar chains much faster than Endo F. Furthermore, Endo M can act on the triantennary complex-type sugar chains, but Endo F cannot act on them at all. Accordingly, Endo M is much more useful for studying the complex-type sugar chains than Endo F.

1-3-2. Endo-α-N-acetylgalactosaminidase. In 1972, Huang and Aminoff reported that the crude exoglycosidase mixture obtained from the culture fluid of *Clostridium perfringens* releases a disaccharide: Galβ1-3GalNAc from pig submaxillary mucin.[35] However, this enzyme had not been purified, and its specificity was not elucidated. A similar enzyme was found in the culture fluid of *Diplococcus pneumoniae*, and was purified 180 fold by using a human erythrocyte glycoprotein, glycophorin, containing the [^{3}H]Galβ1-3GalNAc residues, as substrate.[21] This enzyme releases the Galβ1-3GalNAc groups, which are linked to either serine or threonine residues of glycoproteins. However, Rα1-Ser and Thr groups, in which R represent the Fucα1-2Galβ1-3GalNAc, the Galβ1-3(NeuGlyα2-6)GalNAc and the Fucα1-2Galβ1-3(NeuGlyα2-6)GalNAc groups, do not work as substrate of the enzyme indicating that substitution of any hydroxyl groups of the Galβ1-3GalNAc group by other sugars abolished the substrate activity of the disaccharide to the enzyme.

The enzyme was effectively used to determine the structure of the serine and threonine-linked sugar chains of a glycopeptide released from bovine plasma high-molecular-weight kininogen by the action of plasma kallikrein.[36]

1-3-3. Endo-β-galactosidases and their application to the studies of the sugar chains of complex carbohydrates. A very interesting endoglycosidase was found in the culture fluid of *Diplococcus pneumoniae*.[23] This enzyme releases the GalNAcα1-3(Fucα1-2)Gal group and the Galα1-3(Fucα1-2)Gal group from blood type A and B substances, respectively, but does not act on blood type H substance. Since the enzyme cleaves β-galactosyl linkages, it was classified as an endo-β-galactosidase. The enzyme also acts on oligosaccharides with blood type A and B determinants. After 1000-fold purification, action of the enzyme on various oligosaccharides was investigated,

Table 2. The substrate specificities of Endo D,[53),54)] Endo H,[53)] and Endo C_{II}.[55)] R represent H, monosaccharides, or sugar chains

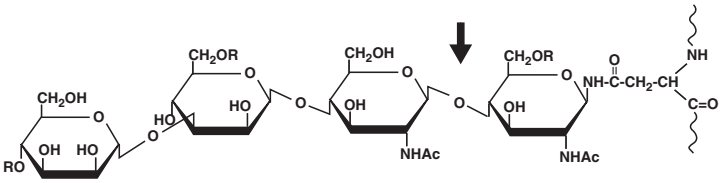
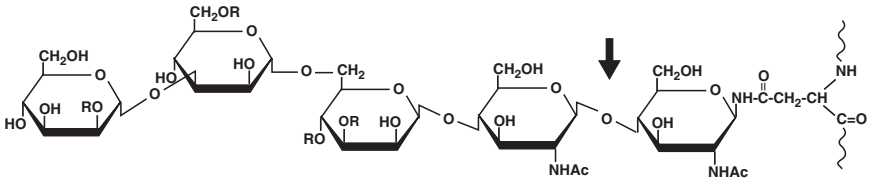
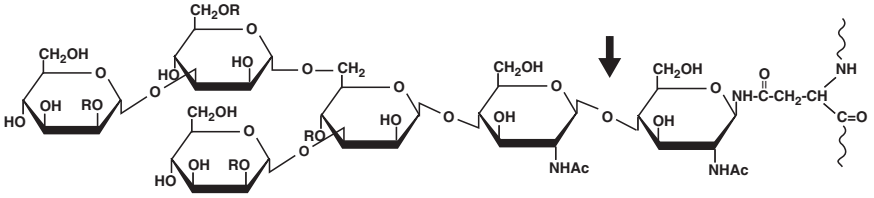
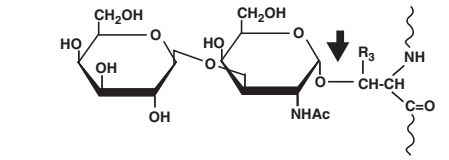
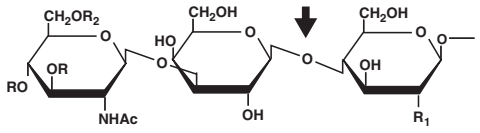
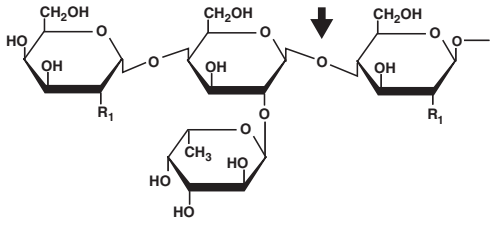
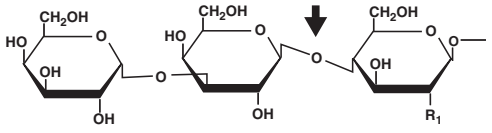
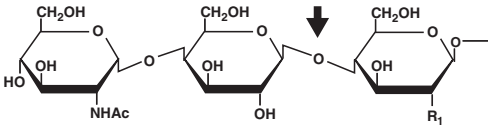
Endo enzymes	Structures of substrates
D	
H	
C_{II}	

Table 3. The substrate specificities of 1, endo-α-N-acetylgalactosaminidase;[21)] 2, Diplococcal endo-β-galactosidase;[23)] 3, *Escherichia freundii* endo-β-galactosidase;[27),45)] 4, endo-β-galactosidase C;[28)] and 5, endo-β-Gal_{GnGa}.[29)] R_1 represents either –OH, or –NHAc, R_2 represents either H, or SO_4, and R_3 represents either –H or $-CH_3$. R represents either H, β-galactosyl residue or sugar chains with β-galactosyl residue at their reducing termini

Enzymes	Structures of substrates	Enzymes	Structures of substrares
1		3	
2		4	
		5	

and finally concluded that the enzyme has the substrate specificity shown in Table 3-2. Several exoglycosidases, which destroy the activities of blood group antigens, were found and had been called blood group-destroying enzymes.[37)–41)] However, these enzymes were either α-galactosidases or α-*N*-acetylgalactosaminidases, which also hydrolyze other α-galactosyl or α-*N*-acetylgalactosaminyl linkages than blood group determinants. In contrast, the diplococcal endo-β-galactosidase hydrolyzes only blood group

A and B determinants and can be called a true blood group-destroying enzyme.

The enzyme was successfully used to characterize the ABO blood group-active glycoproteins of human erythrocyte membrane,[42] and to characterize the ABO blood group determinants with branched core.[43] Another endo-β-galactosidase with quite different specificity was found in the culture fluid of *Escherichia freundii* by Kitamikado and Ueno.[44] This enzyme releases the Neu5Ac-Gal-GlcNAc(SO_4)-Gal group from purified keratosulfate preparations. Fukuda and Matsumura studied the action spectrum of this enzyme on various glycoproteins and oligosaccharides, and found that the enzyme releases the GlcNAcβ1-3Gal and the GlcNAc(6-SO_4)β1-3Gal groups from a mucin isolated from pig, and glucose from Galβ1-3GlcNAcβ1-3Galβ1-4Glc.[27] The exact substrate specificity of this enzyme was elucidated by studying its action on various glycolipids.[45] The enzyme showed no action on the glycosphingolipids of globo and ganglio series. However, it cleaves all the glycolipid with the R-GlcNAcβ1-3Galβ1-4Glc (or GlcNAc-) groups in which R represents either hydrogen or sugars. Further substitution of the galactose residue in the essential trisaccharide group by additional sugars greatly reduces the susceptibility of the sugar chains to the enzyme. Another interesting characteristic of this enzyme is that the presence of a sialyl residue in the R moiety enhances the hydrolyzability of the sugar chains. This is very unusual because sialyl substitution of sugar chains usually reduces their susceptibility to most of the exo- and endoglycosidases. Possibly, the endo-β-galactosidase has cationic peptide group close to the catalytic polypeptide moiety.

In 1987, Muramatsu's group reported the finding of a novel endo-β-galactosidase, which releases the Galα1-3Gal group from the carbohydrate moieties of complex carbohydrates, in the culture fluid of *Clostridium perfringens*, and named it as endo-β-galactosidase C.[28] After cloning the enzyme gene,[46] they performed a series of studies to apply the gene for producing the Galα1-3Gal group free mice as a model to produce the animal suitable for nonprimate-to-primate xenotranplantation.[47–49]

In 2001, Ashida *et al.* found another endo-β-galactosidase, which releases the GlcNAcα1-4Gal group from glycans expressed in the gastric gland mucous cell-type mucin, as a contaminant of commercial *Clostridium perfringens* sialidase. They purified the enzyme in electrophoretically homogeneous form from the culture supernatant of *Clostridium perfringens*, and named it as GlcNAcα1-4Gal-releasing endo-β-galactosidase (Endo-β-Gal_{GnGa}).[29]

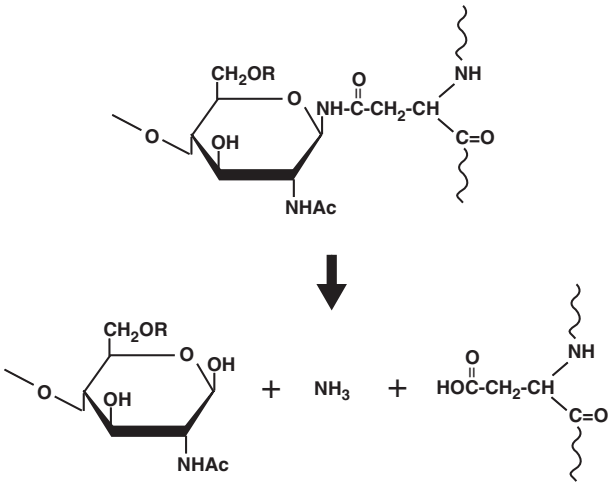


Fig. 5. Mechanism of the release of N-linked sugar chains by PNGase action. R represents either H or Fucα1-. Release of ammonia from glycosylamine occurs spontaneously.

1-3-4. Peptide:N-glycanase. Peptide:*N*-glycanase (PNGase), another group of endoglycosidase acting on the N-linked sugar chains, cleaves the amide bond of the GlcNAcβ1-Asn group located at the linkage region of N-linked sugar chains to the polypeptide portion as shown in Fig. 5. The enzyme was first extracted and purified from Almond emulsion by Takahashi in 1977.[50] Similar enzyme was later found in the culture fluid of *Flavobacterium meningosepticum* by Plummer *et al.*,[51] and has been used for the studies of structures and functions of N-linked sugar chains of glycoproteins.

2. Endoglycosidases found in the animal kingdom and their physiological functions

In 1974, Nishigaki *et al.* found an endo-β-*N*-acetylglucosaminidase in the organs of mammals.[19] Following this, Tarentino and Maley[20] found similar enzyme in hen oviduct, indicating that endo-β-*N*-acetylglucosaminidases occur widely in the animal kingdom. In 1989, DeGasperi *et al.*[52] found two endo-β-*N*-acetylglucosaminidases in human kidney and named them E-β-GNase 1 and 2. E-β-GNase 1 has similar substrate specificity as Endo H, cleaving effectively glycopeptides containing the high mannose-type sugar chains but not those containing the complex-type sugar chains. Furthermore, acetylation or dansylation of the Asn residue of the substrates does not affect susceptibility to the enzyme. Since the GlcNAcβ1-4GlcNAc moieties of Manα1-6(Manα1-

3)Manα1-6(Manα1-2Manα1-3)Manβ1-4GlcNAcβ1-4GlcNAc and Manβ1-4GlcNAcβ1-4GlcNAc were also hydrolyzed by the enzyme, Asn residue is not strictly required for the enzyme activity.

On the contrary, E-β-GNase 2 does not act on the Asn-linked oligosaccharides at all, but hydrolytically cleaves the GlcNAcβ1-4GlcNAc moiety of the high mannose-type oligosaccharides and the complex-type oligosaccharides containing the *N*,*N*′-diacetylchitobiose at their reducing termini. Among these enzymes, E-β-GNase 1 is considered to be the same as that we found in the rat organs,[19] and probably distributes widely in the mammalian organs. E-β-GNase 2 showed the same characteristics as the di-*N*-acetylchitobiase reported by Kuranda and Aronson.[110] As to PNGase, Inoue's group found that this enzyme widely occurs in animal tissues.[56),57] Tadashi Suzuki, who was one of the young scientists in Inoue's group, recently elucidated that PNGases in animal and plant kingdoms are playing important roles in the ER-associated degradation (ERAD) pathway.[58)–60]

All N-linked sugar chains are added as the tetradecasaccharide: $Glc_3{\cdot}Man_9{\cdot}GlcNAc_2$ to the Asn residue constructing the Asn-X-Thr (or Ser) group of the polypeptide chain being translated in the rough endoplasmic reticulum, and then trimmed to the heptasaccharide: $Man_5GlcNAc_2$ until the polypeptide reaches to the Golgi. After addition of an *N*-acetylglucosamine residue to the hexasaccharide, the formed octasaccharide is further trimmed its two α-mannosyl residues to form the hexasaccharide: Manα1-6(GlcNAcβ1-2Manα1-3)Manβ1-4GlcNAcβ1-4GlcNAc by the Golgi resident α-mannosidase. The hexasaccharide is then converted to a series of complex-type and hybrid-type sugar chains in Golgi. During this pathway, the *N*-linked sugar chain of nascent glycoprotein is converted to $GlcMan_9GlcNAc_2$ by the action of α-glucosidases I and II, while the protein still exist in the endoplasmic reticulum. This dodecasaccharide plays important role as a ligand of two chaperons: Calnexin and Calreticulin, which retain the polypeptide within ER for proper folding. When the protein happens to be miss-folded by some reason, the protein will be released from ER to cytoplasm and degraded by proteasome. At this stage, PNGase, which occurs in the cytoplasm, plays an important role in removing bulky *N*-linked sugar chains from a miss-folded nascent protein and helps its entry into proteasome. The oligosaccharides, thus released from miss-folded nascent protein, have the GlcNAcβ1-4GlcNAc group at their reducing termini. As shown in Fig. 6C, one *N*-acetylglucosamine residue is removed from these oligosaccharides by E-β-GNases 1 and 2 found by DeGasperi *et al.*[52] Action of E-β-GNase 2 is indispensable here, because large amount of complex-type sugar chains containing the GlcNAcβ1-4GlcNAc group at their reducing termini must be handled by this pathway as will be described in the story of glycosidase deficiencies later.

The oligosaccharides are then brought into lysosomes after being partially hydrolyzed by cytoplasmic α-mannosidase, and completely hydrolyzed into monosaccharides by the action of lysosomal exoglycosidases.

As described so far, PNGase is considered to play a main role in the removal of *N*-linked sugar chains in the catabolism of glycoproteins. However, there is one important problem we must have in our mind. As described already, there occurs endo-β-*N*-acetylglucosaminidase (which correspond to E-β-GNase 1 of human kidney) in the cytoplasm of cells of animal organs. If this enzyme acts on the glycoproteins within the animal body, various proteins with *N*-acetylglucosamine residues on their Asn residues would be produced as shown in Fig. 6B. However, PNGase cannot release these *N*-acetylglucosamine residues.[61] Therefore, biological actions of such modified glycoproteins are the interesting targets for investigation.

Very useful data for considering the metabolism of the *N*-linked sugar chains of glycoproteins are obtained by the structural studies of oligosaccharides excreted in the urine of patients with glycosidase deficiency. Among the hereditary metabolic diseases, those induce the abnormal metabolic disorder of complex carbohydrates are generically called glycosidase-deficiencies.

A series of these diseases are genetically suffering a loss of one of the exoglycosidases. Among the diseases, GM1-gangliosidosis lacking β-galactosidase, Sandhoff disease lacking β-*N*-acetylhexosaminidase, mannosidosis lacking α-mannosidase and fucosidosis lacking α-fucosidase have been found. Since degradation of a sugar chains by exoglycosidase proceed from non-reducing termini, lack of an exoglycosidase stops the degradation at the monosaccharide residue, which should be removed by the missing exoglycosidase and accumulate in the tissues.

In the case of glycolipid metabolism, such accumulating materials were precisely detected, and the abnormal schemes were clearly elucidated as described in the review of Seyama and Yamakawa.[62]

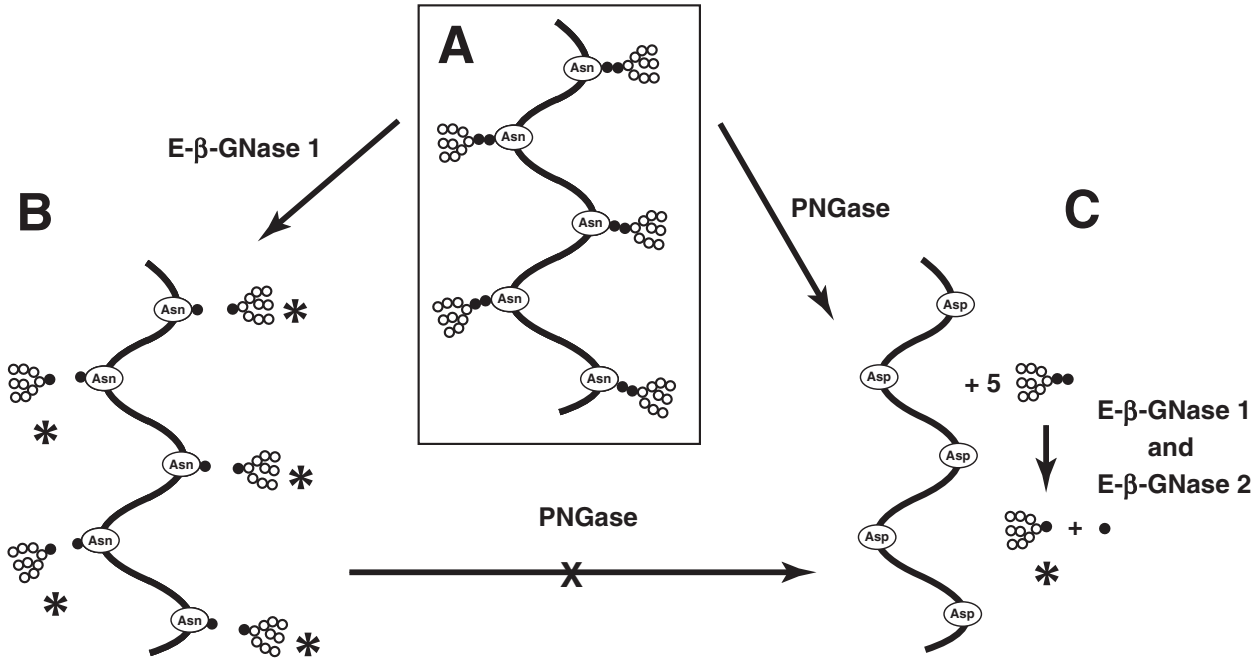


Fig. 6. Metabolic pathway of the N-linked sugar chains of a glycoprotein (A) by the actions of PNGase, E-β-GNase 1 and 2. A high mannose-type undeca-saccharide: Manα1-2Manα1-6(Manα1-2Manα1-3)Manα1-6(Manα1-2Manα1-2Manα1-3)Manβ1-4GlcNAcβ1-4GlcNAc (● indicates GlcNAc and ○ indicates Man) is drawn as the sugar moieties of the glycoprotein in this figure. However, other portions of the undeca-saccharide than the core penta-saccharide: Manα1-6(Manα1-3)Manβ1-4GlcNAcβ1-GlcNAc can be changed to form other high mannose-type, hybrid-type and complex-type sugar chains in pathway C. Only high mannose-type and hybrid-type sugar chains can be included in pathway B. Oligosaccharide with asterisk will be transported to lysosomes after removal of a part of mannose residues by the action of cytoplasmic α-mannosidase, and completely hydrolyzed by lysosomal exoglycosidases.

In contrast, the accumulating materials originated from glycoproteins were not found, and it was considered that the exoglycosidases working for the metabolism of the sugar chains of glycolipids and of glycoproteins might be different, and there was a trend to call the diseases as sphingoglycolipidosis.

We were interested in the preliminary finding that large amount of oligosaccharides are excreted in the urine of patients of exoglycosidase deficiencies, and performed a systematic research of the structures of oligosaccharides accumulated in the urine samples of various exoglycosidase-deficiency patients in collaboration with the Departments of Pediatrics of Osaka University, Kumamoto University and Hokkaido University. It was found that the urine samples of patients with mannosidosis, and those of patients with GM1-gangliosidosis contain the oligosaccharides shown in Fig. 7.[63)–65)] The structural characteristics of these oligosaccharides are that they contain monosaccharides, which should be removed by the missing exoglycosidases, at their non-reducing termini, and contain the Manβ1-4GlcNAc group at their reducing-termini. These characteristics can be easily explained if we estimate the *N*-linked sugar chains of glycoproteins are released from polypeptides by endo-β-*N*-acetylglucosaminidase, and then the sequential digestion from non-reducing termini is stopped at the missing exoglycosidase. The structures of oligosaccharides in the urine samples of GM1-gangliosidosis patients and mannosidosis patients (Fig. 7) indicated that human endo-β-*N*-acetylglucosaminidase hydrolyzes both the high mannose-type sugar chains and the complex-type sugar chains. These results indicated that the oligosaccharides in Fig. 7 are mainly produced by pathway C shown in Fig. 6.

Further information as to the human endo-β-*N*-acetylglucosaminidase was obtained from the structural study of the incomplete degradation products of *N*-linked sugar chains of glycoproteins accumulating in the urine samples of the fucosidosis patients.[66),67)] In contrast to the urine samples of other patients with exoglycosidase-deficiency, the urine sample of patients with fucosidosis contain large amounts of Asn-oligosaccharides (Fig. 8B) together with small amounts of oligosaccharides (Fig. 8A). A noteworthy evidence is that all these Asn-oligosaccharides contain the Fucα1-6(3)GlcNAc-Asn groups. This

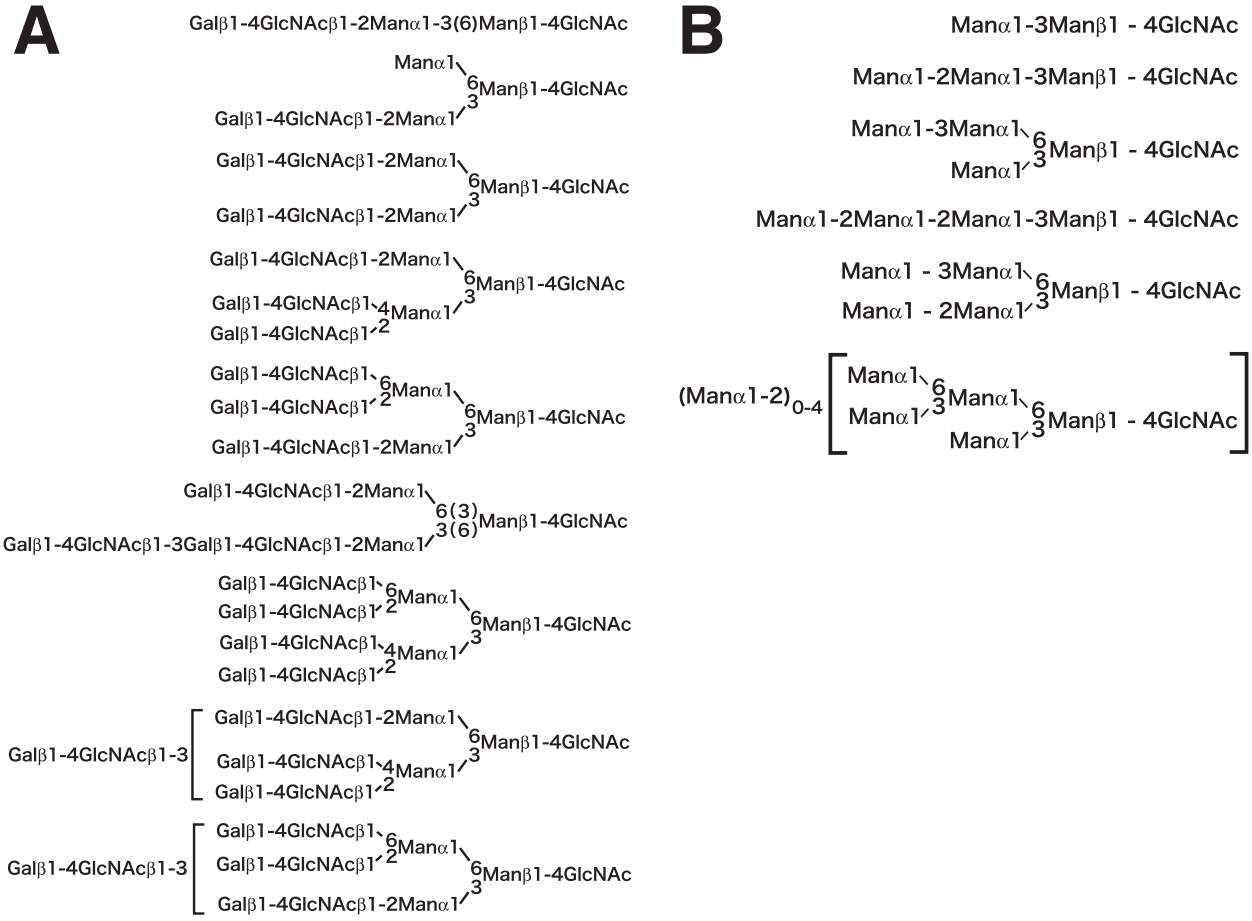


Fig. 7. Structures of oligosaccharides excreted in the urine of GM1-gangliosidosis patients (A), and mannosidosis patients (B).

evidence indicated that human endo-β-*N*-acetylglucosaminidase cannot act on the *N*-linked sugar chains containing a fucose residue linked to the proximal *N*-acetylglucosamine residue. In order to confirm this estimation, we used the homogenates of skin fibroblasts obtained from fucosidosis patients as enzyme sources of endo-β-*N*-acetylglucosaminidase, and investigated their action to various Asn-oligosaccharides. By this study, it was confirmed that both high mannose-type sugar chains and complex-type sugar chains can be cleaved by the enzyme, but none of the oligosaccharides containing a fucose linked to the proximal *N*-acetylglucosamine residue can be cleaved by the enzyme.[68] These results indicated that human skin fibroblasts contain endo-β-*N*-acetylglucosaminidase with wider glycon-specificity than E-β-GNase 1. However, the evidence that the complex-type sugar chains without fucose residue was hydrolyzed at much slower rate: 1/10 of the high mannose-type sugar chains, must be taken into account.

Furthermore, it was also reported by Inoue's group that the glycopeptides containing the Fucα1-6(3)GlcNAc-Asn groups cannot be hydrolyzed by PNGase.[61] Therefore, the above results could be interpreted that it was obtained by the pathway Fig. 6C not by Fig. 6B. If it is, the evidence that the complex-type sugar chains work as substrate can be well explained.

Because *N*-linked sugar chains with the Fucα1-3GlcNAc-Asn group do not exist in human body, occurrence of such sugar chains in the urine of fucosidosis patients may be originated from other living organisms taken as foods.[69]

3. Role of glycosidases of intestinal bacteria for the expression of Bifidus factor activity of human milk oligosaccharides

As already described, many exo- and endoglycosidases were found in the culture fluid and cells of various bacteria and used as very effective reagents to

Fig. 8. Structures of oligosaccharides (A) and Asn-oligosaccharides (B) excreted in the urine of fucosidosis patients.

study the structures and functions of oligosaccharides and the sugar chains of complex carbohydrates.

Recently, these enzymes were found to play important roles in relation to the Bifidus factor, an important topic in the baby nutrition.

It has been known that *Lactobacillus bifidus* becomes a predominant intestinal flora of babies fed with human milk. This bacterium digests lactose, and produces large amount of lactic acid and acetic acid. The acidic condition in the intestine of babies suppresses the growth of many other microorganisms, and may protect babies from harmful intestinal infection.[70)]

Schönfeld found a growth factor of *Lactobacillus bifidus* var. pennsylvanicus in the whey of human milk, and named it **Bifidus factor**.[71)] In collaboration with György, Kuhn started a series of systematic studies to elucidate the chemical entity of Bifidus factor, and found many oligosaccharides in human milk.[72)–74)]

The problem of the chemical entity of Bifidus factor had been considered to be solved, when *Lactobacillus bifidus* var. pennsylvanicus was found to request *N*-acetylglucosamine as a growth factor and only the milk oligosaccharides containing the GlcNAc residue were effective as Bifidus factor. However, the project of Bifidus factor is developing now to a new point of view by the finding that human milk oligosaccharides show a unique growth activity working specifically for various *Bifidus* strains as will be described below.

Most human milk oligosaccharides are considered as soluble fibers, because they are not degraded by exoglycosidases in the digestive tract of suckling

Table 4. Core structures found in human milk oligosaccharides. The Galβ1-3GlcNAc groups, located at the non-reducing termini of the oligosaccharides, are underlined

Names	Structures	References
Lactose	Galβ1-4Glc	
Lacto-*N*-tetraose (LNT)	Galβ1-3GlcNAcβ1-3Galβ1-4Glc	87
Lacto-*N*-*neo*tetraose (LN*n*T)	Galβ1-4GlcNAcβ1-3Galβ1-4Glc	88
Lacto-*N*-hexaose	Galβ1-4GlcNAcβ1-6(Galβ1-4Glc) Galβ1-3GlcNAcβ1-3	89
Lacto-*N*-*neo*hexaose	Galβ1-4GlcNAcβ1-6(Galβ1-4Glc) Galβ1-4GlcNAcβ1-3	90
para-Lacto-*N*-hexaose	Galβ1-3GlcNAcβ1-3Galβ1-4GlcNAcβ1-3Galβ1-4Glc	91
para-Lacto-*N*-*neo*hexaose	Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ1-3Galβ1-4Glc	91
Lacto-*N*-octaose	Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ1-6(Galβ1-4Glc) Galβ1-3GlcNAcβ1-3	92
Lacto-*N*-*neo*octaose	Galβ1-3GlcNAcβ1-3Galβ1-4GlcNAcβ1-6(Galβ1-4Glc) Galβ1-4GlcNAcβ1-3	92
iso-Lacto-*N*-octaose	Galβ1-3GlcNAcβ1-3Galβ1-4GlcNAcβ1-6(Galβ1-4Glc) Galβ1-3GlcNAcβ1-3	93
para-Lacto-*N*-octaose	Galβ1-3GlcNAcβ1-3Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ1-3Galβ1-4Glc	94
Lacto-*N*-decaose	Galβ1-4GlcNAcβ1-6(Galβ1-4GlcNAcβ1-6(Galβ1-4Glc) Galβ1-3GlcNAcβ1-3 Galβ1-3GlcNAcβ1-3)	95
Lacto-*N*-*neo*decaose	Galβ1-4GlcNAcβ1-6(Galβ1-4GlcNAcβ1-6(Galβ1-4Glc) Galβ1-4GlcNAcβ1-3 Galβ1-3GlcNAcβ1-3)	96

babies and reach to colon intact[75)–79)]. Accordingly, these oligosaccharides must first be digested to their monosaccharide constituents in order to be used by intestinal flora as nutrients. However, it is not so easy task for the bacteria settling in colon, because most of the β-galactosidases produced by the intestinal bacteria hydrolyze readily the Galβ1-4GlcNAc group but do not strongly act on the Galβ1-3GlcNAc group. Some of the enzymes do not work on the Galβ1-3GlcNAc group at all as described for the diplococcal β-galactosidase. As already described in my recent review,[80)] human milk contains more than hundreds of oligosaccharides, which are constructed from the thirteen core oligosaccharides summarized in Table 4. As underlined in the table, the Galβ1-3GlcNAc groups are amply located at the non-reducing termini of human milk oligosaccharides. Therefore, many human milk oligosaccharides are not digested by bacterial β-galactosidase. According to the recently published review,[81)] occurrence of oligosaccharides enriched in the Galβ1-3GlcNAc group is a unique phenomenon of human milk among the milk of mammals.

In 1993, Sano *et al.* found in the culture fluid of *Streptomyces* sp. 142 an endoglycosidase, which cleaves the Galβ1-3GlcNAc group from the non-reducing termini of sugar chains, and named it lacto-*N*-biosidase.[82)] Recently, Ando *et al.* reported

that this enzyme is widely distributed in various *Bifidus* strains, but not in *Clostridia*, *Bacteroides* and *Lactobacilli*.[83] While Wada *et al.* found a transporter protein in the plasma membrane of *Bifidus* strains, which takes up the disaccharide released from sugar chains by lacto-*N*-biosidase action.[84] Together with the Galβ1-3GlcNAc group, this transporter also binds to the Galβ1-3GalNAc group, which is released from mucin by the action of endo-α-*N*-acetylgalactosaminidase.[21] Accordingly, it was named galacto-*N*-biose/lacto-*N*-biose I-binding protein (GL-BP).

In 1999, Derensy-Dron *et al.* found in the ultrasonicate of *Bifidobacterium bifidum* DSM 20082 a phosphorylase, which phosphorylates Galβ1-3GlcNAc or Galβ1-3GalNAc to form Galα1-PO_4 and GlcNAc or GalNAc. They partially purified the enzyme, and named it as β-1,3-galactosyl-*N*-acetylhexosamine phosphorylase.[85] Kitaoka *et al.* purified this enzyme and cloned its gene from *Bifidobacterium longum* JCM1217.[86] Furthermore, they suggested that the enzyme plays an important role in the metabolism of the Galβ1-3GlcNAc group released from human milk oligosaccharides by lacto-*N*-biosidase. Namely, collaboration of three proteins: extracellular lacto-*N*-biosidase, GL-BP in the plasma membrane and intra-cellular β-1,3-galactosyl-*N*-acetylhexosamine phosphorylase, can effectively metabolize human milk oligosaccharides containing the Galβ1-3GlcNAc group at their non-reducing termini, even if no β-galactosidase cleaving the disaccharide group is available. Kitaoka further found a series of genes of enzymes responsible for the metabolism of all monosaccharides constructing human milk oligosaccharides in the operon containing the gene of β-1,3-galactosyl-*N*-acetylhexosamine phosphorylase, and presented the whole scheme of the metabolic pathway of human milk oligosaccharides.[97)–99)]

A series of these research results confirmed that *Bifidobacteria* are equipped with a unique mechanism called **GNB/LNB pathway**,[99] and effectively utilizes oligosaccharides having the Galβ1-3GlcNAc group at their non-reducing termini. Accordingly, human milk oligosaccharides, which are enriched in the Galβ1-3GlcNAc group at their non-reducing termini, works as specific nutrients for *Bifidobacteria*.

Presence or absence of fucosyl residues constructing the Fucα1-2Gal, the Fucα1-3GlcNAc, the Fucα1-4GlcNAc and the Fucα1-3Glc groups, and of sialic acid residues constructing the Neu5Acα2-3Gal, the Neu5Acα2-6Gal, and the Neu5Acα2-6GlcNAc groups were the major source to produce over one hundreds oligosaccharides from the thirteen core oligosaccharides in Table 4.[80]

Quite recently, Asakuma *et al.* selected four typical *Bifidus* strains: *B. bifidum* JCM1254, *B. longum* subsp. *infantis* JCM1222, *B. longum* subsp. *longum* JCM1217 and *B. breve* JCM1192, and cultured them in a medium containing 1% human milk oligosaccharides as carbohydrate source. JCM1254, and JCM1222 quickly grew in the medium, while JCM1217, and JCM1192 grew very slowly.[100] They then analyzed the time course alteration of the sugar components in the spent media by using 2-anthranilic acid labeling method followed by HPLC analysis. In the 1 L of original medium, 0.45 g of lactose, 2.88 g of monofucosyllactoses, 1.11 g of lacto-*N*-tetraose (LNT), 0.32 g of lacto-*N*-*neo*tetraose (LN*n*T), 2.45 g of a mixture of mono-fucosyl LNT and LN*n*T, 2.65 g of di-fucosyl LNT and LN*n*T, and few percent of other sugars were detected.

Time course studies of spent media revealed that in the case of two strains growing quickly, all oligosaccharides are used up before the growth of bacteria reach to maximum, and large amounts of the monosaccharides: fucose, galactose and glucose, quickly appeared and then vanished.

In contrast, in the case of two strains growing very slowly, only LNT disappeared but no degradation is observed in other oligosaccharides.

Assay of exoglycosidases, revealed that the two slowly growing strains completely lack α-fucosidases which hydrolyze the Fucα1-2Gal group and the Galβ1-4/3(Fucα1-3/4)GlcNAc groups, while large amounts of such α-fucosidases were detected in the two quickly growing strains. β-Galactosidase, β-*N*-acetylhexosaminidase and GL-BP were detected in all four strains. However, lacto-*N*-biosidase was detected in JCM1254, and JCM1217, but not in JCM1222 and JCM1192. These enzymatic studies revealed that the two slowly growing strains could not use the fucosylated oligosaccharides at all, because of the lack of α-fucosidases, and JCM1217 rely on LNT only by GNB/LNB pathway. Although human milk contains LN*n*T in about 1/4 amount of LNT, the two slowly growing strains could not use the tetrasaccharide. This evidence may indicate that the β-galactosidase in the slowly growing strains acts on lactose but not on the Galβ1-4GlcNAc group. In contrast, the quickly growing strains can use LN*n*T also, indicating that they contain another β-galactosidase acting specifically on the Galβ1-4GlcNAc group, which will be described later in this section.

Anyway, the data presented here clearly indicated that the major oligosaccharides in human milk, which have the Galβ1-3GlcNAc group in their non-reducing termini, are selectively used by *Bifidus* strains. Probably because of this mechanism, *Clostridia*, *Bacteroides* and *Lactobacilli*, which do not have lacto-*N*-biosidase, cannot use human milk oligosaccharides and cannot grow in the colon of breast-fed baby.

Absence of lacto-*N*-biosidase in *B. longum* subsp. *infantis* JCM1222 and *B. breve* JCM1192 indicated that these strains couldn't use the GNB/LNB pathway. Therefore, the fact that these strains can digest LNT was a mystery. Recently, Yoshida *et al.* solved this mystery by finding that *B. longum* subsp. *infantis* takes up directly LNT, and digest it by intra-cellular lacto-*N*-tetraose β-1,3-galactosidase, produced by *Blon_2016* gene.[101] Another interesting evidence is that this strain contains another β-galactosidase made by *Blon_2334* gene, and this enzyme acts on the Galβ1-4GlcNAc group and lactose. Namely, this bacteria is equipped with two kinds of β-galactosidases working specifically to the Galβ1-3GlcNAc group and the Galβ1-4GlcNAc group. These two enzymes now started to be found in other bifidobacteria. Although the metabolism of LNT in *B. breve* JCM1192 has not been investigated, similar mechanism as JCM1222 may work in this strain also.

Concluding remarks

Studies of exo- and endoglycosidases, which have been utilized as useful reagents for the structural studies of complex carbohydrates, are recently expanding to their physiological function in the living organisms producing the enzymes.

Two topics: metabolism of the sugar moieties of glycoproteins within human body, and specific digestion of human milk oligosaccharides by *Bifidus* strains are introduced in this review. Nothing is known about the physiological functions of proteins containing the GlcNAc-Asn residues produced by the pathway of Fig. 6B. However, in view of the various important physiological functions found in the case of proteins containing the GlcNAc-Ser (Thr) residues,[102] the role of GlcNAc-Asn residues may be elucidated as a new field of glycobiology in the future.

As shown in the case of *Bifidobacteria*, glycosidases produced by bacteria are playing important roles for their growth. These facts indicated that a bacterium could acquire a greater adaptation to the circumstances by obtaining a new glycosidase.

Furthermore, a glycosidase, with peculiar substrate specificity, may be useful for the bacterial growth. For example, diplococcal endo-β-galactosidase, listed in Table 1, shows a very interesting specificity cleaving the antigenic determinant trisaccharides from the human blood group A and B substances.[23] Since similar enzyme was recently found in *Clostridium perfringens* ATCC 10543 by Anderson *et al.*,[103] this unique enzyme may widely distribute in many bacteria. Blood group A and B antigenic determinants are distributed at the non-reducing termini of the sugar chains of mucins, which are covering the surface of epithelial cells of human respiratory tract, and digestive tract. These mucins work as the barrier to protect epithelial cells from bacterial invasion. Accordingly, bacteria must destroy this barrier in order to start infection. However, it is not an easy task for a bacterium, because at least four glycosidases: α-*N*-acetylgalactosaminidase, α-galactosidase, α-fucosidase and β-galactosidase, are necessary in order to simply remove the A and B blood group determinants from the sugar chains of mucins. Accordingly, the endo-β-galactosidase, which can remove the two trisaccharides at once, is a very useful weapon for a bacterium.

Similarly, endo-α-*N*-acetylgalactosaminidase,[21] which can remove all of the core disaccharide: Galβ1-3GalNAc at once may work as a useful weapon for an invading bacterium.

Because of the limited space, I did not describe about the reverse reaction of glycosidases for the synthesis of oligosaccharides as the topic of glycosidases. Oligosaccharide synthesis has been traditionally performed by using the methods of organic chemistry. However, this approach has a serious drawback: it is essential to use a vicious cycle of protection-deprotection steps, which often result in a dramatic decrease in reaction yields. By the development of molecular biology, use of glycosyltransferases for the synthesis of oligosaccharides has quickly been developed. Though this new technique is very effective because one can synthesize an aimed oligosaccharide effectively, it requires expensive sugar nucleotides and has a drawback of using fragile enzymes. Therefore, this method is not practical to synthesize oligosaccharides in industrial scale.

Under such circumstances, oligosaccharide synthesis catalyzed by the reversed reaction of exoglycosidases has been developed recently. As already described, exoglycosidases show various aglycon specificities. Since these specificities will be reflected in the reverse reactions also, a particular disaccharide

can be synthesized by selecting a specific exoglycosidase. Furthermore, synthetic method of glycoproteins containing N-linked sugar chains by using the reversed reaction of Endo M by Yamamoto *et al.*[104] has tremendously been developed in recent years,[105),106] and it is now possible to obtain even a glycoprotein, which has no micro-heterogeneity in its sugar moieties, and opening a gate for the comparative study of a glycoprotein with different N-linked sugar chains. I would like to present two representative reviews[107),108] for the readers who are interested in this newly developing field.

Quite recently, even site-directed mutants of glycosidases, in which hydrolytic activities are suppressed, were developed and collectively termed glycosynthases. In addition to these developments in the enzyme side, very effective donors like oligosaccharide oxazoline[109] were also developed.

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Profile

Akira Kobata was born in 1933. Graduated and received Ph.D. from the University of Tokyo. Elucidated the biosynthetic pathway of ABO and Lewis blood group determinants in Victor Ginsburg's laboratory in NIH (1967–1971). In 1971, he became the Professor of the First Department of Biochemistry, Kobe University School of Medicine. From 1982 to 1993, he served as the Professor and Chairman of Department of Biochemistry, Institute of Medical Science, the University of Tokyo. During these 22 years, he developed a series of reliable and sensitive methods for the structural study of the N-linked sugar chains, and investigated functions and pathology of the sugar chains of glycoproteins. Studies on glycosidases, which are introduced in this review, were performed for the purpose of developing enzymatic reagents for the structural studies of the sugar chains of glycoproteins.

He was awarded the Prize for the Promotion of Young Scientists for 1963 from the Pharmaceutical Society of Japan, Science and Technology Prize for 1985 from Toray Science Foundation, PSJ Award for 1992 from the Pharmaceutical Society of Japan, Claude S. Hudson Award for 1992 from American Chemical Society, and also the 1992 Japan Academy Prize. He was a Fogarty Scholar-in-Residence in NIH (1985–1987), Auckland Foundation Visiting Professor in New Zealand in 1988, and also served as the Director of Institute of Medical Science (1990–1992).

In 1993, he was appointed as the Director of Tokyo Metropolitan Institute of Gerontology, and became a Professor Emeritus of the University of Tokyo. In this last carrier as a scientist, he developed a new glycobiology area in the field of aging research. From 2000, he has been the Director Emeritus of Tokyo Metropolitan Institute of Gerontology, and served as the advisor of Seikagaku Kogyo Co., LTD. until 2003. Currently, he is the scientific advisor and a member of the board of directors of the Noguchi Institute, a non-profit institution established for the study of carbohydrate chemistry in Japan.