CRC

HANDBOOK *of* ENDOGLYCOSIDASES *and* GLYCOAMIDASES

Edited by
Noriko Takahashi
Takashi Muramatsu

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CRC Handbook of Endoglycosidases and Glycoamidases

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PREFACE

The carbohydrate moieties of glycoproteins and glycolipids carry important information, some of which plays key roles in cell surface interactions. Structural and functional studies of these carbohydrates are now required in diverse fields of biological science, and enzymes which release oligosaccharides from them are highly useful for such purposes. These enzymes consist of two groups—endoglycosidases and amidases—and this handbook furnishes comprehensive information about these enzymes with many examples of their application. We hope that this handbook will be useful not only to specialists in glycoprotein and glycolipid research, but also to those who have to analyze carbohydrate portions during other investigations.

We are grateful to Professor Y. C. Lee for his helpful suggestions, without which it would not have been possible to produce this handbook.

Noriko Takahashi
Takashi Muramatsu

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Dr. Muramatsu received the Promotion Award from the Japanese Society of Biochemistry and has presented over 15 invited lectures at International Meetings. He has published more than 130 research papers, five review articles in international journals and a monograph, "Cell Surface and Differentiation." His current major research interest is the structural functional relationship of cell surface glycoproteins and mediators of retinoic acid action.

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Chapter 1

INTRODUCTION

Takashi Muramatsu

Glycoproteins and glycolipids, both of which carry covalently bound sugars, are widely distributed in the animal and plant kingdoms. Some of these carbohydrate structures have recently been established as recognition signals in cell-surface interactions.[1-4]

Structural studies of the carbohydrate moieties and elucidation of their structural-functional relationship are now necessary in a variety of research fields, especially in molecular cell biology. Enzymes that release oligosaccharides from the carbohydrate moieties have played important roles and are expected to function even more significantly in these studies. Two types of enzymes, namely endoglycosidases and amidases, which are oligosaccharide-releasing enzymes, are the subjects of this volume. Before entering main chapters, a brief introduction is furnished about carbohydrate biochemistry and the history of these enzymes. Although most endoglycosidases and amidases useful in glycoprotein and glycolipid research are fully covered in this book, two endoglycosidases, endo-*N*-acetylneuraminidase and endo-α-mannosidase are not; such endoglycosidases are briefly mentioned in this introductory chapter.

BIOCHEMISTRY OF GLYCOPROTEINS AND GLYCOLIPIDS

Glycoproteins are abundant in plasma membranes and extracellular matrices.[1] Furthermore, epithelial mucins are carbohydrate-rich glycoproteins, and various blood proteins, except for serum albumin, are glycoproteins. Proteins commonly found, such as ovalbumin milk casein, and silk fibroin are also glycoproteins. Glycolipids are important components of plasma membranes.

Sugars commonly appearing in glycoproteins and glycolipids do not vary much, and they are listed in Table 1; however, a characteristic of most protein- and lipid-bound oligosaccharides is complexity. In addition to the different sugars involved in the structure, a variety of linkages (e.g., 1→3 or 1→4), anomeric forms (α or β), and branching of carbohydrate sequences contribute to the complexity.

Carbohydrate structures of glycoproteins and glycolipids can be described in several ways. As an example, the structure of the asparagine-linked oligosaccharide of ovalbumin, $(Man)_5$ $(GlcNAc)_2$ can be shown in two ways (Figure 1). Although formula II is that recommended by International Union of Pure and Applied Chemists-International Union of Biochemists (IUPAC-

TABLE 1
Monosaccharide Components Typically Found in Glycoproteins and Glycolipids

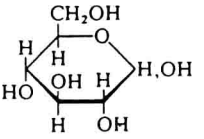
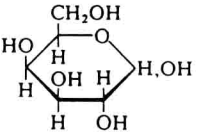
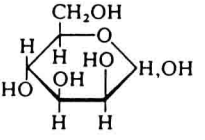
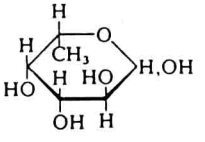
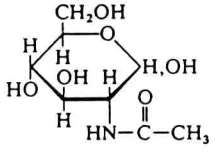
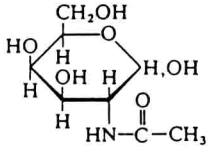
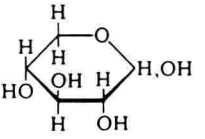
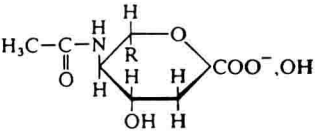
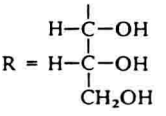
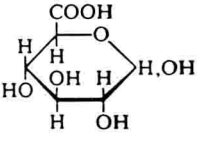
Name (abbreviations)	Structure
Glucose (Glc)	
Galactose (Gal)	
Mannose (Man)	
L-Fucose (Fuc)	
N-Acetylglucosamine (GlcNAc)	
N-Acetylgalactosamine (GalNAc)	

TABLE 1 (continued)
Monosaccharide Components Typically Found in Glycoproteins and Glycolipids

Name (abbreviations)	Structure
Xylose	
N-Acetylneuraminic acid (NeuNAc) (a sialic acid)	
	
Glucuronic acid (GlcA)	

Note: All sugars except for fucose are in D-configuration and are written in pyranose form.

IUB) nomenclature and is convenient for presenting many different structures, formula I is sometimes convenient for visual presentation of substrate structures. Thus, both forms are used in this book.

Glycoproteins are often classified by the structure of their protein-carbohydrate linkages. The most commonly observed are the N-glycosidic linkage between *N*-acetylglucosamine and the β-amido group of asparagine (GlcNAc→Asn), and O-glycosidic linkages between *N*-acetylgalactosamine and the hydroxyl group of serine (GalNAc→Ser) or threonine (GalNAc→Thr) (Figure 2). The former type of sugars are often called asparagine-linked or *N*-linked oligosaccharides.

Asparagine-linked oligosaccharides invariably have a common trimannosyl core and a di-*N*-acetylchitobiose structure. According to the outer sugar chains attached to the core, asparagine-linked oligosaccharides are classified as *N*-acetyllactosamine type (or complex type), oligomannose type (or high

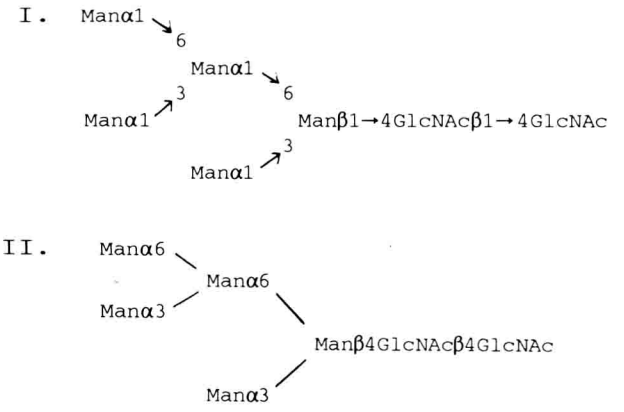
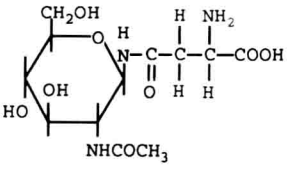
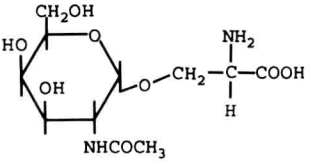


FIGURE 1. Structure of an oligomannose type oligosaccharide written in two different ways.



GlcNAc-Asn



GalNAc-Ser

FIGURE 2. Examples of protein-carbohydrate linkages.

mannose type), and hybrid type. The *N*-acetyllactosamine type has outer chains (antenna) with a sialyl-galactosyl-*N*-acetylglucosamine sequence or a modified form, such as fucosyl-galactosyl-*N*-acetylglucosamine or galactosyl-*N*-acetylglucosamine (Figure 3A). The number of antenna usually varies from two to four; *N*-linked oligosaccharides with two, three, and four antennas are referred to as biantennary, triantennary, and tetraantennary, respectively. *N*-Acetyllactosamine type oligosaccharides frequently have a fucose residue

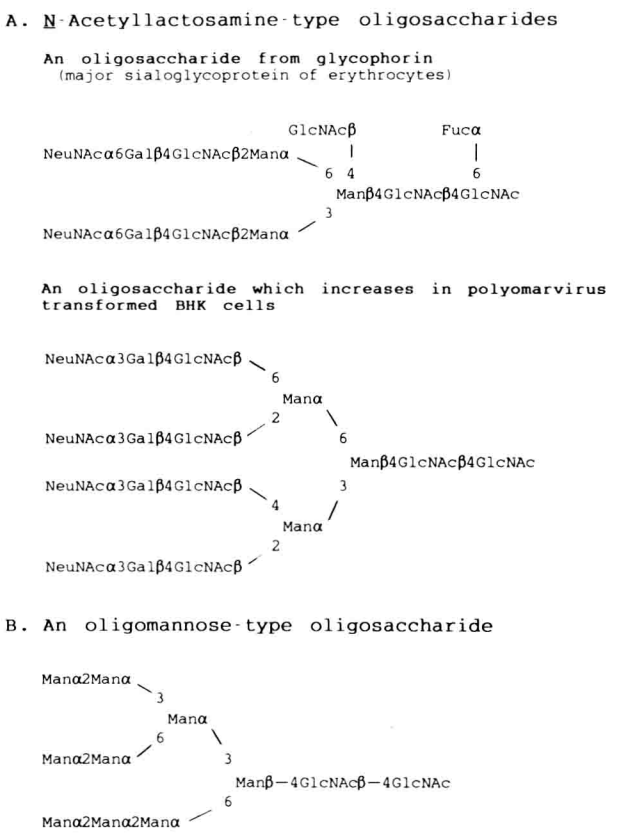


FIGURE 3. Examples of asparagine-linked oligosaccharides. Oligosaccharide structures found in glycophorin[6] and virus transformed BHK cells[7] are based on respective references.

attached to the core *N*-acetylglucosamine residue. Sometimes, *N*-acetyllactosamine units (Galβ1→4GlcNAc) in the antenna are repeated and occasionally branched and the sugar units become polysaccharidic; these glycans are called polylactosaminoglycans or poly-*N*-acetyllactosamines.

In oligomannose-type oligosaccharides, additional mannose residues are attached to the trimannosyl core, and the total number of mannosyl residues usually ranges from five to nine (Figure 3B). Hybrid type oligosaccharides have structures intermediate to the *N*-acetyllactosamine and the oligomannose types. An *N*-acetylglucosamine residue, which is occasionally added to the β-mannosyl residue of asparagine-linked oligosaccharides is called a bisecting *N*-acetylglucosamine.

A variety of structures are also present in oligosaccharides O-glycosidically linked to serine or threonine, whereas a core structure Galβ1→3GalNAc is usually found (Figure 4).

A subclass of glycoproteins consists of proteoglycans, which are

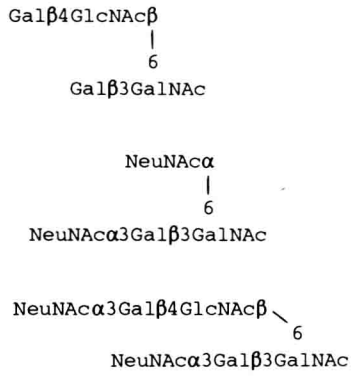


FIGURE 4. Examples of O-linked oligosaccharides.

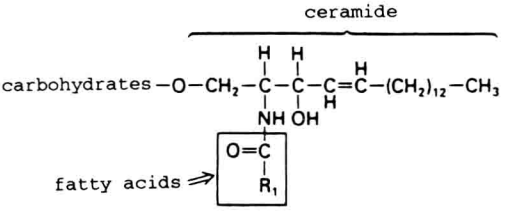


FIGURE 5. General structure of sphingoglycolipids.

glycosylated by glycosaminoglycans. Glycosaminoglycans are acidic polysaccharides basically composed of a linear linkage of repeating units: hyaluronic acid, chondroitin sulfates, heparin, heparan sulfate, and keratan sulfate are typical examples.[5] The carbohydrate-protein linkage region of many proteoglycans is GlcAβ1→3Galβ1→3Galβ1→4Xylβ1→Ser, and this nonrepeating nature shares a characteristic common to usual protein-linked oligosaccharides (see Chapter 5).

The most commonly observed glycolipids are glycosphingolipids, where oligosaccharides are linked to the hydroxyl group of ceramide (acylated sphingosine) (Figure 5). Various structures are also present in glycosphingolipids (Figure 6), while Galβ1→4Glc is the most common core structure. Glycosphingolipids are occasionally classified into the globo-, lacto- (or neo-lacto-series), and ganglio-series, according to the sugar residue attached to the core structure[2] (Chapter 4). On the other hand, gangliosides are all glycosphingolipids with sialic acids.

From a biological viewpoint, carbohydrates in glycoproteins and glycolipids first attracted interest as antigenic epitopes and attachment sites of infectious agents. Classical examples are the elucidation of blood group A and B antigenic epitopes and the demonstration of sialic acid involvement in the adhesion of influenza virus to host cells. Evidence then accumulated slowly

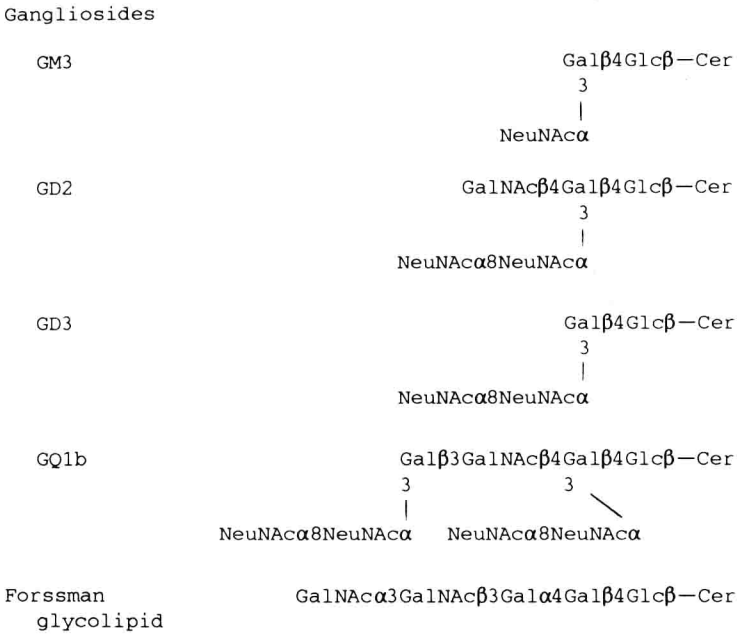


FIGURE 6. Examples of glycolipids.

but steadily to indicate that these carbohydrates serve as recognition signals in various biological phenomena such as fertilization, cell adhesion, induction of differentiation, and immunological reactions.[1-4] Recent clear-cut evidence has indicated that a family of cell adhesion proteins, including a lymphocyte homing receptor, is a group of carbohydrate recognizing proteins.[8] This finding is probably the final stroke to make even the most skeptical investigators believe in the importance of carbohydrates in cell surface recognition.

A BRIEF HISTORY OF ENDOGLYCOSIDASES AND GLYCOAMIDASES

As enzymes acting on carbohydrate moieties of glycoproteins and glycolipids, exoglycosidases were initially characterized and extensively studied. These enzymes act on sugars only when the target sugar is exposed (or at the nonreducing end) and release monosaccharides. Neuraminidase (sialidase), β-galactosidase, β-*N*-acetylglucosaminidase, α-L-fucosidase, α-*N*-acetylgalactosaminidase, α-mannosidase, and β-mannosidase are typical examples of these exoglycosidases. By combined action of exoglycosidases, carbohydrates of glycoproteins and glycolipids usually can be completely degraded.

Endoglycosidases acting on glycoproteins (except for proteoglycans) and glycolipids remained undiscovered for a long time. This was considered somewhat strange because α-amylase acting on starch, chitinase acting on chitin,

and lysozyme acting on bacterial cell walls are all endoglycosidases that cleave sugar chains into large fragments. The failure to discover them was the result of the lack of a proper assay system. Thus, the first endoglycosidase of this category, endo-β-*N*-acetylglucosaminidase D, was found during analysis of radioactively labeled glycopeptides. Soon, another endo-β-*N*-acetylglucosaminidase, called endo-β-*N*-acetylglucosaminidase H was discovered. Both enzymes cleave the di-*N*-acetylchitobiose linkage in asparagine-linked oligosaccharides, and have almost complementary specificities for mannosyl structures in the oligosaccharides. The combined use of the two types of enzymes greatly stimulated studies on asparagine-linked oligosaccharides; a typical example was finding the processing pathway in the biosynthesis of asparagine-linked oligosaccharides (Chapter 2).

Endo-β-galactosidase was initially found to be an enzyme acting on keratan sulfate. The finding that the enzyme can act on glycolipids and carbohydrate chains of ordinary glycoproteins other than proteoglycans was striking, and the enzyme played a critical role in the discovery of polylactosaminoglycans (Chapter 4).

Three endoglycosidases cleave protein-carbohydrate or lipid-carbohydrate linkages: endo-α-*N*-acetylgalactosaminidase, which releases sugars O-glycosidically linked to serine or threonine (Chapter 3); endo-β-xylosidase, which acts on the linkage point of glycosaminoglycans to proteins (Chapter 5); and endoglycoceramidase, which releases intact oligosaccharides from glycosphingolipids (Chapter 6). All are highly useful for studies of these glycoconjugates. The protein-carbohydrate linkage of asparagine-linked oligosaccharides, however, is not cleaved by an endoglycosidase, but by amidases. The first example of this type of enzyme is N^4-β-*N*-(acetylglucosaminyl)-L-asparaginase (EC 3.5.1.26). The enzyme acts only when oligosaccharides are attached to an asparagine residue, which is not linked to other amino acids.[9] Since to free asparagine-linked oligosaccharides from other amino acids is a difficult task, the practical value of the enzyme is low. However, precise elucidation of the mode of enzymatic action formed the basis for characterization of other amidases discovered later. An amidase found in almond emulsin showed properties ideal for application to glycoprotein research; the enzyme can act on oligosaccharides linked to peptides of various sizes (Chapter 7). The almond enzyme was initially called almond glycopeptidase. Another amidase subsequently found in an endoglycosidase preparation of *Flavobacterium* was named *N*-glycanase. The action mechanisms of glycopeptidase and *N*-glycanase are the same, but *N*-glycanase may act on intact glycoproteins more easily. Almond glycopeptidase and *N*-glycanase have become highly important tools in glycoprotein research in many respects, e.g., preparation of oligosaccharides for structural characterization (Chapter 8) and protein deglycosylation (Chapter 9). The xylose-containing structure common to many asparagine-linked oligosaccharides of plant origin was discovered by analysis of glycopeptidase-released oligosaccharides (Chapter 8).

Since almond glycopeptidase and *N*-glycanase belong to the same group of enzymes, Takahashi proposed a new name applicable to both enzymes: glycoamidase (Chapter 7). This nomenclature emphasizes that these enzymes are amidases and not glycosidases.

ENDO-*N*-ACETYLNEURAMINIDASE AND ENDO-α-MANNOSIDASE

ecENDO-*N*-ACETYLNEURAMINIDASE

Bacteriophage KIF recognizes polysialic acid capsule of *Escherichia coli* K1 as a receptor. The receptor destroying enzyme, endo-*N*-acetylneuraminidase (abbreviation Endo *N*) has been purified to homogeneity from the bacteriophage.[10] The molecular weight of the subunit is 105,000, whereas the native molecule has a molecular weight of 328,000. The enzyme acts on poly- or oligo-α-2,8 sialosyl units, when the number of sialic acid residues is five or more. Capsular polysaccharides as well as carbohydrate units on neural cell adhesion molecule (N-CAM)[11] and polysialoglycoproteins of salmon eggs[12] are also substrates of Endo *N*. Sialic acid can exist either in *N*-acetylneuraminic acid or *N*-glycosylneuraminic acid as a substrate.[12] Thus, the enzyme is a useful reagent with which to analyze the structure and function of carbohydrates with polysialo-units.

ENDO-α-MANNOSIDASE

An enzyme found in rat liver Golgi membrane catalyzes the following reaction:[13]

$$Glc_1Man_9GlcNAc \longrightarrow Glc\alpha 1 \longrightarrow 3Man + Man_8GlcNAc$$

Thus, endo-α-mannosidase serves in an alternate processing pathway in the biosynthesis of asparagine-linked oligosaccharides (Figure 7).[14]