Innovative Preparation of Biopharmaceuticals Using Transglycosylation Activity of Microbial Endoglycosidases

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Abstract: Most functional biopharmaceuticals such as antibodies are glycoproteins carrying N-linked oligosaccharides (N-glycans). In animal cells, these glycans are generally expressed as heterogeneous glycoforms that are difficult to separate into a pure form. The structure of these glycans directly affects several biological aspects of the glycoproteins, especially binding affinity. Therefore, the preparation of glycoproteins with well-defined and homogeneous glycoforms is necessary for functional studies and improved efficacy, particularly for biopharmaceuticals. This review describes the recent remarkable progress in the development and production of biopharmaceutical glycan-modified antibodies, through the use of glycan remodeling using microbial endoglycosidases and sophisticated glycoengineering techniques utilizing microbial enzymatic reaction mechanisms.

Key words: immunoglobulin G, endo-β-N-acetylglucosaminidase, transglycosylation, remodeling, glycoengineering

INTRODUCTION

In recent years, enzymatic glycoengineering techniques have been used more frequently to produce effective biopharmaceuticals, such as monoclonal antibodies (mAbs). This engineering technique relies on the conversion of IgG sugar chains by a microbial endo-β-N-acetylglucosaminidase (endo-β-N-GlcNAc-ase, EC 3.2.1.96), which can transfer oligosaccharides from donor substrates to acceptors that carry hydroxyl residues by its transglycosylation activity. Monoclonal antibodies represent effective therapeutic glycoproteins used for the treatment of cancers, inflammatory disorders, and infectious diseases. Most therapeutic mAbs are of the IgG class and contain N-linked oligosaccharides. A typical IgG class antibody is composed of two heavy chains and two light chains that form three distinct domains, including two identical Fab (antigen-binding) domains and an Fc (crystallizable) domain. The Fc domain is a homodimer of the heavy chain that carries conserved sites for N-glycosylation at the Asn-297 position and is usually attached by biantennary, core-fucosylated complex-type sugar chains. This domain is involved in the recruitment of immune cells by binding to Fc receptors (FcγRs) on the immune cell surface, and directly initiates immune responses including phagocytosis, immune cell activation, and cytokine stimulation towards antigen-displaying targets.

The glycosylation of the Fc domain mediates interactions with FcγRs and regulates antibody-dependent effector functions, including antibody-dependent cellular cytotoxicity (ADCC), which involves the activation of natural killer cells to initiate lysis of cancer cells, and complement-dependent cytotoxicity (CDC), which involves the initiation of target cell lysis and deployment of the complement pathway. Previous studies have shown that glycosylation has profound effects on biological function and overall therapeutic efficacy. Core-fucosylation of the Fc domain sugar chain hinders the binding of IgG antibodies with the Fc receptor of the cell, and the lack of the core-fucose in the sugar chain dramatically enhances ADCC. The galactose residue on the non-reducing end of the Fc domain sugar chain also improves ADCC and CDC. Moreover, a study by Washburn et al. has shown that terminal sialic acid moieties (α2,6-linked) of the Fc domain sugar chain are crucial for the anti-inflammatory activity of intravenous immunoglobulin (IVIg) (Fig. 1).

As mammalian cell lines, such as Chinese Hamster Ovary (CHO) cells, are commonly used as hosts, the recombinant mAbs are often glycoproteins with heterogeneous glycoforms. However, most active non-fucosylated glycoforms are usually found as minor fractions within the heterogeneous mixtures. Therefore, it is necessary to develop IgG antibodies with well-defined sugar chains of the Fc domain, and glycoengineering of the sugar chain may become a useful approach to improve the function of IgG antibodies. As a result, the remodeling or replacement of sugar chains
using endoglycosidases has attracted significant attention in this field. Recent studies have shown that glycoengineering using microbial endoglycosidases allows for the efficient production of homogenous glycoforms.

This review describes the recent advances related to an innovative preparation of biopharmaceutical mAbs using microbial endoglycosidases. This review will also detail efficient methods to obtain homogeneous IgG antibodies using recombinant yeast and mutants of endoglycosidases.

APPLICATION OF TRANSGLYCOSYLATION ACTIVITY OF MICROBIAL ENDOGLYCOSIDASES TO THE GLYCOENGINEERING OF BIOPHARMACEUTICALS

Glycan remodeling methods that produce structurally well-defined, homogeneous glycoforms of antibodies are desirable for both functional studies and the development of antibody-based therapeutics. The chemo-enzymatic glycosylation remodeling, which involves endoglycosidase-catalyzed deglycosylation and subsequent glycosylation, has emerged as an elegant and promising method to obtain antibodies with homogeneous glycoforms.

Endo-β-GlcNAc-ase is a useful tool for the enzymatic addition of sugar chains to other compounds. This enzyme is a unique endoglycosidase that hydrolyzes N,N′-di-acetyltrehalosyl linkages in sugar chains bound to asparagine residues of various glycoproteins and glycopeptides, leaving one N-acetylgalactosaminyl residue on the protein or peptide moiety, and is useful for elucidating the structure and function of the glycoprotein or glycopeptide. It is well known that many glycosidases exhibit transglycosylation activity that involves the transfer of a carbohydrate moiety to the hydroxyl groups of various compounds, in addition to hydrolytic activity. The transglycosylation activity of exo-type glycosidases is useful for the enzymatic synthesis of various oligosaccharides through the addition of monosaccharides, whereas that of endo-type glycosidases is useful for adding large oligosaccharides en bloc to various compounds bearing hydroxyl groups. The transglycosylation activity of endo-β-GlcNAc-ase catalyzes the following reaction:

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\text{Oligosaccharide-GlcNAc-GlcNAc-Asn-R (glycoside donor)} + \text{GlcNAc-Asn-R}′ (acceptor) → \text{Oligosaccharide-GlcNAc-GlcNAc-Asn-R}′ (\text{transglycosylation product}) + \text{GlcNAc-Asn-R (R, R′: peptide or protein, GlcNAc: N-acetyl-D-glucosamine) (Fig. 2)}
\]

However, not every endo-β-GlcNAc-ase possesses transglycosylation activity. This type of enzyme is classified into two glycoside hydrolase (GH) families, GH18 and GH85, in the Carbohydrate-Active Enzymes (CAZy) database. The GH18 family includes bacterial endo-β-GlcNAc-ases, such as Endo-H (from Streptomyces pilatus), Endo-F (from Elizabethkingia meningosepticum, previously named Flavobacterium meningosepticum), and Endo-S (from Streptococcus pyogenes), as well as many chitinases. On the other hand, the GH85 family is composed of endo-β-GlcNAc-ases from both prokaryotes and eukaryotes, such as Endo-M (from Mucor hiemalis), Endo-A (from Arthrobacter protophormiae), Endo-D (from Streptococcus pneumoniae, previously named Diplococcus pneumoniae), Endo-CE (from Caenorhabditis elegans), Endo-CC (from Coprinopsis cinerea) and endo-β-GlcNAc-ase from humans. Unlike GH18 enzymes, which generally exhibit only hydrolytic activity, most of the GH85 enzymes possess transglycosylation activity (see Fig. 2).

GH85 enzymes show various substrate specificities for transglycosylation activity. Endo-A is typically used as an enzymatic tool that can act on high-mannose type sugar chains by accepting hydroxyl residues by transferring oligosaccharides from donor glycopeptides which is generally obtained from hen egg white. Endo-M is an enzyme acting on complex-type biantennary oligosaccharides and is generally used for transferring complex-type oligosaccharides onto acceptors from donor substrates, such as sialylglycopeptides of hen egg yolk. Other endo-β-GlcNAc-ases are used depending on their glycan specificities.

Endo-β-GlcNAc-ases are inherent glycosyl hydrolases, and their transglycosylation activity is relatively low. Therefore, the product that is formed by the transglycosylation reaction is quickly hydrolyzed as the substrate of the enzyme, and the amount of the resulting product significantly decreases as it cannot accumulate. To overcome this issue, it was necessary to review the reaction mechanism of this enzyme. Transglycosylation proceeds via a double displacement mechanism that is mediated by two active residues of a catalytic nucleophile and an acid/base. Generally, glycosidases hydrolyze glycosidic bonds following either an inverting or retaining mechanism. Both mechanisms involve an oxocarbenium-ion-like transition state and a pair of carboxylic acids (i.e. aspartate and glutamate residues). However, there are exceptions, such as some N-acetylhexosaminidases that lack a catalytic nucleophile residue and act via a substrate-assisted mechanism.

Endo-β-GlcNAc-ases belonging to the GH85 family also act via a substrate-assisted mechanism involving a 2-acetamido group through the formation of a 1,2-oxazolinium ion intermediate. This suggests that endo-β-GlcNAc-ases of the GH85 family can take synthetic sugar oxazolines as donor substrates for transglycosylation. Sugar oxazolines can serve as an excellent donor substrate for transglycosylation and can be used to activate glycosyl donors for GH85 enzymes.

Although structural analyses of GH18 chitinases and GH20 β-N-acetylhexosaminidases revealed that the proper orientation of the acetamide group may be aided by another carboxylate residue located 1 or 2 amino acid residues upstream from the general acid/base catalytic residue, GH85 endo-β-GlcNAc-ases has no second conserved carboxylate residue near the general acid/base carboxylate residue. Instead, a conserved asparagine (Asn) residue at the second position upstream from the general acid/base catalytic residue is present, and this residue may play a similar role as the second carboxylate in the GH18 and GH20 enzymes. These results suggested that the mutation of this conserved Asn residue causes a complete loss of glycosylation activity.
the hydrolytic activity of the enzyme. If an enzyme such as glycosynthase, which loses its hydrolytic activity but exhibits only unilateral transglycosylation activity, was obtained, the yield of transglycosylation product would be remarkably high. Therefore, our research group attempted to carry out site-directed mutagenesis that alters the corresponding Asn residue of Endo-M to an alanine (Ala) residue. The resulting mutant enzyme (N175A) was able to utilize synthesized sugar oxazolines as a donor substrate for transglycosylation to form a transglycosylation product, but it lacked hydrolysis activity for the product.

36) The corresponding mutants of other GH85 enzymes were also capable of catalyzing the transglycosylation using the sugar oxazoline as the donor substrate (Fig. 3). Furthermore, the mutant in which the Asn residue is replaced with a glutamine (Gln) residue at the corresponding position of GH85 endo-β-GlcNAc-ases (N175Q mutant for Endo-M) was found to possess dramatically enhanced glycosynthase-like activity with sugar oxazolines, and almost no hydrolytic activity. The glycosynthase-like mutant enzymes are considered useful for creating new glycoproteins.

Recently, some endo-β-GlcNAc-ases belonging to the GH18 family were found to possess transglycosylation activity. Endo-S was found to liberate natural oligosaccharides of human IgG. Moreover, Endo-S was capable of transferring biantennary complex-type oligosaccharides onto defucosylated GlcNAc with an inner Fucα1,6GlcNAc or defucosylated GlcNAc, using the corresponding oxazoline as a donor substrate. Endo-F3 mutant was also found to transfer bi- and tri-antennary complex-type oligosaccharides onto deglycosylated IgG with inner Fucα1,6GlcNAc.

USE OF TRANSGLYCOSYLATION ACTIVITY OF ENDOGLYCOSIDASES FOR IgG REMODELING

The importance of Fc domain glycosylation for the biological function of IgG prompted the application of the chemo-enzymatic synthesis methods. These methods use the transglycosylation activity of endo-β-GlcNAc-ase to remodel glycoproteins carrying heterogeneous glycoforms.
into glycoproteins carrying well-defined and homogeneous oligosaccharide structures. The initial trial was reported by Wei et al., who performed the remodeling of *N*-linked oligosaccharides of a recombinant IgG Fc domain using Endo-A and synthetic sugar oxazolines of high-mannose type oligosaccharides (tetrasaccharide Man,GlcNAc) as the donor substrate.\(^{40}\) The process is briefly outlined as follows: the Fc domain produced by recombinant yeast *Pichia pastoris* included heterogeneous *N*-linked oligosaccharides composed of mannos-oligomers, and these oligosaccharides were first removed by Endo-H to give a GlcNAc-Fc domain. Then, tetrasaccharide Man,GlcNAc was added to the GlcNAc moiety of the Fc domain by Endo-A using the corresponding tetrasaccharide oxazoline as donor substrate under mild reaction conditions to give an Fc domain with a homogeneous Man,GlcNAc oligosaccharide. Following this initial study, Zou et al. created many Fc domains carrying various truncated oligosaccharides, by use of Endo-A and various corresponding oligosaccharide oxazolines, and also demonstrated that the presence of a bisecting GlcNAc moiety (a branching GlcNAc residue bound to the core β-mannose residue) of the oligosaccharide enhanced the binding affinity of the Fc domain to the Fc receptor.\(^{41}\) In addition to Endo-A, Endo-D has also been used for transglycosylation using Fc fragments. Fan et al. demonstrated the transglycosylation activity of an Endo-D glycosynthase mutant N322Q, which transfers Man,GlcNAc of the corresponding sugar oxazoline (donor substrate) to the fucosylated GlcNAc-Fc domain prepared by protease digestion and Endo-S treated IgG.\(^{42}\)

In 2012, an interesting report relating to the glycoengineering of an mAb was presented by Goodfellow et al.\(^{39}\) As previously described, they found that Endo-S belonging to the GH18 family possessed a high level of hydrolysis activity specific to complex-type oligosaccharides of Fc domains of human IgG antibodies. Furthermore, the enzyme possessed transglycosylation activity that adds a biantennary complex-type oligosaccharide to the GlcNAc-Fc domain of a full-length IgG with or without fucosylation of the innermost GlcNAc. This report encouraged the use of Endo-S for glycoengineering of intact full-length mAbs. Huang et al. reported the creation of glycosynthase mutants from Endo-S: D233A and D233Q. They demonstrated that these Endo-S mutants were able to transfer complex-type oligosaccharides from the corresponding oligosaccharide oxazolines to deglycosylated mAb (Rituximab) to form a new and homogeneous glycoform of the antibody without hydrolysis of the product, and were also able to perform remodeling of Fc oligosaccharides on intact full-length antibodies.\(^{43}\) This practical method features two steps: (1) trimming of natural glycoforms of IgG with wild-type Endo-S to produce a deglycosylated IgG with an innermost Fucα1,6GlcNAc disaccharide or defucosylated GlcNAc monosaccharide; (2) Endo-S mutant-catalyzed transglycosylation of chemically synthesized oligosaccharide oxazolines as the donor substrate onto Fucα1,6GlcNAc-IgG or GlcNAc-IgG, to produce glycoengineered antibodies with well-defined oligosaccharide structures (Fig. 4). However, these Endo-S mutants cannot transfer high-mannose type oligosaccharides.

The above method has become a typical method for the remodeling of mAbs in glycoengineering. Lin et al. used Endo-S and its mutants to remodel the oligosaccharide of the Fc domain in an intact antibody to generate a series of antibody glycoforms.\(^{44}\) They found that a biantennary oligosaccharide structure with two terminal α2,6-linked sialic acids was a common and optimal structure for the enhancement of ADCC, CDC, and anti-inflammatory activity. Parsons et al. evaluated the reaction conditions for the transglycosylation catalyzed by an Endo-S mutant using Herceptin (a therapeutic mAb).\(^{45}\) They reported that if the reaction conditions were not controlled, some side-reactions between the active oligosaccharide oxazolines (donor substrates) and certain lysine residues on the antibody protein might occur, and non-enzymatic byproducts could be formed. The side-reaction could be minimized by the stepwise addition of oligosaccharide oxazolines and using more enzymes with shorter reaction times. This led to optimal conditions that reduced the formation of byproducts due to “glycation”.

While some groups focused on the remodeling of IgG using known endo-β-GlcNAc-ases and sugar oxazolines, Sjögren et al. found a novel enzyme named Endo-S2, from *Streptococcus pyogenes* of serotype M49.\(^{46}\) This novel enzyme belonging to the GH18 family demonstrated a much broader substrate specificity than Endo-S, which is specific for biantennary complex-type N-glycans on the Fc domain of IgG antibodies in native form. The Endo-S2 enzyme could essentially deglycosylate all major types of *N*-glycans of the Fc domain. Based on this finding, Li et al. identified mutagenesis at Asp-184 as an equivalent to the Asp-233 mutation of Endo-S. They created several mutants including D184M and D184Q.\(^{47}\) These mutants had a much higher catalytic efficiency than the corresponding Endo-S mutants and had a significantly wider substrate specificity, which made it capable of transferring complex-type, high-mannose type, and hybrid type *N*-glycans. The utility of Endo-S2 glycosynthase mutants was exemplified by an efficient remodeling of the mAb *N*-glycans. Moreover, Shivatore et al. found that the T138Q mutant of Endo-S2 also reduced hydrolytic activity, though it maintained transglycosylation activity, and generated antibodies with new glycoforms of various high-mannose, hybrid and complex type oligosaccharides.\(^{48}\) A residue in the proximity of the catalytic domain like Thr-138 might also modulate the glycosynthase activity. These results stimulated further studies on the remodeling of whole bodies of therapeutic mAbs and analyses of their biofunctions. Indeed, the analysis of Rituximab, a glycan-remodeled with the Endo-S2 mutants, demonstrated detrimental effects of core-fucosylation on effector functions.\(^{49}\) Furthermore, the GlcNAc-Herceptin, which is generated by deglycosylating Herceptin with wild-type Endo-S2 and α1,6-fucosidase of *Lactobacillus casei*, was remodeled into Herceptin carrying a sialylated biantennary complex-type oligosaccharide by Endo-S2-D184M glycosynthase using the corresponding oligosaccharide oxazoline as the donor substrate (Fig. 5). The remodeled Herceptin with non-fucosylated glycoforms has been dem
onstrated to have enhanced ADCC.

In 2015, an interesting study regarding the functional differences of various N-glycan structures of the Fc domain was reported by Kurogochi et al. To elucidate the relationships between the structure of N-glycans of the Fc domain and the therapeutic activity of mAb, they used the Endo-S-D233Q mutant and various endo-β-GlcNAc-ases to assemble a relatively large glycoform library of Herceptin produced in a transgenic silkworm cocoon, including both full-sized and truncated N-glycans. Using the glycoform library of Herceptin, the binding of N-glycans to the Fc receptor was assessed and cell-based assays were performed. The results revealed that the glycoform influenced ADCC and proved that sialylation significantly decreased ADCC in the presence of core-fucose, but had no impact in the absence of core-fucose.

Our group also succeeded in replacing heterogeneous oligosaccharides of the recombinant mAb, which was produced by the recombinant methylotrophic yeast *Ogataea minuta*, to sialyl-complex type oligosaccharides using an Endo-M glycosynthase mutant N175H and sialylglycopeptide derived from hen egg yolk as donor substrate (unpublished data) (Fig. 6).

**REMODELING OF CORE-FUCOSYLATED IgG**

As described above, core fucosylation of the Fc domain N-glycans is a modification often found in natural and recombinant glycoproteins that affects N-glycan conformation and regulates the biological activity of IgG. Notably, IgG with fucose-deficient N-glycans in its Fc domain shows an increased binding affinity for the Fc receptor on the effector cells, resulting in highly enhanced ADCC.

The SDS-PAGE in the top panel was blotted with an anti-human IgG antibody, and the SDS-PAGE in the bottom panel was stained with a sialic acid-specific SNA (*Sambucus nigra*) lectin for the heavy chain of IgG. The heavy chain of the recombinant yeast IgG with high-mannose type sugar chains was removed by Endo-H, which was then remodeled into the IgG with a sialo-complex type sugar chain by the transglycosylation reaction of Endo-M N175H mutant with sialyl-glycopeptide oxazoline as glycan donor.
Sylated glycoproteins. These findings broaden the applicability of preparation processes for remodeled IgG. Iwamoto et al. attempted to develop a one-pot transglycosylation by the combined use of Endo-S (D233Q) and Endo-M (N175Q) mutants with intact sialylglycopeptides as the donor substrate, without the use of the glycan oxazoline. This was an attempt to circumvent the use of oxazoline donor substrates by using a combination of endoglycosidase mutants with varying target glycan specificities. In the reaction mixture, an Endo-M-N175Q mutant worked better than the Endo-M wild-type as an auxiliary endoglycosidase to supply active intermediates, presumably because the rate of hydrolytic reaction from an active intermediate to the hydrolysate is slow enough for an Endo-S mutant to utilize it as a substrate.

Tang et al. also reported a one-pot strategy for remodeling IgG using Endo-M to cleave glycopeptides and Endo-S-D233Q to glycosylate the acceptor substrates with sialylglycopeptides (donor substrate). They also successfully carried out a glycoside-specific conjugation to develop new antibody-drug conjugates (ADCs). Li et al. reported the preparation of immobilized endoglycosidases of Endo-S2 and its glycosynthase mutant D184M using a recombinant microbial transglutaminase for chemo-enzymatic glycan remodeling of antibodies. Recently, Giddens et al. reported the site-selective remodeling of the N-glycans of both Fc (attached to Asn-297 of the heavy chain) and Fab (attached to Asn-88 of the heavy chain) of Cetuximab, a therapeutic mAb used for the treatment of a variety of cancers. This work was completed using the high specificity of the Endo-F3-D165A glycosynthase mutant, which only glycosylates core-fucosylated GlcNAc residues and is unable to bind glycans to non-fucosylated GlcNAc residues, and the Endo-S-D233A glycosynthase mutant, which only glycosylates GlcNAc residues at Asn-297 of IgGs. Taking advantage of the substrate specificity of these glycosynthase mutants, together with α1,6-fucosidase from Lactobacillus casei, which only removes the core-fucose from the GlcNAc residues at Asn-297, a homogeneous glycoform of Cetuximab was synthesized. This preparation scheme is very elegant, and the glycoengineered Cetuximab demonstrated an increased affinity for the Fc receptor and significantly enhanced ADCC activity.

**DEVELOPMENT OF PREPARATION PROCESSES FOR REMODELED IgG**

Several processes have been under development for the effective production of remodeled IgG. Iwamoto et al. attempted to develop an effective one-pot transglycosylation by the combined use of Endo-S (D233Q) and Endo-M (N175Q) mutants with intact sialylglycopeptides as the donor substrate, without the use of the glycan oxazoline. This attempt to circumvent the use of oxazoline donor substrates by using a combination of endoglycosidase mutants with varying target glycan specificities. In the reaction mixture, an Endo-S mutant can successfully add glycans onto deglycosylated IgG by exploiting active intermediates as a donor substrate, generated from SGP by the hydrolytic activity of an auxiliary endoglycosidase (Endo-M-N175Q in this case). Note that the Endo-M-N175Q mutant worked better than the Endo-M wild-type as an auxiliary endoglycosidase to supply active intermediates, presumably because the rate of hydrolytic reaction from an active intermediate to the hydrolysate is slow enough for an Endo-S mutant to utilize it as a substrate.

**PERSPECTIVE**

Endo-β-GlcNAc-ase-mediated-remodeling of mAb is an attractive approach that can give various well-defined and homogeneous IgG glycoforms that are difficult to obtain by other previously developed methods. Additionally, this method is promising for the development of more effective therapeutic antibodies. Microbial endo-β-GlcNAc-ases and their variants applied to the remodeling of sugar chains of biopharmaceuticals are summarized in Table 1.

At present, mammalian cell lines such as CHO cells are commonly used as hosts for mAb production. However, the process is relatively expensive, some alternative processes are being considered. Among them, approaches using yeast hosts are regarded as an effective process to realize large-scale production of remodeled antibodies because of its higher production potential and lower cost. However, yeasts express large and varied high-mannose type sugar chains attached to proteins. These sugar chains may cause heavy antigen-antibody reactions in humans and therefore should be replaced with human-compatible ones. To produce human-compatible sugar chains, a recent study expressed human IgG in the yeast host *Pichia pastoris,*
Endo-A

Endo-F3

Endo-D

Endo-S

Endo-S2

Endo-S2


glycan class; HM, high-mannose type; Hyb, hybrid type; C-Bi, biantennary complex type; C-Tri, Triantennary complex type. b The only enzyme capable of transferring triantennary complex type N-glycans. c Highly specific to IgG.

Table 1. Various useful microbial endoglycosidases for the preparation of biopharmaceuticals.

| Enzyme       | Origin                        | GH family           | N-Glycan classa of oxazoline substrate as donors for transglycosylation | Tolerance to core-fucose of N-glycan in acceptors | Remarks                  |
|--------------|-------------------------------|---------------------|-----------------------------------------------------------------------|--------------------------------------------------|--------------------------|
| Endo-A       | Arthrobacter protophormiae   | 85                  | HM/Hyb                                                               | No                                               | Commercially available   |
| Wild-type    |                               |                     | N175Q/A/H                                                            | HM/Hyb/C-Bi                                      | Commercially available   |
| N175Q/W251N  |                               |                     |                                                                       | HM/Hyb/C-Bi                                      |                         |
| Endo-D       | Streptococcus pneumoniae      | 85                  | Truncated HM                                                         | Yes                                              | Commercially available   |
| Wild-type    |                               |                     |                                                                        |                                                  |                         |
| N322Q/A      |                               |                     |                                                                       |                                                  |                         |
| Endo-F3      | Elizabethkingia meningoseptica| 18                | Truncated HM                                                         | Yes                                              |                         |
| D165A        |                               |                     | C-Bi/C-Tri                                                           | Yes                                              |                         |
| Endo-S       | Streptococcus pyogenes        | 18                  |                                                                      |                                                  |                         |
| Wild-type    |                               |                     |                                                                       |                                                  |                         |
| D233A/Q      |                               |                     |                                                                       |                                                  |                         |
| Endo-S2      | Streptococcus pyogenes serotype M49 | 18         |                                                                      |                                                  |                         |
| Wild-type    |                               |                     |                                                                       |                                                  |                         |
| D184M/Q      |                               |                     |                                                                       |                                                  |                         |
| T138Q        |                               |                     |                                                                       |                                                  |                         |

a N-Glycan class: HM, high-mannose type; Hyb, hybrid type; C-Bi, biantennary complex type; C-Tri, Triantennary complex type. b The only enzyme capable of transferring triantennary complex type N-glycans. c Highly specific to IgG. d Endo-S2 and its variants are capable of transferring all classes (high-mannose, hybrid, and biantennary complex types) of N-glycans to IgG regardless of the presence or absence of core-fucose.

followed by the replacement of sugar chains using both hydrolysis and transglycosylation activities of microbial endo-β-GlcNAc-ases. However, the use of these methods is still in its infancy, and future development of such preparation methods would allow for large-scale and efficient production of biopharmaceutical IgG with microbial cells and enzymes.

CONFLICTS OF INTEREST

No potential conflict of interest was reported by the authors.

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