Glycosidase Selection Strategies

Glycosylation is a common post-translational modification in eukaryotic cells, crucial for protein folding, stability, activity, and intercellular signaling. It can be categorized into N-linked and O-linked glycosylation. N-linked glycans are further classified into high-mannose, hybrid, and complex types.

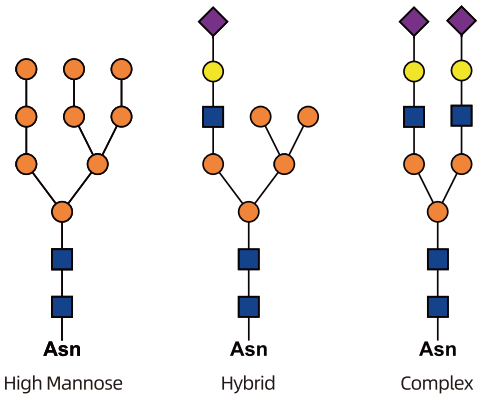

Fig. 1 Three main types of glycans

However, glycosylation poses challenges: (1) it reduces protein homogeneity, hindering crystallization, and (2) abnormal glycosylation can cause diseases. Deglycosylation enzymes are valuable tools for studying glycoproteins and related diseases.

PNGase F

PNGase F is a broad-spectrum N-linked glycosidase that cleaves all three types of N-linked glycans: high-mannose, hybrid, and complex. It is not specific to amino acid sequences and can cleave glycans from denatured proteins, with generally higher efficiency than from native proteins.

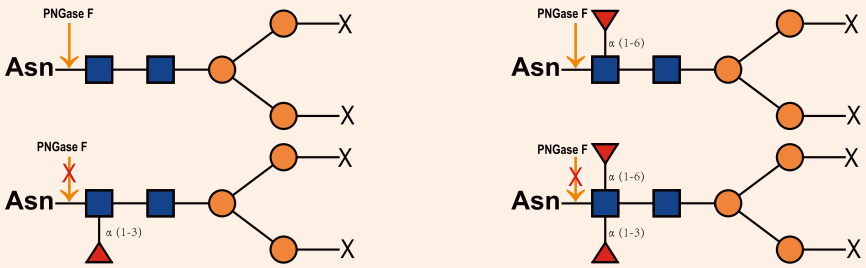

Fig. 2 The types of glycans recognized by PNGase F and its cleavage site

Endo S

Endo S is an endoglycosidase that specifically recognizes and cleaves the complex glycans at the Asn297 site of human IgG.

Fig. 3 The types of glycans recognized by Endo S and its cleavage site

CU43

CU43 is a glycosidase that specifically cleaves glycans at the Asn297 site of human IgG, efficiently targeting both hybrid and complex glycans. In mouse models, CU43 has been shown to effectively prevent autoimmune hemolytic anemia and antibody-dependent enhancement (ADE) effects (Diego et al., Cell, 2024), demonstrating strong in vivo activity.

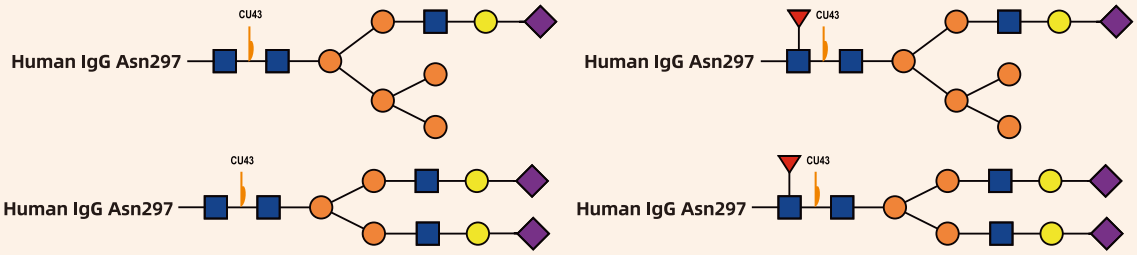

Fig. 4 The types of glycans recognized by CU43 and its cleavage site

Endo S2

Endo S2 is capable of cleaving all three types of glycans—high-mannose, hybrid, and complex—at the Asn297 site of human IgG.

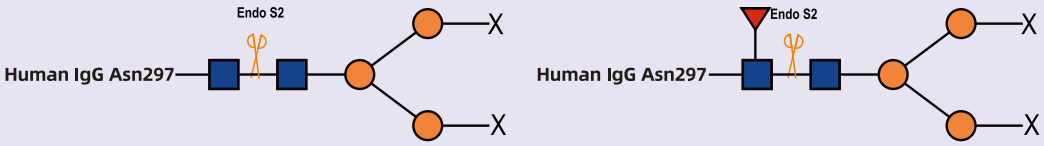

Fig. 5 The types of glycans recognized by Endo S2 and its cleavage site

It is important to note that the deglycosylation activity of Endo S, CU43, and Endo S2 is highly dependent on the intact conformation of human IgG. If human IgG is denatured, they cannot cleave the glycans. Additionally, Endo S, CU43, and Endo S2 are unable to remove glycans from human IgA, IgM, IgE, or IgGs from other species.

The following is a summary of the four enzymes:

Enzyme	Catalog No.	Target Glycan Types	Recognition Site	Dependence on Protein Conformation
PNGase F	JN842012	High-mannose, hybrid, complex	None	Works on both denatured and native proteins
Endo S	JN936012	Complex glycans	Human IgG Asn297	Specific to native human IgG
CU43	JN946012/JN946011	Hybrid, complex glycans	Human IgG Asn297	Specific to native human IgG
Endo S2	JN865012	High-mannose, hybrid, complex	Human IgG Asn297	Specific to native human IgG

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