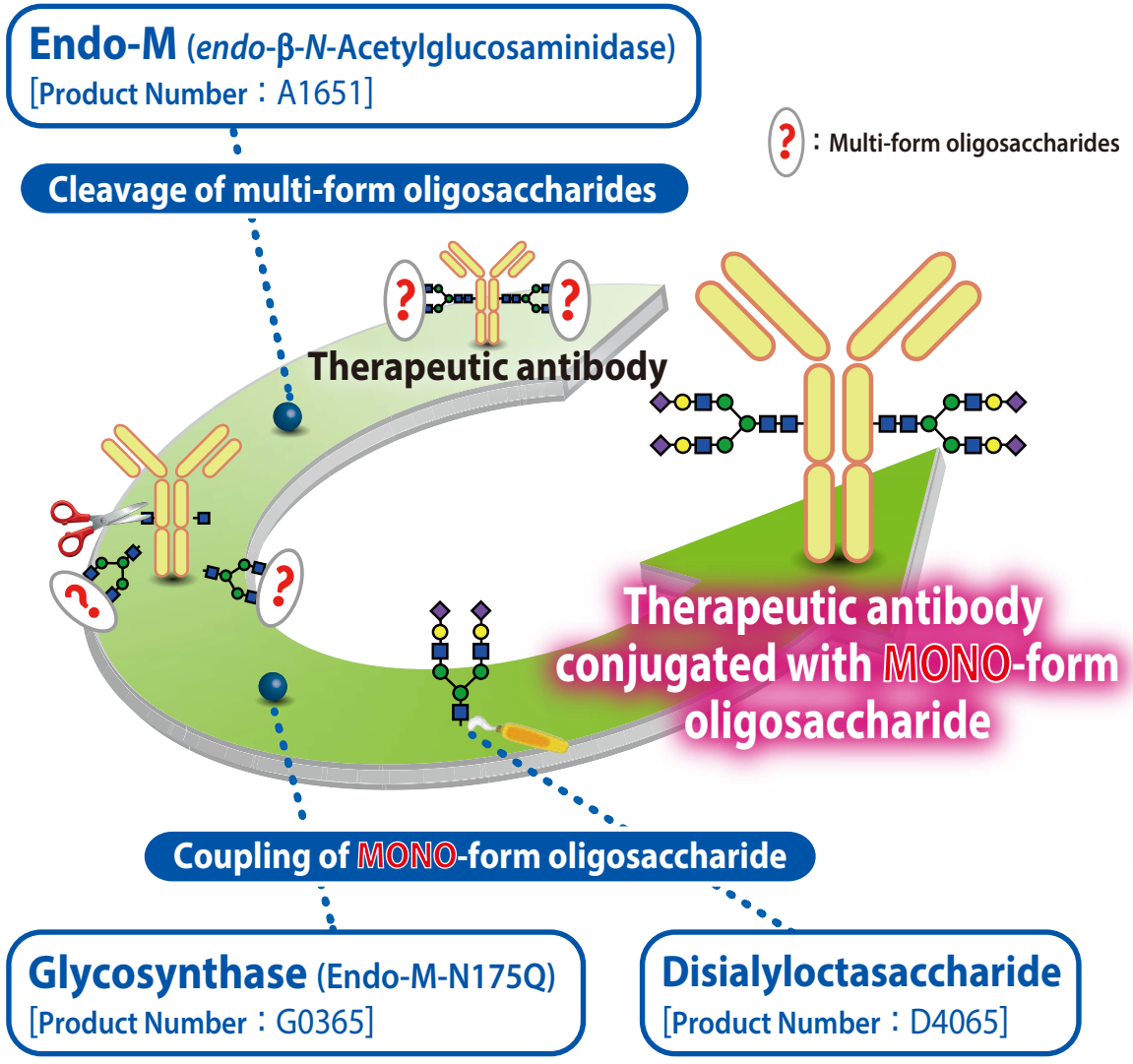
LIFE SCIENCE

Oligosaccharide Replacement of a Therapeutic Antibody

by using Endo-M and Glycosynthase

As a result of chemoenzymatic glycoengineering, multi-form *N*-linked oligosaccharides of a therapeutic antibody were replaced by the structure fine-defined oligosaccharide

In recent years, the expectation of a therapeutic benefit for antibody drugs is growing and the development of industrial technology for antibody production is required. However, heterogeneity of glycosylation of antibody drugs has long been left unsolved. In this context, TCI achieved introduction of a MONO-form oligosaccharide to a therapeutic antibody by using our enzyme products, "Endo-M" and "Glycosynthase".

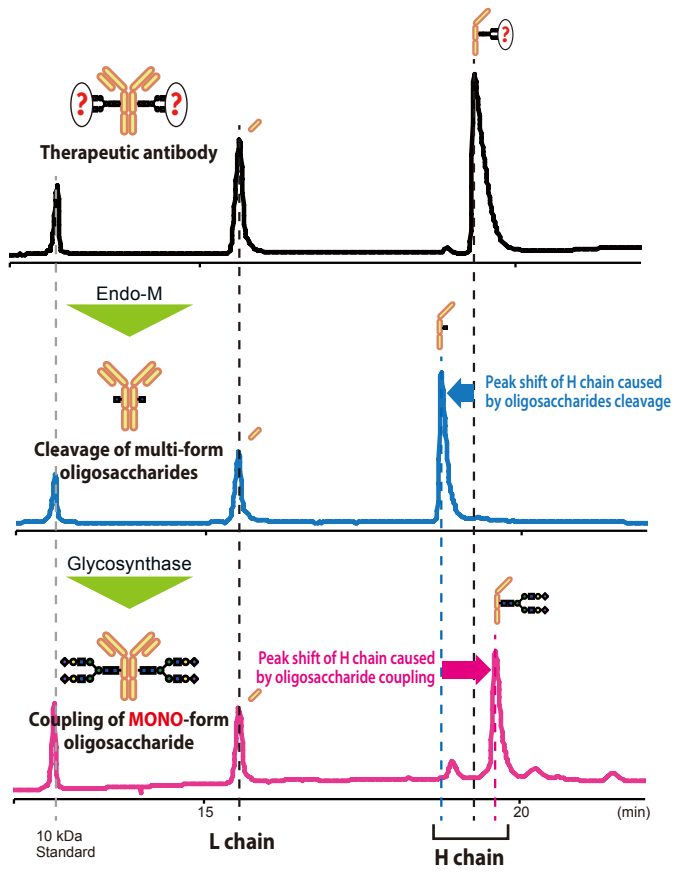


Please harness TCI's [Endo-M], [Glycosynthase] and [Chemically Synthesized Oligosaccharides] for the benefit of your Research & Development.

Oligosaccharide Replacement of a Therapeutic Antibody by using Endo-M and Glycosynthase

Cleavage of multi-form oligosaccharides by Endo-M and coupling of MONO-form oligosaccharide by Glycosynthase were conducted under non-reducing condition. Verification of enzymatic reaction was performed with capillary electrophoresis (Fig. 1) and SDS-PAGE (Fig. 2A). The terminal sialic acid of the chemoenzymatically-transferred *N*-linked oligosaccharide to the therapeutic antibody was detected by lectin blotting using a sialic acid binding lectin (Fig. 2B).

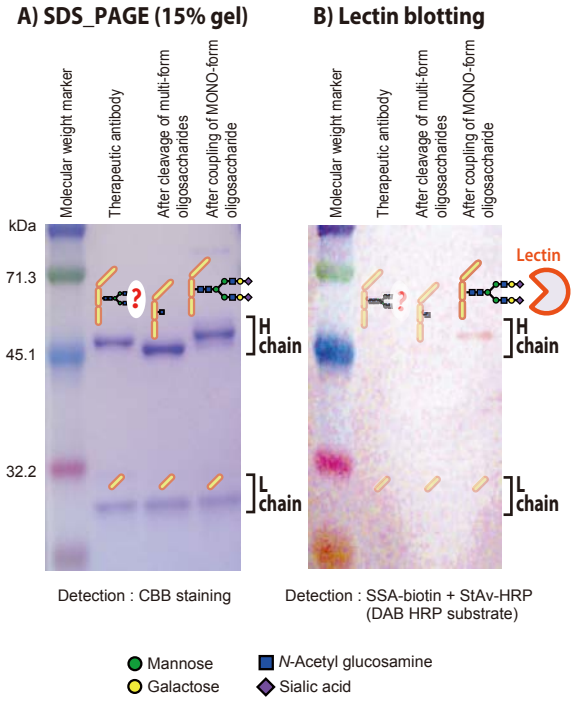
Fig. 1 Verification of enzymatic reaction



Capillary electrophoresis (under reducing condition)
[BECKMAN COULTER PA 800]
Detection : UV214 nm

After coupling of a MONO-form oligosaccharide to the Endo-M-treated antibody, a peak shift of the H chain of the therapeutic antibody was observed while a peak shift of L chain was not.

Fig. 2 Detection of sialylated oligosaccharide



A) SDS-PAGE
After coupling of a MONO-form oligosaccharide to the Endo-M-treated antibody, a band shift of the H chain of the therapeutic antibody was observed while a band shift of L chain was not.

B) Lectin blotting
In this study, SSA (*Sambucus sieboldiana* agglutinin) was used. A band of H chain of the therapeutic antibody coupled with MONO-form oligosaccharide was significantly stained.

Related Products

endo-β-*N*-Acetylglucosaminidase (Endo-M) from *Mucor hiemalis* expressed in *Candida boidinii*	100m units/vial [A1651]
Glycosynthase (Endo-M-N175Q) from *Mucor hiemalis* expressed in *Escherichia coli*	100m units/vial [G0365]
Disialyloctasaccharide	20mg [D4065]
Sialylglycopeptide (SGP)	10mg [S0523]

References K. Yamamoto, S. Kadowaki, J. Watanabe, H. Kumagai, *Biochem. Biophys. Res. Commun.* **1994**, *203*, 244.
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M. Umekawa, C. Li, T. Higashiyama, W. Huang, H. Ashida, K. Yamamoto, L-X. Wang, *J. Biol. Chem.* **2010**, *285*, 511.

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