

Glycosynthase

(Endo-M-N175Q)

An enzyme that adds whole sugar chains without breaking down products.

Increases efficiency of the sugar metastasis reaction

Applicable to Biosimilars

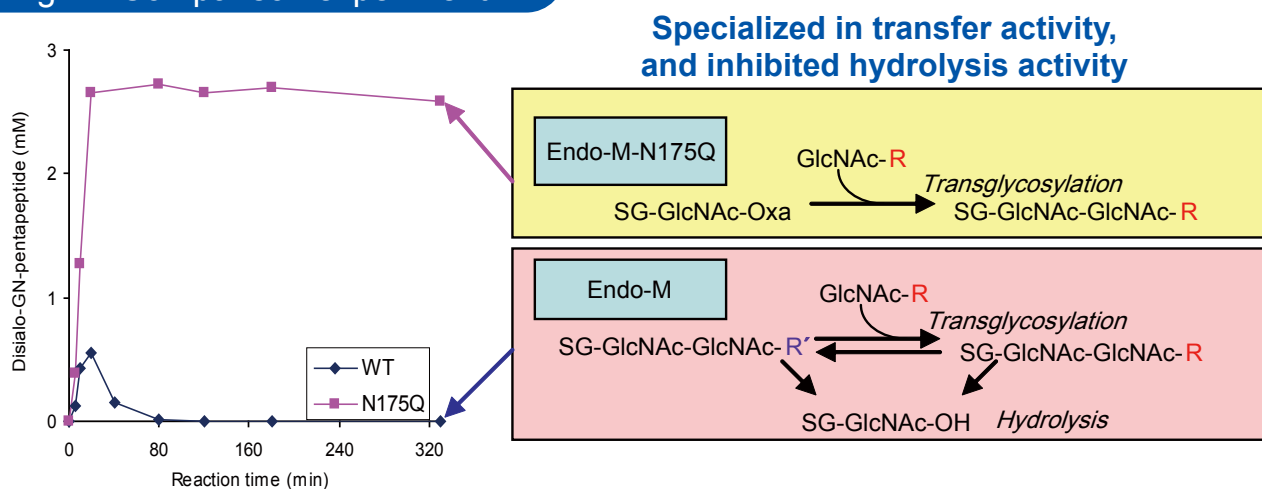
Making of a new functional sugar complex



New tool for Sugar synthesis

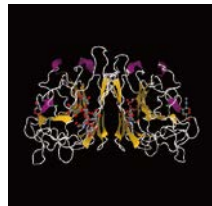
Glycosynthase (Endo-M-N175Q) is an enzyme developed by Yamamoto, Umekawa, *et al.* through site-directed mutation of areas around the active center of Endo-M¹⁾ which is already marketed. Since the feature of Glycosynthase is efficient transglycosylation activity by using oxazoline derivatives as glycosyl donors while suppressing sugar hydrolysis activity, the resulting glycosylated products are obtained in high yield with less digestion of the products by the enzyme. Due to this feature Glycosynthase is expected to be applied as a useful tool in glyco-technology.

Fig. 1 Comparison experiment ²⁾



Sugar chains of clear structure

Glycoprotein authentic sample



Sugar metastasis
Glycosynthase (Endo-M-N175Q)

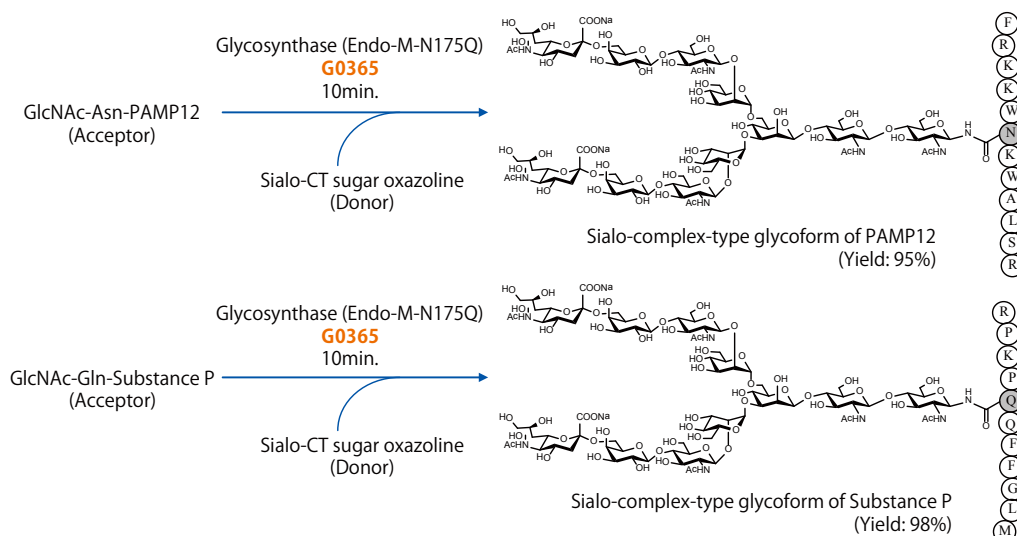
The acquisition of standardized sugar complexes

Biomedicines
Biosimilars

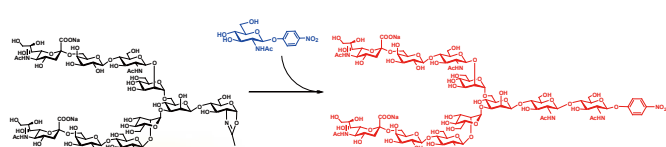


Umekawa and her colleagues caused transglycosylation reactions at the GlcNAc site of sperm antigen CD52 using oxazoline derivatives of the high-mannose type sugar chains or the complex type sugar chains as glycosyl donors²⁾. They succeeded in obtaining glycosylated products in high yield of 84% and 76%, respectively. Moreover, they also achieved transglycosylation reactions using two biologically active blood-pressure-lowering peptides, PAMP12 and Substance P, as glycosyl acceptors and an oxazoline derivative of a complex type sugar chain containing sialic acids as a glycosyl donor in 95% and 98% yield, respectively³⁾.

Fig. 2 Experiment example of the transglycosylation

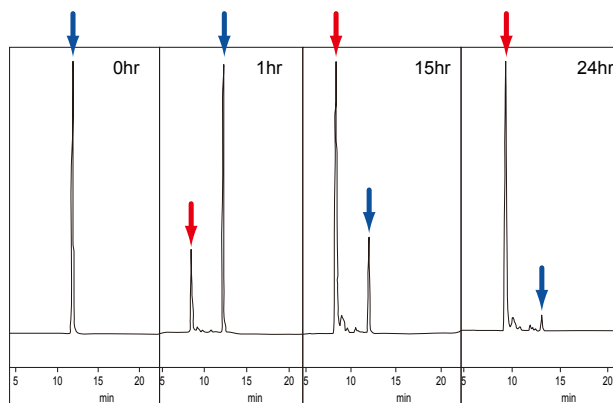


HPLC monitoring of the sugar metastasis reaction



Rate of metastasis
95%

HPLC : ODS
CH₃CN / H₂O
UV260nm



Realizing efficient transglycosylation reactions would also be useful for expansion into glycoprotein synthesis, such as the area of biosimilars. Creation of new functional sugar complexes can be expected also.

Glycosynthase (Endo-M-N175Q)

from *Mucor hiemalis* expressed in *Escherichia coli*

Endo-β-N-Acetylglucosaminidase (Endo-M)

from *Mucor hiemalis* expressed in *Candida boidinii*

Endo-M-W251N Recombinant

from *Mucor hiemalis* expressed in *Escherichia coli*

Sialylglycopeptide (SGP)

Disialyloctasaccharide

100mU/vial [G0365]

100mU/vial [A1651]

500mU/vial [E1339]

10mg [S0523]

10mg [D4065]

1) K. Yamamoto, S. Kadowaki, J. Watanabe, H. Kumagai, *Biochem. Biophys. Res. Commun.* **1994**, 203, 244.

2) M. Umekawa, T. Higashiyama, T. Tanaka, M. Noguchi, A. Kobayashi, S. Shoda, W. Huang, L.-X. Wang, H. Ashida, K. Yamamoto, *Biochim. Biophys. Acta, Gen. Subj.* **2010**, 1800, 1203.

3) 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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