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Carbohydrate Research 339 (2004) 719–722
Carbohydrate RESEARCH

Note

High efficiency of transferring a native sugar chain from a glycopeptide by a microbial endoglycosidase in organic solvents

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Received 11 August 2003; accepted 8 December 2003

Abstract—We examined the transglycosylation reaction by the recombinant endo-β-*N*-acetylglucosaminidase from *Mucor hiemalis* (Endo-M) expressed in *Candida boidinii* in media containing organic solvents. The recombinant Endo-M could transglycosylate a disialo biantennary complex-type oligosaccharide from hen egg yolk glycopeptide to *p*-nitrophenyl *N*-acetyl-β-D-glucosaminide even in the presence of 30% acetone, dimethyl sulfoxide, or methanol. The yield of the transglycosylation product reached 21–34% of the total amount of acceptor, while the yield was only about 14% in aqueous solution.

Keywords: Endo-β-*N*-acetylglucosaminidase; Endo-M; Transglycosylation; Organic solvent; Biantennary complex-type oligosaccharide; Neoglycoconjugates

Endo-β-*N*-acetylglucosaminidases hydrolyze the glycosidic bond in the *N*,*N*′-diacetylchitobiose moiety of N-linked sugar chains in glycoproteins. Among them, Endo-A from *Arthrobacter protophormiae*[1] Endo-M from *Mucor hiemalis*[2] and Endo-CE from *Caenorhabditis elegans*[3] have been found to have transglycosylation activity, that is, they can transfer the oligosaccharide blocks of N-linked sugar chains from a glycopeptide or a glycoprotein to suitable acceptors having an *N*-acetylglucosamine (GlcNAc) residue. Endo-A and Endo-CE can transglycosylate the high-mannose-type oligosaccharides from a glycopeptide or a glycoprotein to the appropriate glycosyl acceptors.[4,5] Endo-M can transfer not only the high-mannose-type oligosaccharides, but also complex-type oligosaccharides to the *N*-acetylglucosamine moieties of acceptors.[6] Endo-A and Endo-M are used as effective tools for the reconstruction and remodeling of oligosaccharides from glycopeptides and glycoproteins.

Fan et al. have investigated the transglycosylation and hydrolytic activities of Endo-A in organic solvents and revealed that the addition of organic solvents, such as acetone, *N*,*N*-dimethylformamide, or dimethyl sulfoxide, to the reaction mixture could enhance the transglycosylation activity and suppress the hydrolytic activity.[7,8]

Using the transglycosylation activity of Endo-M, Haneda et al. succeeded in adding oligosaccharides to chemically synthesized peptides (calcitonin,[9] substance P,[10] and peptide T[11]) having *N*-acetylglucosaminyl-asparagine or -glutamine residues.

However, the transglycosylation activity of Endo-M in the presence of organic solvents has not yet been determined. In this paper, in order to improve the utilization of Endo-M, we investigated the transglycosylation activity of the recombinant Endo-M in organic solvents using H-Lys-Val-Ala-Asn[(NeuAc-Gal-GlcNAc-Man)$_2$-Man-GlcNAc$_2$]-Lys-Thr-OH (SGP) from hen egg yolk as the oligosaccharide donor and *p*NP-β-D-GlcNAc as the acceptor (Scheme 1).

At first, we examined the transglycosylation activity of Endo-M in various organic solvents. The enzymatic reaction was carried out with 10 mM *p*NP-β-D-GlcNAc

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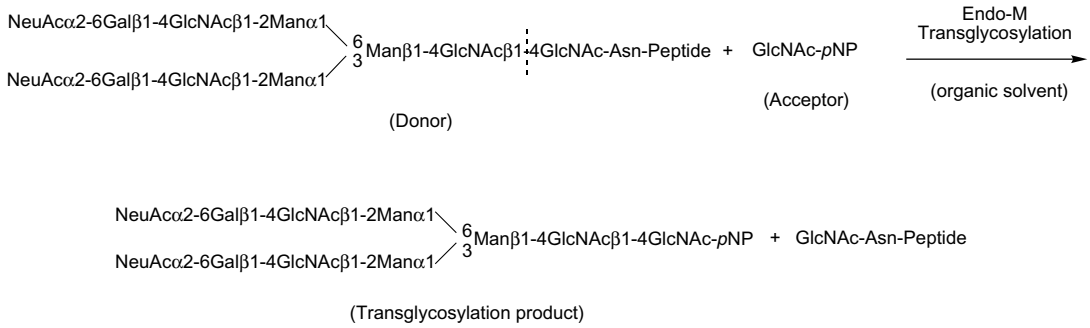
doi:10.1016/j.carres.2003.12.007



Scheme 1.

and 20 mM SGP. The reaction mixture was incubated with Endo-M at 25 °C for 3 h in various 30% organic solvents (v/v) such as acetone, butanone, acetonitrile, dioxane, *N*,*N*-dimethylformamide (DMF), dimethyl sulfoxide (DMSO), methanol, or tetrahydrofuran (THF). These results are shown in Table 1. In 30% acetone solution, the yield of the transglycosylation product was found to reach 34.1% of the total amount of *p*NP-β-D-GlcNAc as the acceptor. Moreover, the yields of the transglycosylation products reached 21.3% and 24.7% in DMSO and methanol, respectively (though the transglycosylation product by the transfer of an oligosaccharide to alcohol might be obtained in the presence of methanol). On the other hand, the yield was only 14.7% for the reaction in aqueous solution. Thus, the transglycosylation activity of Endo-M was shown to be highly promoted in organic solvents. Furthermore, all evidence points to the fact that Endo-M is likely to be extremely stable in these organic solvents.

We investigated the effects of the acetone concentration (0–50%) on the transglycosylation activity of Endo-M. These results are shown in Figure 1. The yields of the transglycosylation product reached about 40% after a 1-h incubation in 10–30% acetone concentrations. Even in 30% DMSO and methanol, the transglycosylation yields attained 31–37% for a 1-h incubation (data not shown). According to the reaction process results, it seemed that the degradation of product scarcely occurred in the organic solvent, although it occurred rather rapidly in aqueous solution.

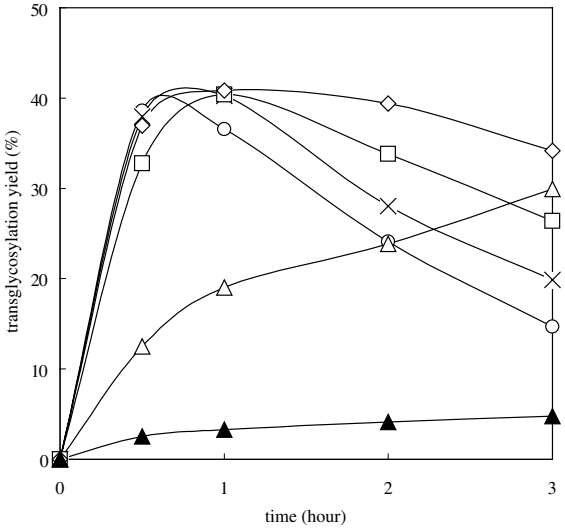


Figure 1. The effect of the acetone concentration in the range of 0–50% (v/v) on the transglycosylation activity. (○; 0%, ×; 10%, □; 20%, ◇; 30%, △; 40%, ▲; 50%).

Next, we examined the effect of the sugar-chain donor concentration on the yield of the transglycosylation product. These results are summarized in Table 2. The incubation with 40 mM SGP in 30% acetone for 2 h raised the transglycosylation yield to about 60%. The maximum yield attained was about 66% after 3 h in each reaction mixture, including 30% acetone, methanol or

Table 1. The yields of the transglycosylation product incubated for 3 h in various 30% organic solvents (v/v)[a]

Organic solvent	Transglycosylation yield (%)
None	14.7
Acetone	34.1
Butanone	0
Acetonitrile	0
Dioxane	0
DMF	0
DMSO	21.3
Methanol	24.7
THF	0

[a] Acceptor: *p*NP-β-D-GlcNAc. Donor: SGP.

Table 2. The effect of various concentrations of SGP on the yields of the transglycosylation product incubated for 2 h in various 30% organic solvents (v/v)[a]

SGP (mM)	Yield (%)		
	Acetone	Methanol	DMSO
20	39.4	32.1	29.2
40	59.7	50	48.3
80	60.1	63	60.3

[a] Acceptor: *p*NP-β-D-GlcNAc. Donor: SGP.

DMSO using 80 mM SGP. We determined the mass value of the transglycosylation product of *p*NP-(GlcNAc)$_2$-Man-(Man-GlcNAc-Gal-NeuAc)$_2$ by MALDI-TOF mass spectrometric analysis. *p*NP-(GlcNAc)$_2$-Man-(Man-GlcNAc-Gal-NeuAc)$_2$: found *m/z* $[M-H]^-$ 2340.7, calcd for $C_{90}H_{141}N_7O_{64}$ $[M-H]^-$ 2342.8.

Next, we investigated the transglycosylation of Endo-M to transfer an oligosaccharide to a compound that is poorly soluble in aqueous solution. We used 2-benzyloxycarbonylamino-2-deoxy-D-glucopyranose[12] (GlcNZ) as an acceptor. GlcNZ is known as a synthetically important GlcNAc derivative and is poorly soluble in water, but easily dissolved in 30% DMSO solution. We found that the enzymatic reaction in 30% DMSO, which was carried out for 2 h with 10 mM of GlcNZ and 20 mM of SGP, successfully produced the transglycosylation product with a 21.1% yield. This result shows that water-insoluble compounds can be used as acceptors of Endo-M for the transglycosylation reaction. We determined the mass value of the transglycosylation product of GlcNZ-GlcNAc-Man-(Man-GlcNAc-Gal-NeuAc)$_2$ by MALDI-TOF mass spectrometric analysis. GlcNZ-GlcNAc-Man-(Man-GlcNAc-Gal-NeuAc)$_2$: found *m/z* $[M-H]^-$ 2312.0, calcd for $C_{90}H_{142}N_6O_{63}$ $[M-H]^-$ 2313.8.

1. Experimental

1.1. Preparation of the enzyme

Recombinant Endo-M was obtained as previously reported from the cell extract of *Candida boidinii* (protease deficient (pep4) strain).[13]

1.2. Materials

Glycosyl donor, a sialoglycopeptide having a disialo biantennary complex-type oligosaccharide: H-Lys-Val-Ala-Asn[(NeuAc-Gal-GlcNAc-Man)$_2$-Man-GlcNAc$_2$]-Lys-Thr-OH, named SGP, was prepared from hen egg yolk according to the reported method.[14] *p*NP-β-D-GlcNAc was purchased from the Seikagaku Corporation. GlcNZ was prepared by the reported method.[12] All other chemicals used were obtained from commercial sources and were of the highest grade available.

1.3. Transglycosylation reaction with Endo-M

The transglycosylation reaction at an analytical level was performed with a reaction mixture composed of 0.1 μmol of *p*NP-β-D-GlcNAc, 0.2 μmol of SGP, and 1.48 mU of Endo-M in a total volume of 10 μL of 60 mM potassium phosphate buffer (pH 6.25) containing 0–50% of various organic solvents (v/v). After incubation for 0.5–3 h at 25 °C, the reaction was terminated by the addition of 490 μL of 0.2% trifluoroacetic acid (TFA) solution.

1.4. HPLC analysis

Analyses of the transglycosylation products were done using an HPLC (Hitachi L-6200 chromatograph equipped with an L-4250 ultraviolet spectrophotometer) on a reversed-phase column (4.6×250 mm, Mightysil RP-18, Kanto Chemical Co. Tokyo). Elution was carried out with a linear gradient of acetonitrile (10–30%) containing 0.1% aqueous TFA in 20 min at a flow rate of 1 mL/min. The reaction products were monitored by absorption at 254 nm. The yields of the transglycosylation products based on the acceptors added were calculated from the ratio (%) of the peaks of the transglycosylation products to the initial ones of the oligosaccharide acceptors. This was based on the assumption that the absorptivity of the transglycosylation products and that of the acceptor are approximately the same. The purification of the transglycosylation products was performed by HPLC, which was same as the analysis methods.

1.5. Mass spectrometry

Matrix-associated laser desorption ionization time-of-flight (MALDI-TOF) mass spectrometry was performed in the negative-ion mode using 2,4,6-trihydroxyacetophenone (THAP) as the matrix on a Voyager Biospectrometry Workstation (PerSeptive Biosystems, USA).

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