Bioscience, Biotechnology, and Biochemistry
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/tbbb20

Increased Transglycosylation Activity of Rhodotorula glutinis Endo-β-Glucanase in Media Containing Organic Solvent

Tadao OIKAWA[ab], Yasuyuki TSUKAGAWA[a], Masashi CHINO[a] & Kenji SODA[ab]
[a] Department of Biotechnology, Faculty of Engineering, Kansai University
[b] Kansai University High Technology Research Center
Published online: 22 May 2014.

To cite this article: Tadao OIKAWA, Yasuyuki TSUKAGAWA, Masashi CHINO & Kenji SODA (2001) Increased Transglycosylation Activity of Rhodotorula glutinis Endo-β-Glucanase in Media Containing Organic Solvent, Bioscience, Biotechnology, and Biochemistry, 65:8, 1889-1892, DOI: 10.1271/bbb.65.1889

To link to this article: http://dx.doi.org/10.1271/bbb.65.1889

Biosci. Biotechnol. Biochem., **65** (8), 1889–1892, 2001

Note

Increased Transglycosylation Activity of *Rhodotorula glutinis* Endo-β-Glucanase in Media Containing Organic Solvent

Tadao OIKAWA,[1,2] Yasuyuki TSUKAGAWA,[1] Masashi CHINO,[1] and Kenji SODA[1,2,†]

[1]*Department of Biotechnology, Faculty of Engineering, Kansai University;* [2]*Kansai University High Technology Research Center, Suita-shi, Osaka 564-8680, Japan*

Received January 22, 2001; Accepted March 21, 2001

The transglycosylation of *p*-nitrophenyl-β-D-cellotrioside to cellotetraose catalyzed by endo-1,4-β-glucanase (cellulase, EC 3.2.1.4) from a psychrotrophic yeast, *Rhodotorula glutinis* KUJ 2731, was increased by addition of a miscible organic solvent in the reaction mixture. Among various organic solvents tested, acetone was most effective. The transglycosylation activity increased with an increase in acetone concentrations, while hydrolysis activity was suppressed. The transglycosylation preferably occurred at acidic pH with the optimum pH at 2 in 10 mM Gly-HCl buffer. The optimum temperature of transglycosylation was found to be 50°C in the presence of 40% acetone.

Key words: transglycosylation; endo-1,4-β-glucanase; *Rhodotorula glutinis*; psychrotrophic yeast

Several endo-glycosidases catalyze either transglycosylation of cellulose and other β-glucans or the reverse reaction, besides their hydrolysis.[1–4] For example, endo-α-*N*-acetylgalactosaminidase (EC 3.2.1.97) from *Diplococcus pneumoniae* shows both activities, and catalyzes the transfer of a disaccharide to various compounds such as glycerol, *p*-nitrophenol, threonine, and serine.[2] Ceramide glycanase from a leech is used for synthesis of neoglycoconjugates.[3] However, the efficiency of transglycosylation and glycoside formation catalyzed by the enzymes is usually low in aqueous solution, because of concomitant hydrolysis of the substrates and products.

Endo-1,4-β-glucanases (EC 3.2.1.4) catalyze the hydrolytic cleavage of cellulose to cello-oligosaccharides or glucose. The enzyme was found in various microorganisms such as *Rhodotorula glutinis* KUJ 2731,[5] *Erwinia chrysanthemi*,[6] *Acetobacter xylinum* KU-1,[7] and *Bacillus* sp. No. 1139.[8] Recently, we showed that the enzyme from a psychrotrophic yeast, *R. glutinis* KUJ 2731, catalyzes transglycosylation of celloorigosaccharides and their *p*-nitrophenyl derivatives, although the hydrolysis is still predominant.[5]

We found that the hydrolytic activity of endo-1,4-β-glucanase from *R. glutinis* KUJ 2731 was suppressed, and the transglycosylation activity was increased, by incubation in media containing organic solvents. We here describe the increased transglycosylation activity of *R. glutinis* endo-1,4-β-glucanase in the reaction mixture containing an organic solvent, and the optimization of the reaction.

Carboxymethyl cellulose sodium salt (CMC) with an average molecular weight of about 135,000 was obtained from Nacalai Tesque Co., Kyoto. Cellobiose, cellotriose, cellotetraose, cellopentaose, cellohexaose, *p*-nitrophenyl-β-D-glucoside (PNPG), *p*-nitrophenyl-β-D-cellobioside ($PNPG_2$), *p*-nitrophenyl-β-D-cellotrioside ($PNPG_3$), and *p*-nitrophenyl-β-D-cellotetraoside ($PNPG_4$) were from Seikagaku Kogyo Co., Tokyo. Kieselgel 60 (silica gel) plates were from E. Merk, Dartmstadt, Germany. Other reagents were used without further purification. The endo-1,4-β-glucanase activity was measured by the procedure previously described.[5] One unit of enzyme was defined as the amount of enzyme that catalyzes production of 1 μmol of D-glucose per minute under the standard assay conditions. Proteins were measured with a protein assay kit (Bio-Rad, Tokyo), and bovine serum albumin was as a standard. The endo-1,4-β-glucanase was purified by the procedure previously described (specific activity: 489 units/mg).[5]

The transglycosylate activity was measured as follows: the standard reaction mixture containing enzyme (4.89 U, 10 μg) and 1.6 mmol of *p*-nitrophenyl-β-cellotrioside in 10 mM acetate buffer (pH 5, 0.16 ml) was incubated at 20°C for 3 h. The incubation time was exactly the same for all reaction mixtures. The reaction products were analyzed by high pressure liquid chromatography (HPLC) and thin layer chromatography (TLC). Celloorigosacharides and their *p*-nitrophenyl derivatives were separated by HPLC on a Shimadzu LC-10 system (Shimadzu, Kyoto) with a Sugar-Pak Oligo 5 column (ϕ7.8 × 300 mm, Millipore, Tokyo) and detected with a

† To whom correspondence should be addressed. Kenji SODA, Fax: +81-6-6388-8609; E-mail: soda@ipcku.kansai-u.ac.jp

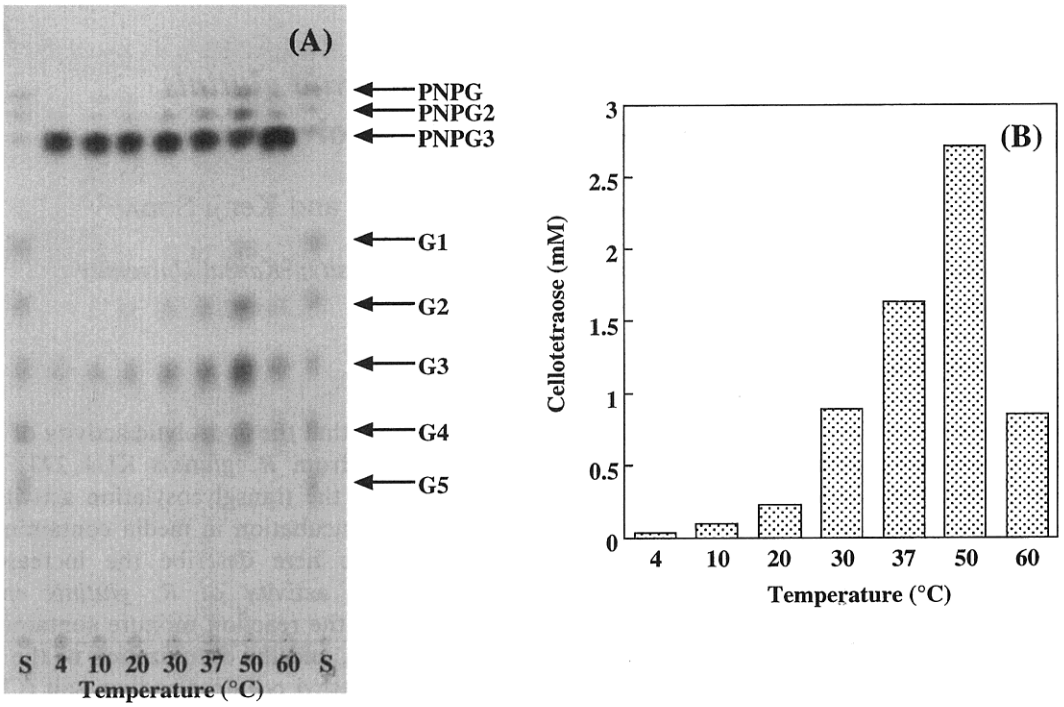

Fig. 1. Effects of Temperatures on Transglycosylation by Endo-β-Glucanase from *Rhodotrula glutinis* KUJ 2731.
(A) Thin-layer chromatograms of transglycosylation products at various temperatures. S: Standard glucose, cello-oligosaccharides (G_1–G_5), *p*-nitrophenyl-β-D-glucoside (PNPG), *p*-nitrophenyl-β-D-cellobioside ($PNPG_2$), and *p*-nitrophenyl-β-D-cellotrioside ($PNPG_3$).
(B) cellotetraose concentrations measured by HPLC.

differential refractive index (RI) detector at 80°C. The mobile phase was deionized water with a flow rate of 0.7 ml/min. Celloorigosacharides and their *p*-nitrophenyl derivatives were also analyzed by TLC with a Kiesel gel 60 sheet and a chloroform-methanol-water (90:65:15 vol/vol) solvent system, and made visible with phenol-containing sulfuric acid.[9] The hydrolyzed product was analyzed by a Flying spot scanner CS-9000 (Shimadzu, Kyoto).

The effects of organic solvents on the hydrolysis activity of the endo-1,4-β-glucanase were examined in the presence of various organic solvents (10% in final concentration: acetone, methyl alcohol, ethyl alcohol, and dimethyl sulfoxide). The reaction mixture used was as follows: the enzyme (4.89 U, 10 μg) and 1.6 mmol of *p*-nitrophenyl-β-cellotrioside in an organic solvent. The enzyme was fairly stable in organic solvents, in particular acetone: the enzyme activity was not influenced by addition of 10% acetone at all (final concentration: 10%). More than 50% of the initial activity was shown even at a final concentration of 40%.

The effects of temperature on cellotetraose production from *p*-nitrophenyl-β-cellotrioside by endo-1,4-β-glucanase were examined at various reaction temperatures (4, 10, 20, 30, 37, 50, and 60°C). The cellotetraose production increased effectively with an increase in temperature, and the optimum temperature was found to be 50°C (Fig. 1). The value agreed well with that of hydrolysis by the enzyme.

The effects of pHs (2–8) on cellotetraose production from *p*-nitrophenyl-β-cellotrioside were also examined. The following 10 mM buffers were used in the experiments: pH 2, 3, 4 Gly-HCl buffer; pH 4, 5, acetate buffer; pH 5, 6 citrate buffer; and pH 6, 7, 8 potassium phosphate buffer. The yield of cellotetraose production was high at the acidic pH with the optimum pH of 2 (0.075 mM). This value was quite different from that of the hydrolysis: the optimum pH for CMC-hydrolysis was pH 4.5.[5] This probably results from differences in basity between water and the cellotrioside moiety of the substrate.

The effects of acetone concentration on transglycosylation and hydrolysis by the enzyme are shown in Fig. 2. The transglycosylation activity increased in proportion to acetone concentration, but the hydrolysis activity was inversely suppressed. It is likely that acetone decreases the activity of water molecule to prevent the substrate from hydrolysis. Alcohols, acetonitrile, dimethyl formamide, or dimethyl sulfoxide were effective to suppress of hydrolysis. Acetone generally denatures and insolubilizes protein. However, the enzyme is not so susceptible to acetone, but functions mainly to decrease the reactivity of water molecule.

Several methods have been developed to increase the transglycosylation activity of various enzymes. The modification of the active center of glycosidases by site-directed mutagenesis changes the transglycosylation activity of the enzymes.[10–12] Hydrophil-

ic organic solvents can be added to the enzyme reaction mixture to decrease the activity of water. This method is useful for exoglycosidase transglycosylation,[13-15] and especially for pepetide synthesis based on by transpeptidation by proteases.[16,17] In fact, when 40% acetone was added to the reaction mixture, about 50% of hydrolysis was suppressed (Fig. 2). Methanol, ethanol, and dimethyl sulfoxide also are effective in suppressing the hydrolytic activity, although they are not so effective as acetone. The rate of enzymatic transglycosylation is affected by the kind of solvents even with the same water content suggests that transglycosylation is not only affected by the apparent water content, but also by the nature of the organic solvent added.

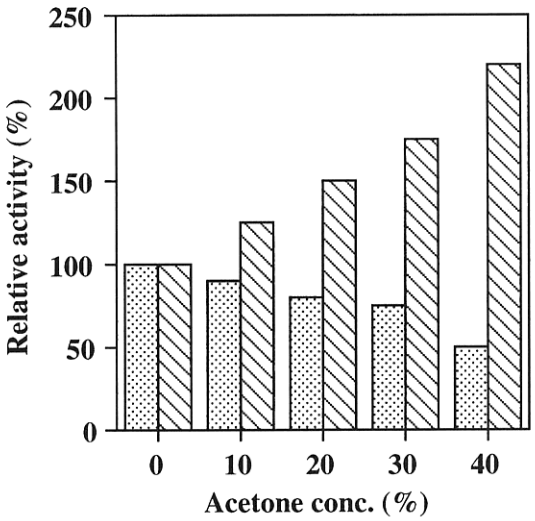

Fig. 2. Effects of Acetone concentrations on Transglycosylation and Hydrolysis Activities of Endo-β-Glucanase from *Rhodotrula glutinis* KUJ 2731.
▨: Transglycosylation activity
▩: Hydrolysis activity

A proposed mechanism of transglycosylation of *p*-nitrophenyl-β-D-cellotrioside to cellotetraose catalyzed by *R. glutinis* KUJ 2731 endo-1,4-β-glucanase is shown in Fig. 3. When $PNPG_3$ was used as a substrate, G_4 and $PNPG_2$ were synthesized. The formation of G_4 and $PNPG_2$ probably proceeds by two steps; the non-reducing glycosyl moiety of $PNPG_3$ is transferred to G_3 produced from $PNPG_3$ to form $PNPG_6$, and then there is hydrolysis of $PNPG_6$ to G_4 and $PNPG_2$.

We are currently studying the transglycosylation of the enzyme with an immobilized enzyme system.

Acknowledgments

This work was supported in part by the Research Grant from Japan Society for the Promotion of Science (Grant-in-Aid for Exploratory Research).

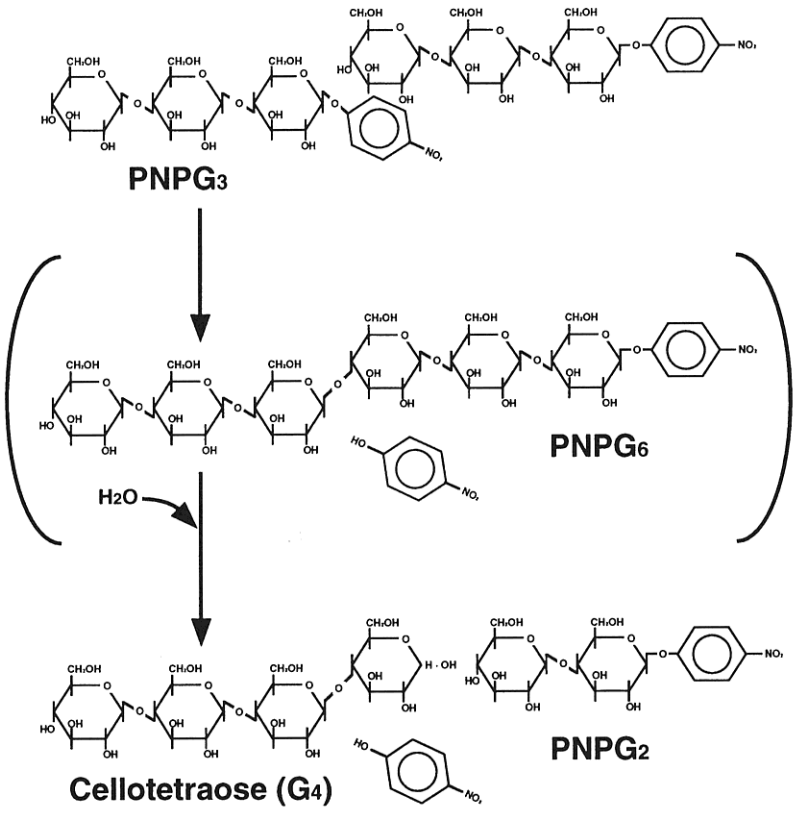

Fig. 3. Proposed Mechanism of Cellotetraose Formation by Transglycosylation of Endo-β-Glucanase from *Rhodotrula glutinis* KUJ 2731.
β-Glucanase from *Rhodotrula glutinis* KUJ 2731.

References

1) Ashida, H., Tsuji, Y., Yamamoto, K., Kumagai, H., and Tochikura, T., Transglycosylation activity of endoglycoceramidase from *Corynebacterium* sp. *Arch. Biochem. Biophys.*, **305**, 559–562 (1993).
2) Bardales, R. M. and Bhavanandan, V. P., Transglycosylation and transfer reaction activities of endo-alpha-*N*-acetyl-D-galactosaminidase from *Diplococcus* (*Streptococcus*) *pneumoniae*. *J. Biol. Chem.*, **264**, 19893–19897 (1989).
3) Li, Y. T., Carter, B. Z., Rao, B. N., and Schweingruber, H., Li, S. C., Synthesis of neoglycoconjugates using oligosaccharide-transferring activity of ceramide glycanase. *J. Biochem.*, **266**, 10723–10726 (1991).
4) Trimble, R. B., Atkinson, P. H., Tarentino, A. L., Plummer, T. H. Jr., Maley, F., and Tomer, K. B., Transfer of glycerol by endo-beta-*N*-acetylglucosaminidase F to oligosaccharides during chitobiose core cleavage. *J. Biochem.*, **261**, 12000–12005 (1986).
5) Oikawa, T., Tsukagawa, Y., and Soda, K., Endo-β-glucanase secreted by a psychrotrophic yeast: purification and characterization. *Biosci. Biotechnol. Biochem.*, **62**, 1751–1756 (1998).
6) Boyer, M. H., Chambost, J. P., Magnan, M. and Cattanéo, J., Carboxymethyl-cellulase from *Erwinia chrysanthemi*. II. Purification and partial characterization of an endo-β-1,4-glucanase. *J. Biotechnol.*, **1**, 241–252 (1984).
7) Oikawa, T., Kamatani, T., Kaimura, T., Ameyama, M., and Soda, K., Endo-β-glucanase from *Acetobacter xylinum*: Purification and characterization. *Current Microbiol.*, **34**, 309–313 (1997).
8) Fukumori, F., Kudo, T., and Horikoshi, K., Purification and properties of a cellulase from alkalophilic *Bacillus* sp. No. 1139. *J. General Microbiol.*, **131**, 3339–3345 (1985).
9) Wood, T. M. and McCrae, S. I., The cellulase of *Trichoderma koningii*. Purification and properties of endoglucanase components with special reference to their action on cellulase when acting alone or in synergism with the cellobiohydrolase. *Biochem. J.*, **171**, 61–72 (1978).
10) Moreau, A., Shareck, F., Kluepfel, D., and Morosoli, R., Alteration of the cleavage mode and of the transglycosylation reactions of the xylanase A of *Streptomyces lividans* 1326 by site-directed mutagenesis of the Asn173 residue. *Eur. J. Biochem.*, **219**, 261–266 (1994).
11) Matsui, I., Yoneda, S., Ishikawa, K., Miyairi, S., Fukui, S., Umeyama, H., and Honda, K., Roles of the aromatic residues conserved in the active center of *Saccharomycopsis* alpha-amylase for transglycosylation and hydrolysis activity. *Biochemistry*, **33**, 451–458 (1994).
12) Nakamura, A., Haga, K., and Yamane, K., The transglycosylation reaction of cyclodextrin glucanotransferase is operated by a Ping-Pong mechanism. *FEBS. Lett.*, **337**, 66–70 (1994).
13) Usui, T. and Murata, T., Enzymatic synthesis of *p*-nitrophenyl alpha-maltopentaoside in an aqueous-methanol solvent system by maltotetraose-forming amylase: a substrate for human amylase in serum. *J. Biochem.* (*Tokyo*), **103**, 969–972 (1988).
14) Shinoyama, H., Kamiyama, Y., and Yasui, T., Enzymatic synthesis of alkyl β-xylosidase from xylobiose by application of the transglycosyl reaction of *Aspergillus niger* β-xylosidase. *Agric. Biol. Chem.*, **52**, 2197–2202 (1988).
15) Shinoyama, H. and Yasui, T., Superiority of *Aspergillus niger* β-xylosidase from the enzymatic synthesis of alkyl β-xylosides in the presence of a variety of alcohols. *Agric. Biol. Chem.*, **52**, 2375–2377 (1988).
16) Nilsson, K. and Mosbach, K., Peptide synthesis in aqueous-organic solvent mixtures with α-chymotrypsin immobilized on tresyl chloride-activated agarose. *Biotechnol. Bioeng.*, **26**, 11465–1154 (1984).
17) Inada, Y., Takahashi, K., Yoshimoto, T., Ajima, A., Matsushima, A., and Saito, Y., Application of polyethylene glycol-modified enzymes in biotechnological processes: organic solvent soluble enzyme. *Trends. Biotechnol.*, **6**, 190–194 (1986).