Available online at www.sciencedirect.com
SCIENCE DIRECT®
Carbohydrate Research 340 (2005) 1089–1096
Carbohydrate RESEARCH

Production of a new sucrose derivative by transglycosylation of recombinant *Sulfolobus shibatae* β-glycosidase

Na-Young Park,[a] Nam-In Baek,[b] Jaeho Cha,[c] Soo-Bok Lee,[d] Joong-Hyuck Auh[e] and Cheon-Seok Park[a,*]

[a]*Department of Food Science and Biotechnology and Institute of Life Sciences and Resources, KyungHee University, Yongin 449-701, Republic of Korea*
[b]*Graduate School of Biotechnology and Plant Metabolism Center, KyungHee University, Yongin 449-701, Republic of Korea*
[c]*Department of Microbiology, Pusan National University, Pusan 609-735, Republic of Korea*
[d]*Department of Food Science and Nutrition, Yonsei University, Seoul 120-749, Republic of Korea*
[e]*Section of Molecular and Cellular Biology, University of California, Davis, CA 95616, USA*

Received 26 November 2004; received in revised form 25 January 2005; accepted 2 February 2005

Abstract—The gene encoding β-glycosidase of the hyperthermophilic archaea *Sulfolobus shibatae* (SSG) was expressed in *Escherichia coli*. Recombinant SSG (referred to as rSSG hereafter) was efficiently purified, and its transglycosylation activity was tested with lactose as a donor and various sugars as acceptors. When sucrose was used as an acceptor, we found a distinct intermolecular transglycosylation product and confirmed its presence by TLC and high performance anion exchange chromatography (HPAEC). The sucrose transglycosylation product was isolated by paper chromatography, and its chemical structure was determined by ^{1}H and ^{13}C NMR. The sucrose transfer product was determined to be β-D-galactopyranosyl-(1→6)-α-D-glucopyranosyl-β-D-fructofuranoside with a galactose molecule linked to sucrose via a β-(1→6)-glycosidic bond.

Keywords: *Sulfolobus shibatae*; β-Glycosidase; Transglycosylation reaction; Sucrose; Sucrose transglycosylation product

1. Introduction

β-Glycosidases (β-glucoside glucohydrolases, EC 3.2.1.21) are a group of biologically important enzymes that catalyze the hydrolysis of β-glycosidic bond, linking carbohydrate residues in aryl-, amino-, or alkyl-β-D-glucosides, cyanogenic glucosides, and oligo-, or di-saccharides. Based on their amino acid sequence and folding similarities, β-glycosidases are classified into family 1 (GH1) and family 3 (GH3) of the glycosyl hydrolases,[1–3] with which the substrates were hydrolyzed while retaining an anomeric configuration via a double-displacement mechanism.[3] These enzymes are found in all living kingdoms including archaea, bacteria, fungi, plants, and animals. Recently, β-glycosidases from hyperthermophilic archaea *Sulfolobus solfataricus* (Sβgly) and *Pyrococcus furiosus* (CelB) has been extensively studied due to their stability to high temperatures and availability of 3D structure.[4–6] The gene coding for β-glycosidase from *S. solfataricus* has been cloned and expressed in *Escherichia coli*. It was found that Sβgly is extremely thermostable by showing a half-life of 48 h at 85 °C and displaying a maximal activity at 95 °C.[7] Likewise, purified CelB shows a remarkable thermostability with a half-life of 85 h at 100 °C and 13 h at 110 °C.[8] Although these two enzymes share very similar properties in thermostability, enzyme kinetics, substrate specificity, and pH optima, they show significant differences in their stability to various denaturing agents. CelB is more stable to temperature, salts, and high pH, but more sensitive to SDS than Sβgly.[4]

There is a growing interest toward β-glycosidases due to their various biological functions, including cellular

* Corresponding author. Tel.: +82 31 201 2631; fax: +82 31 204 8116; e-mail: cspark@khu.ac.kr

0008-6215/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.carres.2005.02.003

signaling, host–pathogen interactions, and degradation of structural and storage polysaccharides, as well as a number of industrial applications such as biomass conversion, flavor enhancing, and production of biodegradable nonionic surfactants.[9–11] In general, β-glycosidases catalyze the hydrolysis of β-glycosidic bonds linking carbohydrate residues. However, the biosynthesis of glycoconjugates can occur under defined conditions. Reverse hydrolysis and transglycosylation are two different modes for the biosynthesis of glycoconjugates. Transglycosylation usually gives higher yields than reverse hydrolysis in the formation of glycoconjugates. In general, transglycosylation is known to be a kinetically controlled reaction, whereas reverse hydrolysis is thermodynamically controlled.[9] However, such reactions are under thermodynamic control if the rate of conversion of the donor into glycosyl–enzyme intermediate is slower than the subsequent attack by the acceptor and/or water. These reactions only come under kinetic control when the donor is highly activated and the rate of formation of the glycosyl–enzyme intermediate is much higher than the rates of the following steps.

The enzymatic synthesis of oligosaccharides represents an interesting alternative to the classical methods due to its regioselectivity and stereochemistry of bond formation. Two groups of enzymes, glycosyl transferases and glycosylhydrolases, are the choices for enzymatic approach in the production of oligosaccharides.[12–14] Among the glycosyl transferases, cyclodextrin glucanotransferase (CGTase) is one of the most extensively studied enzymes that participate in transglycosylation reactions to produce various oligosaccharides.[15–17] It is known that CGTase can transfer a glycosyl residue mainly to the 4-OH group of free glucose or to a glycoysl residue of glycone compounds.[18] Recently, the highly thermostable maltosyltransferase from *Thermotoga maritima* was utilized to modify daidzin, an isoflavone, to make maltosyl–daidzin via transglycosylation.[19] Maltogenic amylases (MAases) from *Bacillus stearothermophilus* and *Thermus* sp. were also applied to modify various natural compounds, such as ascorbic acid, naringin, and sorbitol, with which maltosyl residues were mainly transferred to the acceptor molecules to produce maltosyl-transfer products.[20–22] The modified products had improved oxidative stability, solubility, and bitterness compared to their original counterparts. In addition, various other enzymes including α-L-fucosidases, dextransucrase, and alternansucrase, have been reported to synthesize novel oligosaccharides via their transglycosylation activities.[23–25]

In this paper, we report the overexpression of the gene coding for β-glycosidase from hyperthermophile *Sulfolobus shibatae* JCM 8931 (SSG) in *E. coli* and provide general characteristics on the recombinant enzyme, specifically on the transglycosylation. We also describe the synthesis of a new sucrose derivative using the transglycosylation activity of rSSG and determine the structure of a newly formed sucrose transglycosylation product.

2. Results and discussion

2.1. Expression and purification of rSSG

The gene corresponding to β-glycosidase from hyperthermophile *S. shibatae* JCM 8931 was successfully amplified by PCR as described in Section 3. The absence of error in the nucleotide sequence of the PCR-generated gene was determined with BigDye Terminator Cycle Sequencing Kit for ABI377 PRISM (PerkinElmer, Boston, MA). The resulting PCR fragments were inserted into the *E. coli* expression vector, p6 × His119, to generate p6 × His119-SSG.[26] *E. coli* DH5α cells transformed with p6 × His119-SSG showed their β-glycosidase activities when aryl-glucoside (*p*NPG) was used as a substrate in cell-free extracts, implying that rSSG was successfully expressed in *E. coli*. rSSG could be efficiently purified from cell-free extracts by two steps: heat treatment at 70 °C for 30 min and Ni–NTA affinity chromatography. Furthermore, the yield of rSSG after heat treatment was 91%, suggesting that rSSG was highly thermostable and could be easily separated from most of the heat-labile *E. coli* proteins (Table 1). SDS-PAGE showed that the purified protein was present homogeneously as a single band at 57 kDa (Fig. 1). The analysis by MALDI-TOF MS of purified enzymes revealed that rSSG had molecular masses of 57,403 Da. The molecular mass of rSSG is consistent with the expected size deduced from the primary amino acid sequence of the β-glycosidase from *S. shibatae* having six-histidine tags.

Although CelB (β-glucosidases from *P. furiosus*), Sβgly (β-glucosidases from *S. solfataricus*) and rSSG showed extremely thermostable properties by exhibiting their optimal temperatures over 90 °C, the multiple sequence alignment of three thermostable β-glucosidases from *P. furiosus*, *S. solfataricus*, and *S. shibatae*, revealed that the amino acid sequence identities were extremely high (94%) within the same genus, but they were relatively low between the genus (50%). For instance, a comparison between β-glucosidases from *P. furiosus* and *S. shibatae* showed 65% sequence similarity and 50% identity over their entire length. Both thermostable β-glucosidases belong to family 1 of glycosylhydrolases, in which the typical $(\alpha/\beta)_8$ barrel fold first seen in the structure of triose phosphate isomerase is present. The three-dimensional structures of Sβgly have been solved, and revealed that the active site is placed at the center of the top face of the barrel.[27] It is connected to the surface by a radical channel, with a putative acid/base catalyst located at the end of β-strand 4 and a catalytic nucleophile near the end of β-strand 7. Although