Biosci. Biotechnol. Biochem., **72** (9), 2415–2417, 2008

Note

A New Method of Synthesis of Alkyl β-Glycosides Using Sucrose as Sugar Donor

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Received February 13, 2008; Accepted May 25, 2008; Online Publication, September 7, 2008
[doi:10.1271/bbb.80097]

Cellobiose phosphorylase from *Clostridium thermocellum* catalyzed the β-anomer-selective synthesis of alkyl glucosides from cellobiose. Synthesis of alkyl β-glucoside from inexpensive sucrose using cellobiose phosphorylase and sucrose phosphorylase from *Pseudomonas saccharophilia* was investigated. By combined use of these two phosphorylases, alkyl β-glucoside was anomer-selectively synthesized from sucrose and alkyl alcohol.

Key words: cellobiose phosphorylase; sucrose phosphorylase; β-glucoside; β-anomer-selective transglucosylation

Glycosylated compounds usually become more soluble and stable, and they are exploited for pharmaceuticals and biocompatible materials. Among the glycosides, alkyl glycosides are utilized as surfactants because they show high biodegradability and low toxicity. In biochemical synthesis, alkyl glucosides were usually synthesized using glucosidases.[1,2] These glucosidases show both hydrolysis and glucosyltransfer activity. Hence, the yield of alkyl glucoside is often lowered by hydrolysis activity.

Phosphorylases catalyze reversible phosphorolysis of saccharides, such as glycogen, maltose, sucrose, and cellobiose. They have very strict regiospecificity and catalyze anomer-selective phosphorolysis. Taking advantage of this regiospecificity, they are utilized in the synthesis of oligosaccharides *via* their reverse reactions.[3–5]

Moreover, some sucrose phosphorylases (EC 2.4.1.7; SPase) catalyze α-anomer-selective transglycosylation of compounds having various alcoholic, phenolic, or carboxylic OH groups.[6–8] On the other hand, some maltose phosphorylases (EC 2.4.1.8) catalyze only α-anomer-selective transglycosylation of compounds having alcoholic OH groups.[9] Phosphorylases are useful for the effective synthesis of alkyl α-glycosides because the reaction is biased toward transglycosylation, but there has been no report to the effect that phosphorylase catalyze the β-anomer-selective synthesis of alkyl glycosides. Alkyl β-glycosides are preferred for food additives, cosmetics, and drugs because they are naturally occurring glycosides. Hence, the effective process for alkyl β-glycoside production is desirable.

Among the several phosphorylases, cellobiose phosphorylase (CPase) is known to have β-anomer selective glucosylation activity toward saccharides[10] Hence, we expected that CPase can be utilized to alkyl β-glucoside synthesis, and found that CPase from *Clostridium thermocellum* YM4 (CtCPase, AY072794) catalyzed the β-anomer-selective synthesis of several alkyl glucosides (Table 1). Similarly to the glucosylation of saccharide, CtCPase utilized both cellobiose and α-glucose-1-phosphate (α-G1P) as glucosyl donor (data not shown). Hence, it was concluded that α-G1P

Table 1. Synthesis of β-Glucosides with CtCPase

Acceptor	activity[a]
Methanol	+[b]
Ethanol	+
1-Propanol	+
2-Propanol	−[c]
1-Butanol	+
t-Butanol	−
n-Pentanol	+
n-Hexanol	+
n-Heptanol	+
1,2-Butandiol	+
1,3-Butandiol	+
1,4-Butandiol	−

[a]Activity was investigated under the following conditions: the reaction mixture consisted of CtCPase, 100 mM cellobiose, and 30% alkyl alcohol (v/v) in 100 mM citrate-phosphate buffer (pH 7.0), incubated at 40 °C with shaking at 160 rpm for 20 h.
[b]+, Glucoside was detected in TLC analysis.
[c]−, not detected.

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Abbreviations: CPase, cellobiose phosphorylase; SPase, sucrose phosphorylase; α-G1P, α-glucose-1-phosphate; β-MetGlc, methyl β-D-glucopyranoside

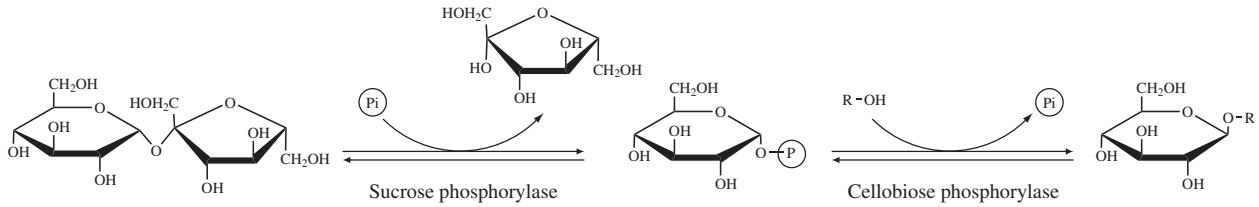

Fig. 1. Alkyl β-Glucoside Production from Sucrose.

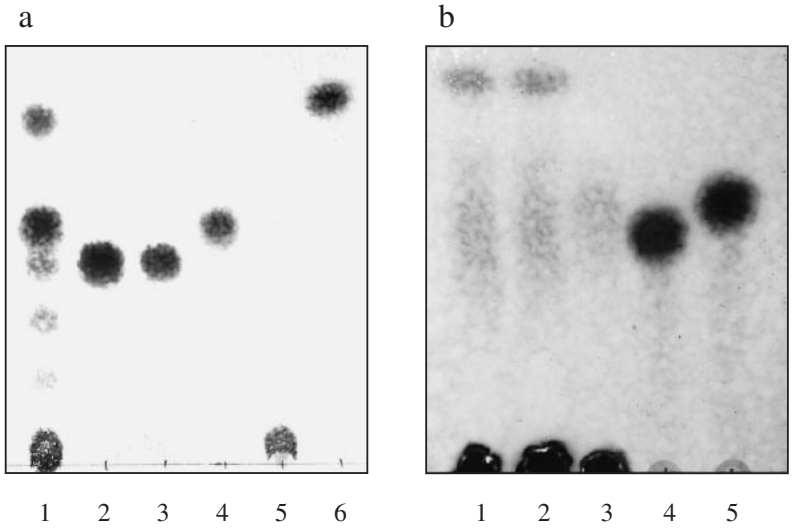

Fig. 2. Determination of the Reaction Product.

TLC analysis of the reaction mixture (a). The reaction mixture consisted of purified PsSPase, CtCPase, 100 mM sucrose, and 30% methanol (v/v), with and without inorganic phosphate. It was incubated at 40 °C with shaking at 160 rpm for 20 h. Lanes: 1, reaction mixture with inorganic phosphate; 2, without inorganic phosphate; 3, sucrose; 4, fructose; 5, α-G1P; 6, β-MetGlc. Treatment with α- and β-glucosidase (b). Lanes: 1, reaction product; 2, α-glucosidase treated; 3, β-glucoside treated; 4, glucose; 5, fructose.

released from phosphorolysis of cellobiose was transferred to alkyl alcohol. Although several alkyl β-glucosides can be synthesized in one pot using CPase, cellobiose is too much expensive for industrial production of alkyl β-glucosides. Hence, it was concluded that effective synthesis of alkyl β-glucoside from an inexpensive disaccharide, such as sucrose, was necessary, and we focused on the combined use of CPase and sucrose phosphorylase (Fig. 1).

It was expected that SPase would phosphorolyze sucrose into α-G1P and fructose, and that subsequently CPase would catalyze the synthesis of alkyl β-glucoside from α-G1P and alkyl alcohol. In this process, SPase should not have transglycosylation activity. The transglycosilation activities of several SPases were tested, and it was found that the recombinant SPase from *Pseudomonas saccharophilia* IAM 14368 (PsSPase, AF158367) had no transglycosylation activity (data not shown). In the reaction with CtCPase and PsSPase, a spot of glucoside was detected using methanol as sugar acceptor (Fig. 2a).

Methanol was transglycosylated with phosphorylases under the following conditions: a reaction mixture containing PsSPase, CtCPase, 100 mM sucrose, and 30% methanol (v/v) in 100 mM citrate-100 mM phosphate buffer (pH 7.0) was incubated at 30 °C with shaking at 160 rpm for 20 h. The reaction product was separated by solvent extraction and then purified with a silica column packed with Wakogel C-280 (Wako, Osaka, Japan).

The structure of the product was determined by enzymatic treatment and NMR analysis (Avance 600 spectrometer, Bruker, Rheinstetten, Germany) in DMSO-d_6 with tetramethylsilane as an internal standard. Under glucosidase treatment, the spot corresponding to glucoside disappeared after treatment with β-glucosidase, but not with α-glucosidase (Fig. 2b). Hence, it was suggested that the product was β-glucoside of methanol. The product was confirmed to be β-glucoside by ^{13}C-NMR (150 MHz) and ^{1}H-NMR (600 MHz). The data suggested that the product consisted of methanol and D-glucose (data not shown). A doublet at 4.2 ppm showed the existence of a β-anomeric proton of the glucosyl moiety, since this signal had a larger coupling constant ($J = 7.7$ Hz) than that for α-glucoside ($J = 3.8$ Hz). Based on these results, the product was identified as methyl β-D-glucopyranoside (β-MetGlc). Thus it was found that β-glucoside could be anomer-selectively synthesized from inexpensive sucrose in an one pot reaction with PsSPase and CtCPase.

Subsequently, the reaction was carried out under several temperatures and pH levels for high yield synthesis of β-MetGlc. The optimal temperature and pH were 10 °C and pH 6.0. The low reaction temperature was preferred for β-MetGlc synthesis because the SPase tended to reconvert α-G1P and fructose to sucrose at high reaction temperature (data not shown). Then the time course of β-MetGlc production from sucrose was investigated under the following conditions: the reaction mixture containing PsSPase, CtCPase, 100 mM sucrose, and 30% methanol (v/v) in 100 mM citrate-100 mM phosphate buffer (pH 6.0) was incubated at 10 °C with shaking at 160 rpm for 20 h. In this reaction, β-MetGlc was selectively synthesized, and no α-MetGlc was detected. The total amount of β-MetGlc reached a maximum (34%, mol/mol) at 18 h. In the case of reactions with other SPases that showed higher phosphorolysis activity than PsSPase, α-MetGlc was synthesized as a by-product. Hence, the fact that PsSPase showed no glucosyltransfer activity was essential to this method.

In this study, we developed a unique method for the synthesis of alkyl β-glucosides from sucrose by combined use of SPase and CPase. This is the first report to describe an effective process for β-anomer-selective synthesis of alkyl glucoside using phosphorylases. It is expected that the method can be applied in effective synthesis of other β-glucosides using other CPases with different acceptor specificities.

Acknowledgments

We are grateful to Dr. Motomitsu Kitaoka of the National Food Research Institute for providing recombinant CtCPase. This research was done at the "Center for Practical Chemical Wisdom" supported by the Global COE program of the Ministry of Education, Culture, Sports, Science and Technology of Japan.

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