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Note

Enzymatic Synthesis of α-Anomer-Selective D-Glucosides Using Maltose Phosphorylase

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A maltose phosphorylase (EC 2.4.1.8; MPase) showed novel acceptor specificity and transferred the glucosyl moiety of maltose not only to sugars but also to various acceptors having alcoholic OH groups. Salicyl alcohol acted as acceptor for MPase from *Enterococcus hirae*, and the product, salicyl-*O*-α-D-glucopyranoside (α-SalGlc) was identified. The yield based on supplied salicyl alcohol was 86% (mol/mol).

Key words: maltose phosphorylase; α-glucoside; transglycosylation

Many glycosides have biological activities, and the acceptor compounds become more soluble and stable with transglycosylation.[1)] Maltose phosphorylase (maltose: orthophosphate 1-β-D-glucosyltransferase, EC 2.4.1.8; MPase) catalyzes the reversible phosphorolysis of maltose to β-D-glucose-1-phosphate (β-G1P) and D-glucose, with inorganic phosphate as cosubstrate.[2)] MPase has been found in various bacterial cells,[3–5)] and is thought to be involved in the metabolism of extracellular sugars. The phosphorolysis reaction proceeds *via* a sequential bi bi mechanism,[6,7)] and in the reverse reaction, MPase has rather broad acceptor specificity and transfers the glucosyl moiety of β-G1P to various sugars.[8,9)] However, as the sugar acceptor, only sugars have been deeply investigated. Among disaccharide phosphorylases, only sucrose phosphorylase (EC 2.4.1.7, SPase) has been reported to show transfer activity not only to sugars but to phenolic compounds.[10,11)] The enzyme catalyzes a reversible phosphorolysis of sucrose and inorganic phosphate to α-D-glucose-1-phosphate and D-fructose, and is different from MPase in reaction mechanism.[1)] In this note, we report for the first time that the some MPases catalyzed anomer-selective transglycosylation of compounds having alcoholic OH groups. Furthermore, the transglycosylation of salicyl alcohol by an MPase from *Enterococcus hirae* was investigated in detail.

Recombinant MPase from *Enterococcus hirae* (Kikkoman, Tokyo), recombinant MPase from *Enterococcus* sp. (Sigma, St. Louis, MO), and purified MPase from bacteria (Oriental Yeast, Tokyo) were investigated. Acceptor compounds and the MPase (25 U/ml) were added to 100 mM citrate-100 mM Na_2HPO_4 buffer (pH 6.0, total volume 2.0 ml) containing 1.0 M maltose, and the reaction mixture was incubated at 40 °C with shaking at 160 rpm for 24 h. TLC was used to detect products using silica gel 60 plates (Merck, Boston, MA) and a solvent system of ethylacetate–acetic acid–water (3:1:1, v/v/v) for phenolic compounds, or 1-propanol–water (17:3, v/v) for aliphatic alcohols. Spots were made visible by spraying with methanol–H_2SO_4–water (40:3:17, v/v/v), followed by heating at 160 °C. As shown in Table 1, the spots of glucosides were detected using aliphatic alcohols, butandiol, cyclohexanol, benzyl alcohol, and salicyl alcohol as sugar acceptor. These results suggest that MPases can catalyze transglycosylation to compounds having an alcoholic OH group, though the acceptors were slightly different, depending on the source of the organisms.

Salicyl alcohol was transglycosylated by recombinant MPase from *Enterococcus hirae* under the following conditions: 100 mM salicyl alcohol and MPase from *Enterococcus hirae* (210 U/ml) were added to 2 ml of 100 mM citrate–100 mM Na_2HPO_4 buffer (pH 6.0) containing 1.0 M maltose, and incubated at 40 °C with shaking at 160 rpm for 24 h. By TLC analysis, one product corresponding to a salicyl glucoside was detected. However, in the reaction mixtures without MPase or phosphate, the product was not detected. Furthermore, the product was detected using β-G1P (Sigma) instead of maltose as sugar donor, but not using D-glucose. Hence it is possible that the product was synthesized from maltose *via* β-G1P.

The product was purified for further study. It was separated by solvent extraction of the reaction mixture and then purified by a silica column packed with Wakogel C-280 (Wako, Osaka). The purified product was obtained as a white powder by lyophilization.

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Abbreviations: α-SalGlc, salicyl-*O*-α-D-glucopyranoside; β-G1P, β-D-glucose-1-phosphate; MPase, maltose phosphorylase

Table 1. Synthesis of Various Glucosides by MPases

Acceptor[a]	Source		
	Enterococcus hirae	*Enterococcus* sp.	Bacteria
Methanol	−	−	−
Ethanol	−	−	+
1-Propanol	+	+	+
2-Propanol	+	+	+
1-Butanol	+	+	+
t-Butanol	−	−	+
n-Pentanol	+	+	+
n-Hexanol	+	+	+
n-Heptanol	+	+	+
1,2-Butanediol	+	N.T.[b]	N.T.
1,3-Butanediol	+	N.T.	N.T.
1,4-Butanediol	+	N.T.	N.T.
Cyclohexanol	+	N.T.	N.T.
Benzyl alcohol	+	N.T.	N.T.
Salicyl alcohol	+	+	+
Catechol	−	−	−
Resorcinol	−	−	−
Hydroquinone	−	−	−

[a]Quantities of acceptor compounds used: aliphatic alcohols, 9 M; butanediols, cyclohexanol, benzyl alcohol, 55 mM; phenol derivatives, 90 mM.
[b]N.T., not tested.
+, detected; −, not detected.

To analyze the structure of the product, α- and β-glucosidase treatment and NMR analysis (Avance 600 spectrometer (Bruker, Rheinstetten, Germany) in DMSO-d_6 with tetramethylsilane as an internal standard) were done. The product (5 mg/ml) was hydrolyzed by treatment with 5 mg/ml of α-glucosidase (from yeast, Biozyme Laboratories, Blaenavon, UK), or 5 mg/ml of β-glucosidase (from almond, Oriental Yeast, Tokyo) at 40 °C for 24 h. By TLC analysis, salicyl alcohol and D-glucose was liberated after treatment with α-glucosidase, but not with β-glucosidase. Hence the product was α-monoglucoside of salicyl alcohol. ^{13}C-NMR (150 MHz), ^{1}H-NMR (600 MHz), ^{1}H–^{1}H correlation spectroscopy (COSY, 600 MHz), ^{1}H-detected multiple quantum coherence (HMQC, 600 MHz), and hetero-nuclear multiple bond coherence (HMBC, 600 MHz) spectra were obtained. The data suggested that the product consisted of salicyl alcohol and D-glucose (data not shown). A doublet signal at 4.7 ppm showed the existence of an α-anomeric proton of the glucosyl moiety, since this signal had a smaller coupling constant ($J = 3.8$ Hz) than that for β-glucoside ($J = 7.9$ Hz). Moreover, a sequence of correlation at the C-l (64 ppm)/H-1′ (4.7 ppm) position was clearly detected on the HMBC spectrum, indicating that D-glucose was bonded to the C-l position of salicyl alcohol. Based on these results, the product was identified as salicyl-*O*-α-D-glucopyranoside (α-SalGlc). Hence, as shown in Fig. 1, β-G1P was synthesized through phosphorolysis of maltose, and then α-SalGlc was synthesized from β-G1P and salicyl alcohol with the second inverting of the anomer.

Figure 2 shows the time course of the synthesis of α-SalGlc under the following conditions: the reaction mixture (2 ml) containing 100 mM salicyl alcohol, 1.0 M maltose, and MPase (210 U/ml) in 100 mM citrate–100 mM Na_2HPO_4 buffer (pH 6.0) was incubated at 40 °C with shaking at 160 rpm for 240 h. The amounts of α-SalGlc and salicyl alcohol were measured by HPLC LC-8020 with a differential refractmeter (Tosoh, Tokyo) with a TSK-Gel ODS 80-TS column (4.6 × 250 mm, Tosoh). Elution was done with methanol–water (3:7, v/v) at a flow rate of 1 ml/min at 40 °C. The amounts of D-glucose and maltose were measured by HPLC with an Asahipak NH2P-50 4E column (4.6 × 250 mm, Showa Denko, Tokyo), and the elution was done with acetonitrile–water (7:3, v/v) at a flow rate of 1 ml/min at 40 °C. Under these conditions, only α-SalGlc was synthesized, and byproducts such as maltotriose, maltotetraose, and β-glucoside were not detected. Production of α-SalGlc increased with a gradual decrease in maltose concentration, and the total amount of α-SalGlc reached a maximum (50 mg) at 240 h. Hence, the enzyme was excellent in thermal stability, and 210 U/ml of highly active MPase made possible a high yield based on supplied salicyl alcohol, 86% (mol/mol).

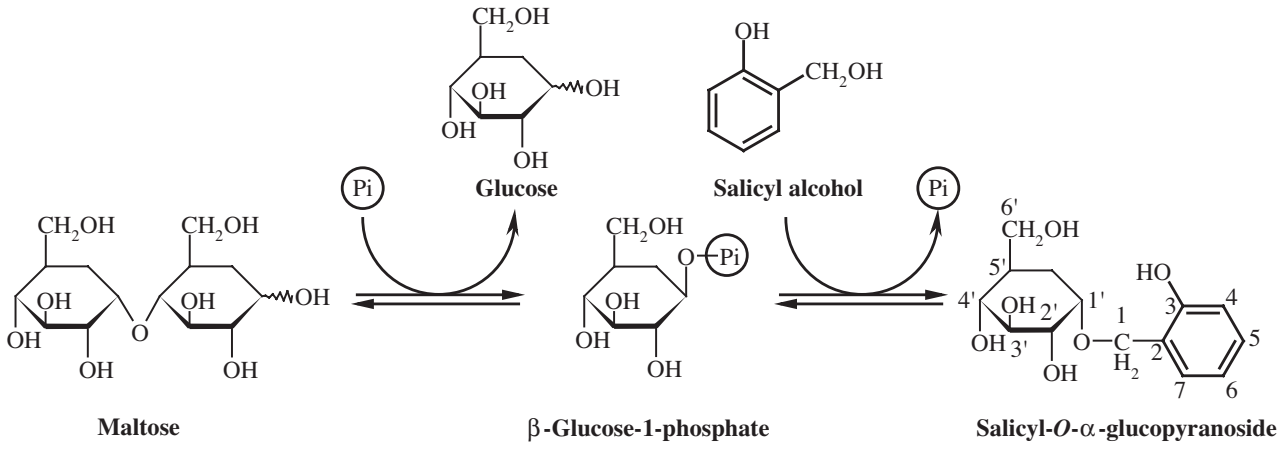

Fig. 1. Synthesis of α-SalGlc by MPase.

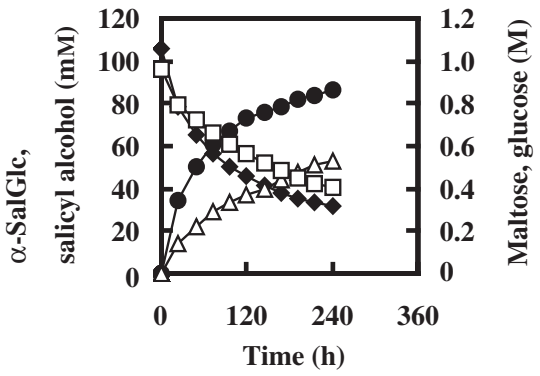

Fig. 2. Time Course of α-SalGlc Synthesis.

A reaction mixuture (2 ml) containing 100 mM salicyl alcohol, 1.0 M maltose, and MPase (210 U/ml) in 100 mM citrate–100 mM Na_2HPO_4 buffer (pH 6.0) incubated at 40 °C with shaking at 160 rpm for 240 h. Symbols: closed circle, α-SalGlc; closed diamond, salicyl alcohol; open triangle, D-glucse; open square, maltose.

In this study, we found that MPase showed transfer activity for compounds having an alcoholic OH group. MPase catalyzed phosphorolysis of maltose to form β-G1P, and then transferred the glucosyl moiety of β-G1P to salicyl alcohol with a second inverting of the anomer. The novel acceptor specificities of MPase should be useful for in synthesis of important anomer-selective glucosides.

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