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Transglycosylation reaction of maltotriose-forming amylase from *Streptomyces griseus* †

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ABSTRACT

A maltotriose-forming amylase from *Streptomyces griseus* produced predominantly *p*-nitrophenyl α-maltotetraoside through a transglycosylation reaction from maltotetraose as a donor and *p*-nitrophenyl α-D-glucopyranoside as an acceptor in an organic co-solvent. With *p*-nitrophenyl β-D-glucopyranoside acceptor, the enzyme catalyzed the formation of an α-(1 → 3)-linked tetrasaccharide (*p*-nitrophenyl 3^1-*O*-α-maltotriosyl-β-D-glucopyranoside) in a yield of 16%, based on the acceptor added with its isomer *p*-nitrophenyl β-maltotetraoside. This was also the case for the formation of *o*-chloro-*p*-nitrophenyl 3^1-*O*-α-maltotriosyl-β-D-glucopyranoside with *o*-chloro-*p*-nitrophenyl β-D-glucopyranoside acceptor. The results shows that the anomeric configuration of the aryl group in the glucosyl acceptors had an effect on the position of transglycosylation.

INTRODUCTION

Maltooligosaccharides and their chromogenic derivatives have been useful not only for the investigation of the catalytic behavior of alpha amylase, but also for the measurement of alpha amylase activity in human serum[1–7]. We have already reported an efficient synthesis of *p*-nitrophenyl α- and β-maltopentaosides from maltopentaose and respectively *p*-nitrophenyl α- and β-D-glucopyranoside, under catalysis by the maltotetraose-forming amylase from *Pseudomonas stutzeri* in an organic co-solvent system[8,9]. Further study of this mode of glycoside synthesis by enzymic transglycosylation was undertaken using the maltotriose-forming amylase from *S. griseus* (G_3-amylase)[10], the normally hydrolytic enzyme whose activity in transglycosylation has been almost ignored.

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† Abbreviations: G_3-amylase, maltotriose-forming amylase from *S. griseus*; pNP-α-G or β-G, *p*-nitrophenyl α- or β-D-glucopyranoside; CNP-β-G, *o*-chloro-*p*-nitrophenyl β-D-glucopyranoside.

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This paper describes an efficient synthesis of *p*-nitrophenyl α-maltotetraoside by utilizing the G_3-amylase-catalyzed transglycosylation in an organic co-solvent and the capacity of the enzyme to catalyze preponderantly the α-(1 → 3)-linked tetrasaccharide glycoside by using a β-D-glucoside acceptor.

EXPERIMENTAL

Materials.—G_3-amylase from *S. griseus* was purified by the method of Wako et al[10]. A series of *p*-nitrophenyl α- or β-maltooligosides (degree of polymerization, 2–5) was purchased from Calbiochem-Behring Corp. *o*-Chloro-*p*-nitrophenyl β-D-glucopyranoside (CNP-β-G) was generously given by Sapporo Breweries Ltd. Reduced short-chain amylose was prepared by treatment of commercial Amylose A from corn starch (MW ~ 2900, Nakarai Tesque Co. Ltd.) with $NaBH_4$. Other chemicals used were of reagent grade and available commercially.

Amylase assay.—G_3-amylase was assayed by using a substrate solution containing 0.5% reduced short-chain amylose in 0.9 mL of 0.1 M acetate buffer (pH 6.0). After incubation of the substrate solution for 3 min at 40°C, 0.1 mL of suitable diluted enzyme solution was added, and the amount of reducing sugar liberated by the enzyme action for 30 min at 40°C was measured by the Somogyi–Nelson method[11,12]. One unit (U) was defined as the amount of the enzyme liberating one μmol reducing-sugar equivalent as glucose per minute under the assay conditions.

Analytical methods.—HPLC was performed with a YMC AQ-312 ODS (6.0 × 150 mm) in a Hitachi L-6000 pump equipped with a Hitachi L-4000 UV detector and a Hitachi L-3000 RI monitor (differential refractometer). ^{13}C and ^{1}H NMR spectra were determined with a Varian XL-500 spectrometer operating at 125.7 MHz in the pulsed Fourier-transform mode with complete proton decoupling and 499.84 MHz, respectively. Chemical shifts are expressed in ppm relative to sodium 4,4-dimethyl-4-silapentanoate as an internal standard. FABMS spectra of the oligosaccharides were recorded with a Jeol DX-303 HF mass spectrometer, operating at the full accelerating potential (3 kV) and coupled to a Jeol DA-500 mass-data system. The sample (3 μL) in distilled water was added to the glycerol matrix, and 0.1 M HCl (1 μL) was added. The molecular weight of the sample was estimated from the *m*/*z* value of the quasi-molecular-ion $[M + H]^+$ peak. Specific rotations were determined with a Digital Automatic Polarimeter PM-101 apparatus (Union Giken Corp., Ltd).

Preparation of maltotetraosides.—*(a)* p-*Nitrophenyl α-maltotetraoside* (**1**) *and* p-*nitrophenyl* 3^1-O-*maltotriosyl-α-D-glucopyranoside* (**2**). Maltotetraose (100 mg) and *p*-nitrophenyl α-D-glucopyranoside (pNP-α-G, 90 mg) were dissolved in 1 mL of 20 mM acetate buffer (pH 6.0) containing 50% Me_2SO (v/v). The mixture was incubated with 2.4 U of G_3-amylase for 4 h at 40°C, treated with two volumes of 1.0 M acetic acid, and boiled for 5 min. After evaporation under diminished pressure (0.1 torr) at 50°C, the syrupy residue was dissolved in 2 mL of 3:1 water–MeOH and applied to a column (2.2 × 95 cm) of Toyopearl HW-40S as in

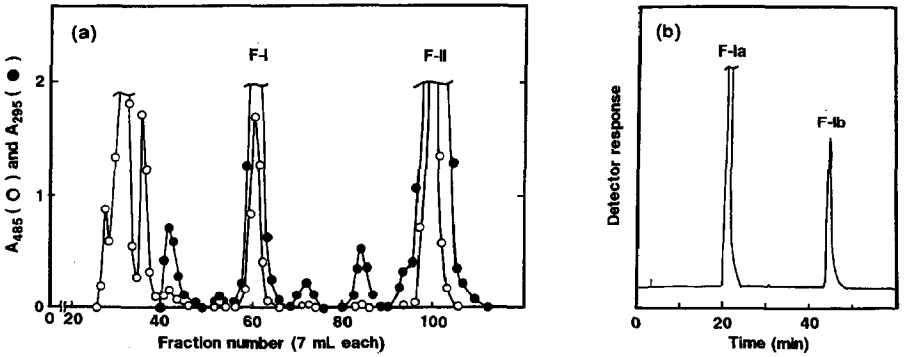

Fig. 1. Chromatographic separation of transglycosylation products by the action of G_3-amylase on maltotetraose and pNP-α-G: (a) Chromatography of carbohydrates was performed on a column (2.2×95 cm) of Toyopearl HW-40S at room temperature. The column was eluted with 3:1 water–methanol at a flow rate of 40 mL/h: (b) HPLC was performed with a YMC-packed AQ-323 S-5 120A (ODS) column (10×250 mm). Elution was effected with 9:1 water–methanol.

Fig. 1a. The column was eluted with (3:1) water–MeOH at a flow rate of 52 mL/h at room temperature. Elution was monitored by measurement of the *p*-nitrophenyl group at 295 nm and at 485 nm (phenol–H_2SO_4 method[13], from which the carbohydrate content is calculated). The eluate (7-mL fractions) showed two main peaks (F-I and F-II) for which the absorption at 295 nm coincided with that at 485 nm. The first peak (F-I, tubes 57–64) was presumed to contain transfer products and the second peak (F-II, tubes 98–105) to contain residual pNP-α-G. The F-I fraction was concentrated to 0.5 mL, 0.1 mL of which was resolved by HPLC. One main peak (F-Ia) and one minor peak (F-Ib) were obtained (Fig. 1b). The eluates corresponding to F-Ia and F-Ib peaks were in each case combined, concentrated, and lyophilized. The remainder of F-1 was similarly worked up. F-Ia and F-Ib were obtained as compounds **1** (15.3 mg) and **2** (1.1 mg), respectively.

Compound **1** had $[\alpha]_D^{25}$ +207.5 (*c* 1, H_2O) and *m/z* 788. ^1H NMR data (D_2O): 8.276 and 7.328 (2m, 2 2 H, aromatic), 5.832 (d, 1 H, *J* 3.5 Hz, H-1), 5.445 (d, 1 H, *J* 3.5 Hz, H-1′), 5.386 (d, 2 H, *J* 3.5 Hz, H-1″, −1‴). The ^{13}C NMR spectra could be superposed on those of authentic *p*-nitrophenyl α-maltotetraoside.

Compound **2** had $[\alpha]_D^{25}$ +206.4 (*c* 1, H_2O) and *m/z* 788. ^1H NMR data (D_2O): 8.277 and 7.328 (2m, 2 2 H, aromatic), 5.839 (d, 1 H, *J* 3.5 Hz, H-1), 5.427 (d, 1 H, *J* 3.5 Hz, H-1′), 5.401 (d, 1 H, *J* 3.5 Hz, H-1″), 5.380 (d, 1 H, *J* 3.5 Hz, H-1‴). The ^{13}C NMR data of isomeric forms are shown in Table I.

(b) p-*Nitrophenyl β-maltotetraoside* (**3**) *and* p-*nitrophenyl 3′-O-maltotriosyl-β-*D-*glucopyranoside* (**4**). Maltotetraose (100 mg) and *p*-nitrophenyl β-D-glucopyranoside (pNP-β-G, 90 mg) were dissolved in 1 mL of 20 mM acetate buffer (pH 6.0) containing 50% Me_2SO. Other conditions were the same as those in (*a*) except for the incubation time of 24 h. The transfer products were separated by chromatography on a Toyopearl HW-40S column as before. The eluate (5-mL fractions) showed three main peaks (F-I′, tubes 83-89; F-II′, tubes 91-99; F-III′, tubes

TABLE I

^{13}C-Chemical shifts of compounds **1**, **2**, **3**, and **4** in D_2O solution

α-Glc-(1 → 4)-α-Glc-(1 → 4)-α-Glc-(1 → 4)-α-Glc-pNP (**1**) α-Glc-(1 → 4)-α-Glc-(1 → 4)-α-Glc-(1 → 3)-α-Glc-pNP (**2**)
IV III II I IV III II I

α-Glc-(1 → 4)-α-Glc-(1 → 4)-α-Glc-(1 → 4)-β-Glc-pNP (**3**) α-Glc-(1 → 4)-α-Glc-(1 → 4)-α-Glc-(1 → 3)-β-Glc-pNP (**4**)
IV III II I IV III II I

Compound		C-1	C-2	C-3	C-4	C-5	C-6	*m*-Ar	*o*-Ar	*p*-Ar	*c*-Ar
1	I	99.207	73.554	75.662	79.337	74.322	62.956				
	II	102.368	74.005	76.074	79.610	74.340	63.207				
	III	102.463	74.016	76.114	79.839	74.526	63.269				
	IV	102.550	74.176	76.114	72.119	75.502	63.770				
								128.826	119.536	145.160	164.123
2	I	99.485	72.462	82.251	72.338	75.427	62.775				
	II	101.805	73.147	75.656	79.753	74.338	63.139				
	III	102.274	73.955	76.053	79.607	74.527	63.270				
	IV	102.540	74.272	76.133	72.109	75.489	63.270				
								128.809	119.519	145.150	164.011
3	I	101.794	75.139	78.362	78.937	77.411	62.920				
	II	101.979	73.751	75.394	79.291	73.970	62.960				
	III	102.169	73.751	75.776	79.524	74.072	63.008				
	IV	102.263	74.250	75.860	71.850	75.234	63.008				
								128.616	119.012	145.150	164.175
4	I	102.340	74.268	84.637	72.513	78.759	62.964				
	II	101.808	73.128	75.698	79.640	74.359	62.971				
	III	102.808	73.980	76.100	79.640	74.545	63.066				
	IV	102.562	74.250	76.151	72.134	75.510	63.371				
								128.907	119.333	145.445	164.519

TABLE II

^{13}C-CHemical shifts of compounds **5**, **6**, and **7** in D_2O solution

α-Glc-(1 → 4)-α-Glc-(1 → 4)-α-Glc-(1 → 4)-β-Glc-CNP (**5**) α-Glc-(1 → 4)-α-Glc-(1 → 4)-α-Glc-(1 → 3)-β-Glc-CNP (**6**)
IV III II I IV III II I

α-Glc-(1 → 4)-α-Glc-(1 → 4)-α-Glc-(1 → 4)-β-Glc-(1 → 4)-α,β-Glc (**7**)
V IV III II I

Compound		C-1	C-2	C-3	C-4	C-5	C-6	*m*-Ar	*o*-Ar	*p*-Ar	*c*-Ar
5	I	102.554	75.255	78.620	79.097	77.837	63.175				
	II	102.255	74.038	75.673	79.558	74.249	63.237				
	III	102.456	74.038	76.059	79.818	74.351	63.291				
	IV	102.554	74.534	76.140	72.134	75.513	63.291				
								126.120	118.396	145.222	160.033
								127.078	128.972		
6	I	102.965	74.143	84.545	72.450	78.896	62.992				
	II	101.965	73.127	75.684	79.688	74.358	63.054				
	III	102.288	73.979	76.099	79.668	74.555	63.287				
	IV	102.568	74.267	76.143	72.137	75.513	63.298				
								126.182	118.498	145.240	160.139
								127.118	128.957		
7	Iα	94.636	73.750	74.627	81.573	72.918	62.745				
	β	98.562	77.092	78.975	81.427	77.588	62.876				
	II	105.203	76.698	78.727	79.808	77.384	63.300				
	III	102.225	74.027	75.676	79.599	74.363	63.300				
	IV	102.444	74.027	75.866	79.559	74.538	63.300				
	V	102.561	74.261	76.144	72.144	75.516	63.300				

150-160) for which the absorption at 295 nm coincided with that at 485 nm. The eluates corresponding to F-I′ and F-II′ were in each case combined, concentrated, and lyophilized to afford compounds **4** (16.4 mg) and **3** (11.4 mg), respectively. F-III′ contained unreacted pNP-β-G.

Compound **3** had $[\alpha]_D^{25}$ +68.5 (*c* 1, H_2O) and *m/z* 788. ^1H NMR data (D_2O): 8.285 and 7.278 (2m, 2 2 H, aromatic), 5.455 (d, 1 H, *J* 3.5 Hz, H-1′), 5.408 (d, 1 H, *J* 3.5 Hz, H-1″), 5.401 (d, 1 H, *J* 3.5 Hz, H-1‴), 5.300 (d, 1 H, *J* 7.0 Hz, H-1). The ^{13}C and ^1H NMR spectra could be superposed on those of authentic *p*-nitrophenyl β-maltotetraoside.

Compound **4** had $[\alpha]_D^{25}$ +71.7 (*c* 1, H_2O) and *m/z* 788. ^1H NMR data (D_2O): 8.297 and 7.283 (2m, 2 2 H, aromatic), 5.431 (d, 1 H, *J* 3.5 Hz, H-1″), 5.415 (d, 1 H, *J* 3.5 Hz, H-1‴), 5.402 (d, 1 H, *J* 3.5 Hz, H-1′), 5.312 (d, 1 H, *J* 7.0 Hz, H-1). The ^{13}C NMR data of isomeric forms are shown in Table I.

(c) o-*Chloro*-p-*nitrophenyl* β-*maltotetraoside* (**5**) *and* o-*chloro*-p-*nitrophenyl* 3^1-O-*maltotriosyl*-β-D-*glucopyranoside* (**6**). Maltotetraose (100 mg) and *o*-chloro-*p*-nitrophenyl β-D-glucopyranoside (pNP-β-G, 90 mg) were dissolved in 1 mL of 20 mM acetate buffer (pH 6.0) containing 50% Me_2SO. Other Conditions were the same as those in (*a*) except for the incubation time of 24 h. The transfer products were separated by the same column as in (*b*). The chromatogram showed three main peaks (F-I″, tubes 72–78; F-II″, tubes 83–88; F-III″, tubes 142–158) for which the absorption at 295 nm coincided with that at 485 nm. The eluates corresponding to F-I″ and F-II″ were in each case combined, concentrated, and lyophilized to furnish compounds **6** (17.6 mg) and **5** (13.2 mg), respectively. F-III″ contained unreacted CNP-β-G as an acceptor.

Compound **5** had $[\alpha]_D^{25}$ +63.3 (*c* 1, H_2O) and *m/z* 823. ^1H NMR data (D_2O): 8.438, 8.245, and 7.412 (m, 3 H, aromatic), 5.467 (d, 1 H, *J* 3.5 Hz, H-1′), 5.415 (d, 1 H, *J* 3.5 Hz, H-1″), 5.412 (d, 1 H, *J* 3.5 Hz, H-1‴), 5.364 (d, 1 H, *J* 9.0 Hz, H-1).

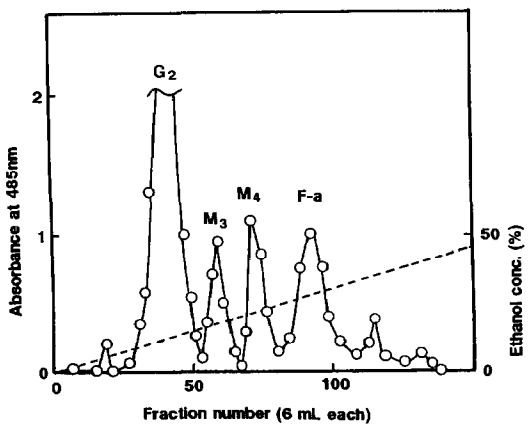

Fig. 2. Charcoal–Celite chromatography of transglycosylation products by the action of G_3-amylase on maltotetraose and cellobiose. Chromatography was carried out on a column (1.5×50 cm) of charcoal–Celite. G_2, cellobiose; M_3, maltotriose; M_4, maltose.

Compound **6** had $[\alpha]^{25}$ +64.5 (*c* 1, H_2O) and *m/z* 823. ^1H NMR data (D_2O): 8.423, 8.240, and 7.418 (m, 3 H, aromatic), 5.435 (d, 1 H, *J* 3.5 Hz, H-1′), 5.4111 (d, 1 H, *J* 3.5 Hz, H-1″), 5.408 (d, 1 H, *J* 3.5 Hz, H-1‴), 5.350 (d, 1 H, *J* 9.0 Hz, H-1). The ^{13}C NMR data of isomeric forms are shown in Table II.

(d) 4-O-β-Maltotriosyl-D-glucose (**7**). Maltotetraose (197.5 mg), and cellobiose (202.5 mg) dissolved in 1 mL of 20 mM acetate buffer (pH 6.0) were incubated with 2.5 U of G_3-amylase for 5 h at 30°C. The mixture was treated with two volumes of 1.0 M AcOH, boiled for 5 min, and loaded onto a column (1.3 × 50 cm) charcoal–Celite. After washing the column with water (500 mL), the absorbates were eluted by a linear gradient of 0 (500 mL)–50% (500 mL) EtOH (Fig. 2). The eluate was monitored by measurement at 485 nm. The F-a fraction, which was eluted at ~ 30% concentration of EtOH (tubes, 85–100), was concentrated to low volume (5 mL), and then subjected to rechromatography. The corresponding F-a′ fractions eluted (tubes, 88–97) by 30% EtOH were combined, concentrated, and lyophilized to give 52 mg.

Compound **7** had $[\alpha]_D^{25}$ +133.5 (*c* 1, H_2O) and *m/z* 829. ^1H NMR data (D_2O): 5.415 (d, 2 H, *J* 3.5 Hz, H-1″, H-1‴), 5.403 (d, 1 H, *J* 3.5 Hz, H-1⁗), 5.237 (d, 1 H, *J* 3.5 Hz, H-1), 4.674 (d, 1 H, *J* 8.0 Hz, H-1), 4.540 (d, 1 H, *J* 8.0 Hz, H-1′).

RESULTS AND DISCUSSION

The G_3-amylase-catalyzed transglycosylation in a buffer containing 50% Me_2SO was efficient, allowing accumulation of **1** in preparative amounts. The yield of **1** was ~ 15.3% of the G_3-catalyzed net decrease of maltotetraose, although the enzymic reaction was not completely regioselective and gave a small amount of the undesired **2**. Conditions for maximal yield of the desired compound were established by investigating the effects of various conditions on transglycosylation as shown next.

Effect of solvent, temperature, and pH on production of 1.—The effects of various conditions on G_3-amylase-mediated formation of **1** at high substrate concentration were investigated by HPLC. The time-course of accumulation of **1** from maltotetraose incubated at different Me_2SO concentrations was first examined (Fig. 3a). Use of a water–Me_2SO system (Fig. 3a) in this reaction not only ensured a sufficient solubility of pNP-α-G, but also resulted in a remarkable increase in the formation of **1**. Thus, pNP-α-G shows much higher solubility (8.4%) in a medium containing 50% Me_2SO than that (1.5%) in the buffer free from Me_2SO. The maximum production of **1** at 50% Me_2SO was 4.5 times that in the absence of Me_2SO (data not shown). The time for maximum formation of compound **1** was ~ 1 h at 40°C, but the amount decreased remarkably during the next 12 h. A similarly large amount of **1** was first found to be present at ~ 2 h in the digest with 50% Me_2SO; in this case, the amount declined much more slowly. At 60% Me_2SO, the transferase activity was remarkably diminished. Fig. 3b shows the effect of temperature in a buffer containing 50% Me_2SO at pH 6.0. Reaction

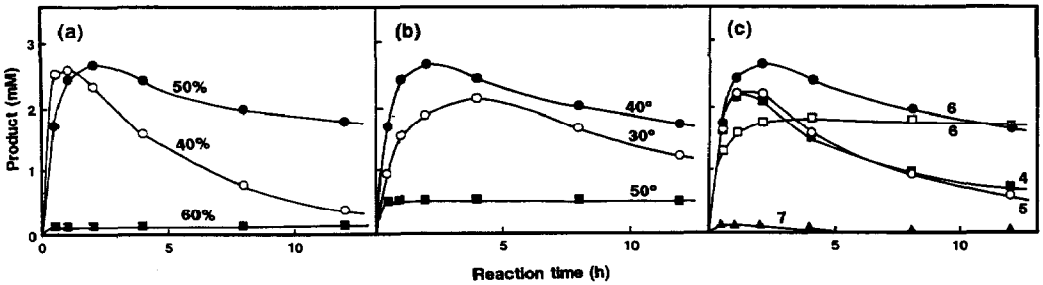

Fig. 3. Effects of Me_2SO concentration, temperature, and pH: (a) The enzyme reaction was performed with maltotetraose (100 mg) and pNP-α-G (90 mg) at 40°C in 1 mL of 20 mM acetate buffer (pH 6.0) containing different Me_2SO concentrations (40, ○; 50, ●; 60%, ■). G_3-amylase (2.4 U) was added and samples were taken during incubation for analysis by HPLC: (b) Substrates were dissolved in 1 mL of the same buffer containing 50% Me_2SO at 30 (○), 40 (●), and 50°C (■), respectively. Other conditions were the same as those in (a): (c) Substrates were dissolved in 1 mL of acetate buffer (pH 4.0, ■; pH 5.0, ○; pH 6.0, ●) and phosphate buffer (pH 6.0, □; pH 7.0, ▲) containing 50% Me_2SO at 40°C, respectively. Other conditions were the same as those in (a).

at 40°C was best suited for the production of **1**; 50°C was wholly unsuited. As shown in Fig. 3c, the formation was highly dependent on pH. Production of **1** was largest at pH 6.0 (acetate buffer); pH 7.0, the transferase activity was not observed.

In the reaction between maltotetraose and pNP-α-G production of **1** certainly predominated in the aqueous Me_2SO system during the entire course of reaction, but there was only a small proportion of α-(1 → 3)-linked isomer. When production of **1** reached a maximum, the molar ratio of **1** and **2** was 10:1.

Acceptor effects on G_3-amylase-mediated tetrasaccharide glycoside formation.— The regioselectivity of G_3-amylase-catalyzed formation of tetrasaccharide glycosides may be greatly changed by using the corresponding β-D-glucoside acceptors with hydrophobic aglycons as shown in Fig. 4. Thus, the α-(1 → 4)-linked tetrasaccharide glycoside (**1**) was a main product during the entire course of reaction when pNP-α-G was an acceptor. However, with pNP-β-G acceptor, the α-(1 → 3)-linked product (**4**) is formed in appreciable amounts, along with the α-(1 → 4)-linked homotetrasaccharides (**3**). This is also the case for the formation of **5** and **6** in the reaction with CNP-β-G acceptor. In the both cases, more α-(1 → 4)-linked product than the α-(1 → 3)-linked one was found in the initial stage of reaction, but the relation between this yield was reversed in the later stage of the reaction. The yields of α-(1 → 3)-linked isomers **4** and **6** were ~ 16.4 and 17.6% of the amylase-catalyzed net decrease of maltotetraose, respectively. Furthermore, when cellobiose was the acceptor instead of pNP-β-G, the enzymic reaction gave exclusively 4-*O*-β-maltotriosyl-D-glucose (**7**). Nakakuki et al. have reported that in the reaction with maltotetraose as substrate G_3-amylase produces maltoheptaose by transferring the maltotriosyl residue from the reducing end of maltotetraose to the 4-position of another maltotetraose molecule[14]. The present findings indicate that the configuration of the aryl group (α or β) in the glucosyl acceptors had an effect

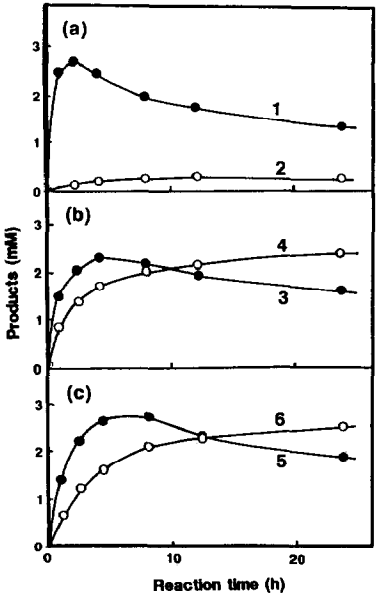

Fig. 4. Time-course of G_3-amylase-mediated isomer formation from a maltotetraose donor and some glucoside acceptors. (a) The enzyme reaction was performed with maltotetraose (100 mg) pNP-α-G (90 mg) at 40°C in 1 mL of 20 mM acetate buffer (pH 6.0) containing 50% Me_2SO. Other conditions were the same as those in Fig. 3(a). Products **1** (•) and **2** (○) were formed. (b) The reaction with pNP-β-G acceptor. Other conditions were the same as those in (a). Products **3** (•) and **4** (○) were formed. (c) The reaction with CNP-β-G acceptor. Other conditions were the same as those in (a). Products **5** (•) and **6** (○) were formed.

on the position of transglycosylation. Thus, with the enzyme, the α-(1 → 3)-linked tetrasaccharide glycoside was produced more than the α-(1 → 4)-linked isomer when pNP-β-G or CNP-β-G was acceptor, whereas with the corresponding pNP-α-G, the α-(1 → 4)-linked product preponderated and there was only a small proportion of α-(1 → 3)-linked isomer. We have already reported that a maltotetraose-forming amylase from *P. stutzeri* transfers a maltotetraosyl group from maltopentaose exclusively to the 4-position of α- and β-D-glucopyranosidic acceptors with *p*-nitrophenyl group in an organic co-solvent system, regardless of the anomeric configuration.[9] Nilsson has demonstrated that the regioselectivity of glycosidase-catalyzed formation of disaccharides may be changed by using α- or β-glycosyl acceptors with various acceptors[15,16]. While glycosidases do exhibit some regioselectivity for the hydroxyl linkage to the acceptor, this selectivity is less predictable and lower than that of such polysaccharide hydrolases as amylase[14], amylomaltase[17], cellulase[18], and lysozyme[19,20].

An interesting result from these studies is the capacity of the G_3-amylase to catalyze preponderant formation of the α-(1 → 3)-linked tetrasaccharide glycoside the glycosidase-catalyzed formation, by using β-D-glucoside acceptors in mixtures containing 50% Me_2SO.

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REFERENCES

1 B.K. Gillard, H.C. Markman, and S.A. Feig, *Clin. Chem.*, 23 (1977) 2279–2282.
2 R.F. Foery, *Clin. Chem.*, 27 (1981) 1047.
3 E.O. Hagele, E. Schaich, E. Rauscher, P. Lehmann, H. Burk, and A.W. Wahlefeld, *Clin. Chem.*, 28 (1982) 2201–2205.
4 K. Omichi and T. Ikenaka, *J. Biochem.*, 100 (1986) 1353–1358.
5 S. Satomura, T. Iwata, Y. Sakata, K. Omichi, and T. Ikenaka, *Carbohydr. Res.*, 176 (1988) 107–115.
6 H. Matsui, H. Kawagishi, and T. Usui, *Biochim. Biophys. Acta*, 1035 (1990) 90–96.
7 T. Usui, K. Ogawa, H. Nagai, and H. Matsui, *Anal. Biochem.*, 202 (1992) 61–67.
8 T. Usui and T. Murata, *J. Biochem.*, 103 (1988) 969–972.
9 K. Ogawa, T. Murata, and T. Usui, *Carbohydr. Res.*, 212 (1991) 289–294.
10 K. Wako, S. Hashimoto, S. Sato, S. Kubomura, K. Yokota, K. Aikawa, and J. Kanaeda, *J. Jpn. Soc. Starch Sci.*, 26 (1979) 175–181.
11 M. Somogyi, *J. Biol. Chem.*, 195 (1952) 19–23.
12 N. Nelson, *J. Biol. Chem.*, 153 (1944) 375–380.
13 M. Dubois, K.A. Gilles, J.K. Hamilton, P.A. Rebers, and F. Smith, *Anal. Chem.*, 28 (1956) 350–356.
14 T. Nakakuki, K. Azuma, and K. Kainuma, *Carbohydr. Res.*, 128 (1984) 297–310.
15 K.G.I. Nilsson, *Biocat. Org. Med.*, (1987) 369–374.
16 K.G.I. Nilsson, *Carbohydr. Res.*, 167 (1987) 95–103.
17 T.N. Palmer, *B.E. Ryman, and W.J. Whelan, Eur. J. Biochem.*, 69 (1976) 105–115.
18 S. Kobayashi, K. Kainuma, T. Kawasaki, and S. Shoda, *J. Am. Chem. Soc.*, 113 (1991) 3079–3084.
19 T. Usui, Y. Hayashi, F. Nanjo, and Y. Ishido, *Biochim. Biophys. Acta*, 953 (1988) 179–184.
20 T. Usui, H. Matsui, and K. Isobe, *Carbohydr. Res.*, 203 (1990) 65–77.