Characterization of Acid-Cyclodextrin Glycosyltransferase of an Alkalophilic Bacillus sp.

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Cyclodextrin glycosyltransferase (EC 3.2.1.19, CGTase) which catalyzes the formation of cyclodextrins from starch and related carbohydrates such as amylose, amylopectin, glycogen, etc., is known to be produced by some certain species of Bacillus, i.e., B. macerans, B. circulans, B. stearothermophilus, and B. megaterium.

In the previous works of this series, the authors have isolated many alkalophilic microorganisms capable of growing in high alkaline pH media containing 1% Na₂CO₃, and many alkaline enzymes were produced by these bacteria. By the analogous method, we have also isolated a CGTase-producing bacterium from soil. The culture filtrate of this bacterium (alkalophilic Bacillus sp., ATCC 21783) mainly produced β-cyclodextrin from starch in the range of pH 4~10 with somewhat high yield. From this result, we considered that some different CGTases, which were tentatively named as acid-, neutral- and alkaline-CGTase, were present in the culture filtrate.

Although these enzymes were tried to purify, we could not do it except acid-CGTase which was most active at pH 4.5~5.0 and 45°C. To compare its specificity with those of the enzymes from other strains, the formation ratio of α-, β- and γ-cyclodextrin at early stage of the reaction and minimum substrate for the enzyme action were determined. As shown in Fig. 1, β-cyclodextrin was mainly formed from starch, and α- and γ-dextrin were gradually produced after prolonged incubation.

**Fig. 1.** Formation of α-, β- and γ-Cyclodextrin from Starch by Acid-CGTase at Early Stage of the Reaction.

Reaction mixture containing 0.2 ml of starch-¹⁴C (5 μCi, 10 mg) and 50 μl of 0.5 M acetate buffer (pH 4.6) was incubated with 10 μl (8.4 μg) of acid-CGTase at 40°C for various periods. Aliquots (15 μl) were spotted on a filter paper (Toyo Roshi No. 51-A) and dried in hot air with hair dryer to inactivate the enzyme. After the area around of spotting line was sprayed with 1 mg/ml of glucoamylase (pure grade, 22 U/mg, Seikagaku Kogyo Co.) solution, the paper was kept in a damp atmosphere at 50°C for overnight to hydrolyze saccharides which were not converted to cyclodextrins. Then the paper was developed 3 times by ascending method with a solvent of 65% (v/v) of n-propanol. After the radioautogram was prepared by contacting with Fuji X-ray film and the paper for 5 hr, the spots of α-, β- and γ-dextrin which correspond with those of the X-ray film were cut out and determined those radioactivities, respectively, in Beckmann LS 230 scintillation counter. The yield of each cyclodextrin was calculated as described by Kitahata and Okada. Symbols express that (○) α-, (●) β- and (×) γ-cyclodextrin.

**Fig. 2.** Minimum Substrate for the Enzyme Action of Acid-CGTase.

About 5 mg of glucose, maltose and maltotriose in 1 ml of 0.1 M acetate buffer (pH 4.6) were incubated with 3 μl (53 μg) of acid-CGTase at 40°C for various periods, respectively. Aliquots (0.1 ml) were withdrawn and kept in a boiling water bath for 10 min to stop the reaction. Then the reaction mixture was incubated with 0.25 mg of glucoamylase in 1 ml of the same buffer described above at 40°C for 15 hr. Glucose thus formed was determined by 3, 5-dinitrosalicylic acid method. The yield of cyclodextrins was calculated from the difference of glucose formed by glucoamylase before and after the CGTase action as described by Kobayashi et al. Symbols express that (×) glucose, (○) maltose and (●) maltotriose.
incubation. After 10 and 40 min incubation, the ratios of α-, β- and γ-dextrin formed were 1%: 15%: 2% and 2%: 22%: 3%, respectively. This result strongly suggested that β-dextrin would be preferentially formed from starch. Furthermore, the ratio of β-dextrin/α- and γ-dextrin was very higher than those obtained by the enzymes from other strains. To make its difference from other CGTases more clear, the enzyme was also incubated with glucose, maltose and maltotriose to determine the minimum substrate for cyclodextrins formation. As shown in Fig. 2, about 18% of maltotriose and 7% of maltose were converted to cyclodextrins, whereas glucose was not converted. This result showed that the enzyme could attack maltooligosaccharides larger than maltose.

As a result, we concluded that the specificity of acid-CGTase from alkalophilic Bacillus sp. was similar to those of the enzymes from B. circulans and B. megaterium in regard to minimum substrate and main product except the formation ratio of α-, β-, and γ-cyclodextrins, and different from that of B. macerans as to those points described above.

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