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Enzymatic Properties of Cyclodextrin Glycosyltransferase from Alkalophilic *Bacillus* sp. YC-335

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호알칼리성 *Bacillus* sp.가 생산하는 Cyclodextrin Glycosyltransferase의 효소적 특성

정용준 · 정명호 · 유주현
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Abstract

The enzymatic properties of purified CGTase from alkalophilic *Bacillus* sp. YC-335 have been examined. Apparent Vmax values of the enzyme in transferring glycosyl residues α-, β- and γ-cyclodextrin (CD) to sucrose were 16.13, 21.8 and 9.8 μmoles/min/mg protein, respectively and Km values of the corresponding CD were 1.68, 0.33 and 0.37 mM, respectively. A number of saccharides, specially starch hydrolyzates such as glucose and maltose, could activate the dextrinizing activity of the enzym. However, the dextrinizing activity was inhibited by β-CD. It was found from Lineweaver-Burk plot that the inhibition of CGTase by β-CD was noncompetitive. High performance liquid chromatographic analysis showed that the enzyme has three kinds of activity ; transglycosylation and disproportionation as well as cyclization

Key words : cyclodextrin glycosyltransferase, alkalophilic *Bacillus* sp., enzymatic properties

Introduction

Cyclodextrin glycosyltransferase(CGTase, EC 2.4, 2.19) is an enzyme that produces cyclodextrin, designated as α-, β- and γ-cyclodextrin. CGTase has three kinds of activity ; cyclization, coupling and disproportionation. Therefore, the mechanism of CGTase is more complicated than that of amylases[1,2]. The action of CGTase has been extensively studied and its characteristics and reaction mechanism are becoming well understood[3~6].

In previous work, we have isolated a CGTase-producing bacterium, *Bacillus* sp. YC-335 capable of growing in high alkaline pH media contaning 1% Na_2CO_3[7], and we reported the purification and general properties of the enzyme[8].

The present paper describes some enzymatic properties and the reaction mode of the CGTase.

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Materials and Methods

Preparation of purified enzyme

The CGTase produced by alkalophilic *Bacillus* sp. YC-335 was prepared, as described previously[7,8]. The preparations were homogeneous on SDS-polyacrylamide gel electrophoresis[9].

Assay of enzyme

CGTase activity was determined by the glucoamylase method, as described previously[7]. One unit of enzyme is defined as the amount of enzyme to form 1 μmole of glucose per min under given conditions. Dextrinizing activity was measured according to the method of Fuwa[11] with slight modification. The reaction mixture containing 20 μl of the enzyme and 0.3 ml of 0.2%(w/v) soluble starch in 0.05 M acetate buffer(pH 6.0) was incubated at 40℃ for 10 min. The reaction was stopped by adding 1 ml of 0.5 M acetic acid and 0.5 ml of iodine reagent(0.02% I_2 and 0.2% KI). Absorbance at 700 nm was measured. One unit of enzyme was defined as the amount of enzyme which produces 10% reduction in the intensity of blue color of starch-iodine complex

Table 1. Effect of cosubstrate on dextrining activity of CGTase

Cosubstrate	Concentration(%,w/v)	Ralative acitivity(%)
Control	—	100
Arabinose	0.05	157
Fructose	0.05	158
Galactose	0.05	157
Glucose	0.05	184
Lactose	0.05	145
Maltose	0.05	227
Melibiose	0.05	97
Sorbitol	0.05	165
Sucrose	0.05	156
Xylose	0.05	145
β-Cyclodextrin	0.3	80

per minute under given conditions.

Determination of protein content

Protein concentration were determined by the method of Lowry *et al*[10]. with bovine serum albumin as standard.

Determination of total sugar content

The amount of total sugar was measured by phenol-H_2SO_4 method[12]. To 1 m*l* of the reaction mixture was added 1 m*l* of trichloroethylene(TCE), sentrifuged vigorously for 1 min, and after standing at 4℃ for 60 min, centrifuged at 5,000 rpm for 10 min. The amount of cyclodextrin precipitated with TCE was calculated by substracting the amount of total sugar in the supernatant from that present in the original solution.

Analysis of CDs by high performance liquid chromatography(HPLC)

The amount of α-, β- and γ-CD, and maltooligosaccharides were determined by HPLC under the following conditions : column, Carbohydrate analysis column(3.9 mm×30 cm, Waters) : solvent system, acetonitril/water=65 : 35 : flow rate, 1 m*l*/min ; pump, Waters 402 type ; detector, differential refractormeter (Waters R401 type). The amount of various CDs in the reaction mixture of starch was measured after pretreatment by glucoamylase, 20 or 50 μ*l* of the reaction mixture was taken for HPLC analysis after Sep-Pak treatment.

Results and Discussion

Determination of Vmax and Km values for glycosyl residues transfer

In order to determine maximum velocity(Vmax) and

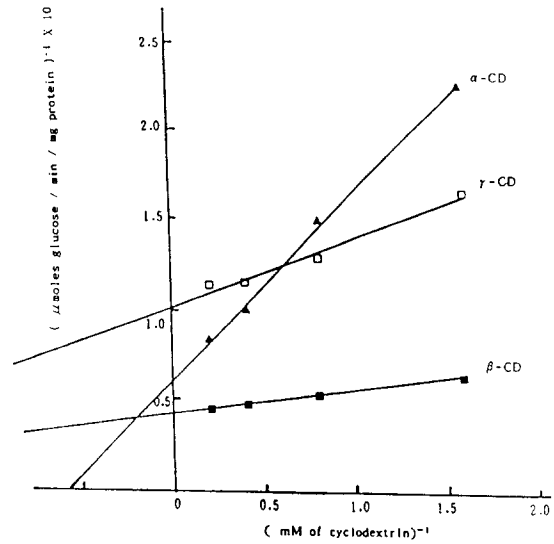

Fig. 1. Lineweaver-Burk plot to determine Vmax and Km of CGTase for transferring glycosyl resldues of α-, β- and γ-CD to sucrose

Michaelis constant(Km) of the CGTase to transfer glycosyl residues from CDs to sucrose, initial velocity was measured at the varying concentration of CDs. It was found from Lineweaver-Burk plot that the apparent Vmax values for α-, β- and γ-CD at a fixed concentration of starch(25 mM) were 16.13, 23.80 and 9.80 μmole glucose/min/mg protein, respectively, and Km values for the corresponding CD were 1.68, 0.33 and 0.37 mM, respectively.

Effect of co-substrate on dextrinizing activity

The effect of various co-substrate on the dextrinizing activity was examined by monitoring the decrease of blue color of starch-iodine complex. As shown in Table 1, the rate of the reaction was accelerated by the addition of various saccharides except melibiose and β-CD. Starch hydrolyzates such as maltose and glucose were more effective. On the other hand, the CGTase was slightly inhibited by β-CD. This is consistent with *Bacillus macerans* CGTase which also inhibited by β-CD. In case of alkalophilic *Bacillus* sp. ATCC 21783 CGTase, however, the enzyme was reported not to be inhibited by β-CD[13].

Inhibiton of dextrinzing activity of CGTase by β-CD

To investigate the inhibition type of CGTase by β-CD, the dextrinizing rate was measured in the prese-

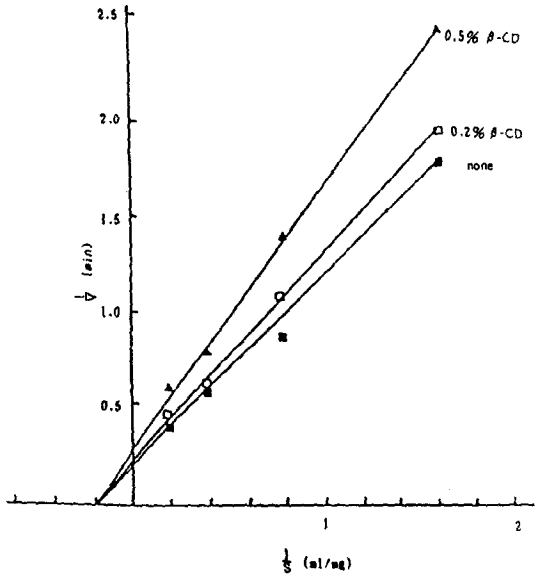

Fig. 2. Lineweaver-Burk plots showing the inhibition of dextrinizing activity of CGTase by β-CD on the rate of starch hydrolysis by the CGTase

nce of β-CD. As is evident from Lineweaver-Burk plot of 1/velocity vs 1/substrate concentration(Fig. 2), increasing β-CD concentration decrease Vmas with Km unchanged. This indicates that β-CD is a noncompetitive inhibitor of CGTase in dextrinizing property.

Transglycosylation of CDs to co-substrate

The enzyme was incubated with 2.5% starch solution at 40℃ in the presence of sucrose. At certain intervals, 2 m*l* aliquots of the reaction mixture were removed to analyze the decrease in iodine color and the amounts of CD precipitated with TCE. The bars of Fig. 3 shows the amounts of CDs formed by CGTase from starch. The total amount of α-, β- and γ-CDs generally decreased as sucrose concentration increased, suggesting that the enzyme is inhibited by sucrose or that the CDs formed is transglycosylated to sucrose as soon as they are formed. When a similar experiment was carried out with 5% starch solution, HPLC analysis showed that a major product formed by CGTase was β-CD(Fig. 4). If the reaction was performed in the presence of sucrose, CD peaks was gradually disappearing with increasing amount of sucrose. In addition, other peaks, which were identified as maltooligosaccharides, were newly formed, suggesting that the CDs formed by CGTase were transglycosylated to sucrose to form various maltooligosaccharides.

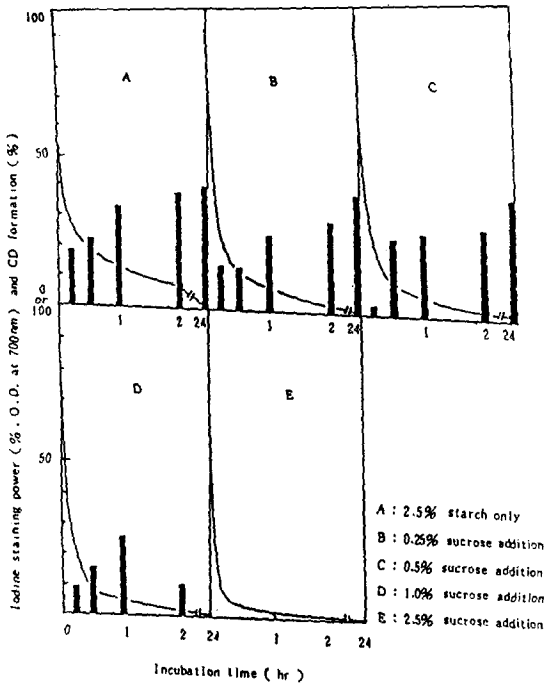

Fig. 3. Action of CGTase on starch in the presence of various concentration of sucrose as acceptor
▌: Amounts of CD precipitated with TCE
—: Dextrinizing activity

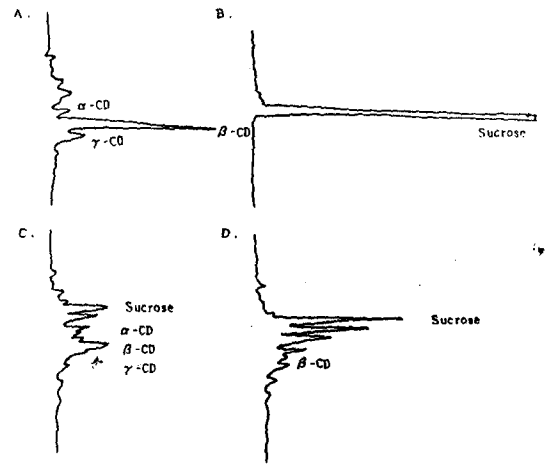

Fig. 4. HPLC chromatograms showing the formation of CDs from starch by CGTase in the absence and presence of sucrose
A : 5% starch only
B : 5% starch+2.5% sucrose(no enzyme treatment)
C : 5% starch+1% sucrose
D : 5% starch+2.5% sucrose

Table 2. Distribution of disproportionation products formed by CGTases with maltotriose

Reaction time (min)	Enzyme source									
	Bacillus sp. YC-335				*B. megaterium* *B. circulans* ATCC 9995		*B. macerans* IFO 3490		*B. stearothemophilus*	
	10	30	60	120	20	180	20	180	20	180
G_1	7.9	19.6	23.1	26	2.1	7.0	−	2.5	4.3	13
G_2	28.6	30.4	25.0	24	18	27	3.5	15	16	23
G_3	23.8	23.2	21.2	18	65	24	96	75	63	22
G_4	30.2	14.3	15.2	14	6.1	17	−	5.9	4.6	18
$G_4<$	9.5	12.5	15.4	18	8.6	25	0.8	1.6	12	24

[a] G_1 : glucose, G_2 : maltose, G_3 : maltotriose, G_4 : maltotetrose, $G_1<$: oligosaccharides larger than maltotetrose

Distribution of disproportionation of CGTase

Maltose is split by CGTase with concomitant transfer of glycosyl residues to acceptor to produce member of maltooligosaccharides[14]. To examine this disproportionation reaction of the CGTase, the enzyme was incubated with 2% maltotrise at 40℃. At various times an aliquots(0.2 m*l*) was removed, and then 10 μ*l* of 0.2 N hydrochloric acid were added to inactivate the enzyme. The amount of each maltooligosaccharide was analyzed by HPLC. The distribution of reaction products as a function of reaction time are shown in Table 2. At the early state of reaction, maltose, maltotriose and maltotetrose were dominant saccharides. As the reaction time was passed, however, they were degraded to glucose monomer. For example, the glucose content after 2 hrs of reaction is 26%, which is much higher amount than that liberated by CGTase form *B. stearothermophilus*, *B. megaterium*. *B. circuins* and *B. macerans*. It appears then that our enzyme is a novel type of CGTase.

요 약

호알칼리성 *Bacillus* sp. YC-335가 생산하는 CGTase의 효소학적 특성 및 작용반응을 살펴보았다. α-CD, β-CD 및 γ-CD로부터 glucosyl residues를 설탕으로 전이시키는 반응에 대한 효소의 최대 반응속도, Vmax 값은 각각 16.13, 21.8, 9.8 μmoles glucose/min/mg protein이었으며 Km 값은 각각 1.68, 0.33, 0.37 mM이었다. 효소의 전분 가수분해활성은 여러 당류에 의해 촉진되었으며 특히 전분 가수분해 산물인 maltose와 glucose에 의한 효과가 가장 좋았다. 이 효소는 β-CD에 의해 효소의 전분 분해활성이 저해되었으며 비경쟁적 저해형식을 보였다. 또한 전분으로부터 효소작용에 의해 생성된 산물을 총당량법 및 HPLC 분석을 통해 조사한 결과 이 효소는 cyclization 작용 뿐만 아니라 transglycosylation 작용과 disproportionation 작용을 가지는 것으로 확인하였다.

Acknowledgment

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