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단보 (Note)

Purification of Cyclodextrin Glucanotransferase by Affinity Chromatography

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Affinity Chromatography를 이용한 Cyclodextrin Glucanotransferase의 정제

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Abstract — The cyclodextrin glucanotransferase (CGTase) of a mutant of *Bacillus stearothermophilus* was purified in one step by affinity chromatography. The recovery was 95%. The specific activity of the CGTase increased from 26.2 U/mg protein to 485.5 U/mg protein. The purified CGTase was almost homogeneous by SDS-polyacrylamide gel electrophoresis. The one-step purification proved to be feasible with the mutant in contrast to the parent strain, which required pre-purification step of ammonium sulfate precipitation.

Cyclodextrin bound epoxy-activated Sepharose 6 B has been used to purify the CGTase from *Bacillus macerans* (1) and from *Bacillus stearothermophilus* (2). In the previous report (2), the CGTase of a parent strain of *Bacillus stearothermophilus* was purified by affinity chromatography on β-cyclodextrin bound epoxy-activated Sepharose 6B after ammonium sulfate precipitation. The ammonium precipitation was required because otherwise the CGTase did not bind to the β-cyclodextrin bound to epoxy-activated Sepharose 6B. It seemed that some inhibitory substance(s) present in the culture broth interfered with the binding between the CGTase and the ligand, and the inhibitory substance(s) might have been removed by ammonium sulfate precipitation.

In this report, we purified the CGTase of a MNNG (N-methyl-N′-nitro-N-nitrosoguanidine) mutant of the parent strain by the same affinity chromatography without ammonium sulfate precipitation step.

Key words: Cyclodextrin glucanotransferase, affinity chromatography
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Enzyme Activity and Protein Content

The CGTase activity was measured by Kitahata and Okada's method (3) at 40℃. The protein content was measured by the Lowry-Folin's method (4) using bovine serum albumin as the standard protein.

Enzyme Preparation

The stock culture of the mutant of *B. stearothermophilus* was cultivated at 55℃ for 48 hrs in the medium of 2% sucrose, 0.7% defatted soybean meal, 0.1% $NaH_2PO_4 \cdot 2H_2O$ and 0.015% $CaCl_2$ (pH 7.0). Crude enzyme was obtained by the centrifugation at 8,000g for 30 min at 4℃. The activity of the crude enzyme was 50.4 U/m*l*.

Affinity Chromatography and SDS-PAGE

The 50 m*l* crude enzyme was loaded on to 10× 100 mm column as described in the previous report (1). After loading the crude enzyme, the column was washed with 10 mM sodium acetate buffer (pH 6.0) until unwanted proteins were removed. The

Table 1. One-Step Purification of CGTase by Affinity Chromatography

Step	Volume (m*l*)	Total activity (U/m*l*)	Specific activity (U/mg protein)	Yield (%)	Purification fold
Culture broth	50	2520	26.2	100	1
Purified enzyme	16.8	2406	485.5	95	18

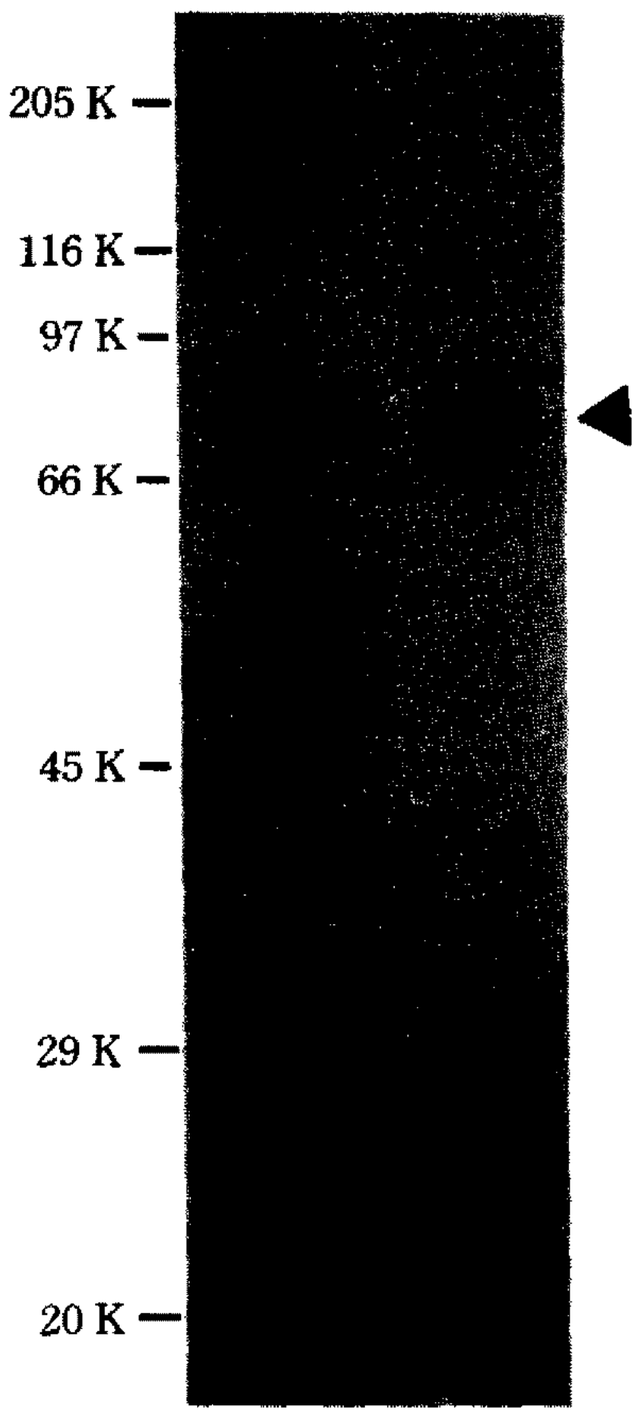

Fig. 1. SDS-polyacrylamide gel electrophoresis (12.5%) of the purified CGTase.
Lane A; Culture Broth Concentrate
Labe B; Purified CGTase

elution of the bound enzyme was carried out by 10 mg/m*l* solution of β-cyclodextrin in 10 mM sodium acetate buffer (pH 6.0).

As shown in Table 1, 95% of the crude enzyme was recovered by the affinity chromatography. The homogeneity of the purified enzyme was determined by SDS-polyacrylamide gel electrophoresis (5). The figure shows a single band, which suggests the homogeneity of the purified enzyme (Fig. 1). The mutation seemed to have changed the physiology of the microorganism so that the inhibitory substance(s) mentioned beforehand might have been absent in the culture broth of the mutant.

요 약

*Bacillus stearothermophilus*의 mutant가 생산하는 cyclodextrin glucanotransferase(CGTase)를 affinity chromatography법을 이용하여 정제하였다. 이 CGTase의 회수율은 95%이었고 specific activity는 26.2 U/mg protein에서 485.5 U/mg protein으로 증가하였다. 정제된 CGTase는 SDS-polyacrylamide gel 전기영동 결과 단일 band로 나타났다. CGTase는 affinity chromatography를 이용하여 one-step으로 정제할 수 있었다.

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