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Effect of Various Additives and Solvents on Thermostability of Cyclodextrin Glucanotransferase from *Bacillus stearothermophilus*

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여러 첨가물과 용매가 *Bacillus stearothermophilus*가 생산하는 Cyclodextrin Glucanotransferase의 열안정성에 미치는 영향

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Abstract — The influence of ethylene glycol, glycerol, sorbitol and sucrose on the thermostability of *Bacilus stearothermophilus* cyclodextrin glucanotransferase (CGTase) was investigated. Glycerol, sorbitol and sucrose had effect on thermostability of the CGTase. The effects appeared to be strongly dependent on concentration of additives. The thermostability of CGTase also was assayed in organic solvents such as n-butanol, 1,4-dioxane, n-octane. The thermostability of CGTase increased in 1,4-dioxane and n-octane. Particularly, in n-octane, the CGTase retained the 81% of the initial activity after incubation at 75℃ for 90 min.

The thermostability of industrial enzymes is important in conducting their reations at high temperature. Under this condition, the productivity is improved and microbial contamination reduced (1).

Various methods to enhance the thermostability of the *Bacillus licheniformis* alpha-amylase have been extensively studied by addition of different external compounds (2). It has been reported that the environmental factors-especially water- play important roles in enzyme thermostability. Water has the dual effect on enzymatic system: it is essentional for acequisition and maintenance of enzyme's catalytically active conformation, and it is required for most enzyme inactivation processes (3). Additives that modify the structure of water or strengthen hydrophobic interactions inside protein molecules are known as stabilizing agents (4). It has been known for many years that sugars, polyhydric alchols, and organic solvents have effects on the thermal stability of enzyme (2, 5, 6). And, generally, enzymes increase its thermostability in the presence of substrates, products, and inhibitors at high concentrations (7).

In this report, we examined the thermostability of cyclodextrin glucanotransferase (CGTase) from *Bacillus stearothermophilus* in the presence of various additives and in organic solvents.

Key word: Cyclodextrin glucanotransferase, thermostability
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Materials and Methods

Materials

Sucrose, sorbitol and glycerol were purchased from Sigma Chemical Co. Ethylene glycol, n-butanol, 1,4-dioxane and n-octane were purchased from Kanto Chemical Co., Inc.

Enzyme

Cyclodextrin glucanotransferase from *Bacillus stearothermophilus* was purified by the method in the previous report (8).

Enzyme activity

The CGTase activity was measured by Kitahata and Okada method (9) at 55℃.

Study of thermal stability

CGTase thermal denaturation was measured by incubating the enzyme (at final concentration of 6 units per milliliter) in 100 mM sodium acetate buffer (pH 6.0) containing different additives at the desired temperatures. After 10 min incubation, the enzyme was removed, cooled on ice and remaining activity was determined.

Thermal inactivation in organic solvent was done as follows. An aqueous solution of enzyme was evaporated in Brinkmann rotary vaccum evaporator. The enzyme was dispersed in organic solvent. After incubation at 75℃, the sample was removed from bath and cooled. The solvent was evaporated as described above. The enzyme was resuspended in 100 mM sodium acetate buffer (pH 6.0) and measured the remaining activity.

Results and Discussions

The effects of ethylene glycol, glycerol, sorbitol and sucrose on the CGTase thermostability were investigated respectively. After incubation of the CGTase at 75℃ for 10 min in the presence of above additives at various concentrations, the remaining activity was measured by the method described previously. In the absence of any additives, the CGTase was completely denatured at 75℃ for 10 min. Glycerol, sorbitol and sucrose had postive effects on thermostability of the CGTase (Fig. 1). The CGTase thermostability increased with increasing concentrations, respectively. No inactivation occurred at 5 M sorbitol and 2 M sucrose, respectively. About 90% of enzyme activity was maintained in the presence of 10 M glycerol. Ethylene glycol had no effect on the thermal stability of CGTase even at very high concentration (15 M).

The effect of 10 M glycerol, 5 M sorbitol and 2 M sucrose on the CGTase thermostability was investigated respectively at the temperature from 60℃ to 95℃ (Fig. 2). The CGTase retained its full activity at the temperature up to 70℃ in the presence of 10 M glycerol, up to 80℃ in the presence of 5 M sorbitol and up to 75℃ in the presence of 2 M sucrose, respectively. After 10 min at 95℃ in the presence of 5 M sorbitol, about 70% of the initial activity remained, while no activity was detected in the presence of 2 M sucrose and 10 M glycerol, res-

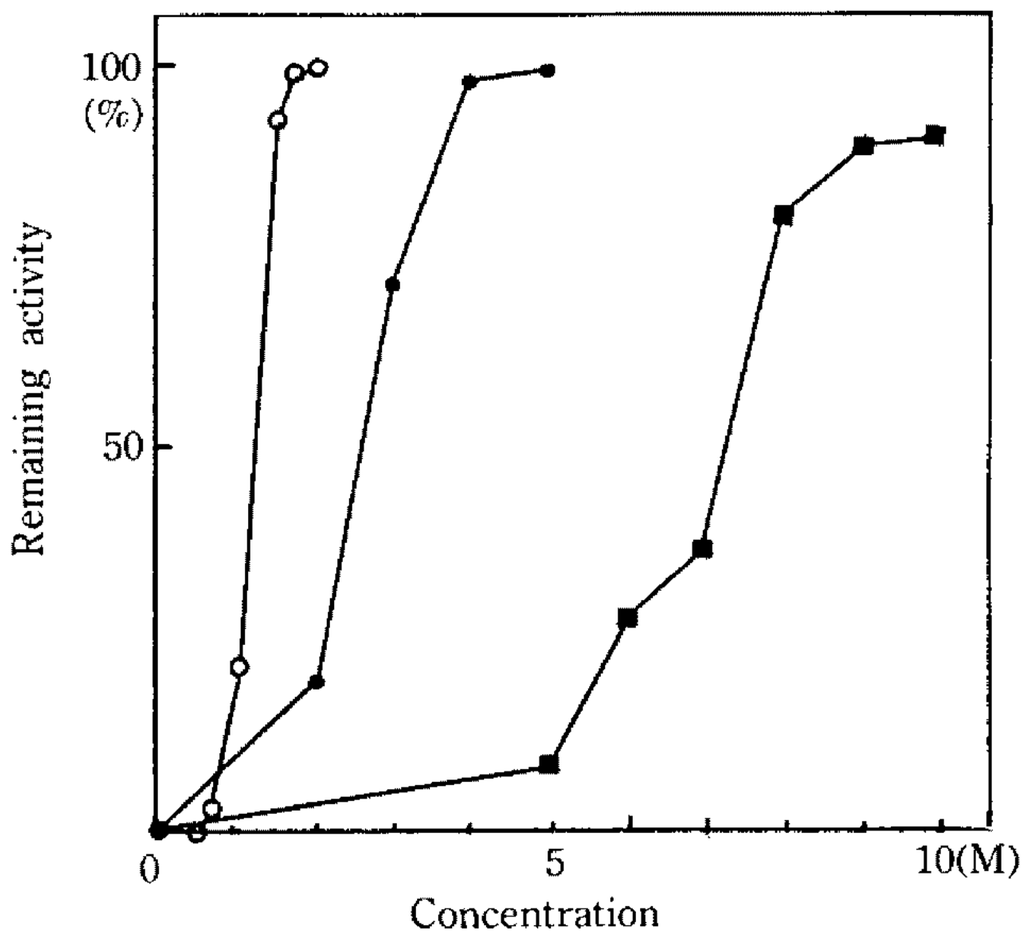

Fig. 1. Effect of sucrose (○—○), sorbitol (●—●) and glycerol (■—■) on CGTase thermostability at 75°C.

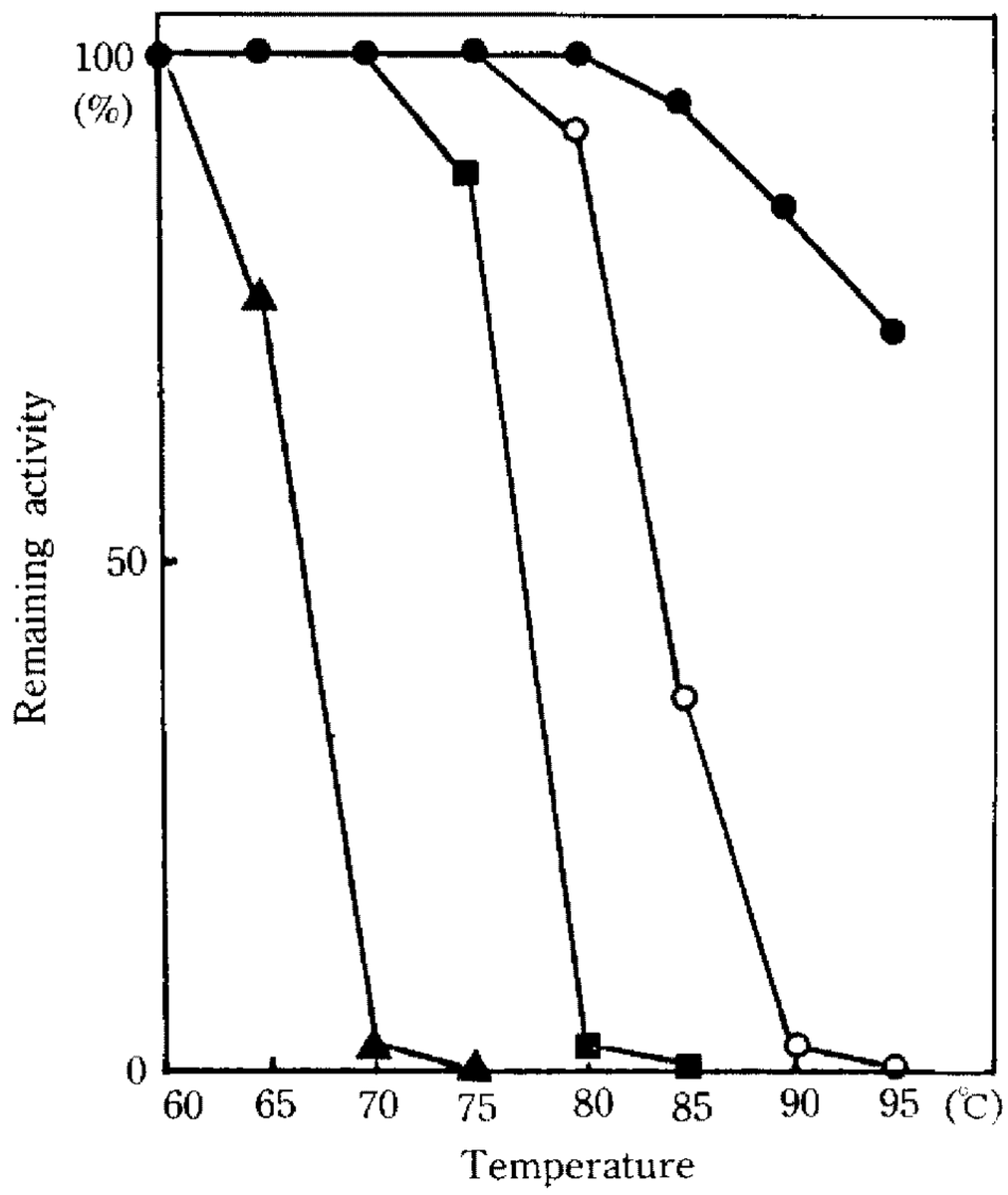

Fig. 2. Effect of temperature on CGTase thermostability in the presence of additives.
The CGTase was heated for 10 min at various temperatures (from 60℃ to 95℃) in the presence of 10 M glycerol (■—■), 5 M sorbitol (●—●), 2 M sucrose (○—○) or in the absence of any additive (▲—▲).

pectively. In the case of 10 M glycerol, the incubation at 80℃ for 10 min made the CGTase completely inactive. The CGTase maintained its 37% activity in the presence of 2 M sucrose after incubation at 85℃ for 10 min. The 5 M sorbitol had the most stabilizing effect on the thermostability of CGTase.

The protective effect of above additives on thermostability was known in the *Bacillus licheniformis* alpa-amylase (2) and *Aspergillus oryzae* alpa-amylase (9). It has been found that the stabilizing effect of above additives increased with their concentration and the sorbitol played the effective role in the thermostability of above alpa-amylases. But, in the case of glucose oxidase (10), different result was observed. Therefore, the intensity of the stabilization effect and the nature of the most effective additives seems to depend greatly on the enzyme tested and be not the absolute effect (9).

The mechanism by which sugars and polyhydroic alcohols stabilize proteins against heat denaturation may be through their effects on water, which, in turn, determines the strength of hydrophobic interactions (5). Solute exclusion (11) or solute interaction with the protein surface (12) has also suggested as the mechanism of stabilization or destabilization, respectively.

Several enzymes were known to have higher thermostability in organic solvents than in water (3, 13-15). In this study, we examined thermostability of the CGTase in various organic solvents such as n-butanol, 1,4-dioxane, n-octane. The enzyme was suspended in n-butanol, 1,4-dioxane and n-octane, respectively and incubated at 75℃. The thermostability of CGTase depended on the nature of solvent. n-Butanol completely inactivated the CGTase after incubation at 75℃ for 10 min (data not shown). 1,4-Dioxane and n-octane increased the thermostability of the CGTase. About 42% of the initial activity remained after incubation at 75℃ for 10 min in 1,4-dioxane (Fig. 3). n-Octane was most effective on the thermostability of the CGTase. The CGTase retained the 81% of the initial activity after incubation at 75℃ for 90 min (Fig. 3). The results suggested that thermostability seems to be related to the hydrophobicity of the solvent as in previous reports (3, 15). Hydrophobic solvent seems to be more effective on thermostability than hydrophilic solvent. It is not clear why the thermostability is increased in organic solvents. It seems that the conformational mobility of the enzyme is hindered by dehydration in organic solvents (2).

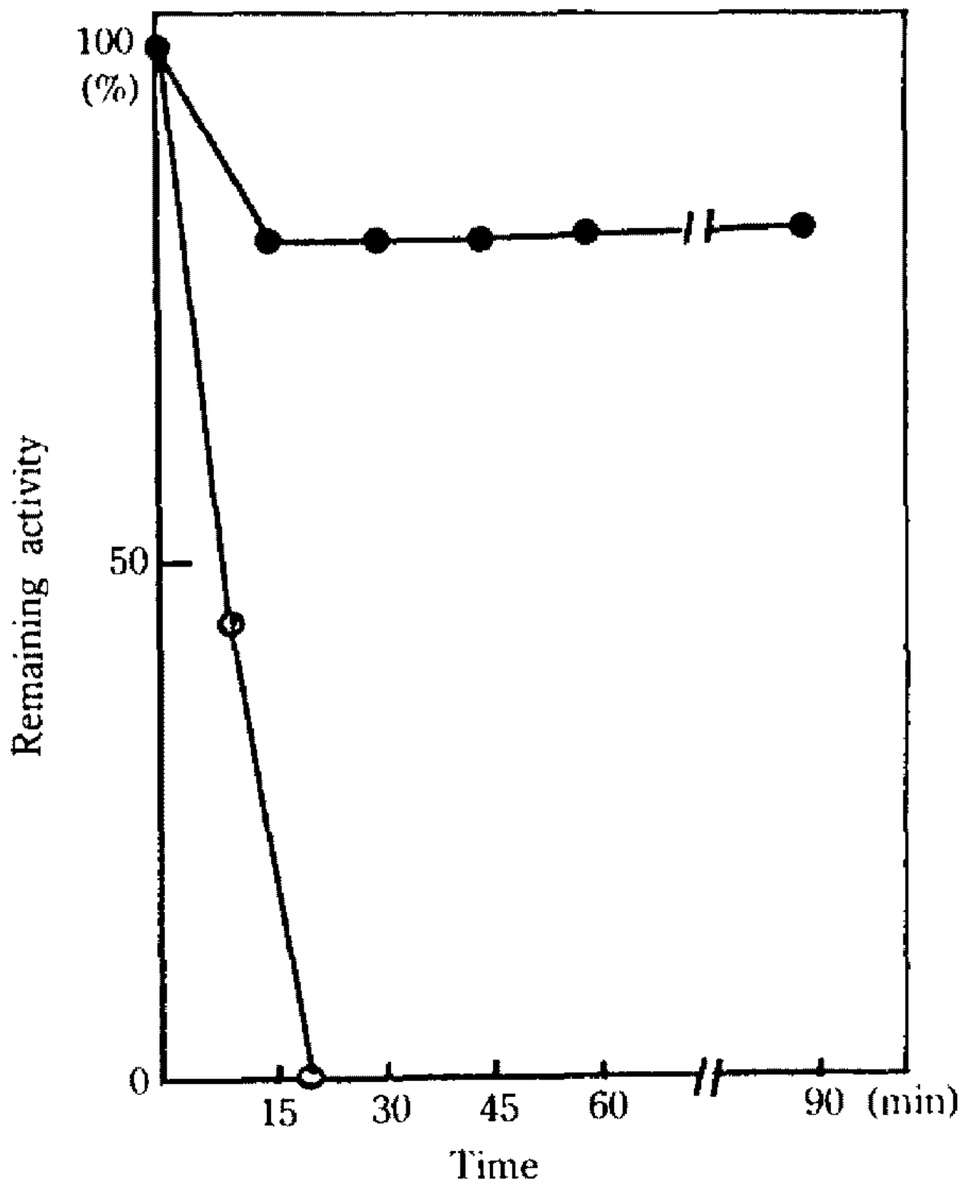

Fig. 3. The CGTase thermostability in n-octane (●—●) and 1,4-dioxane (○—○) as a function of time at 75°C.

요 약

Ethylene glycol, glycerol, sorbitol 그리고 sucrose가 *Bacillus stearothermophilus*가 생산하는 cyclodextrin glucanotransferase(CGTase)의 열안정성에 미치는 영향을 조사하였다. Glycerol, sorbitol 그리고 sucrose가 CGTase의 열안정성에 효과가 있었다. 이 효과는 첨가물의 농도와 밀접한 관계가 있었다. n-Butanol, 1,4-dioxane 그리고 n-octane과 같은 유기 용매에서 CGTase의 열안정성을 조사하였다. 1,4-dioxane과 n-octane은 CGTase의 열안정성을 증가시켰다. 특히, n-octane에서 CGtase를 75℃에서 90분간 정치시킨 후에도 원래 효소활성의 81%를 유지하였다.

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