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Purification of Streptodornase from *Streptococcus equisimilis* and Its DNA-Induced Conformational Change

Minjung Lee and Kyung Bin Song[1]
Department of Food Science and Technology, College of Agriculture, Chungnam National University, Taejon, 305-764, Korea

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Extracellular streptodornase was purified from fermentation media of *Streptococcus equisimilis* by stepwise carboxymethyl-Sepharose column chromatography. The active enzyme fraction was eluted with phosphate buffer containing 0.2 M NaCl. The purified enzyme showed a homogeneity on SDS-PAGE and had a subunit molecular weight of 35 kDa. Conformational change of streptodornase by binding to calf thymus DNA was examined by circular dichroism (CD). CD study clearly showed a DNA-induced conformational change in the secondary structure of streptodornase, resulting in a decrease of α-helical content of the enzyme. © 1997 Academic Press

Streptococci produce various proteins and enzymes into the extracellular environment (1-2). Especially, group C streptococci produce extracellular nuclease, streptodornase, which degrades both the RNA and DNA endonucleolytically and produces oligonucleotides (3). Streptodornase has been used commercially as a therapeutic agent in combination with streptokinase, especially for breaking down the blood clots. The pharmaceutical industry has much difficulty in scale-up production of streptodornase due to low yield and impurity. Compared to many studies on streptokinase (4-7), there are few reports available on streptodornase (8). Thus, better understanding on molecular properties of streptodornase is needed.

There have been many studies on the DNA-induced conformational changes in the protein using circular dichroism to elucidate DNA-protein interaction and examine the DNA binding site of the protein (9-14). In this study, streptodornase was purified from fermentation media of *Streptococcus equisimilis* by stepwise CM-Sepharose column chromatography. To better understand molecular properties of streptodornase and to examine the DNA binding region of streptodornase, the secondary structures of streptodornase in the absence and presence of the DNA were examined. We find that streptodornase has a molecular weight of 35 kDa and DNA-inducedconformational change of streptodornase was significant, resulting in change of the secondary structure of the enzyme.

[1] To whom correspondence should be addressed. Fax: 82-42-825-2664. E-mail: kbsong@hanbat.chungnam.ac.kr.

Abbreviations: CD, circular dichroism; CM-Sepharose, carboxymethyl Sepharose; SDS-PAGE, sodium dodecylsulfate-polyacrylamide gel electrophoresis.

EXPERIMENTAL PROCEDURE

Purification of streptodornase. After anaerobic fermentation of *Streptococcus equisimilis,* the fermentation medium was centrifuged, micro-filtered, ultra-filtered, and applied to CM-Sepharose column (1.5 × 30 cm) equilibrated with 20 mM phosphate buffer (pH 7.5). The enzyme was eluted with stepwise of 0 to 1 M NaCl in the same buffer. The enzyme fraction with the highest specific activity was pooled and lyophilized.

Assay of streptodornase activity. Activity of streptodornase was assayed as described by Kunitz (15). To the reaction mixture containing calf thymus DNA, 1 mM $MgSO_4$, 1 mM $CaCl_2$ in 0.1 M glycine buffer (pH 9.0), 10 μl of enzyme solution was added and incubated at 37 °C for 10 min. The reaction was stopped by the addition of 80 μl of 5 N perchloric acid and kept at −20 °C for 5 min. After centrifugation (12,000 rpm, 10 min), the amount of acid-solubilized fraction was determined by optical density of the supernatant at 260 nm.

Circular dichroism study. Circular dichroism (CD) spectroscopy was performed on a spectropolarimeter (JASCO J-720). Streptodornase samples were prepared in a buffer containing 50 mM Tris-HCl buffer(pH 7.5) in the absence and presence of calf thymus DNA and were scanned at 20°C A cell pathlength of 1 mm was used. Far UV CD spectra were obtained from the average of six scans after subtraction of a buffer blank and DNA sample.

RESULTS AND DISCUSSION

Purification of streptodornase was performed in this study since there have been much difficulty in purifying the streptodornase. After anaerobic fermentation of *Streptococcus equisimilis,* the broth was centrifuged, micro-filtered, ultra-filtered, and applied to CM-Sepharose column (1.5 × 30 cm) equilibrated with 20 mM

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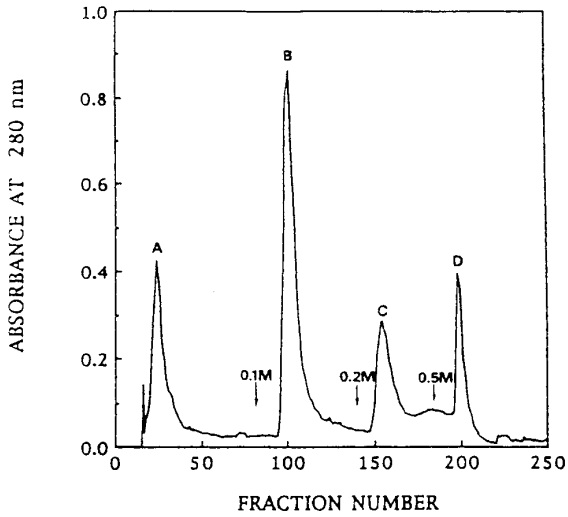

FIG. 1. CM-Sepharose column chromatography profile of crude streptodornase extracts. Crude streptodornase solution was applied on a CM-Sepharose column (1.5 × 30 cm) equilibrated with 20 mM phosphate buffer, pH 7.5. The active enzyme was eluted with a stepwise salt gradient from 0 to 1M. The specific activities (× 1000 units/mg) of elution peaks were as follows: peak A, 21; B, 37; C, 487; D, 58.

phosphate buffer (pH 7.5). CM-Sepharose column elution profile indicates that 0.2 M elution peak has the highest enzyme activity (Figure 1). Also, SDS-PAGE pattern clearly indicates that 0.2 M fraction has a single band, while 0.1 M elution peak contains 47 kDa protein and 30 kDa protein (Fig. 2). The stepwise elution could eliminate impurities resulting in pure streptodornase by 0.2 M elution. Therefore, this method is suggested for scale-up production of streptodornase in pharmaceutical industry where there have been many problems such as low yield and impurity in purifying streptodornase. Also, the subunit molecular weight of streptodornase was determined to be about 35 kDa (Fig. 2).

To elucidate the binding property of streptodornase

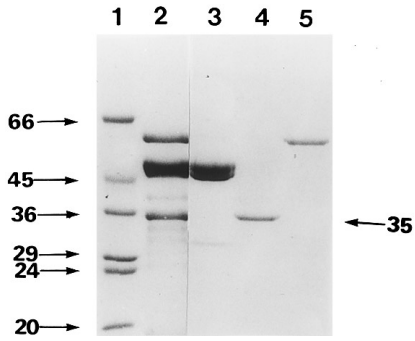

FIG. 2. SDS-PAGE profile of streptodornase isolated from Streptococcus equsimilis. Lane 1, molecular weight marker; 2, crude extracts; 3, 0.1 M elution; 4, 0.2 M elution; 5, 0.5 M.

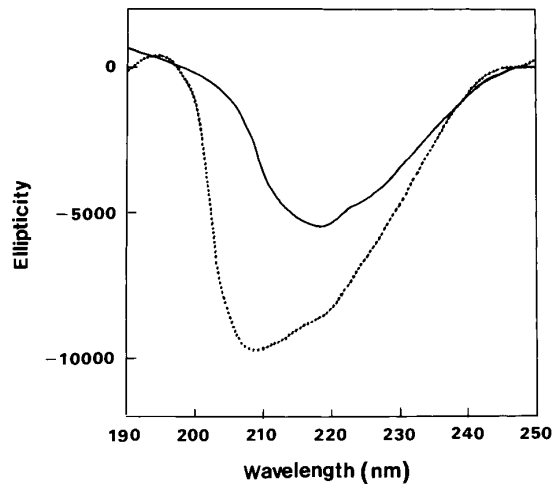

FIG. 3. Circular dichroic spectra of the purified streptodornase in the absence (---) and presence (—) of calf thymus DNA.

to DNA and to examine DNA-protein interaction, CD study was performed using the purified enzyme. Far-UV CD spectra of streptodornase are shown in Fig. 3. The spectrum of streptodornase in the absence of DNA had a negative minimum ellipticity value at 207 nm with a shoulder at 222 nm, indicating the presence of α-helical structure. To further examine the secondary structure content of the enzyme, the spectrum was further anayzed by the method of Chang et al (16). The secondary structure estimation of streptodornase showed 25% α-helix, 5% β-sheet, 30% β-turns and 40% aperiodic structure. However, in the presence of calf thymus DNA, addition of DNA to the enzyme caused decrease in molar ellipiticity values between 205 and 225 nm, which suggests a loss of α-helical secondary structure. This difference is not attributed to DNA itself or a change in DNA structure, since DNA spectrum showed only negligible ellipiticty values and was subtracted. Also, the spectrum in the presence of DNA had a negative minimum ellipticity value at 217 nm, indicating the increase of β-sheet content of the enzyme. Addition of DNA also caused about 35% decrease in molar ellipticity at 222 nm, indicating a decrease in α-helical content. Indeed, the estimation of the secondary structure of the enzyme in the presence of DNA showed 10% α-helix, 55% β-sheet, 10% β-turns and 25% aperiodic structure. These results suggest that the addition of DNA to the enzyme was accompanied by a conformational change in the enzyme and resulted in a decrease in α-helix content with concurrent increase in β-sheet content of the enzyme.

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