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Note

Molecular Properties of Streptococcal Nuclease Isolated from *Streptococcus* sp.

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Molecular properties of streptococcal nuclease purified from *Streptococcus* sp. were examined. The purified enzyme was stable in the range of pH 7 to 10 and easily inactivated above 60°C. Atomic spectroscopy analysis indicated that the enzyme contains Ca, Mg, Zn. Circular dichroism study showed 25% α-helix, 15% β-sheet and 30% β-turns.

Streptococci produce various proteins into the extracellular environment (11, 12). Especially, group C *streptococci* produce extracellular nuclease (streptodomase), which degrades both the RNA and DNA endonucleolytically and produces oligonucleotides of which the average chain length is about 5 as a product(5). Streptodomase (SD) plays an active role in the lysis of materials that is caused by organic disorders in mammals and acts directly on a substrate of nucleic acid and nucleoprotein, which are the major constituents of the sediment of purulent exudates. Commercially, SD is used therapeutically in combination with streptokinase, especially for breaking down the blood clots. While there have been many studies on streptokinase due to its commercial importance(1, 2, 4, 10), there are very few reports available on SD.

DNase activity is observed in the supernatant from cultures of the streptococci group A, B, C, D, E (1, 7, 13). Regarding the streptococci group C, even though the isolation and nucleotide sequence of the SD gene was reported (14), there are no reports available on the molecular properties of SD.

In this study, to better understand SD, the molecular properties of SD were examined using a purified SD. We report that SD is indeed a metallo-enzyme and contains considerable amount of ordered secondary st ructure.

Purification of Streptodornase

After fermentation of *Streptococcus* sp., the broth was pretreated to remove cell debris and applied to CM-Sepharose column. The active enzyme fraction was eluted with stepwise NaCl gradient of 0.2 M, pooled, and lyophilized (8). The molecular weight of the purified SD was determined to be about 35,000 by the SDS-PAGE (Fig. 1). The purity of SD used in this study was more than 95%.

Assay of Streptodornase Activity

Activity of SD was assayed as described by Kunitz (6). To the reaction mixture (0.8 ml) with calf thymus DNA in sterilized distilled water, 0.69 ml of 0.1 M glycine buffer (pH 9.0) containing 0.8 μmol $MgSO_4$, 0.8 μmol $CaCl_2$ and 10 μl of enzyme solution was addded and incubated at 37°C for 10 min. The reaction was stopped by adding 80 μl of 5 N perchloric acid and was kept at -20°C for 5 min. After centrifugation (12,000 rpm, 10 min), the amount of acid-solubilized fraction was determined by the optical density of the supernatant at 260 nm.

pH and Temperature Stability

The optimum pH and temperature for the purified enzyme were pH 9.0 and 50°C, respectively (8). SD was stable in the range of pH 7 to 8. However, it was not stable at an acidic pH (Fig. 2). Thermal inactivation kinetics study of SD (Fig. 3) showed that at 40°C SD was relatively stable. However, at 50°C after 10 min, half of its activity was lost. Also, incubation of SD at 60°C for 10 min inactivated SD up to 90%. This clearly indicates that SD is not heat resistant.

Molecular Properties

To examine the secondary structure of SD, Circular Dichroism (CD) study was performed using a spectropolarimeter (JASCO J-600). Far UV-CD spectrum of SD is shown in Fig. 4. The spectrum had a negative minimum ellipticity value at 217 nm, indicating the presence of β-sheet structure. To further examine the secondary structure content of SD, the spectrum was further anayzed

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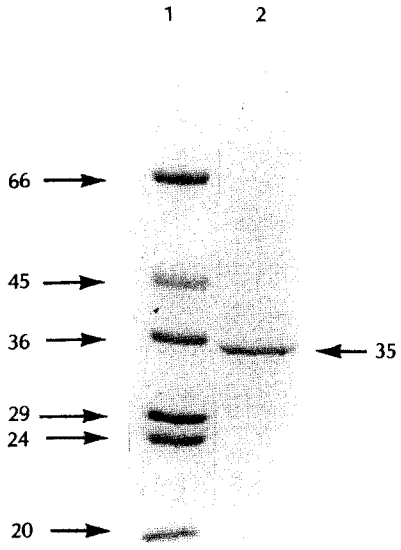

Fig. 1. SDS-PAGE of SD isolated from *Streptococcus* sp. Lane 1; molecular weight marker, 2; purified SD.
The following marker proteins were used; BSA (66 kDa), egg albumin (45 kDa), phosphate dehydrogenase (36 kDa), carbonic anhydrase (29 kDa), trysinogen (24 kDa), trypsin inhibitor (20 kDa).

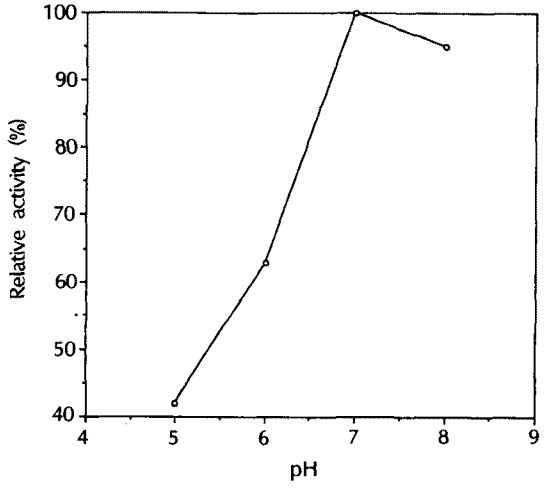

Fig. 2. pH stability of the purified SD.
The enzyme activity was determined after incubation of the enzyme solution at the given pH for 1 hour.

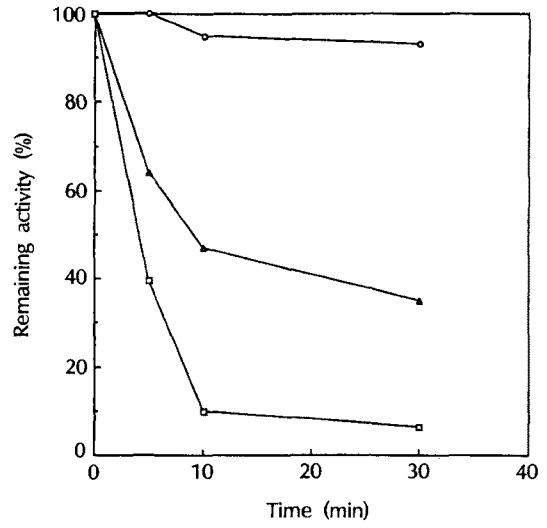

Fig. 3. Temperature stability of the purified SD.
The enzyme solution was incubated at the given temperature. The remaining enzyme activity was measured after taking out the aliquot of sample at various time interval. Key: ○, 40°C; △, 50°C; □, 60°C.

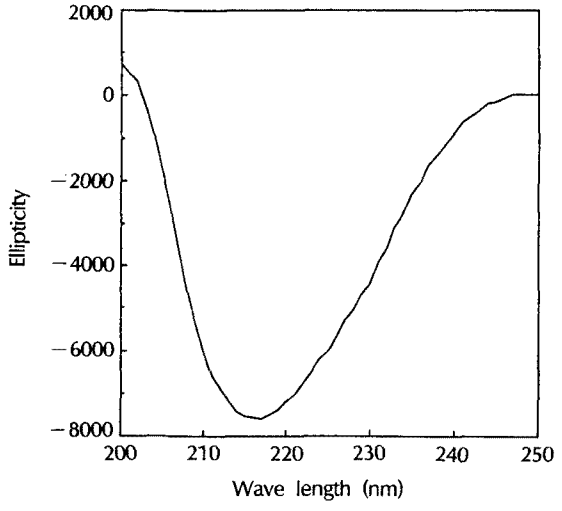

Fig. 4. Circular dichroic spectrum of the purified SD.
Far-UV CD spectrum was obtained using a JASCO J-600 spectropolarimeter. Protein concentration was 0.0046% and path-length was 1 mm.

by the method of Chang et al (3). The secondary structure estimation of SD showed 25% α-helix, 15% β-sheet, 30% β-turns and 30 % aperiodic structure.

In view of the theory that SD requires Ca and Mg to activate (9), a hypothesis that SD may be a metalloenzyme, could be deduced. In order to confirm this, metal analysis was performed using a ICP type atomic spectrometer (Leeman Labs PS1000). Among the metal checked, SD contained 0.77 mole of Zn, 5.76 mole of Ca and 1.98 mole of Mg per mole of an enzyme. This indicates that SD is indeed a metalloenzyme, which contains a considerable amount of Ca and Mg and small amount of Zn. The roles of these metals requires further study.

To determine the amino acid content of SD, amino acid analysis was performed after acid hydrolysis at 110°C for 24 h (Picotag amino acid analysis system, Waters Association Co.). Table 1 shows the amino acid composition of SD. Compared to the sequence of SD previously reported in the sequence of SD reported in the literatures, Gly content was unusually high, while other amino acid contents were in relatively good agreement. However, slight variation may be attributed to the difference in the strain of streptococcal species.

Table 1. Amino acid composition of the purified streptodornase

	sequence*	amino acid analysis (%)
Asx	12.2	11.36
Glx	10.4	10.08
Ser	8.6	7.34
Gly	6.4	19.73
His	1.5	0.97
Arg	4.3	3.71
Thr	8.3	7.03
Ala	5.2	4.44
Pro	3.1	2.67
Tyr	6.4	3.87
Val	9.5	6.8
Met	1.8	1.29
Ile	5.8	4.61
Leu	4.9	4.86
Phe	3.4	2.69
Lys	7.6	7.38

*obtained from reference 14.

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