New Amylase Inhibitor (S-AI) from *Streptomyces diastaticus* var. *amy-* *lostaticus* No. 2476

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Many investigations on amylases were carried out and a large amount of information has been obtained. But the studies on amylase inhibitor have been limited; proteinaceous substances or high molecular organic acids were found to be present as amylase inhibitors in such plants as buckwheat,¹⁾ wheat,²⁾ rye,²⁾ some of sorghums^{2,3)} and potato tuber.⁴⁾ No amylase inhibitor produced by microorganisms had been found, but recently Niwa *et al.* reported that "Nojirimycin"⁵⁾ (5-amino-5-deoxy glucopyranose) had an inhibitory activity on some amylases, and Ueda *et al.* found another inhibitor,⁶⁾ a peptide like substance with the molecular weight of about 2000.

The authors tried to isolate new amylase inhibitor-producing microorganisms and were successful in obtaining a strain which produced amylase inhibitor in the culture broth. The morphological and physiological characteristics of this strain were taxonomically studied according to "The Actinomycetes" Vol. 2⁷⁾ edited by Waksman. This strain was identified as a variety of *Streptomyces diastaticus* and was named as *Streptomyces diastaticus* var. *amylostaticus* No. 2476. This amylase inhibitor was abbreviated as S-AI.

The assay system for S-AI was as follows; a half ml of 0.002% bacterial liquefying α -amylase ($3\times$ recrystallized, Seikagaku Co.) dissolved in 0.2 M acetate buffer (pH 5.5) was incubated with 0.5 ml of S-AI solution at 37°C for 10 min, and the residual amylase activity was assayed by the modified blue value method.⁸⁾ One inhibitor unit was defined as

the amount by which the α -amylase was inhibited 50% of its original activity in this assay system.

When the strain was aerobically cultured in a shaking flask, containing 100 ml of a medium which consisted of 4% corn starch, 2% soy bean flake extract, 0.3% NaCl, 0.1% K_2HPO_4 , 0.05% $MgSO_4 \cdot 7H_2O$, 0.001% $FeSO_4 \cdot 7H_2O$, 0.0001% $CuSO_4 \cdot 5H_2O$, 0.0001% $ZnSO_4 \cdot 7H_2O$ and 0.0001% $MnSO_4 \cdot nH_2O$ (pH 7.0) at 30°C, the highest activity of S-AI was obtained after 72~80 hr cultivation. The amount of total S-AI in the culture filtrate was about 900 mg/liter.

In order to isolate S-AI, the culture filtrate was adjusted to pH 3.0, and active carbon (3% w/v) was added to the filtrate to adsorb S-AI on it. Then S-AI was eluted from active carbon with 60% (v/v) ethanol solution. The S-AI containing solution was concentrated and desalted by gel filtration on Sephadex G-25. After the crude S-AI was incubated with crystalline bacterial liquefying α -amylase at 37°C in 0.005 M acetate buffer (pH 5.5), the incubation mixture was applied on a Sephadex G-75 column, previously equilibrated with the same buffer. This gel filtration pattern is given in Fig. 1. The enzyme-inhibitor complex fractions (No. 20~32) were collected and cold acetone was added to a final concentration of 66% (v/v). The precipitate was dissolved in water, and this complex solution was adjusted to pH 1.8. After being kept at

FIG. 1. Column Chromatogram of Amylase-inhibitor Complex on Sephadex G-75.

○—○, inhibitory activity; ●—●, protein (O.D. at 280 nm); ◐—◐, sugar (O.D. at 490 nm).

TABLE I. EFFECT OF S-AI ON VARIOUS GLUCOSIDE HYDROLASES

A half ml of each enzyme solution was incubated with 0.5 ml of S-AI solution (60 μ g/ml) at 37°C for 10 min and the residual enzyme activity was assayed. Inhibitory ratings used were as follows: +, 80~100%; -, 0% inhibition of enzyme activity.

Enzyme (Origin)	pH	Inhibition
α-Amylase		
Bacterial liquefying amylase	5.5	+
Bacterial saccharifying amylase	5.5	+
Taka-amylase A	5.5	+
<i>Asp. niger</i> , acid stable amylase	5.5	+
<i>Asp. niger</i> , acid unstable amylase	5.5	+
<i>Bacillus stearothermophilus</i> , amylase	5.5	+
Malt amylase	5.5	+
Saliva amylase	7.0	+
Hog pancreas amylase	7.0	+
β-Amylase		
Wheat	5.5	-
Soy bean	5.5	-
Gluco-amylase		
<i>Rhizopus niveus</i>	5.5	+
Isoamylase		
<i>Aerobacter aerogenes</i>	5.5	-
α-Glucosidase		
<i>Saccharomyces cerevisiae</i>	6.8	-
Dextranase		
<i>Penicillium</i> sp.	5.5	-
Cellulase		
<i>Tricoderma</i> sp.	4.5	-
Laminalinase		
<i>Arthrobacter luteus</i>	7.5	-
β-Glucosidase		
Armond	5.5	-

37°C for 15 hr, the complex solution was adjusted to pH 5.5 and filtered. S-AI was separated from the E-I complex through this acid denaturation method, and was also separated from the complex by heating and acidic 8 M Urea denaturation method. After being desalted, the S-AI solution was adjusted to pH 2.0 and applied on a Dowex 50W $\times$ 2 (H-type) column, previously equilibrated with a solution of pH 2.0, then S-AI was eluted with the same solution. S-AI containing fractions were collected, desalted, and lyophilized. Thus S-AI was obtained as white powder. Homogeneity of this S-AI was examined by means of TLC with Kieselgel 60 (Merck Co.), where S-AI gave a single spot in two solvent

systems (*n*-butanol: DMSO*: water =2:1:1, ethyl acetate: methanol: water =37:40:23).

Effect of S-AI on various glucoside hydrolases was examined, and results are shown in Table I. S-AI had inhibitory activity on α -amylases and glucoamylase, but had not on β -amylase, which was found to hydrolyze S-AI.

S-AI is a very stable substance, as it retained 100% of its original inhibitory activity after being kept for 30 min at 100°C in a pH range between 3.0 and 10.5. The molecular weight of S-AI was estimated to be about 1500 from the data by gel filtration on Sephadex G-25 or Biogel P-6.

S-AI was regarded to be an oligosaccharide which was mainly composed of glucose in an amount of about 80~85%. Minor component (15~20%) are now under investigation. The structure of glucose-polymer parts in S-AI was examined by the method of periodate oxidation⁹⁾ and O-methylation,¹⁰⁾ and it was presumed that S-AI was composed of about eight moles of glucose and this oligomer had 1, 4 linkage in it. As mentioned above, S-AI was hydrolyzed by the action of β -amylase. The hydrolyzate was assayed by means of TLC, where two moles of maltose were released from one mole of S-AI. This fact suggested that at least four glucosidic linkages in S-AI was of α -form. Further, from the GLC analysis of the reduced S-AI, the glucosidic residue of reducing terminal in S-AI seems to be glucose.

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* Dimethyl sulfoxide.

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