Short Communication

New Proteinous Inhibitor (Haim) of Animal α -Amylase from *Streptomyces griseosporus* YM-25Sawao MURAO, Akira GOTO,
Yoshihiro MATSUI* and Kunio OHYAMA**Department of Agricultural Chemistry,
College of Agriculture,
University of Osaka Prefecture,
Sakai, Osaka 591, Japan

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Various amylase inhibitors have been obtained from microbial origins. They are composed of amino sugar,¹⁾ oligosaccharide,^{2,3)} or glycopeptide.⁴⁾ Therefore, they should be considered to belong to substrate analogs. They have a relatively broad specificity to show the inhibitory effect on various glucoside hydrolases, such as α -amylases produced from animals, plants, or microbes. On the other hand, proteinous amylase inhibitors are known to occur in many plants^{5~9)} and most of them are specific for animal α -amylases. But there has been no report on inhibitors of microbial origins, specific for animal α -amylase. Recently we isolated microorganism which produced inhibitors highly specific for animal α -amylase. The strain, YM-25, selected was tentatively identified as *Streptomyces griseosporus*. The α -amylase inhibitor produced by this strain was a proteinous substance and was designated as Haim (Hog pancreatic α -amylase inhibitor of microbe). This paper describes the purification and some properties of Haim.

The assay system of Haim used was the similar procedure as reported previously,²⁾ except for using hog pancreatic α -amylase ($2 \times 10^{-4}\%$; Sigma, twice cryst.; liberating 1 g

maltose from soluble starch/mg/min at 37°C) instead of BLA. One unit of inhibitor activity was defined as the quantity of inhibitor required to reduce the amylase activity by 50% under the above condition. The strain YM-25 was aerobically grown at 30°C for 24 hr in a medium consisting of 5% glycerol, 0.5% peptone, 0.2% meat extract, 0.1% yeast extract, 0.4% $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$, 0.1% KH_2PO_4 , and 0.05% $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ (pH 7.3). Haim was purified from culture broth as follows: The culture filtrate was brought to 80% saturation with ammonium sulfate and resulting precipitate was dissolved in water and dialyzed against 50 mM phosphate buffer (pH 6.3) containing 100 mM NaCl. The dialyzed solution was passed through a DEAE-cellulose column equilibrated with the same buffer as described above. In this procedure, most of pigments were adsorbed. The active fraction was dialyzed against 5 mM phosphate buffer (pH 6.3). The dialyzed solution was adsorbed on a DEAE-cellulose column equilibrated with the same buffer. Then Haim was eluted with a linear gradient of NaCl with a concentration from 0 to 0.5 M in the same buffer. The active fraction of α -amylase inhibitor dialyzed against 5 mM phosphate buffer (pH 6.3) was applied to a TEAE-cellulose column and the inhibitor adsorbed was eluted by a linear gradient of NaCl with a concentration from 0 to 0.3 M in 5 mM phosphate buffer (pH 6.3). The typical chromatographic pattern is shown in Fig. 1. The inhibitory activity was separated into two fractions, I and II. Each fraction was individually re-chromatographed on TEAE-cellulose columns under the same conditions as described above. Fractions I and II were then purified by gel filtration using a column of Sephadex G-50 equilibrated with distilled water. The specific activities for these final preparations were 4650 units/mg for Haim I and 4800 units/mg for Haim II. The final inhibitor preparations were submitted to polyacrylamide gel disc electrophoresis at pH 2.3 and pH 8.0. A single band was obtained for each inhibitor.

Effects of Haim I and II on various

* Present address: Glico Dairy Co. Ltd., 300 Jyogawara, Akishima, Tokyo 196, Japan.

** Present address: Toyo Jozo Co. Ltd., Research Laboratories, Ohito-cho, Tagata-gun, Shizuoka 410-23, Japan.

FIG. 1. Chromatogram of Haim on a TEAE-Cellulose Column.

Crude Haim was applied on a column (2.6 × 40 cm) of TEAE-cellulose equilibrated with 5 mM phosphate buffer (pH 6.3). Haim was eluted with NaCl linear gradient.

○—○, inhibitory activity; -----, absorbance at 280 nm; -----, NaCl concentration.

TABLE I. EFFECTS OF HAIM I AND II ON VARIOUS GLUCOSIDE HYDROLASES

A mixture of 0.5 ml of the enzyme solution indicated and 0.5 ml of Haim I or II solution (each 20 µg/ml) was incubated at 37°C for 5 min, and the residual enzyme activity was assayed. The degrees of inhibition: +, 80~100%; —, no inhibition.

Enzyme (Origin)	pH	Inhibition	
		Haim I	Haim II
α -Amylase			
Hog pancreas	7.0	+	+
Hog blood	7.0	+	+
Human saliva	7.0	+	+
Human pancreatic juice	7.0	+	+
Human urine	7.0	+	+
Horse pancreas	7.0	+	+
Cow pancreas	7.0	+	+
Cat pancreas	7.0	+	+
Bacterial liquefying	5.5	—	—
Bacterial saccharifying	5.5	—	—
Taka-amylase A	5.5	—	—
Malt	5.5	—	—
β -Amylase			
Wheat	5.0	—	—
Sweet potato	5.0	—	—
Glucoamylase			
<i>Rhizopus niveus</i>	5.5	—	—
Pullulanase			
<i>Aerobacter aerogenes</i>	5.5	—	—
Cellulase			
<i>Trichoderma</i> sp.	4.5	—	—
Dextranase			
<i>Penicillium</i> sp.	5.5	—	—

glucoside hydrolases is summarized in Table I. Haim I and II showed the inhibitory activity toward α-amylases of animal origins, but showed no activity toward α-amylases of plant and microbial origins. Therefore, Haim was considered to be specific for animal α-amylase.

The molecular weight of Haims was estimated to be about 8,500 for both of I and II by the gel filtration method on Sephadex G-50 and SDS-polyacrylamide gel electrophoresis. The isoelectric points of I and II of Haim were determined to be pH 4.0 and pH 3.8, respectively, by means of isoelectric focusing. Amino acids analysis revealed that I and II were very similar in the composition: Both Haim I and II contained a large amount of aspartic acid (15.4; 15.0 mol%) and alanine (12.8; 12.5 mol%), and contained neither lysine nor methionine. As mentioned above, Haim was different from α-amylase inhibitors from plants^{5~9)} in the molecular weight and amino acid composition. From the negative color reaction in the phenol-sulfuric acid and Elson-Morgan's test, Haim was concluded not to be a glycoprotein. Therefore, the inhibition mechanism of Haim may be attributed to protein-protein interaction, contrary to that of substrate analog inhibitors.

These results suggest Haim to be a novel inhibitor for animal α-amylase. More detailed investigation on Haim will be published elsewhere.

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