Some Characteristics of an Alpha-amylase Inhibitor from *Phaseolus vulgaris* (Cultivar Great Northern) Seeds

Yukio Furuichi,† Masaharu Takeamura, Naoko Uesaka, Kazuo Kamemura, Shigeru Shimada, Hiroshi Komada,* Hisataka Ohta,* Yasuhiko Itoh,** Hayato Umekawa, and Takao Takahashi

Laboratory of Nutrition Chemistry, Department of Agricultural Chemistry, Faculty of Biosources, Mie University, Mie 514, Japan

*Department of Biology, Suzuka University of Medical Science and Technology, Mie 510–02, Japan

**Laboratory of Microbiology, Faculty of Medicine, Mie University, Mie 514, Japan

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Many edible plant seeds contain substances that inhibit protease or amylase action. In contrast to trypsin inhibitors, amylase inhibitors are not as well understood, and little is known about their structural features. For instance, the complete primary structure of no *Phaseolus vulgaris* α-amylase inhibitor has been identified to date.

Among a large number of legumes investigated, the largest amount of anti-amylase activity was found in some cultivars of *P. vulgaris*. Marshall and Lauda purified an α-amylase inhibitor from Great Northern beans and reported some information, but did not mention fundamental properties such as subunit structure or isoelectric point. It has been shown that the characteristics of amylase inhibitors are considerably variable among cultivars of *P. vulgaris*. In this paper we describe some characteristics of a purified α-amylase inhibitor from Great Northern beans and compared it with those of *P. vulgaris* amylase inhibitors from cultivars such as black bean, white bean, cranberry bean, red kidney bean, Kintoki bean, Greensleaves bean, white kidney bean, and Tendersgreen bean.

An α-amylase inhibitor was isolated from powder of Great Northern beans by water extraction at pH 2.0, ammonium sulfate fractionation (0.25–0.50 satn), ion-exchange chromatography on DEAE-cellulose (DE52, Whatman) and finally by gel filtration on Sephacryl S-200 (Pharmacia, Uppsala, Sweden) as summarized in Table 1. The purified inhibitor proved to be homogeneous by both alkaline and acidic polyacrylamide gel electrophoresis as shown in Fig. 1 (A) and (B).

A purified porcine pancreatic α-amylase was obtained from commercial pancreatin by glycogen-amylase complex formation as described by Loyter and Schramm. The molarity of the α-amylase solution was calculated from the absorbance at 280 nm, on the basis of a molecular mass of 52 kDa and \( A = 24.0 \) (1%, 280 nm) by the method of Elodi et al.

Amylase and amylase inhibitory activities were measured essentially by the method of Dahlqvist using soluble starch as a substrate. One unit of inhibitor was defined as the amount of inhibitor that gave 50% inhibition of the activity of 1 μg of purified porcine pancreatic amylase.

The isoelectric point of the purified inhibitor was found to be 4.42 by isoelectric focusing done by the method of Vesterberg and Svensson with a carrier ampholyte of pH 4–6. Our result is very close to the value that had been obtained by Lajoro and Fillo with black bean inhibitor (4.35) and lower than that observed by Pick and Wöber with white bean inhibitor (5.20). Kotaru et al. obtained 4.68 with cranberry bean.

Table 1. Purification of α-Amylase Inhibitor from Great Northern Beans

<table>
<thead>
<tr>
<th>Purification step</th>
<th>Protein (mg)</th>
<th>Unit (U)</th>
<th>Specific activity (U/mg)</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude extract</td>
<td>45,700</td>
<td>130,000</td>
<td>2.84</td>
<td>100</td>
</tr>
<tr>
<td>(NH₄)₂SO₄ fractionation</td>
<td>3,000</td>
<td>44,000</td>
<td>14.7</td>
<td>33</td>
</tr>
<tr>
<td>DEAE-cellulose</td>
<td>130</td>
<td>3,500</td>
<td>26.9</td>
<td>2.65</td>
</tr>
<tr>
<td>Sephacry-S-200</td>
<td>49</td>
<td>3,300</td>
<td>67.3</td>
<td>2.52</td>
</tr>
</tbody>
</table>

(from 450 g Great Northern beans)

† To whom correspondence should be addressed.

Fig. 1. Electrophoretic Profiles of *P. vulgaris* α-Amylase Inhibitor from Great Northern Beans.

(A) Polyacrylamide gel electrophoresis under alkaline conditions
(B) Polyacrylamide gel electrophoresis under acidic conditions
(C) SDS-PAGE: Protein was stained with Coomassie brilliant blue.
(D) SDS-PAGE: Carbohydrate was stained with periodic acid-Schiff’s reagent (PAS).

SDS-PAGE was done by the method of Schägger and von Jagow. Molecular mass marker protein kit was from LKB-Produktber, AB, Bromma, Sweden: myoglobin II, 6.2 kDa; myoglobin I, 8.2 kDa; myoglobin I & II, 14.5 kDa; myoglobin, 17.0 kDa, bovine carbonic anhydrase, 31.0 kDa. The electrophoresis was done in the presence of 2-mercaptoethanol.
chromatography as derivatives of aldito acid acetates showed that the inhibitor contains mannose exclusively. Wilcox and Whitaker\textsuperscript{19} also found a high-mannose type amylase inhibitor in red kidney beans. Yamaguchi\textsuperscript{19} reported that one of the two subunits of white kidney bean inhibitor has a large amount of mannose.

Subunit structure of the amylase inhibitor was analyzed by the tricine-sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), suitable for the separation of lower molecular mass polypeptides, according to Sch{"a}gger and Jagow.\textsuperscript{40} The subunits proteins in the gel were transferred to a poly(vinylidene difluoride) membrane (Immobilon, Millipore) and stained for protein and carbohydrate.\textsuperscript{23} The amylase inhibitor was found to be composed of at least four different kinds of subunit of 16.0, 15.5, 13.5, and 12.5 kDa as shown in Fig. 1 (C). Two lower molecular mass subunits are shown clearly to be glycoproteins as shown by PAS staining (Fig. 1 (D)). The carbohydrate contents of other subunits were shown to be very low, since they were stained only slightly by PAS. The highest molecular weight component (corresponds to the molecular mass of 30.0 kDa) seems to be aggregates of some subunits. Moreno and Chrispeels\textsuperscript{23} found that a purified α-amylase inhibitor from Greensleeves seeds dissociates into five different kinds of subunit (15–19 kDa) by SDS-PAGE, four of which have covalently attached glycans.\textsuperscript{23} Further, they showed that the inhibitor is composed of two kinds of subunit after the deglycosylation and N-terminal amino acid analysis. Accordingly, it seems necessary to characterize further each subunit of our Great Northern inhibitor with regard to N-terminal primary structure analysis after deglycosylation, structure of the sugar chain, and other physicochemical properties. In this study, the absence of 2-mercaptoethanol on SDS-PAGE did not affect the results (data not shown), indicating that the inhibitor is made up of subunits which are not linked by disulfide bonds. Our Great Northern inhibitor may be considered to be composed of three of the four subunits, since the apparent native molecular mass is about 42 kDa. Which subunit is responsible for the formation of native inhibitor must be discovered. \textit{P. vulgaris} amylase inhibitors so far reported are composed of one to five kinds of subunit as discussed below. Powers and Whitaker\textsuperscript{19} separated red kidney bean inhibitor into three different kinds of subunit by SDS-PAGE. The presence of three kinds of subunit in \textit{P. vulgaris} amylase inhibitor has also been reported by Kotaru and Yoshikawa\textsuperscript{40} with Kintoki beans, Kotaru et al.\textsuperscript{40} with cranberry beans, and Lajolo and Filho\textsuperscript{12} with black beans. Kotaru et al.\textsuperscript{24} observed that only one of the three subunits was glycophore. Recently, Yamaguchi\textsuperscript{19} reported that a purified α-amylase inhibitor from white kidney beans was composed of two different kinds of subunit by size-exclusion high performance liquid chromatography under dissociating conditions. On the other hand, Pick and Wöber\textsuperscript{25} suggested that an inhibitor from white beans had homologous (identical) subunits.

After the Western blotting, the band corresponding to the molecular mass of 15.5 kDa was cut out and the N-terminal amino acid sequence of the subunit was analyzed on an automated gas-phase protein sequencer (Model 473A, Applied Biosystems). The sequence was found to be Ser-Ala-Val-Gly-Leu-Asp-Phe-Val-Leu-Val (SAGVDFLVLY). This N-terminal amino acid sequence agrees with the results obtained by Yamaguchi\textsuperscript{19} on white kidney beans and Moreno and Chrispeels\textsuperscript{23} on Greensleeves seed α-amylase inhibitors. These results suggest that highly homologous primary structures are maintained in the α-amylase inhibitors from \textit{P. vulgaris} family. Further, this sequence is found in a complete amino acid sequence of Tendergreen seed lectin-like protein deduced from the nucleic acid sequence.\textsuperscript{25,26}

We have observed that one of the monoclonal antibodies prepared against the purified α-amylase inhibitor from Great Northern beans cross-reacts with purified lectins from the same seeds (unpublished data). This observation agrees with the suggestion made by Chrispeels and Raikhel\textsuperscript{27} that highly homologous structures are present between α-amylase inhibitor and lectin in the \textit{P. vulgaris} family. Detailed characterizations of the α-amylase inhibitor and lectin in Great Northern beans are now in progress in our laboratory.

References


