The homogeneity of the purified dialysable proteinase inhibitor from eggplant exocarp

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Summary : The homogeneity of the protein-like dialysable proteinase inhibitor purified from eggplant exocarp was investigated by gel chromatography on Sephadex G-50 and by disc and SDS-polyacrylamide gel electrophoreses. These analyses showed this inhibitor preparation was homogeneous protein. The molecular weight of this inhibitor was estimated to be approximately 6000 by the methods of gel chromatography, SDS-polyacrylamide gel electrophoresis and equilibrium centrifugation.

Introduction

The protein-like substances which inhibit the function of proteinase have been widely distributed in plants as well as in animals. Several trypsin inhibitors from leguminous plant seeds have been purified and characterized but the studies on the proteinase inhibitors from non-leguminous plants are few.

In previous papers[2,3], we have reported the occurrence and some characteristics of a trypsin inhibitor in eggplant (*Solanum melongena L.*). In the course of purification step, it was found that some of the inhibitor passed through the cellulose tube during dialysis[4]. This dialyzable inhibitor was purified[5]. This paper deals with the homogeneity and molecular weight of this purified dialyzable inhibitor.

Materials and Methods

All materials and methods used in this study are the same as those described previously[5].

Results and Discussion

Analytical gel chromatography of the purified inhibitor.

The purified inhibitor from eggplant exocarp prepared by the methods reported in the previous paper[5] was chromatographed on Sephadex G-50. The sample (62 mg) was dissolved in 2.0 ml of 50 mM aonium formate solution and this solution was applied on a 2.5×83 cm column of Sephadex G-50 equilibrated with 50 mM ammonium formate solution, followed by elution with the same solution at a flow rate of 30 ml/h. The trypsin inhibitory activity

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of 5μl of each fraction was measured. The protein peak was shown as a single peak and it was completely coincided with the activity peak as shown in Fig. 1.

Homogeneity of the purified inhibitor.

Homogeneity of the purified inhibitor was checked by disc and SDS (sodium dodecyl sulfate)-polyacrylamide gel electrophoreses. Disc electrophoresis was carried out at pH 9.4 in the gel concentration of 7.5 %[5]. A current of 2 mA per tube was supplied for 150 min at 5°. SDS-polyacrylamide gel electrophoresis was performed with 10 % gel[7]. A current of 8 mA per tube was supplied for 5 h at room temperature. As shown in Fig. 2, the inhibitor exhibited

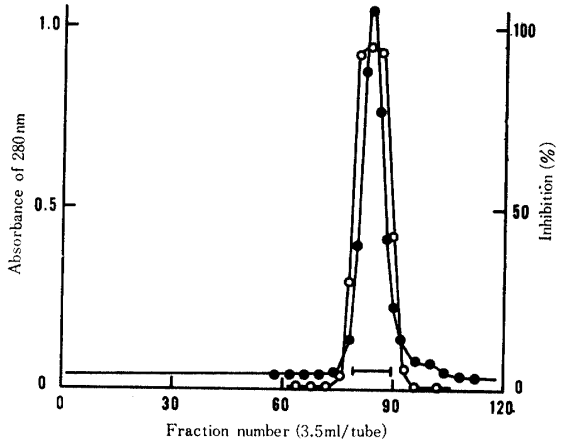
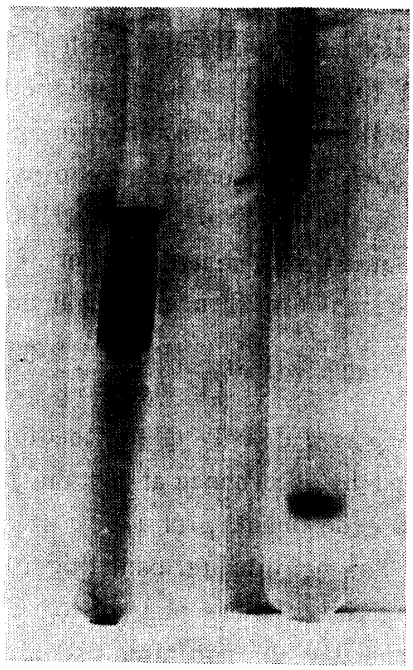


Fig. 1. Analytical gel chromatography of the purified inhibitor on Sephadex G-50. ●, absorbance; ○, inhibition.



(a) (b)

Fig. 2. Electrophoretic patterns of the purifed inhibitor. (a) Disc electrophoresis (b) SDS-polyacrylamide gel electrophoresis.

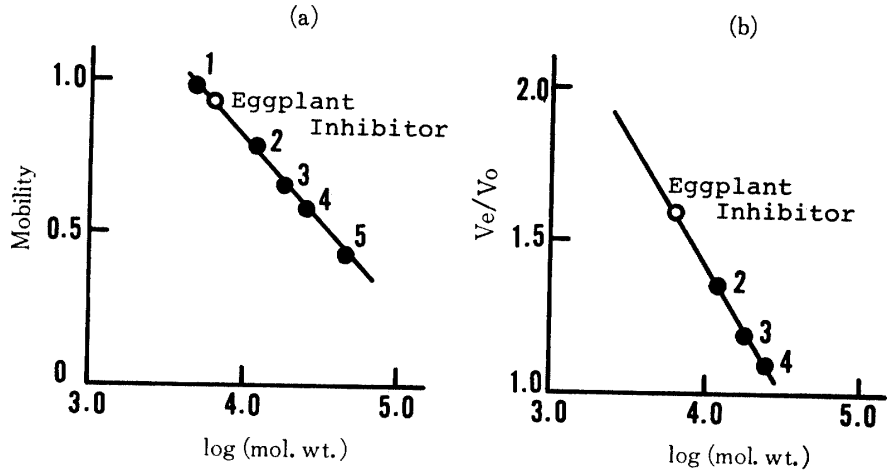


Fig. 3. Molecular weight determination of the purified inhibitor. (a) SDS-polyacrylamide gel electrophoresis (b) Gel filtration on Sephadex G-50. The marker proteins are; 1, bovine insulin (M. W. 5730): 2, horse cytochrome C (M. W. 12400): 3, whale myoglobin (M. W. 17800): 4, bovine chymotrypsinogen A (M. W. 25000): 5, ovalbumin (M. W. 45000). Ve/Vo, ratio of elution volume to void volume.

homogeneous behaviour as a single protein band in these analyses.

Molecular weight of the purified inhibitor.

Fig. 3 illustrates the results of molecular weight determination of the purified inhibitor by SDS-polyacrylamide gel electrophoresis and gel filtration on Sephadex G-50. Electrophoresis was performed under the same condition as in the experiment shown in Fig. 2. Gel filtration was carried out as follows; a protein solution was applied on a column (1.5×83 cm) of Sephadex G-50 and eluted with 0.1 M KCl in 50 mM Tris-HCl buffer (pH 7.5) at a flow rate of 10 ml/h. Molecular weight was estimated to be approximately 6000 in both cases.

In addition, the molecular weight was determined by equilibrium centrifugation with a Hitachi UCA-1A centrifuge equipped with a UV spectrophotometer. The inhibitor was dissolved in 10 mM NaH_2PO_4-Na_2HPO_4 containing 0.1 M NaCl to give a 0.1 % solution and this solution was centrifuged at 34450 rpm for 28 h at 20°. A calculation of the molecular weight from the ultracentrifugal data was carried out using the following equation;

$$\text{mol. wt.} = \frac{2RT}{(1-\bar{v}\rho)\omega^2} \cdot \frac{2.303 d(\log C)}{dr^2}$$

Where ρ is a density of the medium, r is a radial distance, C is a protein concentation represented by absorbance at 280 nm, $\bar{v}$ is a partial specific volume and ω is an angular velocity. In this experiment, ρ of 1.00 g/cm³ and $\bar{v}$ of 0.73 cm³/g, which are presumed values, were used. The equilibrium centrifugal data indicated the molecular weight of 6200. Furthermore, the linearity of these plots of log C versus r^2 means that the inhibitor preparation is a homogeneous protein. This result is shown in Fig. 4.

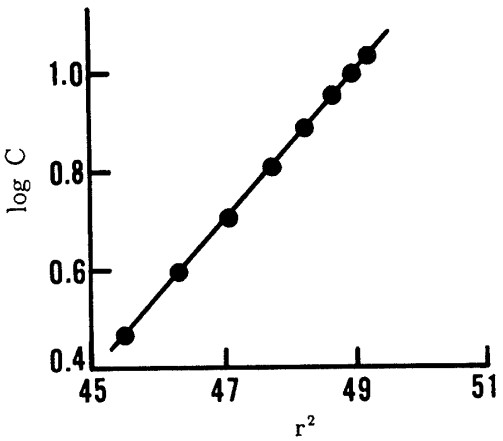


Fig. 4. The plots of log C versus r^2 in the equilibrium centrifugal analysis.

If it is assumed that 1 mole trypsin combines with 1 mole inhibitor, the molecular weigh of the eggplant inhibitor was calculated to be 5500-5800. On the basis of molecular weight of 6000, the inhibibor was composed of 57 amino acid residues and the molecular weight of 6076 was obtained. The detailed results were shown in previous paper[5].

It is concluded that the eggplant trypsin inhibitor has at present, the smallest molecular weight among the trypsin inhibitor isolated from various plants.

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要 旨：ナスビ果皮より単離精製されたトリプシン・インヒビターの均一性をセファデックス G-50 によるゲル口過やデイスクおよび SDS ポリアクリルアミドゲル電気泳動によって，しらべた。このインヒビター標品は完全に均一な蛋白質であることが判明した。またこのインヒビターはゲル口過，SDS ポリアクリルアミドゲル電気泳動および沈降平衡法によって，分子量約 6000 であることが明らかになったが，この値は，植物由来のインヒビターとして最小のものである。