Optical studies on the purified eggplant trypsin inhibitor

Masao Kanamori Toshio Asao[1] Makoto Kotaru[2],
Akihiko Katsurada and Fumio Ibuki

The studies on trypsin inhibitors extracted from eggplant exocarp have been carried out extensively in our laboratory. The studies on the occurrence[1], purification[2], foundamental properties[3], the reactive site of it[4,5] and so on were reported already. This paper deals with the opitical properties of the purified eggplant trypsin inhibitor. A lot of investigations concerning the optical properties of protease inhibitors from various sources have been reported. Quite recently, the optical studies on trypsin inhibitor purified from black-eyed peas[6], chickpea[7], lima bean[8] and soybean[7,9] were reported.

The purified egglant trypsin inhibitor was prepared in our laboratory by the method descrbied previously[2].

1. Ultraviolet absorption spectrum.

The spectrum of the inhibitor was determined at various pH values with a spectrophotometer, Union Giken SM-401, at 25°C. The inhibitor was dissolved in distilled water to give a concetration of approximately 1%. The inhibitor solution was diluted to 10-fold with each of the following buffer (0.1M): acetate buffer, pH 3 and 5; phosphate buffer, pH 7; carbonate buffer, pH 9, pH 10 and pH 11. The inhibitor contains two residues of tyrosine and two residues of phenylalanine per molecule[3] as the only chromophores absorbing to an appreciable extent above 250 nm.

Figure 1 shows the ultraviolet spectrum of the inhibitor at each pH. The spectra were identical in the pH range from 3.1 to 9.4. The ripples at 259 nm, 265nm and 269nm are characteristic of phenylalanine. The maximum at 277 nm and shoulder at 285 nm correspond to the absorption of tyrosine residues. The absence of tryptophan in the inhibitor is confirmed by this spectrum. The marked change in the spectrum was observed at pH 10.1 and pH 10.9, due to ionization of tyrosine residues. Gorbunoff[10] have reported the similar changes of ultraviolet spectrum of lima bean inhibitor which contains no tryptophan, one tyrosine and two phenylalanine per molecule. In his study, the maxima of the difference

Laboratory of Nutritional and Food Chemistry, Department of Agricultural Chemistry, Faculty of Agriculture, Kyoto Prefectural University, Kyoto 606, Japan.
1 Department of Food Science, Faculty of Home Economics, Mukogawa Women University, Nishinomiya 662, Japan.
2 Present address, Laboratory of Food Chemistry, Faculty of Home Economics, Koka Women Junior College, Kyoto 615, Japan.
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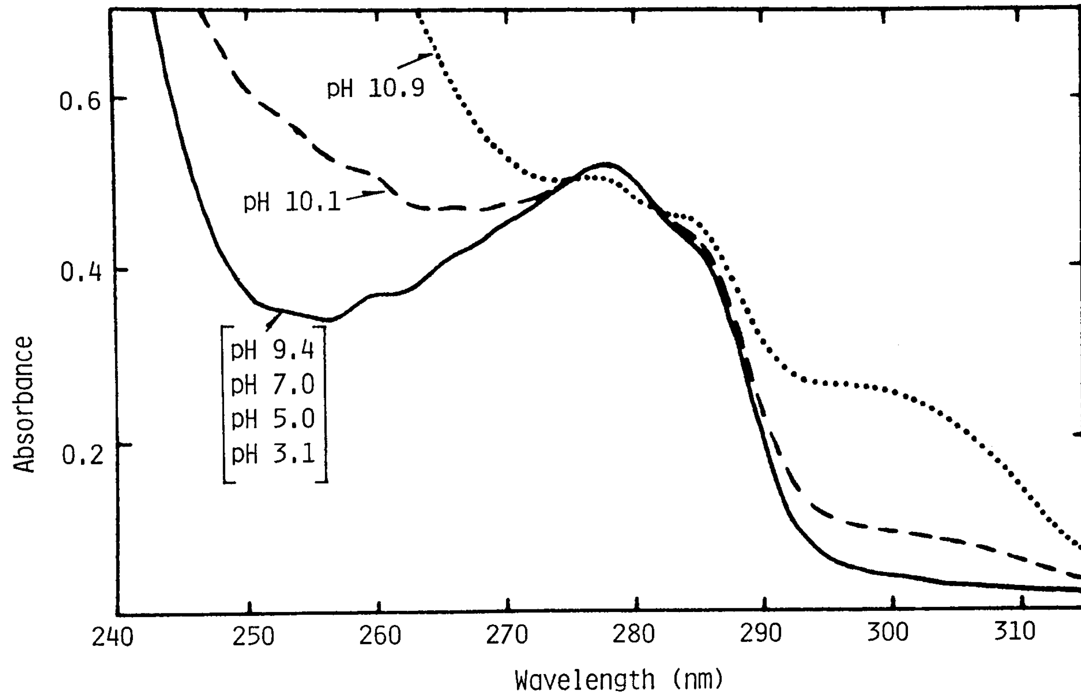

Fig. 1. Ultraviolet absorption spectrum of the inhibitor.

spectrum between pH 7 and pH 13 were at 245 nm and 296 nm, and he has concluded that its difference is due to ionization of tyrosine residue.

The extinction coefficients, $E^{0.1\%}_{1\ cm}$, were determined to be 0.714 at 277 nm and 0.662 at 280 nm for the purified inhibitor dried in vacuo over P_2O_5 to constant weight, in 0.05 M sodium acetate buffer, pH 5.5. Therefore, the optical factor converting absorbance at 280 nm to mg of protein per ml was calculated to be 1.51 for this inhibitor.

2. Fluorescence spectrum.

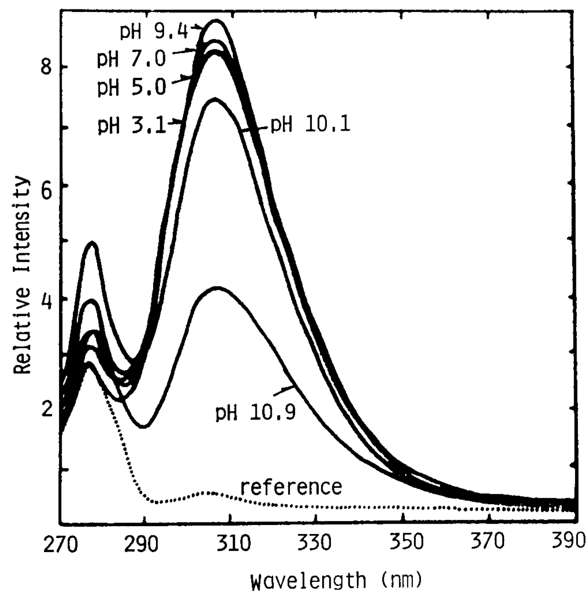

Fig. 2. Fluorescence spectrum of the inhibitor.

Fluorescence spectra were measured with a spectrofluorometer, Union Giken FS-401, at 25°C using the same inhibitor solution as used for determination of the ultraviolet spectrum.

Fugure 2 shows the fluorescence spectra. By excitation at 275 nm, the fluorescence maximum was observed at 307 nm which is characteristic of tyrosine fluorescence[11]. The fluorescence intensity is constant between pH 3.1 and pH 9.4, and it decreases with pH above 10.

3. Circular dichroism spectrum.

Circular dichroism (CD) measurements were done at 25°C with a Govin-Ivon Dichrograph Mark III-J. Spectra in the region above 240 nm were obtained with 0.1% and 0.02 % inhibitor solutions with proper overlap. Figure 3 shows the CD spectra of the inhibitor

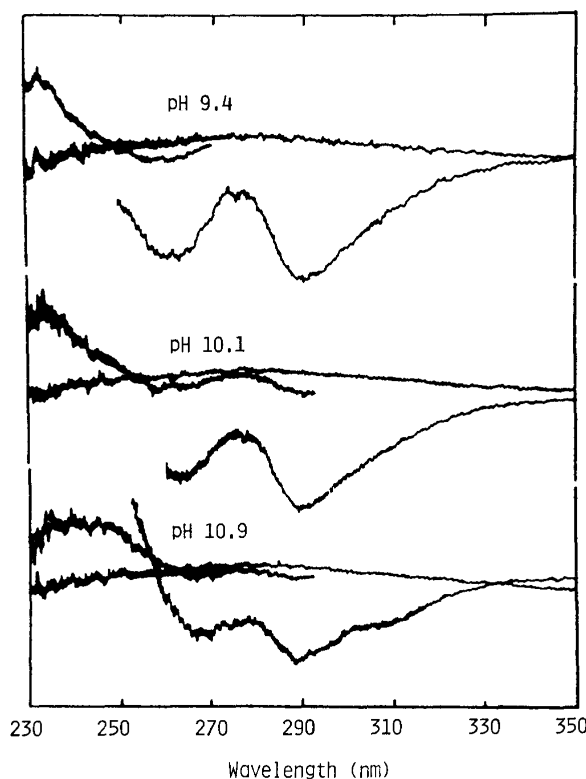

Fig. 3. Circular dichroism spectrum of the inhibitor.

at pH 9.4, pH 10.1 and pH 10.9 The CD in the pH range from 3.1 to 9.4 were essentially identical and characterized by a positive band with a maximum near 235 nm and a negative band between 260 nm and 310 nm with two maxima at 261 nm and 290 nm. The positive band and the negative band with a maximum at 261 nm may be due to four disulfide bonds in the molecule. Another negative band with a maximum at 290 nm may be due to two tyrosine residues. When the inhibitor was dissolved at pH 10.1 or pH 10.9, the intensities of these bands were slightly decreased in comparison with those at pH below 9.4. CD spectrum in the far ultraviolet region was obtained with 6.1% inhibitor in 0.1 M phosphate buffer, pH 7, in cell with 0.01 cm path length. In far ultraviolet region (Fig. 4), the negative band with a maximum at 198 nm and a cross-over point at 192 nm suggests

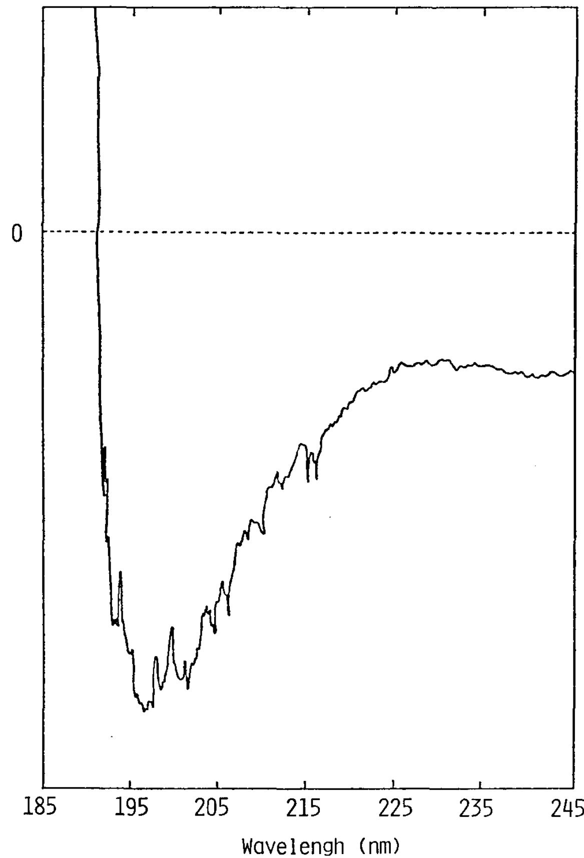

Fig. 4. Circular dichroism spectrum of the inhibitor in far ultraviolet region.

that the inhibitor has neither helix nor β-structure to a detectable extent.

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精製ナストリプシンインヒビターの光学的性質

金森 正雄・浅尾 俊夫・小垂　真・桂田 昭彦・伊吹 文男

要旨：ナス果皮より均一に精製したトリプシン・インヒビターについてその光学的性質を検討した。各種 pH において，紫外吸収スペクトルを測定したが pH 3.1 から9.4の範囲では，チロシンおよびフェニールアラニン残基による同じスペルトルを示したが，pH 10 以上ではチロシン残基のイオン化による著しい変化が認められた．またアミノ酸分析で確認されているようにトリプトファンを含まないことは，紫外吸収スペルトルからも明らかであった．275 nm で励起した蛍光スペクトルが測定されたが，pH 3.1 から9.4の範囲では蛍光強度は，ほゞ一定であり10以上で減少した。また CD スペクトルの測定結果はこのインヒビターが，ほとんどヘリックス構造や β-構造を含まないことを示している。