PHOTOCHEMICAL OXIDATION OF YELLOW SNAKE GOURD PROTEASE

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PHOTOCHEMICAL OXIDATION OF YELLOW SNAKE GOURD PROTEASE

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Abstract

Yellow snake gourd protease was rapidly inactivated by methylene blue catalysed photooxidation at pH 7.8 and 25°C. The rate of inactivation was pH-dependent and became slower at lower pH values, suggesting the involvement of some histidine residues in the inactivation. Changes in amino acid composition occurred only with histidine residues. One mole or more of histidine residues in the molecule are of essential importance in the catalytic function of yellow snake gourd protease.

Introduction

Photooxidation of enzymes in the presence of dyes has often been used to obtain information on the amino acid residues essential for the catalytic activitity of an enzyme. It is well known that the photooxidation of enzyme in the presence of methylene blue causes a rapid inactivation by the destruction of essential amino acid residues involved in active site (1–4). Yellow snake gourd protease was isolated from yellow snake gourd, *Trichosanthes Kirilowii* Maxim. var. Japonica Kitam. in our laboratory. Among proteases of plant derivation, this protease is unique because typical plant proteases so far isolated have belonged mainly to the cysteine protease group. This report shows that yellow snake gourd protease is sensitive to dye-sensitized photochemical oxidation, and suggests that destruction of histidine is responsible for the inactivation.

Experimental

Yellow snake gourd protease was isolated from the sarcocarp of yellow snake gourd according to the procedure of Kaneda (5). The protease activity of the enzyme was determined by a modified Kunitz method (5) using casein as a substrate.

The pH dependence of the rate of inactivation of the enzyme by methylene blue catalysed photooxidation was tested as follows. To 5 ml of a buffer solution (0.2 M phosphate buffer, pH 5.4–8.2) containing 12 mg of the enzyme, 5 ml of a 0.015% methylene

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blue aqueous solution was added and the mixture was irradiated from a distance of 12 cm with a 100 W incadescent lamp at 25°C. Aliquots of 50 μl were withdrawn at appropriate times and used for assay of the enzymatic activity, and at the same time 1 ml aliquots were withdrawn for amino acid analysis. Photooxidized protein was freed from the reagents by passage through a column (1.8×42 cm) of Sephadex G-25 equilibrated and eluted with 0.1 M formic acid. The protein fractions were pooled and lyophilized. Native and photooxidized enzyme were hydrolysed with 6 M HCl at 110°C for 24 hr in evacuated, sealed tubes and analysed with an amino acid analyser.

Results and Discussion

As can be seen in Fig. 1, yellow snake gourd protease was rapidly inactivated by methylene blue catalysed photooxidation. The inactivation was complete after 120 min at pH 8.2. The rate of inactivation became slower in the lower pH regions. After 100 min at pH 6.8, the enzyme retained nearly 50% of its original activity. The plot of the activity loss as a function of pH is of sigmoidal shape and has an inflection point near pH 6.8 as shown in Fig. 2. This pH dependency indicates that some amino acid residues which have a pK value of around neutrality are involved in the inactivation. Since only the photooxidation of histidine and its derivatives has been reported to show a pH-dependence similar to that observed here (6–9), the result strongly suggests that photooxidation of histidyl residue(s)

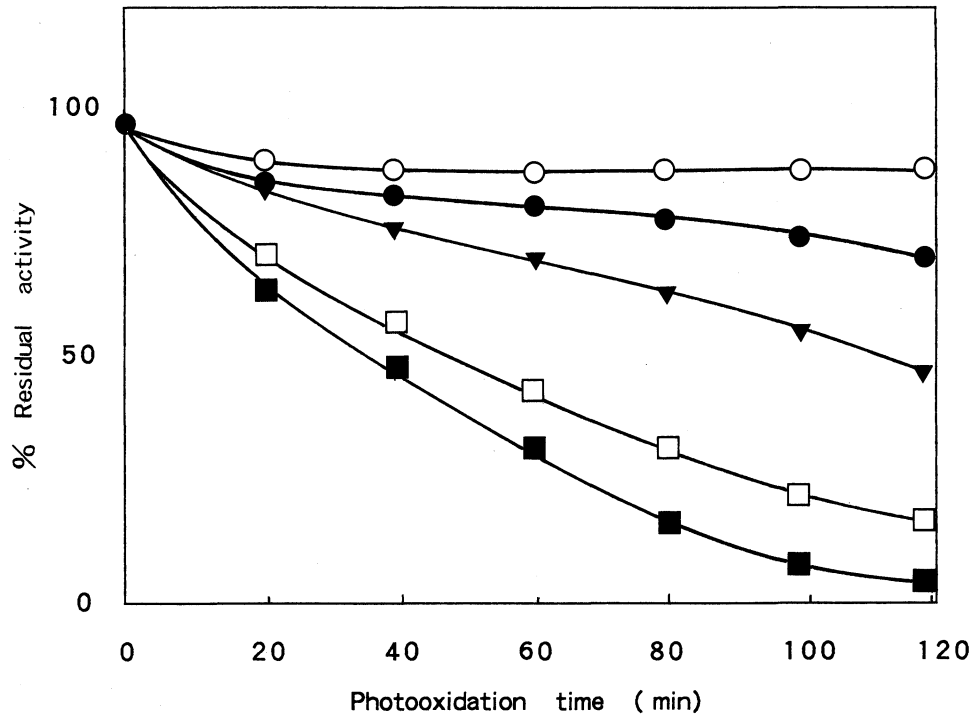

Fig. 1 Rates of inactivation of yellow snake gourd protease by photooxidation at different pH values in the presence of methylene blue. Protease solution was irradiated from a distance of 12 cm with a 100 W incadescent lamp in the presence of methylene blue at 25°C. ○ pH 5.4; ● pH 6.4; ▼ pH 6.8; □ pH 7.4; ■ pH 8.2.

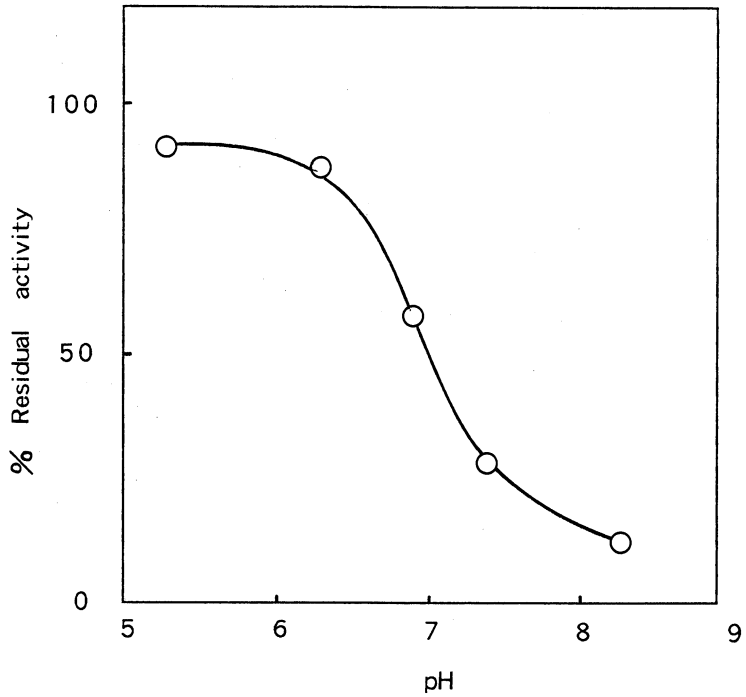

Fig. 2 Effect of pH on photooxidation of yellow snake gourd protease. The rate of photooxidative inactivation is expressed as the percentage of that at 100 min.

Table 1. Amino acid composition of acid hydrolysates of yellow snake gourd protease photooxidized in the presence of methylene bule at pH 8.2. The values are given in terms of the molar ratios of the amino acids assuming the number of alanine residues to be 45.0. No correction has been made for losses resulting from decomposition during acid hydrolysis.

Amino acid	Native enzyme	Photooxidized enzyme 20 min	120 min
Aspartic acid	48.8	48.7	48.0
Threonine	38.7	38.5	38.0
Serine	72.7	72.2	72.0
Glutamic acid	17.7	17.5	17.5
Proline	31.5	31.7	31.4
Glycine	41.6	41.9	42.1
Alanine	45.0	45.0	45.0
Cysteine	+	+	+
Valine	36.7	37.4	37.1
Methionine	6.4	6.4	5.9
Isoleucine	25.4	25.3	25.9
Ieucine	31.5	30.9	31.2
Tyrosine	16.7	16.5	16.4
Phenylalanine	19.4	19.1	19.2
Lysine	17.9	17.6	18.0
Histidine	7.5	5.0	2.7
Arginine	24.4	24.6	24.4
Remaining activity (%)	100	20	0.5

of the enzyme is responsible for the activity loss. In the dark, the enzyme was quite stable in the presence of methylene blue. After removing methlene blue from the reaction mixture by gel filtration, the amino acid composition of photooxidized enzyme was analysed and compared with that for native enzyme (Table 1). The results showed that the amino acid residue which suffered a significant change on photooxidation was only histidine and that the other residues remained almost intact within the limit of experimental error. Figure 3 shows the relationship between the histidine residues lost and the enzymatic activity remaining when the photooxidation was carried out at pH 8.2. About four of the seven histidine residues in the enzyme were lost when complete inactivation took place. The results show that the photooxidation of no more than four histidine residues is directly responsible for the inactivation of yellow snake gourd protease.

In conclusion, the results of photooxidation experiments described here indicate that histidyl residue(s) is involved in the catalytic center of yellow snake gourd protease.

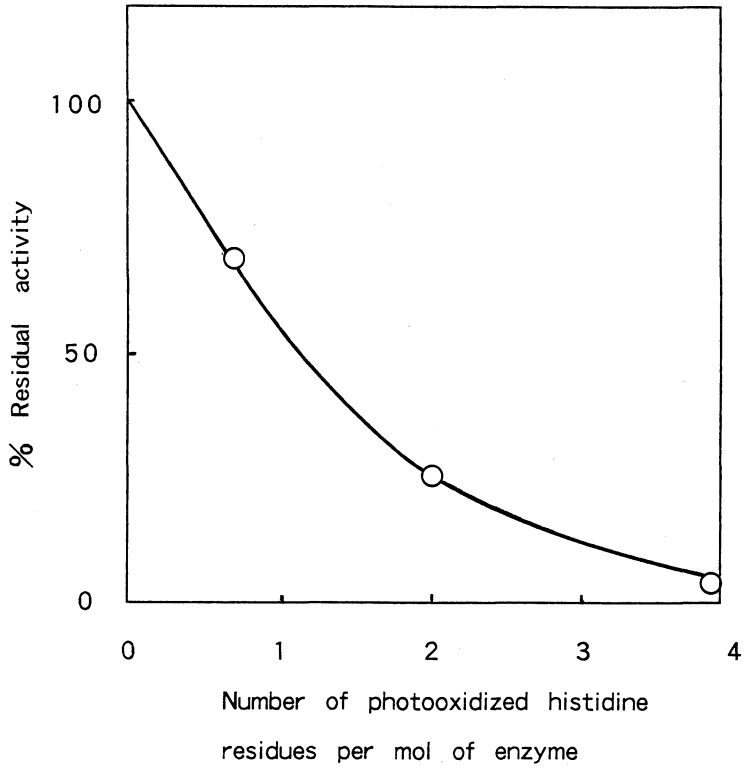

Fig. 3 Relationship of activity of yellow snake gourd protease to the extent of photooxidation of histidine residues at pH 8.2.

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