PHOTOCHEMICAL OXIDATION OF WHITE-GOURD PROTEASE, A SERINE PROTEASE

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PHOTOCHEMICAL OXIDATION OF WHITE-GOURD PROTEASE, A SERINE PROTEASE

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Abstract

White-gourd protease was rapidly inactivated by methylene blue catalysed photooxidation at pH 8.0 and 20°. 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 the amino acid composition occurred only with histidine residues. One mole or more of histidine residues in the molecule are essential importance in the catalytic function of white-gourd protease.

INTRODUCTION

Dye-sensitized photochemical oxidation of enzymes in the dyes has often been used to obtain information on the amino acid residues essential for the catalytic activity of an enzyme. It is well known that the photooxidation of proteins in the presence of methylene blue causes a rapid destruction of histidine and tryptophan residues and a slower destrution of tyrosine, cysteine and methionine residues (*1-4*). However, Martinez-Carrion studied a histidine-specific oxidation with aspartic aminotransferase (*5*). White-gourd protease isolated from the sarcocarp of white-gourd, *Benincasa cerifera* savi, by Kaneda et al. is a serine proteinase (*6*). Among proteases of plant derivation, white-gourd protease is unique because typical plant proteases so far isolated have belonged mainly to the thiol protease group. This report shows that white-gourd protease is sensitive to dye-sensitized photochemical oxidation, and suggests that destruction of histidine is responsilble for the inactivation.

EXPERIMENTAL

White-gourd protease was isolated from the sarcocarp of white-gourd, *Benincasa cerifera* savi, according to the procedure of Kaneda (*6*). The protease activity of white-gourd protease was determined by a modified Kunitz method (*7*) using casein as a substrate.

The pH dependence of the rate of inactivation of enzyme by methylene blue cataly-

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sed photooxidation was tested as follows. To 5 ml of a buffer solution (0.1 M phosphate buffer, pH 5.2−8.0) containing 25 mg of enzyme, 5 ml of a 0.015% methylene blue aqueous solution was added and the mixture was irradiated from a distance of 12 cm with a 100 W incandescent lamp at 20°. Aliquots of 50 μl was withdrawn at appropriate times and used for assay of the enzymatic activity, and at the same time 1 ml aliquots were withdrawn for amino acid analysis.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 free from the reagents by passage through a column of Sephadex G-25 equilibrated and eluted with 0.1 M acetic acid. The protein fractions were pooled and lyophilized. Native and photooxidized enzyme were hydrolysed with 6M HCl at 110° for 24 hours in evacuated, sealed tubes and analysed with an amino acid analyzer, model PICO·TAG HPLC system.

Results and Discussion

As can be seen in Fig. 1, white-gourd protease was rapidly inactivated by methylene blue catalyzed photooxidation. The inactivation was complete after 100 min at pH 8.0. The rate of inactivation became slower in the lower pH regions. The plot of the activity loss as a function of pH is of a sigmoidal shape and has an inflection point near pH 6.6 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.

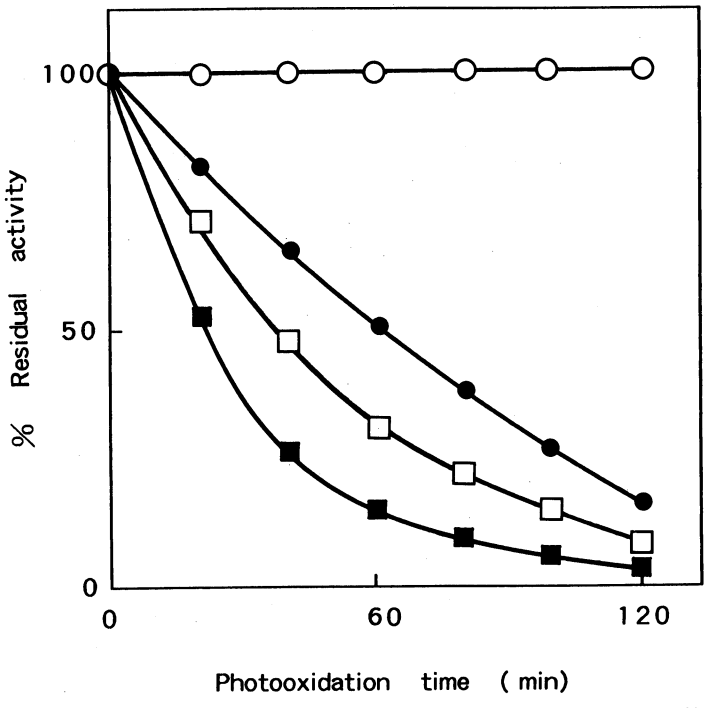

Fig. 1. Rate of inactivation of white-gourd protease by photooxidation at different pH values in the presence of methylene blue. White-gourd protease (0.25% solution) was irradiated for a distance of 12 cm with a 100 W incandescent lamp in the presence of 0.0075% methylene blue at 20°.
○, pH 5.2; ●, pH 6.8; □, pH 7.4; ■, pH 8.0

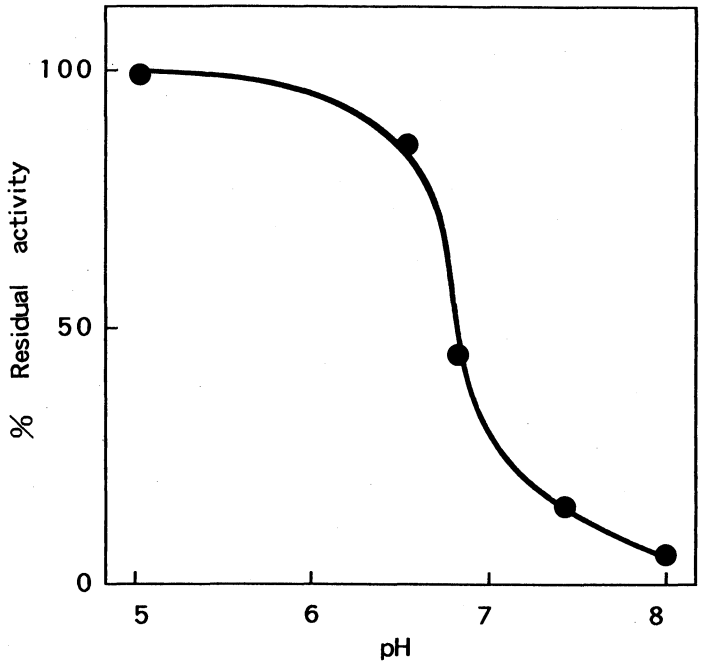

Fig. 2. Effect of pH on photooxidation of white-gourd protease. The rate of photooxidative inactivation is expressed as the percentage of that at pH 8.0 after exactly 60 min.

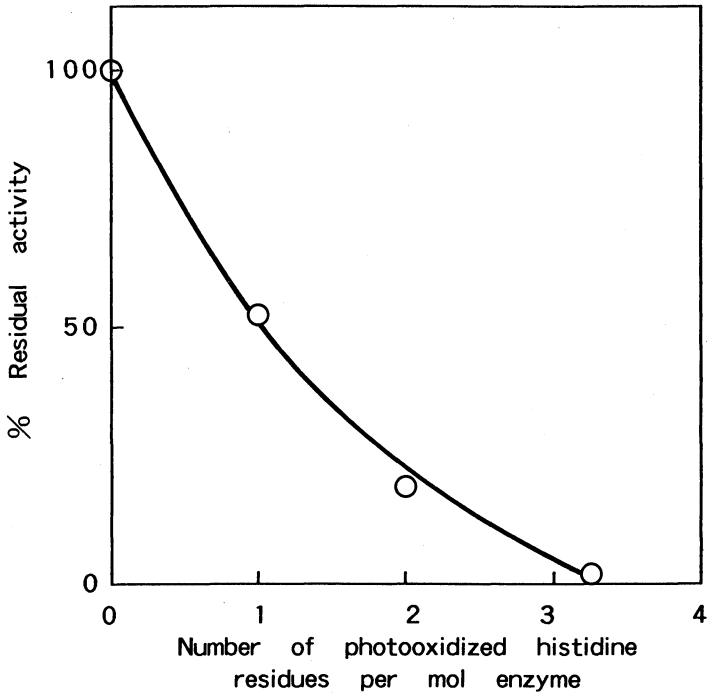

Fig. 3. Relationship of activity of white-gourd protease to the extent of photooxidation of histidine residues at pH 8.0.

Since only the photooxidation of histidine and its derivatives has been reported to show a pH-dependence similar to that observed here (*5*, *8*—*11*), the result strongly suggests that photooxidation of histidyl residue(s) 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 methylene blue from the reaction mixture by gel filtration, the amino acid composition of photooxidized enzyme was analyzed and compared with that for native enzyme. The results showed the amino acid residue which suffered a significant change on photooxidation was only histidine and that the other residues remained almost intact whithin 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 8.0. About three 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 three histidine residues is directly responsible for the inactivation of white-gourd protease.

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

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