EFFECTS OF SUBSTRATE ANALOGUES ON THE PHOTOOXIDATION OF SNAKE-GOURD PROTEINASE A2

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EFFECTS OF SUBSTRATE ANALOGUES ON THE PHOTOOXIDATION OF SNAKE-GOURD PROTEINASE A_2

By

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Abstract

Snake-gourd proteinase A_2 was rapidly inactivated by methylene blue-catalyzed photooxidation at pH 6.8 and 20°C. The rate of inactivation decreased in the presence of substrate analogues. Substrate analogues such as benzyloxycarbonyl-Glu-Tyr-Gly clearly protected the enzyme from the inactivation that is caused by photooxidation.

Introduction

Snake-gourd, Karasu-uri, proteinase A_2 isolated from the sarcocarp of the snake gourd, *Trichosanthes cucumeroides* Maxim, by Kaneda *et al.* is a serine proteinase. Among proteases of plant derivation, snake-gourd proteinase is unique because typical plant proteases so far isolated have belonged mainly to the thiol protease group.

In photooxidation study of snake-gourd proteinase A_2 using methylene blue as a photosensitizer, we have found that the decrease in enzymatic activity was accompanied by a concomitant decrease in histidine residue, suggesting the importance of one or more histidine residues for the catalytic activity (*1*).

This report shows that some substrate analogues of snake-gourd proteinase protect the enzyme from methylene blue-catalyzed photooxidation.

Materials and Methods

Snake-gourd proteinase A_2 was isolated from the sarcocarp of snake groud according to the procedure described in the previous paper (*2*). All substrates were obtained from Peptide Institute, Inc.

The proteinase activity of snake-gourd proteinase was determined by a modified Kunitz method (*2*) using casein as a substrate.

The rate of inactivation of the enzyme by methylene blue-catalyzed photooxidation was measured as follows. To 5.0 ml of a buffer solution(0.2 M phosphate buffer, pH 6.8) containing 25 mg of enzyme, 5.0 ml of a 0.015 % methylene blue aqueous solution was

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added and the mixture was irradiated from a distance of 12 cm with a 100 W incandescent lamp at 25°C. Aliquots of 50 μl were withdrawn at appropriate time intervals and used for assay of the enzymatic activity.

Results and Discussion

As can be seen in Fig. 1, snake-gourd proteinase A_2 was rapidly inactivated by methylene blue-catalyzed photooxidation. When enzyme was photooxidized at pH 6.8 and 25°C for 120 min, it lost 95% of its activity. However, when a 200-fold molar excess of substrate analogues for snake-gourd proteinase A_2, such as Z-Glu-Tyr-Gly or Z-Gly-Glu-Tyr, was present in the reaction mixture, these substrates could effectively protect the enzyme from photoinactivation. It may be observed from Fig. 2 that a sequence of Glu-Tyr-Gly is more effective than Gly-Glu-Tyr in this protection from photoinactivation. It is known that these two substrates are not hydrolyzed easily by snake-gourd proteinase A_2 . That is, these are poor substrates. By these substrates, however, the proteinase was effectively protected from photoinactivation. It appears that these substrates are able to bind readily to the active center of the proteinase. The effect of the length of a peptide chain can be seen in Fig. 3. This proteinase seems to prefer the tripeptide substrate to dipeptide one.

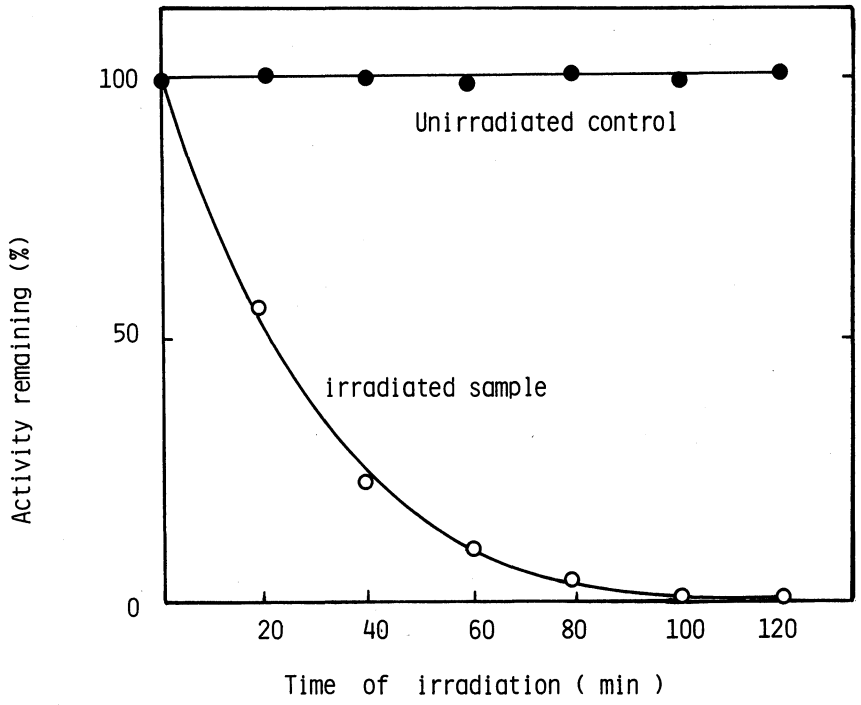

Fig. 1. Inactivation of snake-gourd proteinase A_2 by photooxidation in the presence of methylene blue. Snake-gourd proteinase A_2 (0.25% solution) was irradiated from a distance of 12 cm with a 100 W incandescent lamp in the presence of 0.0075% methylene blue. The experiment was carried out in a 0.2 M phosphate buffer, pH 6.8 at 25°C.

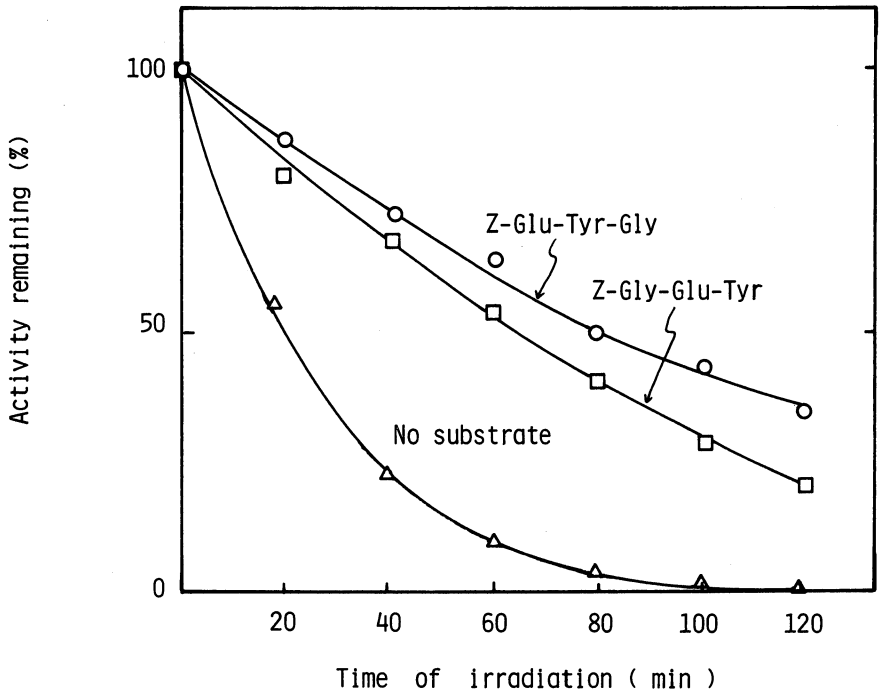

Fig. 2 Effect of substrate analogues on the rate of inactivation of snake-gourd proteinase A_2 by methylene blue-catalyzed photooxidation.

Photooxidation was performed with 0.25% enzyme, 0.0075 % methylene blue, and a 200 molar excess substrate in a 0.2 M phosphate buffer, pH 6.8 at 25°C.

Z, benzyloxycarbonyl.

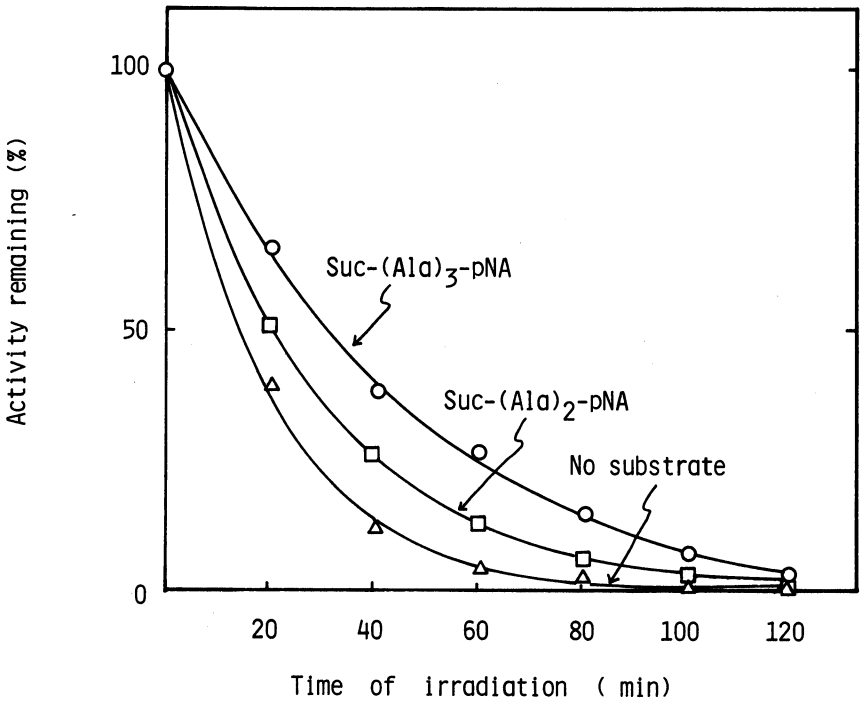

Fig. 3. Effect of substrate analogues on the rate of inactivation of snake-gourd proteinase A_2 by methylene blue-catalyzed photooxidation.

Photooxidation was performed under similar conditions to that in Fig. 2. Suc, succinyl; pNA, p-nitroanilide.

References

1. Kaneda. M., Kamachi, M., and Tominaga, N. (1986) *Phytochem.* **25**, in press.
2. Kaneda, M., Sobue. A., Eida, S., and Tominaga, N. (1986) *J. Biochem.* **99**, 569-577.