Note

Partial Purification and Some Properties of Protease from Wheat Germ and Flour

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Wheat protease has been studied by many authors because it is a factor affecting the quality of dough.^{1,2)} The protease was partially purified from wheat grain and some enzymatic properties were characterized by Skupin and Warchalewski.³⁾ However, no information on the effect of the protease on dough softening is available. In this study, we purified the enzyme from wheat germ and flour, and studied some of its properties.

Protease activity was assayed with casein (Hammarsten) labeled with fluorescein isothiocyanate (FTC-casein), which was prepared by the method of Maeda *et al.*,⁴⁾ as a substrate. Enzyme (0.1 ml) was mixed with 3.9 ml of 1% FTC-casein in 0.1 M phosphate buffer, pH 6.0. The mixture was incubated for 30 min at 35°C, the reaction was stopped by the addition of 5 ml of 12% trichloroacetic acid, and the mixture was filtered. After adding an equal volume of 2 M sodium carbonate to the filtrate, the fluorescence intensity was measured (excitation, 490 nm; emission, 520 nm). One

unit of protease activity was defined as the amount of enzyme producing in 10 min a hydrolyzate, the fluorescence intensity of which was the same as that of a solution of fluorescein isothiocyanate (1 µg/ml) in 1 M sodium carbonate. As shown in Fig. 1, sensitivity and linearity were significantly improved compared with method of using azocasein as a substrate.⁵⁾

Purification was done at 5°C in the presence of 5 mM 2-mercaptoethanol. One hundred grams of germ (supplied by Nippon Flour Mills Co., Ltd.) was extracted with 500 ml of 0.1 N acetic acid. The extract was fractionated with ammonium sulfate (0.3 to 0.7 saturation, pH 6.8), and the precipitate was dialyzed against 0.1 M potassium phosphate buffer, pH 6.8. The dialyzate was put on a hydroxyapatite column (2.5 × 16 cm), and the protease was eluted with the buffer containing 0.5 M sodium chloride. The protease fraction was dialyzed against 0.005 M acetate buffer, pH 4.6, and chromatographed using on affinity column (1.6 × 5 cm) packed with phenylalanine-substituted Sepharose 4B prepared by the method of Cuatrecasas *et al.*⁶⁾ After the column was washed with the buffer containing 0.02 M sodium chloride to remove impurity proteins, prorease was eluted with the buffer containing 0.1 M sodium chloride. The protease was dialyzed against 0.005 M potassium phosphate buffer, pH 7.2, put on an ω -

Table I. PURIFICATION OF PROTEASE FROM WHEAT GERM

	Total activity (unit)	Total protein (mg)	Specific activity (units/mg)	Recovery (%)
Extract ^a	33.6	5391	0.0062	100
Ammonium sulfate fractionation	52.7	1869	0.028	157
Hydroxyapatite	42.8	123	0.348	127
Phenylalanine-Sepharose 4B	15.0	6.1	2.46	44
ω -Aminohexyl Sepharose 4B	9.4	2.2	4.27	28

^a From 100 g of germ.

Table II. INHIBITORY EFFECTS ON PROTEASE OF WHEAT GERM

Inhibitor	Concentration (µM)	Inhibition (%)
N-Ethylmaleimide	10	27
	100	72
p-Chloromercuribenzoate	50	86
E-64	100	0
Diisopropyl fluorophosphate	10	0
	100	4
EDTA	100	13

Fig. 1. Hydrolysis of FTC-casein and Azocasein with Trypsin.

—●—, FTC-casein; —×—, azocasein.

Fig. 2. Consistency of Glutens Digested with Wheat Germ Protease.

Gluten (0.5 g) was incubated with 2 ml of the enzyme at 35°C for 2 hr. a, gluten; b, gluten incubated with 2.1 units of protease; c, 4.4 units of trypsin; d, 44 units of trypsin.

aminohexyl Sepharose 4B column (1.6×1.6 cm), and eluted with a linear gradient of sodium chloride (0 to 1 M in the same buffer). The protease was eluted as a single peak at 0.37 M sodium chloride. The result of the purification is summarized in Table I. The recovery was 28% with a purification factor of 690. Further purification was difficult because the enzyme became increasingly labile as it was purified. The molecular weight of the purified protease was estimated to be 42,000 by the gel filtration method with a Sephadex G-100 column.

Total activity of protease from wheat flour was about one-sixth of that germ on the dry basis. The flour protease differed from that of germ protease, because the flour protease was not adsorbed on the hydroxyapatite column.

Some properties of the germ protease were studied. As shown in Table II, the enzyme was inhibited by SH-blocking reagents such as NEMI and pCMB, so the enzyme was classified as a thiol-protease. However, the

germ protease seemed to be different from papain and cathepsin B because of no inhibition with a thiol-protease inhibitor (E-64).⁷⁾ The germ protease had an optimum pH of 5.6 with FTC-casein as a substrate, but effectively hydrolyzed the high molecular weight subunit of glutenin at pH 4.0–5.0.

The rheological effects of the protease on gluten were tested. Dried gluten (0.5 g) was incubated with 2 ml of the protease (2.1 units) at 35°C for 2 hr by the method of Kruger.⁸⁾ The gluten was molded into a ball by hand and the consistency was measured with a Tensipresser (Takemoto Inc.) as described in our previous paper.⁹⁾ As shown in Fig. 2, the consistency was decreased. The ratio of gluten and protease in whole grain flour was about one-tenth of the above ratio, so no appreciable softening of flour dough may have occurred.

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References

- 1) J. Hanford, *Cereal Chem.*, **44**, 499 (1967).
- 2) D. R. Grant and C. C. Wang, *Cereal Chem.*, **49**, 201 (1972).
- 3) J. Skupin and J. Warchalewski, *J. Sci. Food Agric.*, **22**, 11 (1971).
- 4) H. Maeda, N. Ishida, H. Kawauchi and K. Tuzimura, *J. Biochem.*, **65**, 777 (1969).
- 5) J. E. Kruger, *Cereal Chem.*, **50**, 122 (1973).
- 6) P. Cuatrecasas, M. Wilchek and C. B. Anfinsen, *Proc. Natl. Acad. Sci. U.S.A.*, **61**, 636 (1968).
- 7) K. Hanada, M. Tamai, M. Yamagishi, S. Ohmura, J. Sawada and I. Tanaka, *Agric. Biol. Chem.*, **42**, 523 (1978).
- 8) J. E. Kruger, *Cereal Chem.*, **48**, 121 (1971).
- 9) C. Yoshida and G. Danno, *Agric. Biol. Chem.*, **53**, 121 (1989).