CYSTEINE PROTEASE INHIBITORS FROM BANANA (*MUSA PARADISIACA*)

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ABSTRACT

Inhibitors of cysteine proteases were found in ripened and unripened banana (*Musa paradisiaca*). Extract from unripened banana inhibited the proteolytic activities of papain, ficin and bromelain. Extract from ripened banana inhibited the proteolytic activities of papain and ficin only. Both ripened and unripened banana extracts inhibited caseinolytic and amidolytic activities of papain. The inhibitory activity of ripened banana extract was stable to extreme acidic pH and neutral pH and unstable to extreme alkaline pH. The inhibitory activity of ripened and unripened banana extracts was stable after heating to 100°C for 10 min.

INTRODUCTION

CYSTEINE protease inhibitors from chicken egg white[1], human serum[2], bovine colostrum[3] and microorganisms[4] have been reported. Papain, a plant cysteine protease evolutionarily related to animal cysteine proteases, was used in these studies to identify the inhibitors. While the mammalian inhibitors of cysteine proteases have been extensively investigated, studies on similar factors from plant sources are limited[5,6]. In view of this an attempt was made to identify inhibitors of papain from plant sources. This communication reports the presence of cysteine protease inhibitors in ripened and unripened banana (*Musa paradisiaca*) and some of their properties.

MATERIALS AND METHODS

Ripened and unripened bananas (*Musa paradisiaca*, *Musa cavendish*, *Musa sapientum* and Nendran) were procured from local sources. Twice-crystallized papain (EC 3.4.22.2), twice-crystallized ficin (EC 3.4.22.3), bromelain (EC 3.4.22.4), *N*α-benzoyl-DL-arginine-*p*-nitroanilide (BAPNA), bovine serum albumin and casein were obtained from Sigma Chemical Company, USA. Other reagents were analytical grade commercial chemicals.

Preparation of banana extract

Twenty-five g of ripened or unripened banana without the outer skin was homogenized in 25 ml of 0.02 M borate buffer, pH 7.6. The homogenate was centrifuged at 10,000 *g* for 20 min at 4°C. The supernatant was collected and tested for inhibitory activity. Protein content of the supernatant was measured by the method of Lowry *et al.*[7] using bovine serum albumin as standard.

The activities of papain, ficin and bromelain were measured with casein as substrate by a method modified from Kunitz[8]. The assay system consisted of 100 μmol of borate buffer *p*H 7.6, 5 μmol of EDTA, 10 μmol of β-mercaptoethanol, 10 mg of casein and water in a volume of 1.8 ml. The reaction was started by the addition of 0.2 ml enzyme solution. After a 15 min incubation at 37°C, the reaction was arrested by adding 3 ml of 5% trichloroacetic acid. After standing at room temperature for 30 min, the contents were centrifuged at *2000 g for 10 min. The concentration of trichloro*-acetic acid-soluble peptides was determined in 1.0 ml of the clear supernatant by the method of Lowry *et al.*[7] Under the assay conditions 8 μg of papain, 21.5 μg of bromelain and 25 μg of ficin liberated peptides equivalent to an absorbance of 0.60 from casein. Papain was also assayed using BAPNA as substrate by a method modified from Erlanger *et al.*[9] The assay system consisted of 100 μmol of borate buffer pH 7.6, 5 μmol of BAPNA, 5 μmol of EDTA and 10 μmol of β-mercaptoethanol in a volume of 2.8 ml. The reaction was started by addition of 0.2 ml of enzyme solution. After a 30 min incubation at 37°C the reaction was arrested by adding 1 ml of 30% acetic acid. The yellow colour that developed was measured at 410 nm. Under the assay conditions 54 μg of papain was used to get an absorbance of 0.60.

Estimation of papain inhibitory activity

Suitable aliquots of banana extracts were included in the assay system described above. Reduction in

papain activity was the measure of inhibition. One unit of inhibitory activity (in the caseinolytic and amidolytic methods) was the amount that suppressed hydrolytic activity by one absorbance unit.

Effect of pH on the inhibitory activity of banana

Ripened banana extract (0.1 ml, containing 400 μg protein) was incubated with 0.1 ml of different buffers for 72 h at 4°C: 0.1 M HCl (pH 1.0), 0.1 M HCl/KCl (pH 2.0), 0.1 M acetate (pH 4.5), 0.1 M borate (pH 7.0 and 8.0), 0.1 M bicarbonate (pH 10.0), and 0.2 M NaOH (pH 12.5). After the incubation 0.4 ml of 0.2 M borate buffer pH 7.6 was added and the extract assayed for inhibitory activity against caseinolytic activity of papain.

Effect of temperature on the inhibitory activity of banana

Four ml of ripened or unripened banana extract was subjected to heat treatment at 70, 90 and 100°C for 10 min. After cooling, aliquots were assayed for papain inhibitory activity.

Controls without banana extract were run simultaneously in all cases.

RESULTS

Crude extracts of ripened and unripened banana were found to inhibit several cysteine proteases. Data for inhibition of hydrolytic activities of three cysteine proteases by banana inhibitor are presented in table 1. The caseinolytic and amidolytic activities of papain were maximally inhibited by ripened banana inhibitor. The ripened banana extract showed moderate inhibition of caseinolytic activity of ficin and had no action against caseinolytic activity of bromelain. Unripened banana inhibited the caseinolytic activities of papain and ficin to some extent and bromelain weakly. The inhibition of caseinolytic activities of papain and ficin by ripened banana extract were much more than that of unripened banana extract. However, the latter showed inhibition of caseinolytic activity of bromelain, which was not shown by ripened banana inhibitor. Inhibition of amidolytic activity of papain by both ripened and unripened banana extracts was two times more than inhibition of caseinolytic activity.

The papain inhibitory activity of ripened banana extract as a function of concentration is shown in figure 1. Inhibition of caseinolytic activity of papain was linear up to 33%; beyond this the inhibition was not linear and sluggish, and maximum inhibition obtained at very high inhibitor concentration was only about 80%. In contrast, inhibition of amidolytic activity of papain by ripened banana inhibition was linear up to 70% and complete inhibition was obtained at high inhibitor concentration. Amount of protein (inhibitor) required to cause 50% inhibition was 600 μg for caseinolytic activity (calculated by extrapolation of the linear range) and 300 μg for amidolytic activity.

The stability of inhibitory activity of ripened

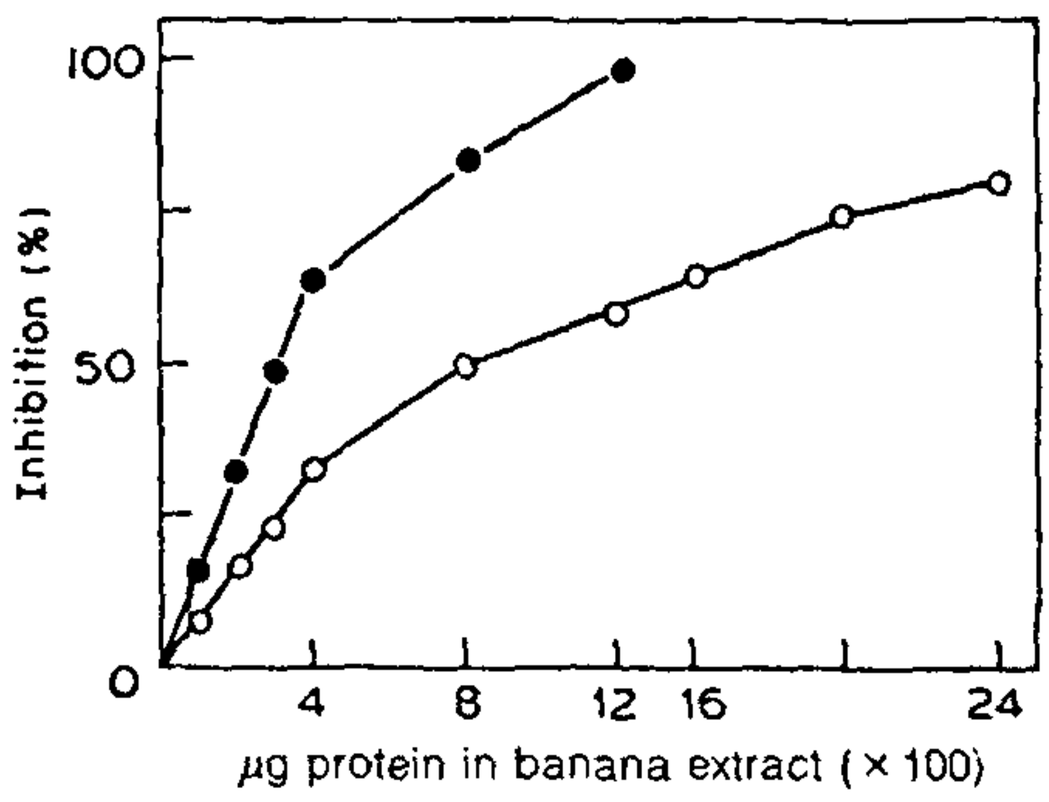

Figure 1. Inhibition of hydrolytic activities of papain by ripened banana extract. ○---○, Inhibition of caseinolytic activity; ●---●, inhibition of amidolytic activity.

Table 1 *Inhibition of proteolytic activity of cysteine proteases by banana extract*

	Inhibition (inhibitory units/ml)			
	Papain		Ficin	Bromelain
Extract	Caseinolytic activity	Amidolytic activity	Caseinolytic activity	Caseinolytic activity
Ripened banana	2.00	4.00	1.00	0.00
Unripened banana	1.20	2.40	0.60	0.30

banana inhibitor to acidic, neutral and alkaline pH was studied and the result is shown in figure 2. The inhibitory activity was stable to acidic and neutral pH and was unstable to alkaline pH. At pH 10 there was 30% loss of activity of inhibitor and complete loss of inhibitory activity was observed at pH 2.5.

The inhibitory activity in ripened and unripened banana was highly heat-stable. No loss of inhibitory activity was noticed even after exposure to 100°C for 10 min.

Papain inhibitory activity was also found in plantain (*M. cavendish*), vegetable banana (*M. sapientum*) and Nendran banana.

DISCUSSION

This report establishes the presence of cysteine protease inhibitors in plants, especially in a storage organ like the fruit. It is known that cysteine protease inhibitors of animal origin are inactive against bromelain[10]. Unlike the animal cysteine protease inhibitors, the unripened banana inhibitor was active against bromelain. It has been reported that the magnitude of inhibition of serine proteases by plant tuber inhibitors depends on the substrate employed[11]. The higher inhibition by banana extract of BAPNA hydrolysis by papain compared to casein hydrolysis suggests that magnitude of inhibition of cysteine proteases by plant inhibitors also depends on the substrate used.

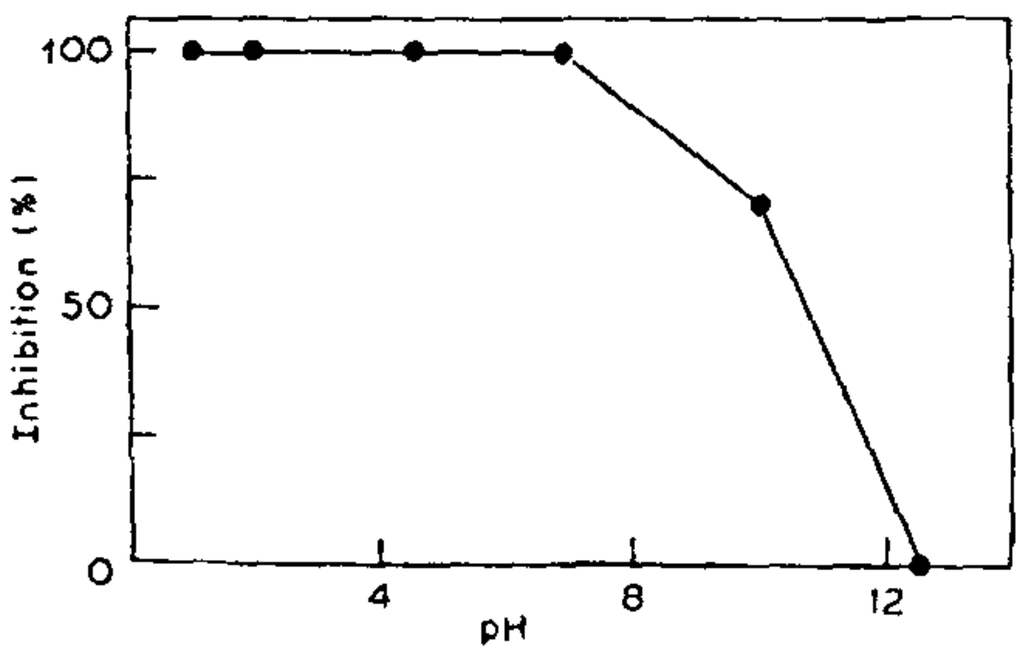

Figure 2. Effect of pH on stability of banana inhibitor of papain caseinolytic activity.

The extreme heat stability and alkaline-sensitive nature of banana inhibitors indicate that the factors responsible for inhibition may be thiol-dependent proteins which probably inhibit the activity of cystein proteases by a disulphide exchange mechanism[1,12].

The exact biological role of cysteine protease inhibitors in plants is not known at present. But the low inhibitor levels in unripened banana compared to ripened banana suggests that the synthesis of cysteine protease inhibitor increases as ripening advances.

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1. Anastasi, A., Brown, M. A., Kembhavi, A. A., Nicklin, M. J. H., Sayers, C. A., Sunter, D. C. and Barrett, A. J., *Biochem. J.*, 1983, **211**, 129.
2. Sasaki, M., Taniguchi, K. and Minakata, K., *J. Biochem.*, 1981, **89**, 169.
3. Hirado, M., Ninobe, M. and Fujii, S., *J. Biochem.*, 1984, **96**, 51.
4. Umezawa, H., *Annu. Rev. Microbiol.*, 1982, **36**, 75.
5. Brzin, J., Popovic, T., Drobnickosorok, M., Kotnik, M. and Turk, V., Hoppe-Seyler's, *Biol. Chem.*, 1988, **369S**, 233.
6. Oliva, M. L. V., Sampaio, M. U. and Sampaio, C. A. U., Hoppe-Seyler's, *Biol. Chem.*, 1988, **369S**, 229.
7. Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J., *J. Biol. Chem.*, 1951, **193**, 265.
8. Kunitz, M., *J. Gen. Physiol.*, 1974, **30**, 291.
9. Erlanger, B. F., Kokowski, N. and Cohen, W., *Arch. Biochem., Biophys.*, 1961, **95**, 271.
10. Barrett, A. J., *Trends. Biochem. Sci.*, 1987, **12**, 193.
11. Rao, N. M. and Pattabiraman, T. N., *J. Food. Sci. Technol.*, 1988, **25**, 296.
12. Kopitar, M., Brzin, J., Locnikar, P. and Turk, V., Hoppe-Seyler's, *Z. Physiol. Chem.*, 1981, **362**, 1411.