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SURVEY OF PLANT INHIBITORS OF POLYMORPHONUCLEAR LEUKOCYTE ELASTASE, PANCREATIC ELASTASE, CATHEPSIN G, CATHEPSIN B, HAGEMAN FACTOR FRAGMENTS, AND OTHER SERINE PROTEINASES

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Abstract—Various flower bulbs and vegetable and legume seeds were tested for inhibitors of polymorphonuclear leukocyte elastase, pancreatic elastase, cathepsin G, cathepsin B, trypsin, α-chymotrypsin, Hageman factor fragments, plasma kallikrein, and plasmin. Calla bulbs contained a 33,000 dalton polymorphonuclear leukocyte elastase inhibitor and a 4,000 dalton cathepsin G inhibitor. Seeds of some members in the *Cruciferae* family, such as radish and broccoli, were found to contain one or more 2,500–4,000 dalton inhibitors which inhibited cathepsin G, trypsin, Hageman factor fragments, and plasmin, but not plasma kallikrein. These seeds also contained a 1,000 dalton cathepsin B inhibitor. The above inhibitors were probably polypeptides which inhibited proteinases by making an enzyme–inhibitor complex, with the exception of the cathepsin B inhibitor. These newly found inhibitors with their characteristic profiles of inhiibition should be useful in biochemical and pathophysiological studies on granulocyte proteinases and enzymes of the coagulation and fibrinolytic pathways.

PMN-elastase‖ (EC 3.4.21.37) and cathepsin G (EC 3.4.21.20) have been implicated in the pathogenesis of pulmonary emphysema, inflammatory diseases, and several other conditions [1]. Proteinase inhibitors from plants, animals and microorganisms, as well as synthetic inhibitors, have been valuable tools in the study of these enzymes and their involvement in disease processes [1–4].

We recently reported on protein proteinase inhibitors in plant materials, some of which were found in hitherto unrecognized sources such as flower bulbs and pumpkin seeds and showed remarkably narrow profiles of inhibition [5–8]. The biological importance of the granulocyte proteinases has led us to survey a wide variety of plants as potential sources of their specific inhibitors. In the present study, thirty kinds of plant materials were tested against seven to nine enzymes including PMN-elastase, cathepsin G and cathepsin B (EC 3.4.22.1), and some were further purified by gel filtration to obtain more detailed profiles of inhibition of the enzymes. In addition, inhibitory activities of several purified plant and animal inhibitors of granulocyte enzymes were determined.

MATERIALS AND METHODS

Substrates. MeO-Suc-Ala-Ala-Pro-Val-*p*NA and Suc-Ala-Ala-Pro-Phe-*p*NA, Suc-Ala-Ala-Ala-*p*NA, and Bz-Pro-Phe-Arg-*p*NA were purchased from Vega Biochemicals (Tucson, AZ), Boehringer-Mannheim Biochemicals (Indianapolis, IN), and the Sigma Chemical Co. (St. Louis, MO) respectively. Bz-Ile-Glu-Gly-Arg-*p*NA, D-Pro-Phe-Arg-*p*NA, and MeO-Suc-Arg-Pro-Tyr-*p*NA were from the Kabi Group, Inc. [^{3}H]Elastin and D-Arg-Val-Tyr-*p*NA were the gifts of Dr. R. D. Crystal, NIH, Bethesda, MD, U.S.A., and Dr. P. Friberger, Kabi, Sweden, respectively.

Enzymes. Human PMN-elastase and cathepsin G were purified from purulent sputum of an emphysema patient by a combined method of those reported by Twumasi and Liener [4] and Baugh and Travis [9]. The purified enzymes were homogeneous by sodium dodecyl sulfate–polyacrylamide gel electrophoresis. Purified cathepsin B from human liver was a gift of Dr. A. J. Barrett, Strangeways Research Laboratory, England [10]. Porcine pancreatic elastase (crystal.) (EC 3.4.21.36), papain (crystal.) (EC 3.4.22.2) and bromelain (crystal.) (EC 3.4.22.4) were obtained from Boehringer-Mannheim Biochemicals. Bovine trypsin-TPCK (EC 3.4.21.4) and bovine α-chymotrypsin (EC 3.4.21.1) were from the Worthington Biochemical Corp. (Freehold, NJ), and

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‖ Abbreviations: PMN, polymorphonuclear leukocyte; MeO, methoxy; Suc, succinyl; *p*NA, *p*-nitroanilide; Bz, benzoyl; BSA, bovine serum albumin; HF_f, Hageman factor fragments; STI, soybean trypsin inhibitor; LTI, lima bean trypsin inhibitor; PTI, pancreatic trypsin inhibitor; and IU, inhibitor unit.

human plasmin (EC 3.4.21.7) was from the Kabi Group, Inc. HF_f (EC 3.4.21.38) [11] and plasma kallikrein (EC 3.4.21.34) [12] were purified from human plasma.

Inhibitors. Plant materials were obtained from local groceries or nurseries. STI (Kunitz), LTI and bovine PTI (Kunitz) were the products of the Worthington Biochemical Corp. STI (Bowman–Birk type; C-I inhibitor), which is homologous with C-II inhibitor [13], was supplied by Drs. S. Odani and T. Ikenaka, Niigata University, Japan. Trypsin-HF_f inhibitors from corn seeds (inhibitor with a pI of 6.3) [5] and pumpkin seeds (with a pI of 8.3) [7] were prepared as previously described.

Preparation of plant extracts. Usually 2–5 g of tissue or seeds was homogenized at room temperature for 2–3 min in a Sorvall Omni-Mixer (Ivan Sorvall, Inc.) with a 10-fold weight of buffer, 0.05 M Tris–HCl/0.2 M NaCl/0.1% sodium azide, pH 7.5. Seeds had been pre-swollen overnight at 4° in the buffer. The homogenates were centrifuged at 24,000 *g* for 30 min, and the supernatant fractions were tested.

Inhibitor assays with synthetic substrates. The plant extracts and stock solutions of purified inhibitors were tested at various dilutions in 0.1 M Tris–HCl, pH 7.5. In the cathepsin B inhibition assay, the extracts and purified inhibitors were diluted with 0.1 M potassium-disodium phosphate/1 mM EDTA, pH 7.5 or 0.2 M phosphate buffer/2 mM EDTA, pH 5.5

Equal volumes of PMN-elastase (30 μg/ml), pancreatic elastase (30 μg/ml), cathepsin G (60 μg/ml), or cathepsin B (30 μg/ml) and sample or appropriate buffer for control were mixed, preincubated at 25° for 20 min, and stored in an ice bath until assayed. The enzymes had been diluted from 0.3 to 2 mg/ml stock solutions with water, and cathepsin B had been activated with 1 mM L-cysteine for 10 min. In the cases of trypsin and chymotrypsin inhibitions, 1 part of sample, 2 parts of 0.05 M Tris–HCl/0.15 M NaCl/0.02% BSA, pH 7.5, and 1 part of trypsin (40 μg/ml in 1 mM HCl) or chymotrypsin (50 μg/ml) were mixed and preincubated at 25° for 10 min.

Remaining activities of PMN-elastase, pancreatic elastase, and cathepsin G were determined at 25° for 1 min with 50 μl of the preincubated mixtures and the respective enzyme substrates, 0.5 mM MeO-Suc-Ala-Ala-Pro-Val-*p*NA, 0.5 mM Suc-Ala-Ala-Ala-*p*NA, and 0.5 mM Suc-Ala-Ala-Pro-Phe-*p*NA, in 0.05 M Tris–HCl, pH 7.5, from the increase of absorbance at 405 nm. The assay volume was 0.5 ml. Cathepsin B activity was determined at 25° with 50 μl of preincubates and 0.5 mM Bz-Pro-Phe-Arg-*p*NA in 0.1 M phosphate buffer/1 mM EDTA/2 mM cysteine, pH 7.5 or pH 5.5, in 0.5-ml final volume. The assay times were 0.5 min at pH 7.5 and 1 min at pH 5.5. Activities of trypsin and chymotrypsin were determined for 0.5 or 1 min with 20 μl preincubate and 0.1 mM Bz-Ile-Glu-Gly-Gly-Arg-*p*NA and 0.1 mM D-Arg-Val-Tyr-*p*NA or 0.1 mM MeO-Suc-Arg-Pro-Try-*p*NA, respectively, in 0.05 M Tris–HCl/0.01 M $CaCl_2$, pH 7.5, in 1-ml final volume.

Inhibition assays of other enzymes were as follows. Preincubation of inhibitor samples with HF_f (20 μg/ml), plasma kallikrein (20 μg/ml), plasmin (50 μg/ml), papain (30 μg/ml) or bromelain (500 μg/ml) was performed as described for PMN-elastase. The first three enzymes were in the Tris–saline–BSA buffer, and the latter two enzymes had been activated with 1 mM cysteine. Remaining activities of the first three enzymes were determined with 0.1 mM D-Pro-Phe-Arg-*p*NA, 0.2 mM D-Pro-Phe-Arg-*p*NA, and 0.2 mM D-Val-Leu-Lys-*p*NA, respectively, in 0.05 M Tris–HCl, pH 7.5, and the activities of the latter two enzymes were with 0.2 mM and 0.5 mM Bz-Pro-Phe-Arg-*p*NA, respectively, in 0.1 M phosphate buffer/1 mM EDTA/2 mM cysteine, pH 7.5. In these assays, 20–50 μl aliquots of preincubation mixture were assayed for 0.5 to 1 min, in a final volume of 0.5 ml. Sephadex G-75 fractions were assayed as indicated in the legend to Fig. 1.

Assay with [^{3}H]elastin. A 30 μl portion of PMN-elastase (10 μg/ml) or pancreatic elastase (10 μg/ml) was mixed with 30 μl of sample, 50 μl of 0.3 M Tris–HCl, pH 7.5, and 50 μl of the elastin suspension (2 mg/ml; total releasable cpm/mg was 1.5×10^6), and incubated for 24 hr at 37° with shaking. The mixture was then centrifuged, and the radioactivity contained in 100 μl of the supernatant fraction was counted. The percent inhibition was calculated from calibration curves of elastin hydrolysis which were made with various amounts of the enzymes.

Determination of protein concentrations of enzymes and inhibitors. Concentrations of PMN-elastase (mol. wt 30,000), cathepsin G (25,000) and HF_f (31,000) were determined by the Lowry method with BSA (Reheis Chemical Co.) as standard. Pancreatic elastase (mol. wt 25,000), α-chymotrypsin (25,000), plasma kallikrein (87,000), and trypsin (24,000) concentrations were determined spectrophotometrically using $E^{1\%}_{280\,nm}$ of 20.2, 20.4 and 10.0, respectively, and active site titration with *p*-nitrophenyl-*p'*-guanidinobenzoate. Plasmin (mol. wt 80,000) was determined assuming that 1 mg of the enzyme has 15 casein units. Salt-free preparations of STI (Kunitz; mol. wt 20,000), LTI (9,000), corn inhibitor (14,000) [5, 14], pumpkin inhibitor (3,300) [7, 8] and PTI (Kunitz; 6,500) were weighed, whereas the concentration of STI (Bowman–Birk type; 8,000) was measured using $E^{1\%}_{280\,nm}$ of 4.6.

Inhibitor unit (IU). One IU was defined as the amount of inhibitor which inhibited 1 mg of enzyme. Values were determined in the range where inhibition was proportional to inhibitor amount (10–50% inhibition).

Estimation of molecular weight. Two milliliters of sample and reference proteins were separately filtered through a 2.2 × 63 cm column of Sephadex G-75 (Pharmacia Fine Chemicals, Piscataway, NJ) equilibrated with 0.1 M Tris–HCl/0.45 M NaCl/0.02% sodium azide, pH 7.5, at 4° with a flow rate of 15 ml/hr. Elution of inhibitors was followed by inhibition assays. Reference compounds were Blue Dextran 2000, BSA, ovalbumin, chymotrypsinogen A, ribonuclease A, cytochrome *c*, PTI, and bradykinin. Elution volumes of the first six standards were determined by absorbance at 280 nm, 412 nm, or 630 nm and those of PTI and bradykinin by trypsin inhibition and contraction of the rat uterus respectively.

Isoelectric focusing. Sample was electrofocused in a 110-ml LKB column with pH 3.5–10 Ampholine

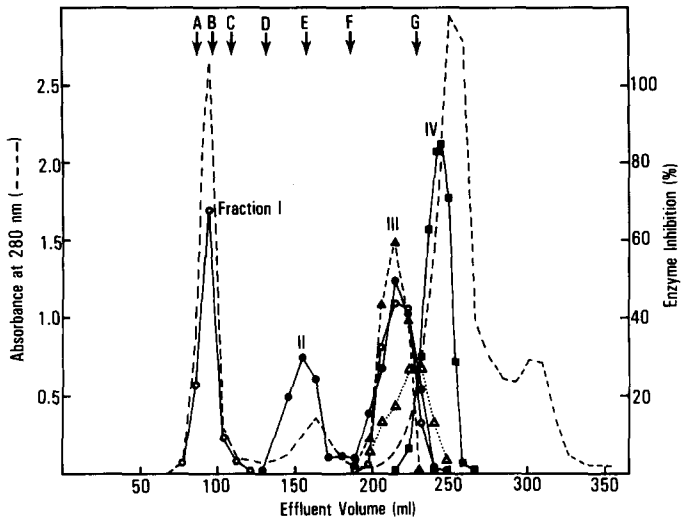

Fig. 1. Elution of proteinase inhibitors in radish seeds on Sephadex G-75 filtration. Two milliliters of the seed extract, which contained 0.2 g seeds and 158 A_{280}, was applied to a 2.2 × 63 cm column of Sephadex G-75. The effluent was preincubated with the enzymes with the following volume ratios: (○) cathepsin G, 60 μg/ml:sample = 1:3; (●) trypsin, 40 μg/ml:0.05 M Tris–HCl/0.15 M NaCl/0.02% BSA, pH 7.5:sample = 1:1:2; (▲) HF_f, 20 μg/ml:sample = 1:1; (△) plasma kallikrein, 20 μg/ml:sample = 1:3; and (■) cathepsin B, 30 μg/ml:sample = 1:1. Reference proteins: A = Blue Dextran 2000; B = BSA (mol. wt 67,000); C = ovalbumin (43,000); D = chymotrypsinogen A (25,000); E = ribonuclease (13,700) and cytochrome *c* (12,400); F = PTI (6,500); and G = bradykinin (1,060).

(1%) for 32–35 hr at 4° and 500 V. After pH measurements of 2-ml fractions at 4°, 2 ml of 0.2 M Tris–HCl, pH 8.0, was added to the fractions.

RESULTS AND DISCUSSION

Extracts of calla (plant No. 1) and ranunculus bulbs (No. 7) and seeds of okra (No. 13), lettuce (No. 17) and several legumes (No. 20–26) were rich sources of inhibitors of PMN-elastase and cathepsin G (Table 1). Relatively high inhibition of pancreatic elastase was observed only in extracts of lily bulbs (No. 2) and soybeans (No. 20).

Calla bulb extracts also strongly inhibited trypsin, chymotrypsin, plasma kallikrein and plasmin but negligibly inhibited pancreatic elastase, cathepsin B and HF_f (Table 1). The PMN-elastase inhibitor was the previously reported inhibitor of trypsin, chymotrypsin, kallikrein and plasmin with mol. wt of 33,000 [6] and a pI of 5.6. In Sephadex G-75 gel filtrations and Ampholine isoelectrofocusings of a bulb extract and a partially purified* preparation, PMN-elastase inhibition was superimposed on inhibitions of those four enzymes. However, it is of interest that this 33,000 dalton inhibitor did not inhibit cathepsin G, a chymotrypsin-like enzyme. In the assay of PMN-elastase with [^{3}H]elastin, the calla bulb showed 2.5 IU/g. This value is less than the value (27 IU/g) obtained in the assay with MeO-Suc-Ala-Ala-Pro-Val-*p*NA, due to the long incubation time with the elastin (24 hr) during which the enzyme–inhibitor complex was partially dissociated, the inhibitor having been displaced by the substrate. Pancreatic elastase was not inhibited in the assay with [^{3}H]elastin as assayed with Suc-Ala-Ala-Ala-*p*NA. On the other hand, on Sephadex G-75 filtration of the bulb extract, a 4,000 dalton cathepsin G inhibitor was found. This inhibitor did not inhibit PMN-elastase, trypsin, chymotrypsin, HF_f, plasma kallikrein or plasmin. The inhibitor was also found to be dialyzable: 25% of cathepsin G inhibitory activity of the bulb extract (2 ml) was found in 8 ml of dialysis buffer, 0.05 M Tris–HCl/0.15 M NaCl/0.02% sodium azide, pH 7.5, after 20 hr of dialysis at 4°.

Of all the plant materials tested, radish seed extracts (plant No. 8) had the highest activity against cathepsin B (Table 1). Fractionation of the extract on a Sephadex G-75 column revealed four peaks of inhibitory activity with different specificities (Fig. 1): Fraction I eluted between Blue Dextran 2000 and BSA, Fraction II with mol. wt *ca.* 15,000, Fraction III with mol. wt *ca.* 2,500, and Fraction IV after bradykinin elution. Fraction I inhibited cathepsin G and plasmin (not shown in Fig. 1) but none of the six other enzymes tested. Fraction II inhibited only trypsin and plasmin. The inhibitor in this Fraction appeared to have an enzyme inhibition profile similar to that of the trypsin inhibitor (mol. wt 8,000) from radish seeds reported by Ogawa *et al.* [15]. Fraction III inhibited chymotrypsin (not shown) and plasmin in addition to inhibiting cathepsin G, trypsin and HF_f. A plasma kallikrein inhibition peak was

* The partially purified calla bulb inhibitor was prepared as follows: 14 g of the bulb was homogenized with 50 ml of the extraction buffer, centrifuged, and applied to a Sephadex G-75 column (5.4 × 45 cm) equilibrated with 0.05 M ammonium acetate, pH 7.5. Trypsin inhibitory fractions (M_r 33,000) were pooled and freeze-dried (130 mg dry weight). One milligram of the preparation inhibited 0.5 mg trypsin.

Table 1. Proteinase inhibition by plant extracts

No.	Common name	Genus	IU/g tissue or seeds									
			PMN elastase*	Pancreatic elastase*	Cathepsin G	Cathepsin B (pH 7.5)	Cathepsin B (pH 5.5)	Trypsin	Chymotrypsin	HF_f	Plasma kallikrein	Plasmin
	Bulbs											
1	Calla	*Zantedeschia aethiopica*	27	0†	17	0.04	0	43	15	0.07	91	120
2	Lily	*Lilium sp.*	4.5	1.6	8.6	0.07	0	11		1.8	0.72	
3	Hyacinth	*Hyacinthus orientalis*	1.7	0.12	3.2	0	0	1.8		0.60	1.5	
4	Grape hyacinth	*Muscari botryoides*	4.5	0.14	6.0	0	0	0.65		0.18	0.36	
5	Tulip	*Tulipa greigii*	4.4	0	15	0	0	1.5		1.9	0.29	
6	Iris	*Iris reticulata*	1.6	0.06	9.2	0.04	0	4.8		2.4	0	
7	Ranunculus	*Ranunculus asiaticus*	11	0.55	15	0.06	0.12	4.1	0.82	0	0	
	Seeds											
8	Radish	*Raphanus savitus*	0.48	0	6.1	3.9	2.1	2.7	0.73	0.50	0.35	4.7
9	Broccoli	*Brassica oleracea italica*	7.7	0	6.5	0.42	0.32	3.7	0.11	0.58	0	8.7
10	Cauliflower	*Brassica oleracea botrytis*	0.60	0	4.3	0.28	0.22	2.6	0.26	1.6	0	6.0
11	Cabbage	*Brassica oleracea capitata*	0.72	0	11	0.66	0.44	14	0.55	5.1	0	31
12	Turnip	*Brassica rapa*	0.60	0	3.2	0.03	0.03	2.2	0.52	0.66	0	3.3
13	Okra	*Hibiscus esculentus*	9.1	0	36	0.05	0.04	9.8	4.4	0.72	0.08	4.8
14	Beet	*Beta vulgaris*	4.9	0.03	11	0.36	0.13	0.03	0.04	0	0	
15	Celery	*Apium graveolens*	7.9	0	8.6	0.30	0.21	0	0	0.11	0.12	
16	Parsley	*Petroselinum crispum*	2.5	0	4.6	0.26	0.12	0	0	0	0.05	
17	Lettuce	*Lactuca sativa*	20	0	34	0.26	0	0.11	0	0	0	
18	Asparagus	*Asparagus officinalis*	1.1	0	8.2	0.34	0.08	0.04	0	0	0	
19	Spinach	*Spinacia oleracea*	4.7	0	9.6	0.28	0.36	0.27	0.05	0.08	0.30	
20	Soybean	*Glycine soja*	56	1.4	140	0.09	0	46		0	35	
21	Lima bean	*Phaseolus limensis*	49	0.06	78	0.07	0.03	38		0.34	0	
22	Red kidney bean	*Phaseolus vulgaris*	19	0.34	89	0.13	0.04	19		0	0	
23	Adzuki bean	*Phaseolus angularis*	16	0.29	63	0.16	0.10	27		0	0	
24	Lentil	*Lens esculenta*	20	0.06	63	0.06	0.12	2.5		0	0	
25	Sweet pea	*Lathyrus odoratus*	14	0	37	0.22	0.20	7.8		0	0	
26	Peanut (albumen)	*Arachis hypogaea*	9.6	0	15	0.11	0.09	2.0		0.62	0.03	
27	Corn	*Zea mays*	4.5	0.04	9.6	0.05	0	0.67	0.08	0.40	0	
28	Pumpkin	*Cucurbita maxima*	2.7	0	6.9	0.05	0.04	0.68	0.03	0.35	0	
	Others											
29	Potato (tuber)	*Solanum tuberosum*	1.7	0.09	4.5	0.05	0.09	1.2		0.55	1.7	
30	Sweet potato (root)	*Ipomoea batatas*	1.9	0	13	0.07	0.03	0.73		1.6	0.06	

* Data from assays with synthetic substrates.
† 0 IU/g was less than 0.03 IU/g.

Table 2. Inhibition profiles of purified proteinase inhibitors

Inhibitors	IU/mg inhibitor					
	PMN-elastase	Pancreatic elastase	Cathepsin G	Cathepsin B (pH 7.5)	Cathepsin B (pH 5.5)	Trypsin
STI (Bowman–Birk)	2.5	0.20	3.3	0	0	3.6
STI (Kunitz)	0.20	0.01	1.2	0	0	1.4
LTI	0.14	0	3.7	0	0	2.4
Corn inhibitor	0	0	0	0	0	1.3
Pumpkin inhibitor	0	0	0.77	0	0	6.0*
PTI (Kunitz)	0.01	0	0.07	0	0	3.4

* Cited from Ref. 7.

observed near Fraction III but not coincident with it (Fig. 1). The HF_f inhibitory activity in Fraction III was partially dialyzed: 16% of the activity in the seed extract was found in the dialysis buffer after 20 hr of dialysis. On the other hand, it is noteworthy that Fraction IV inhibited only cathepsin B and other thiol-proteinases, papain and bromelain (not shown), and that it had an estimated mol. wt of only 1,000. The inhibitor of this fraction was rapidly dialyzed. Through the whole Sephadex G-75 fractionation, there was little or no inhibitory activity toward PMN-elastase.

Extracts of broccoli, cauliflower, cabbage and turnip seeds (No. 9–12) inhibited HF_f but not plasma kallikrein. This was observed previously with extracts of corn and pumpkin seeds and iris bulbs [5–7]. Cabbage seeds had the highest HF_f inhibitory activity, 5.1 IU/g, among tested materials. Seed extracts of broccoli, cauliflower, cabbage and turnip were fractionated on the Sephadex G-75 column and divided into four fractions which had molecular weights similar to those obtained with radish seeds. Inhibition profiles of these fractions also resembled those of radish seeds (Fig. 1): Fraction I (mol. wt more than 67,000) inhibited only cathepsin G and plasmin; Fraction II (mol. wt 17,000–19,000) only trypsin and plasmin; Fraction III (mol. wt 2,500–4,000) trypsin, HF_f and plasmin, and also weakly or negligibly cathepsin G, chymotrypsin and PMN-elastase; Fraction IV (mol. wt 1,000) only papain (used instead of cathepsin B). The HF_f inhibitory activities of these seeds also were dialyzable: 7–17% of the activities appeared in the dialysis buffer after 20 hr of dialysis. In addition, inhibitor contents of edible parts of radish, broccoli, etc. were much less than the contents found in seeds.

Okra seed extracts (No. 13) inhibited HF_f, with negligible inhibition of plasma kallikrein, 0.08 IU/g (Table 1). However, an 18,000 dalton fraction obtained by Sephadex G-75 filtration, which inhibited HF_f, cathepsin G, trypsin, chymotrypsin and plasmin (but not PMN-elastase), weakly inhibited plasma kallikrein. Another inhibitor fraction just after the column exclusion volume weakly inhibited cathepsin G and plasmin.

Beet, celery, parsley, lettuce, asparagus and spinach seeds (No. 14–19) weakly or negligibly inhibited trypsin and several other proteinases. However, these seeds exhibited fairly strong inhibition against PMN-elastase (1.1 to 20 IU/g) and cathepsin G (4.6 to 34 IU/g). There may be inhibitors specific to these two enzymes in these seeds. The strong inhibitory activities of soybeans (No. 20) and lima beans (No. 21) toward PMN-elastase and cathepsin G can be ascribed to their well-known inhibitors, since the purified inhibitors inhibited the enzymes stoichiometrically or weakly as shown in Table 2 and as previously reported [3, 4, 16–18]. Corn trypsin-HF_f inhibitor [5, 14] did not inhibit any other enzymes tested (Table 2). Interestingly, however, pumpkin trypsin-HF_f inhibitor (mol. wt 3,300) [6–8] weakly inhibited cathepsin G (Table 2). Although the pumpkin seed inhibitor does not inhibit α-chymotrypsin [6, 7, 19] (or plasma kallikrein), its cathepsin G inhibition suggests that it may have a structural similarity to the 2,500–4,000 dalton HF_f inhibitors of certain *Cruciferae* members (No. 8–12) which inhibited cathepsin G but not plasma kallikrein. PTI (Kunitz) (which is identical to Trasylol [20]) weakly inhibited PMN-elastase (Table 2) as has previously been reported [2, 17], although lack of inhibition also has been reported [3]. This inhibitor also weakly inhibited cathepsin G, as previously reported [18].

Of the numerous inhibitors observed in this study perhaps of greatest interest are the PMN-elastase inhibitor (mol. wt 33,000) and the specific, 4,000 dalton cathepsin G inhibitor in calla bulbs and the 2,500–4,000 dalton HF_f inhibitor and the 1,000 dalton thiol-proteinase inhibitor in seeds of some members of the *Cruciferae* family. The first two inhibitors are to be compared with previously reported non-specific inhibitors of PMN-elastase and cathepsin G [2–4, 16–18], and human plasma α_1-proteinase inhibitor [4, 9, 16].

As to the nature of the proteinase inhibitors from plant extracts, the following should be considered: peptides which make a complex with enzymes; proteases which destroy the activity of the proteinases; small cell organelles and membrane fragments which are not precipitated by centrifugation, bind assay proteinases and reduce their activities; substance which could be the substrates of the proteinases; and oxidizing enzymes or oxidants which could inactivate proteinases. Although the nature of the inhibitors described in this study has not been identified, we consider many of the inhibitors to be polypeptides which inhibit proteinases by forming complexes (based on kinetic inhibition studies, etc.). The cathepsin B inhibitor may be a thiol-reactive substance or a thiol-oxidant. Further studies on those

newly found inhibitors may lead to useful reagents for biochemical and pathophysiological studies on specific granulocyte enzymes as well as enzymes in the coagulation and fibrinolytic pathways. The HF_f inhibitor from corn [5] has already proved useful [21–24], e.g. in demonstrating [21] that HF_f was the cause of the sometimes life-threatening hypotension associated with the administration of implicated lots of plasma protein fraction (a plasma volume expander commonly used in surgery) [25].

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