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Partial Purification and Characterization of Two Peptide Hydrolases from Pea Seeds

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ABSTRACT

Two peptide hydrolases have been found in pea seeds (*Pisum sativum* var. Greenfeast) and extensively purified by ion exchange chromatography using benzoyl-DL-arginine-*p*-nitroanilide as substrate. The enzymes which both have molecular weights of 65,000 can be separated by anion exchange chromatography but are otherwise virtually identical in the properties tested. They did not hydrolyze several common protease substrates but readily hydrolyzed small peptides containing basic amino acids on the carboxyl side of these residues. They are completely inhibited by diisopropylfluorophosphate and are inhibited to varying extents by thiol reagents.

The seeds of various plants are useful sources of dietary protein and those of legumes are particularly good in this respect. The bulk of the proteins of legume seeds are storage proteins, and these are mobilized by enzymic hydrolysis during germination of the seed providing a source of amino acids for the embryonic plant.

An understanding of the processes involved in the mobilization of the storage proteins would be useful information for plant geneticists aiming to improve the quality of these proteins. To this end it will be first necessary to characterize the proteinases and peptidases involved in this enzymic hydrolysis. Proteinases and peptidases have been partially purified from a number of plant seeds (1, 3, 9, 14, 20, 22, 28), but as yet no such enzymes have been purified from legume seeds to an extent sufficient for characterization with the exception of the aminopeptidases from pea (9).

This paper describes the isolation from pea seeds of two peptide hydrolases active against the trypsin substrate BAPA[1] and their extensive purification and characterization.

MATERIALS AND METHODS

Peas (*Pisum sativum* var. Greenfeast) were purchased from Brunnings Ltd., Melbourne, Australia. Broad beans (*Vicis faba*) and soybeans (*Glycine max*) were supplied by the CSIRO Divisions of Plant Industry and Tropical Agronomy, respectively. Malted wheat was a gift from Joe White, Maltings Ltd., Melbourne, Australia.

BANA, BAPA, alanine-*p*-nitroanilide and glycyl-L-phenylalanine-β-naphthylamide were obtained from Cyclo Chemical Corp.; other peptide-β-naphthylamides and L-BAPA were from Bachem Fine Chemicals Inc.; BAEE was from Nutritional Biochemicals Corp.; the amino acid β-naphthylamides, benzyloxycarbonylglycyl amino acids and special enzyme grade ammonium sulfate were products of Schwarz/Mann. Peptide substrates were kindly supplied by F. H. C. Stewart of this laboratory. Fast Garnet GBC was from G. T. Gurr Ltd., London.

Standard proteins used in the mol wt determination were: whale skeletal muscle myoglobin, mol wt 17,600 (Mann Research Laboratories), bovine erythrocyte carbonic anhydrase, mol wt 31,000 (Schwarz/Mann), ovalbumin, mol wt 43,000 (Mann Research Laboratories), BSA and its dimer, mol wt 69,000 and mol wt 138,000 (Commonwealth Serum Laboratories), and calf intestine alkaline phosphatase mol wt 100,000 (Calbiochem.).

Casein (Hammarsten) was from Hopkin and Williams Ltd.; salmine was from Sigma Chemical Co. The lupin globulin preparation was kindly supplied by R. J. Blagrove of this laboratory. The SCM-proteins were prepared according to the method of Crestfield *et al.* (7), the fowl feather keratin being a gift from E. F. Woods of this laboratory. All other chemicals used were of analytical grade.

ENZYME ASSAYS

BAPAase Activity. Activity against BAPA was estimated by a slight modification of method 2 of Erlanger *et al.* (10). A suitable aliquot of enzyme was incubated for 30 min at 37 C in 0.05 M phosphate, pH 6 (final volume 2 ml), containing 1 mM BAPA. The reaction was stopped with 20% acetic acid (0.5 ml), and the absorbance at 410 nm was measured. The extinction coefficient for *p*-nitroaniline under these conditions was found to be 9650 M^{-1} cm^{-1}. When the reaction mixture was turbid, the *p*-nitroaniline was extracted into an equal volume of ethyl acetate and the absorbance at 385 nm was measured (27). A unit of activity was defined as that amount of enzyme which hydrolyzed 1 μmole of BAPA/min. The assay was carried out at pH 6, the pH of maximum stability of the enzyme.

Aminopeptidase Activity. Aminopeptidase activity was followed during the purification using alanine-*p*-nitroanilide as substrate, the other conditions being as described for BAPAase activity except that the pH was 6.8. In examining the final enzyme preparations for traces of aminopeptidase activity, 1 mM amino acid β-naphthylamides were used with a 2-hr incubation. The liberated β-naphthylamine was coupled to Fast Garnet GBC at pH 4.5, and the absorbance at 520 nm was measured (21).

Carboxypeptidase Activity. Benzyloxycarbonylglycyl amino acids (1 mM) were used as substrates during 4-hr incubations in 0.05 M potassium phosphate, pH 7, at 37 C. The extent of hyrolysis was estimated by a colorimetric ninhydrin procedure (25).

Proteinase Activity. Activity against casein (17) was used as an indication of proteinase activity. An enzyme aliquot in 0.05 M

[1] Abbreviations: BAPA: benzoyl-DL-arginine-*p*-nitroanilide; L-BAPA: benzoyl-L-arginine-*p*-nitroanilide; BANA: benzoyl-DL-arginine-β-naphthylamide; BAEE: benzoyl-L-arginine ethyl ester; Z-: benzyloxycarbonyl-; SCM-: S-carboxymethyl; DFP: diisopropylfluorophosphate; pCMB: *p*-chloromercuribenzoic acid; NEMI: N-ethylmaleimide.

phosphate, pH (final volume 2 ml), containing 20 mg of casein was incubated for 2 hr at 37 C. The reaction was stopped with 5% trichloroacetic acid (3 ml), and after 30 min the precipitated protein was filtered off and the absorbance at 276 nm was measured. The SCM-proteins and the lupin globulin preparation were tested in the same way; when salmine was tested as a potential substrate the extent of hydrolysis was measured by the colorimetric ninhydrin procedure (25).

Peptide Hydrolysis. The peptide (2 mM) in 0.04 M phosphate, pH 7 (0.25 ml), was incubated with enzyme solution (0.05 unit) for 4 to 24 hr at 37 C. The extent of hydrolysis was qualitatively assessed using high voltage electrophoresis at pH 3.5 on Whatman No. 3MM paper (23). In the case of the peptide β-naphthylamides, liberated β-naphthylamine was determined with Fast Garnet GBC in the usual way (21).

Protein Assay. Protein concentration was monitored during the purification by measurement of the absorbance at 276 nm. When specific activity measurements were being made, the method of Lowry *et al.* (19) was used with BSA as standard.

Polyacrylamide Gel Electrophoresis. Discontinuous polyacrylamide gel electrophoresis was carried out using the method of Davis (8), with 7.5% polyacrylamide. Protein bands were stained with Amido black. Enzymic activity was located by incubation of the gel in 0.1 M phosphate, pH 6, containing 2.5 mM BANA for 30 min at room temperature followed by treatment with Fast Garnet GBC in 1 M acetate, pH 4.2, for 15 min (21).

Molecular Weight Determination. Molecular weights were determined by gel filtration on a Sephadex G-150 column (2.2 × 112 cm) at 4 C with 0.1 M tris-HCl, 0.05 M KCl, pH 7.4, as eluting buffer (2). BAPAases were located in the eluted fractions by their enzymic activities.

Michaelis Constant. The hydrolysis of 1.7 μM to 1 mM L-BAPA in 0.05 M phosphate buffer, pH 6, by an amount of enzyme containing approximately 0.15 unit of activity was followed in a Cary 15 recording spectrophotometer at 410 nm. The initial velocities were estimated from the traces so obtained, and the K*m* was determined using the Lineweaver Burk equation (18).

RESULTS

BAPAase Levels during Germination. The total BAPAase activity was virtually unchanged in extracts of peas which had been allowed to germinate for up to 11 days, and comparable levels of activity were also obtained after germination in the presence of actinomycin D or cycloheximide, inhibitors of RNA and protein synthesis, respectively. That the enzymes are probably present in active form in the dry peas was shown by extraction of a dry powder prepared from peas without the normal overnight soaking.

Purification of Enzymes. Peas (500 g) were freed from fungicide by washing in 95% methanol for 3 min, rinsed in distilled H_2O, and then soaked overnight in H_2O at 4 C. All subsequent procedures were carried out at 4 C. The peas were homogenized in a Waring Blendor with 0.01 M tris-HCl, pH 7.4 (1700 ml), and the homogenate was filtered and centrifuged, giving approximately 1900 ml of crude extract (fraction 1, Table I).

Ammonium sulfate fractionation was used as a means of reducing the total volume. The fraction from 35% to 60% saturation was redissolved in 0.01 M tris-HCl, pH 7.4 (fraction 2).

The solution was dialyzed extensively against 0.01 M acetate buffer, pH 5.2, and the copious precipitate was removed by centrifugation and discarded. The supernatant (fraction 3) was then dialyzed against 0.01 M tris-HCl, pH 7.4, and chromatographed on a DEAE-cellulose column with this buffer and a linear salt gradient from 0 to 0.2 M KCl. The bulk of the BAPAase activity eluted between 0.12 M and 0.15 M KCl and was separated from a considerable amount of protein as well as all the caseinolytic activity present in the crude extract; much of the aminopeptidase activity eluted with it however. A minor peak of BAPAase activity which eluted earlier was ignored. The active fractions were pooled, concentrated in a Diaflo apparatus (UM 10 membrane) (fraction 4) and dialyzed against 0.01 M acetate buffer, pH 5.2. The solution was then chromatographed on DEAE-cellulose at pH 5.2 with a linear gradient from 0.01 M to 0.10 M acetate, pH 5.2, commenced after the protein eluting at the front had been removed. The major BAPAase peak eluted with 0.03 M to 0.04 M acetate there being two minor peaks of BAPAase activity which eluted before commencement of the gradient and which were not further investigated. Most of the aminopeptidase was separated from the BAPAase as a large peak of activity following the BAPAase although a small aminopeptidase peak was coincident with the latter. The major BAPAase activity was pooled, adjusted to pH 7.4, concentrated (fraction 5), and dialyzed against 0.01 M tris-HCl buffer, pH 7.4, 0.07 M in KCl. Chromatography on a third DEAE-cellulose column with 0.01 M tris-HCl, pH 7.4, and a linear salt gradient from 0.07 M to 0.17 M KCl resolved the BAPAase activity (Fig. 1) into two peaks, BAPAase I and II, eluting between 0.075 M and 0.1 M KCl, both of these being almost completely separated from the aminopeptidase activity. Fractions corresponding to the two peaks of BAPAase activity were pooled and concentrated to give BAPAase I (90–98) and BAPA-ase II (109–117). These preparations (fraction 6) were used in all subsequent experiments unless otherwise indicated.

The preparations were examined by discontinuous polyacrylamide gel electrophoresis. Both BAPAase I and II gave a number of protein bands including one major band common to both preparations. However the BAPAase activity (indicated in the gel by activity against BANA) was not associated with this major band but was a diffuse band, with the same mobility for BAPAase I and II, which could not be correllated with any one protein band.

Crude extracts of other seeds were prepared by the same method as described above for peas.

pH Optima of Enzymes. The variations of activity with pH for BAPAases I and II are virtually identical and show an optimum pH of 7.

Molecular Weights. The mol wt of BAPAases I and II were determined on a calibrated Sephadex G-150 column. Both en-

Table I. *Purification of BAPAases*

Step	Volume	Total Protein	Specific Activity	Total Activity	Recovery	Purification
	ml	*mg*	*units/mg*	*units*	%	
1. Crude extract	1890	48,400	0.063	3030	100	1
2. 35-60% $(NH_4)_2SO_4$ fraction	500	17,000	0.069	1180	39	1.1
3. pH 5.2 precipitation	480	10,300	0.086	890	29	1.4
4. DEAE-cellulose, pH 7.4	61	530	1.28	680	22	20
5. DEAE-cellulose, pH 5.2	15.4	15.0	15.1	227	7.5	240
6. DEAE-cellulose, pH 7.4						
I	2.3	0.17	114	19.3	0.6	1810
II	2.8	0.19	243	46.2	1.5	3860

zymes gave the same value of 65,000 daltons.

Michaelis Constants. The Michaelis constants, determined at pH 6 were 45 μM for BAPAase I and 30 μM for BAPAase II.

Inhibitors. The effects of various inhibitors of proteinase and peptidase activity on the activity of BAPAases I and II are summarized in Table II. Both enzymes were completely inhibited by DFP and iodoacetic acid and to a lesser extent by other thiol reagents. Protein inhibitors of trypsin from various sources did not inhibit the enzymes but stimulated them. BAPAase activities present in crude extracts of pea, broad bean, soybean, and wheat were all completely inhibited by DFP.

Specificity. Proteinase activity, as indicated by digestion of casein, was monitored during the purification and found to be clearly separated from the BAPAase activity by the first DEAE-column. The final preparations, BAPAases I and II, also had no detectable proteinase activity when incubated with the following proteins: SCM-BSA, SCM-fowl feather keratin, salmine (the arginine-rich protamine from salmon), and a lupin globulin prepration.

Three aminopeptidases have been shown to be present in pea extracts (9) and their absence in the BAPAase preparations was demonstrated by their failure to hydrolyze the β-naphthylamides of glycine, alanine, leucine, phenylalanine, methionine, proline, glutamic acid, and lysine. Arginine β-naphthylamide was hydrolyzed to a very small extent. Carboxypeptidase substrates tested and not hydrolyzed were hippuryl-Gly-Gly, Z-Gly-Ala, Z-Gly-Leu, Z-Gly-Phe, Z-Gly-Tyr, Z-Gly-Trp, Z-Gly-Glu, hippuryl-Lys, and hippuryl-Arg. These results indicate that there is no exopeptidase activity in these preparations.

BAPAases from peanut (5) and soybean (14) show activity against the esterase substrate BAEE; however, the pea BAPAases, when assayed under the same conditions against this substrate, showed no such activity.

A number of peptides and peptide derivatives were tested against the BAPAase preparations (Table III). It can be seen that the enzymes preferred as substrates peptides of the general formula X-(Arg or Lys)-Y where X may be an amino acid or other blocking group and Y an amino acid or other leaving group.

Table III. *Peptide Hydrolysis by BAPAases*

BAPAases I and II were tested separately and gave the same results.

Peptide	Extent of Hydrolysis[1]	Peptide	Extent of Hydrolysis
Acetyl-Gly-Lys-NA	***	Ile-Val-Gly	+
Gly-Arg-NA	***	Z-Leu-Leu-Gly	0
Pro-Arg-NA	***	Z(p-NO_2)-Ile-Gly	0
Z-Gly-Gly-Arg-NA	***	Leu-Thr-Gly	++
Gly-Phe-NA	0	Acetyl-Gly-Lys-Gly	+
		Z-Arg-Gly	++
Z-Gly-Gly	0	Tosyl-Arg-Gly	+
Val-Gly-Gly	0	Leu-Gly-Phe	0
Z-Ala-Gly	0	Gln-Gly-Ala-Gly	0

[1] The extent of hydrolysis of the β-naphthylamides (indicated by *) cannot be compared with that of the other peptides (indicated by +) because of the different methods of assessment involved.

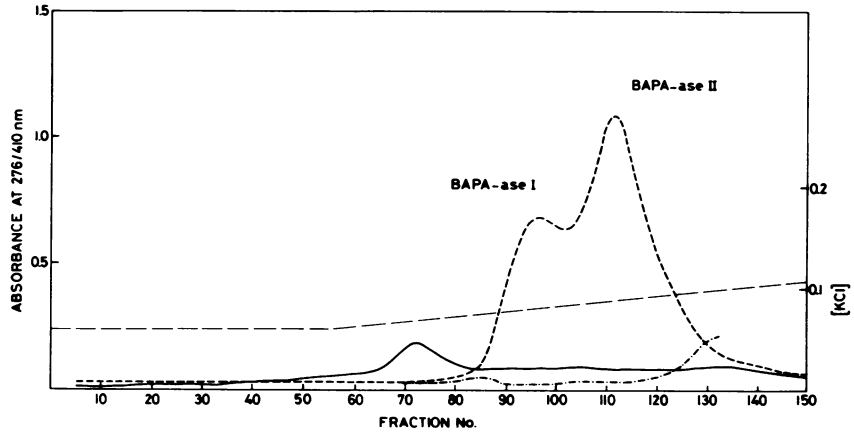

FIG. 1. Elution of BAPAase activity from DEAE-cellulose, pH 7.4 (step 6, Table I). Protein concentration, A_{276} (——); BAPAase activity, A_{410} (-----); activity against alanine-*p*-nitroanilide, A_{410} (-·-·-); KCl concentration (- - -); fraction size: 5 ml.

Table II. *Effect of Inhibitors on BAPAase Activity*

Inhibitor	Conditions of Preincubation				Activity[1]	
					BAPAase I	BAPAase II
					%	
pCMB	1 mM	pH 8	2 hr	R.T.[2]	33	30
NEMI	1 mM	pH 8	2 hr	R.T.	30	33
Iodoacetic acid	1 mM	pH 8	2 hr	R.T.	0	0
Mercaptoethanol	1 mM	pH 6	2 hr	R.T.	108	100
EDTA	1 mM	pH 6	2 hr	R.T.	108	119
1:10 Phenanthroline	1 mM	pH 6	1 hr	R.T.	ND[3]	103
DFP	1 mM	pH 6	1.5 hr	R.T.	0	2
Salmine	0.3 mg/ml	pH 6	30 min	37 C	4	4
Lima bean trypsin inhibitor	0.025 mg/ml	pH 6	30 min	37 C	124	154
Soybean trypsin inhibitor	0.025 mg/ml	pH 6	30 min	37 C	131	162
Ovomucoid trypsin inhibitor	0.025 mg/ml	pH 6	30 min	37 C	123	150

[1] Activity against BAPA was determined by normal assay at pH 6 after a preincubation carried out under conditions listed in the table and is expressed as a percentage of the activity present in the absence of inhibitor. Preincubations at pH 8 were carried out at low volume to allow for adjustment back to pH 6 and a volume of 2 ml for the assay. Approximately 0.04 unit of activity was used.

[2] Room temperature.

[3] Not determined.

The nonbasic peptides Ile-Val-Gly and Leu-Thr-Gly were also cleaved, and in each case it was the N-terminal amino acid that was cleaved off as shown by end group analysis by dansylation (12).

DISCUSSION

Using as substrate BAPA, two enzymes BAPAase I and BAPAase II have been isolated and extensively purified from pea seeds. These enzymes can be separated by chromatography on DEAE-cellulose at pH 7.4 but apart from this they are similar. Neither of the enzyme preparations was pure as shown by the criterion of analytical polyacrylamide gel electrophoresis, but they were virtually free of other proteinase and peptidase activities. Two BAPAases have also been found in peanut (20).

An attempt at further purification of the enzymes by affinity chromatography was made following the observation that BAPAase activity was inhibited by salmine (Table II). Using a partially purified preparation a significant increase in specific activity was obtained after elution of BAPAase from salmine-Sepharose with pH 5 buffer; also the BAPAase activity was clearly separated from an aminopeptidase present in this preparation. However, the enzyme was still far from pure, as shown by polyacrylamide gel electrophoresis, and as the recovery from the column was very low, this approach was not pursued further.

BAPAases I and II both gave mol wt of 65,000 daltons on a calibrated Sephadex column; the Michaelis constants were determined to be 45 μM and 30 μM, respectively. Their behavior with a number of common inhibitors of proteinase and peptidase activity (Table II) suggests that they are serine peptidases with requirement for a thiol group in the molecule. Other peptidases with this same pattern of inhibition have recently been described (11, 13, 15, 26). No requirement for metal ions was demonstrated.

The enzymes are not inhibited by several protein inhibitors of trypsin but are significantly activated by them. A similar level of activation was obtained with BSA suggesting that this is a nonspecific effect.

Assays using a number of common protein substrates showed no endopeptidase activity in either enzyme so that there is little doubt that the two BAPAases are not proteinases but peptide hydrolases. Seventeen small peptides and peptide β-naphthylamides were tested as potential substrates (Table III). Both enzymes hydrolyzed peptides on the carboxyl side of basic amino acids that were not N-terminal, and appeared to have no intrinsic aminopeptidase activity. The small amount of hydrolysis of arginine-β-naphthylamide was taken to be due to traces of an arginine specific aminopeptidase. The situation was complicated further by the hydrolysis observed with the tripeptides Leu-Thr-Gly (after Leu) and Ile-Val-Gly (after Ile), but it seems probable that this was due to traces of aminopeptidase not detected by assays using amino acid β-naphthylamides, as otherwise one might have expected hat Gly-Phe-NA would also be hydrolyzed.

The two BAPAases from pea seem to be typical of those isolated from other plants. The mol wt of 65,000 is comparable with the values of 70,000 for the vetch BAPAases (28), 63,000 for the soybean enzyme (14), 59,000 for the wheat enzyme (16) and 60,000 for both peanut enzymes (20).

The behavior of the various BAPAases described suggests that these enzymes from plant seeds are all of the "active serine" type with a requirement for a thiol group. Thus the pea, soybean, vetch, and wheat enzymes all show a similar pattern of inhibition with thiol reagents while the pea, broad bean, soybean, wheat (3), and peanut (20) enzymes are all inhibited by DFP.

Unfortunately no BAPAase has been purified sufficiently to allow a definitive study of specificity although it has been clearly demonstrated that these enzymes have no proteolytic activity (4–6, 14, 16, 28). It appears from the present study and that on the vetch enzyme that the BAPAases are specific for small peptides containing basic amino acids and cleave them on the carboxyl side of the basic residue. The reported wider specificities for other BAPAases (6, 14, 22, 24) are probably due to the presence of other peptidases.

The pea BAPAases can be extracted from seeds even after H_2O imbibition in the presence of actinomycin D or cycloheximide, inhibitors of RNA and protein synthesis, respectively, suggesting that the enzymes are present, in potentially active form, in the dormant seed. The function of the BAPAases in the metabolism of plants is at present obscure and further advances in this field will require a more complete knowledge of their substrate specificity and perhaps, also, knowledge of the specificity of the proteinases of seeds.

LITERATURE CITED

1. Altschul, A. M., L. Y. Yatsu, R. L. Ory, and E. M. Engleman. 1966. Seed proteins. Annu. Rev. Plant Physiol. 17: 113–136.
2. Andrews, P. 1964. Estimation of the molecular weights of proteins by Sephadex gel-filtration. Biochem. J. 91: 222–233.
3. Belitz, H.-D. and F. Lynen. 1974. The proteolytic activity of wheat: occurrence of a trypsin-like enzyme. Chem. Mikrobiol. Technol. Lebensm. 3: 60–64.
4. Burger, W. C., N. Prentice, J. Kastenschmidt, and M. Moeller. 1968. Partial purification and characterization of barley peptide hydrolases. Phytochemistry 7: 1261–1270.
5. Cameron, E. C. and M. Mazelis. 1971. A nonproteolytic "trypsin-like" enzyme. Purification and properties of arachain. Plant Physiol. 48: 278–281.
6. Catsimpoolas, N., S. K. Funk, J. Wand, and J. Kenney. 1971. Isoelectric fractionation and some properties of a protease from soybean seeds. J. Sci. Food Agric. 22: 79–82.
7. Crestfield, A. M., S. Moore, and W. H. Stein. 1963. The preparation and enzymatic hydrolysis of reduced and S-carboxymethylated proteins. J. Biol. Chem. 238: 622–627.
8. Davis, B. J. 1964. Disc electrophoresis. II. Method and application to human serum proteins. Ann. N.Y. Acad. Sci. 121: 404–427.
9. Elleman, T. C. 1974. Aminopeptidases of pea. Biochem. J. 141: 113–118.
10. Erlanger, B. F., N. Kakowsky, and I. W. Cohen. 1961. The preparation and properties of two new chromogenic substrates for trypsin. Arch. Biochem. Biophys. 95: 271–278.
11. Gleeson, P., L. G. Sparrow, and C. W. Ward. 1976. Aryl acylamidase activity in larvae of the webbing clothes moth, *Tineola bisselliella*. Comp. Biochem. Physiol. 53B: 187–190.
12. Gray, W. R. 1967. Dansyl chloride procedure. Methods Enzymol. 11: 139–151.
13. Hayashi, R. and T. Hata. 1972. Action of yeast proteinase C on synthetic peptides and poly-α, L-amino acids. Biochim. Biophys. Acta 263: 673–679.
14. Hoagland, R. E. and G. Graf. 1974. The purification and properties of an amidohydrolase from soybean. Can. J. Biochem. 52: 903–910.
15. Jones, S. R. and T. Hofmann. 1972. Penicillocarboxypeptidase-S, a non-specific SH-dependent exopeptidase. Can. J. Biochem. 50: 1297–1310.
16. Kruger, J. E. 1971. Purification and some properties of malted-wheat BAPA-ase. Cereal Chem. 48: 512–522.
17. Kunitz, M. 1947. Crystalline soybean trypsin inhibitor. II. General properties. J. Gen. Physiol. 30: 291–310.
18. Lineweaver, H. and D. Burk. 1934. The determination of enzyme dissociation constants. J. Am. Chem. Soc. 56: 658–666.
19. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193: 265–275.
20. Mainguy, P. N. R., R. B. van Huystee, and D. B. Hayden. 1972. Form and action of a protease in cotyledons of germinating peanut seeds. Can. J. Bot. 50: 2189–2195.
21. Marks, N., R. K. Datta, and A. Lajtha. 1968. Partial resolution of brain arylamidases and aminopeptidases. J. Biol. Chem. 243: 2882–2889.
22. Moeller, M., W. C. Burger, and N. Prentice. 1969. Purification of a barley peptide hydrolase by disc electrophoresis. Phytochemistry 8: 2153–2156.
23. Naughton, M. A., F. Sanger, B. S. Hartley, and D. C. Shaw. 1960. The amino acid sequence around the reactive serine residue of some proteolytic enzymes. Biochem. J. 77: 149–163.
24. Prentice, N., W. C. Burger, J. Kastenschmidt, and M. Moeller. 1970. Peptide hydrolase in wheat embryo. Phytochemistry 9: 41–47.
25. Rosen, H. 1957. A modified ninhydrin colorimetric analysis for amino acids. Arch. Biochem. 67: 10–15.
26. Visuri, K., J. Mikola, and T.-M. Enari. 1969. Isolation and partial characterization of a carboxypeptidase from barley. Eur. J. Biochem. 7: 193–199.
27. Ward, C. W. 1973. Spectral data for modified assay of benzoylarginine-*p*-nitroanilide-hydrolysing enzymes in crude extracts. Analyt. Biochem. 56: 596–600.
28. Zemchik, E. I., A. D. Shutov, and J. A. Weintraub. 1973. Partial purification and characterization of arylamidase from vetch seeds. Biokhimiya 38: 964–970.