Aminopeptidases of Pea

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Studies of crude extracts of pea seeds (*Pisum sativum*, var. Green feast) revealed the presence of three enzymes that hydrolyse the amide bond of aminoacyl β -naphthylamides. They differ in their specificity towards the aminoacyl moiety; one is proline-specific, whereas the other two hydrolyse the β -naphthylamides of primary amino acids. Of the latter, one is highly specific for hydrophobic aminoacyl residues whereas the other has a broader, somewhat complementary specificity, showing preferential hydrolysis of non-hydrophobic aminoacyl residues. These latter two aminoacyl- β -naphthylamidases have been separated and partly characterized with regard to substrate specificity and antagonism by inhibitors. Both are true aminopeptidases, requiring the presence of a free amino group and hydrolysing the amide bonds of amino acid amides, dipeptides and oligopeptides consecutively from the *N*-terminal end.

Legumes are easily grown from seeds and have only a minimal requirement for nitrogenous fertilizer. They thus offer a cheap and convenient source of dietary protein, especially the seeds which are rich in stored protein. Unfortunately the reserve proteins of these seeds are deficient in certain essential amino acids and it is hoped to alter the composition to improve the content of these amino acids. A better understanding of these reserve proteins and their mobilization by degradation during germination is useful to determine if such changes are feasible if new varieties, both biologically viable and more nutritionally adequate, are to be created.

This paper is concerned with an initial characterization of the aminopeptidases present in pea seeds.

Methods and Materials

Materials

Peptides and L-aminoacyl β -naphthylamides were purchased from Cyclo Chemical Corp., Los Angeles, Calif., U.S.A.; β -naphthylamine was from Sigma Chemical Co., St. Louis, Mo., U.S.A.; Fast Garnet GBC was from G. T. Gurr Ltd., London S.W.14, U.K. All reagents used were of analytical grade. Ion-exchange celluloses were obtained from Whatman Biochemicals Ltd., Kent, U.K. Hydroxyapatite (Bio-Gel HTP) was from Bio-Rad Laboratories, Richmond, Calif., U.S.A. Peas of the variety Green feast were purchased from Brunnings Ltd., Melbourne, Australia. 3-Phenylpropionyl-leucine and its amide were kindly prepared by Dr. F. H. C. Stewart of this laboratory.

Activity determinations

The enzymic hydrolysis of β -naphthylamides was measured by coupling the released β -naphthylamine

with a stabilized diazonium salt, Fast Garnet GBC, to produce a red azo dye which was estimated spectrophotometrically at 520nm (Marks *et al.*, 1968). Enzymic hydrolyses were carried out in 1ml volumes of 0.05M-potassium phosphate-0.001M-EDTA, pH6.8, at 37°C. Reactions were terminated by addition of 100 μ l of 20% (v/v) acetic acid. A solution (0.5ml) of 1.0M-sodium acetate, pH4.2, containing 0.1% Fast Garnet GBC and 5% (w/v) Brij 35 (to accelerate colour development and keep the azo dye in solution) was added and colour development was allowed to proceed to an optimum value, which was reached after 10min and was stable for several hours. The mixture was diluted to 5ml with 1M-acetate buffer, pH4.2, and the E_{520} was determined. Standard curves were prepared from β -naphthylamine; 0.185 μ mol of β -naphthylamine yielded a ΔE_{520} of 1.0 in the assay, which was linear to beyond this value.

The above assay of Marks *et al.* (1968) was used in all cases except the assessment of the effect of thiol on the activity of the enzymes, since thiol compounds interfere with this method. In this case alone was the method of Barrett (1972) used which involves the addition of a thiol-blocking agent to a new coupling reagent (1ml) composed of equal parts of (a) 0.05% Fast Garnet GBC in 4% (w/v) Brij 35, and (b) 10mM - *p* - chloromercuribenzoate - 50mM - EDTA, pH6.0. The azo dye was extracted from the turbid mixture with an equal volume of butan-1-ol and the E_{520} was determined.

For routine assays during the isolation procedure 1mM - L-leucine β -naphthylamide or L-alanine β -naphthylamide in a 1ml reaction volume was used as substrate. For measurement of kinetic parameters 0.01% (w/v) bovine serum albumin was added to the reaction mixture to obtain consistent results.

Calculation of kinetic parameters

A slight modification of the Lineweaver-Burk equation was used, in which the form is retained but the instantaneous initial velocity is replaced by the change of substrate concentration divided by the time interval and the initial substrate concentration is replaced by the arithmetic mean of the substrate concentration during this time-interval (Lee & Wilson, 1971). This permitted the use of the lower substrate concentrations required in K_m determinations but allowed enough reaction to give a measurable change in reactants or products. This method is valid only if the reaction is irreversible and there is no inhibition by products. Initial studies with methionine β -naphthylamide (0.5mM) showed no significant fall in rate after 30% of the substrate had been consumed, indicating that the method was valid. The extent of reaction was shown to be directly proportional to both time and the concentration of enzyme over the ranges used to determine kinetic parameters.

Peptide hydrolysis

The hydrolysis of peptides by enzymes was performed at 37°C in 0.05M-potassium phosphate-0.001M-EDTA buffer, pH6.8, containing 0.01% bovine serum albumin and with 0.4ml of 2.5mM-substrate and 2 μ l of 'preparation AP1'. (The K_m of Leu-Ala was determined as approx. 1mM.) The extent of hydrolysis after 3½h was determined by amino acid analysis with a Beckman Spinco amino acid analyser. Qualitative assessment of hydrolysis was made by subjecting the samples to high-voltage electrophoresis at pH3.5 on Whatman 3MM paper (Ambler, 1963).

Estimation of protein

Protein concentrations were determined by the method of Lowry *et al.* (1951) with bovine serum albumin as standard.

Polyacrylamide-gel electrophoresis

Slab gels of dimensions 7cm×7cm×0.3cm were used. The discontinuous system of Ornstein & Davis (1964a,b) was used with a running gel pH of 9.5 and a voltage gradient of 20V/cm. Runs were of 4h duration. Proteins were stained with 0.5% Nigrosin in 5% (w/v) trichloroacetic acid-10% (v/v) acetic acid, and gels were destained overnight in the same solvent. Enzymes were located by incubation of the gels for 15min at room temperature in 0.5mM-aminoacyl β -naphthylamide solution in phosphate buffer, pH6.8, followed by immersion in 0.33M-sodium acetate (pH4.2) containing 0.1% Fast Garnet GBC and 1.7% Brij 35. Generally, sufficient enzyme to release

approx. 0.5 μ mol of β -naphthylamine/min under the conditions of routine assay was applied over a gel surface area of 200mm². The heavily loaded gels had twice this amount.

Results

Isolation of enzymes

Peas of the strain *Pisum sativum* var. Green feast were surface-sterilized by soaking in 1% (w/v) sodium hypochlorite for 10min, washed in distilled water and then soaked overnight in water at 37°C. The seed coat and axis were removed and the cotyledons were homogenized in a Waring blender in 0.05M-Tris-HCl-0.002M-EDTA at pH7.5 (150ml per 100g of cotyledons); preliminary studies had shown no inhibition of the aminopeptidases by EDTA. All subsequent procedures were performed at 5°C. The homogenate was centrifuged and filtered and the supernatant dialysed overnight against 0.01M-Tris-HCl-0.002M-EDTA, pH7.5. Anodic slab gel electrophoresis at pH9.5 showed this crude extract to contain 3 separate activities when tested with a variety of aminoacyl β -naphthylamides (Plate 1). The most anionic of these three enzymes, designated AP1, most readily degraded substrates of high hydrophobicity; the next enzyme designated AP2 was of a broader somewhat complementary specificity, degrading non-hydrophobic substrates preferentially; and the least anionic degraded only proline β -naphthylamide.

Proteins showing activity with leucine β -naphthylamide and alanine β -naphthylamide as substrates, were further purified. These enzymes were precipitated from the crude extract at between 35 and 100% saturation with (NH₄)₂SO₄. The precipitate was redissolved, dialysed at pH7.5 and reprecipitated between 35 and 52% saturation. Residual (NH₄)₂SO₄ was removed by dialysis.

The solution was dialysed at pH5.5 for several days in 0.005M-sodium acetate-0.001M-EDTA buffer, producing a copious precipitate which was removed. The supernatant was applied to a column (25cm×2cm) of CM-cellulose (CM52) equilibrated with the pH5.5 buffer. The proteins showing activity with the β -naphthylamides of leucine and alanine were eluted unretarded by the same buffer, whilst much unwanted protein remained on the column (Table 1). The active fraction was dialysed against 0.005M-Tris-HCl-0.05M-NaCl, pH7.4, concentrated to a small volume in a Diaflo apparatus (with a UM 10 membrane) and applied to a column (40cm×2.5cm) of DEAE-cellulose (DE 52) equilibrated with the same buffer. A linear gradient (4 litres) from 0.05M- to 0.25M-NaCl was used to elute and separate the enzymes (Fig. 1). The preparations were concentrated to small volumes in a Diaflo apparatus. 'Preparation AP2'

EXPLANATION OF PLATE I

Discontinuous polyacrylamide-slab-gel electrophoresis of 500 μ l of crude extract from pea seeds applied over 200 mm² surface
The gels were sliced and stained for activities with aminoacyl β -naphthylamides as described in the text. The amino acids marked on the Plate refer to the aminoacyl β -naphthylamides tested as substrates.

EXPLANATION OF PLATE 2

Discontinuous polyacrylamide-slab-gel electrophoresis of protein AP2 as isolated from DEAE-cellulose

The gels were stained for activity with aminoacyl β -naphthylamides. The loading was sufficient to release 1 μ mol of β -naphthylamine/min in the routine assay with alanine β -naphthylamide. The amino acids marked on the Plate refer to the aminoacyl β -naphthylamides tested as substrate.

Table 1. Yields and activity with leucine β -naphthylamide at different stages of purification of protein AP1 from 600g of swollen pea cotyledons

Activity is expressed as μmol of β -naphthylamine released/min in a 1 ml reaction volume. The conditions of assay were as described in the text.

Stage	Vol.	Total activity ($\mu\text{mol}/\text{min}$)	Specific activity ($\mu\text{mol}/\text{min}$ per mg)	Yield (%)
Crude extract	1.2 litre	1166	0.039	100
35–52% $(\text{NH}_4)_2\text{SO}_4$	96ml	1000	0.142	86
pH 5.5 precipitation	100ml	777	0.222	67
CM-cellulose	25ml	740	1.97	63
DEAE-cellulose	5.2ml	481	54.4	41
Sephadex G-200	4.4ml	315	137	27
Hydroxyapatite	3.6ml	154	285	13

Fig. 1. Elution profile from DEAE-cellulose (DE 52) of activities with the β -naphthylamides of leucine and alanine

Samples ($10\mu\text{l}$ per tube) were used in the routine assay for 15 min. Activity with leucine β -naphthylamide is indicated by $\bullet$. Activity with alanine β -naphthylamide is indicated by $\cdots$. E_{280} is indicated by —. A flow rate of 1 ml/min was used and 16 ml fractions were collected.

(the combined fractions active with alanine β -naphthylamide as substrate) was not further purified and was stored at -15°C in a 50% solution of glycerol.

The combined fractions active with leucine β -naphthylamide were concentrated to a small volume and fractionated on a column ($120\text{cm} \times 2.5\text{cm}$) of Sephadex G-200 in 0.01 M-Tris-HCl-0.001 M-EDTA-0.5 M-NaCl. The active fraction was once again concentrated, dialysed against 0.01 M-potassium phosphate, pH 6.8, and fractionated on a column ($40\text{cm} \times 1.5\text{cm}$) of hydroxyapatite (Bio-Gel HTP) with a linear gradient (1 litre) from 0.01 M- to 0.2 M-potassium phosphate, pH 6.8. The active fraction,

Table 2. K_m and V_{max} values for the hydrolysis of L-amino-acyl β -naphthylamides by protein AP1

V_{max} values are expressed relative to the V_{max} for hydrolysis of methionine β -naphthylamide of $0.051\mu\text{mol}/\text{min}$ per μl ($0.05\mu\text{g}$) of preparation AP1, for a 1 ml reaction volume. The conditions of assay are as described in the text. Values of K_m for proline and tryptophan are approximations since they were obtained by extrapolation from substrate concentrations higher than 0.02 mM, i.e. well above their K_m values. No lower concentration could be used in these studies since at least $0.01\mu\text{mol}$ of β -naphthylamine must be produced to yield a ΔE_{520} of 0.054, and even then the accuracy of this ΔE_{520} is poor ($\pm 6\%$).

Amino-acyl group	K_m (mM)	V_{max} (relative %)	V_{max}/K_m (relative %)
Met	0.04	100	100
Leu	0.04	80.6	87
Phe	0.06	94.6	69
Trp	≈ 0.01	37.2	≈ 133
Tyr	0.13	233	77
Ile	0.04	3.1	3.2
Val	0.07	2.2	1.4
Ala	0.42	12.1	1.2
Gly	1.07	0.5	0.02
Thr	0.42	0.2	0.02
Ser	1.16	0.4	0.01
His	0.39	1.4	0.15
Lys	0.59	0.1	0.008
Arg	0.56	0.02	0.001
Glu	3.2	0.015	0.0002
Pro	≈ 0.005	2.5	≈ 21
Asp	No reaction		

designated 'preparation AP1' was concentrated and stored at -15°C in a 50% solution of glycerol.

Purity of the preparations

The protein showing activity with alanine β -naphthylamide, AP2, was eluted from the DEAE-cellulose column completely free from contamination with the other β -naphthylamidases, as judged by heavily loaded disc gel electrophoresis (Plate 2), although it was still highly impure as regards other proteins. The protein with activity with leucine β -naphthylamide, AP1, was chromatographed on hydroxyapatite to remove the last traces of AP2 activity as judged by heavily loaded disc gel electrophoresis. The AP1 preparation had at this stage been purified 7000-fold from the initial extract (Table 1) and contained only two protein bands, one of which coincided with the activity with leucine β -naphthylamide although this was not the major protein.

Hydrolysis of peptides and peptide analogues by protein AP1

Purified protein AP1 was found to cleave the β -naphthylamides of L-amino acids with a particular

Table 3. *Hydrolysis of peptides by protein AP1*

Values indicated are the percentage hydrolysis of the *N*-terminal peptide bond in 3½ h at 37°C by 2 µl (0.1 µg) of preparation AP1 under the conditions described in the text.

Dipeptides		Tripeptides		Other peptides	
Phe-Ala	100	Phe-Gly-Gly	100	Leu-NH ₂	100
Phe-Gly	100	Leu-Gly-Gly	100	Phe-Gly-Ala-Gly-Phe-Gly-Gly	100
Phe-Arg	100	Met-Ala-Ser	100	Pro-Leu-Gly-Gly	0
Phe-Glu	75	Val-Gly-Gly	0	Gln-Gly-Ala-Gly	0
Phe-Pro	0	Ala-Gly-Gly	0	Formyl-Met-Ala	0
Leu-Ala	99	Ala-Ala-Ala	0	Benzoyloxycarbonyl-Phe-Leu	0
Leu-Gly	28	Ala-Phe-Gly	28	Benzoyloxycarbonyl-Phe-Leu-NH ₂	0
Leu-Pro	0	Ala-Gly-Phe	20		
L-Leu-D-Leu	4	Ala-Gly-Leu	0	-CH ₂ CH ₂ CO-Leu	0
D-Leu-L-Leu	0	Ala-Gly-Ile	0		
Met-Ala	95	Ala-Gly-Val	0	-CH ₂ CH ₂ CO-Leu-NH ₂	0
Lys-Ala	0				
Glu-Ala	0				
Ile-Ala	≤ 2				
Ile-Ile	6				
Ile-Phe	22				
Ile-Leu	57				
Ala-Ala	0				
Ala-Phe	0				
Ala-Leu	15				
Gln-Leu	0				
Glu-Phe	0				
Pro-Phe	0				
Pro-Leu	0				

preference for those of hydrophobic amino acids, except for valine and isoleucine. The kinetic parameters determined for the hydrolysis of these derivatives at pH 6.8 are shown in Table 2.

A generally similar specificity is also demonstrated in the hydrolysis of dipeptides (Table 3), a hydrophobic amino acid being necessary in position 1 for hydrolysis of the peptide bond. A hydrophobic amino acid in position 2 in dipeptides and tripeptides and an aromatic amino acid in position 3 in tripeptides increased the rate of hydrolysis of less favoured amino-terminal residues, such as alanine and isoleucine.

Protein AP1 has an absolute requirement for an L amino group since D-leucyl-L-leucine, peptides with substituted amino groups, deaminated peptides (e.g. 3-phenylpropionyl is equivalent to phenylalanyl less the amino grouping) and prolyl peptides were not hydrolysed. Glutarylphenylalanine β-naphthylamide was not hydrolysed with 100 times the enzyme used in a standard assay of phenylalanine β-naphthylamide. Only bonds with L amino acids in position 2 were cleaved to any considerable extent, and bonds formed by the imino group of proline were not cleaved.

The proximity of the C-terminal carboxyl group interfered with the hydrolysis of the *N*-terminal peptide bond since leucine amide and leucylglycylglycine are more rapidly hydrolysed than leucylglycine.

Hydrolysis of peptides and peptide analogues by protein AP2

Protein AP2 showed a broad specificity in hydrolysis of β-naphthylamides of amino acids as demonstrated by gel electrophoresis (Plate 2). Glutarylphenylalanine β-naphthylamide was not detectably hydrolysed under conditions using 100 times the enzyme required for hydrolysis of phenylalanine β-naphthylamide, but benzoylarginine β-naphthylamide was hydrolysed at a rate similar to arginine β-naphthylamide. This activity with benzoylarginine β-naphthylamide was, however, completely inhibited by di-isopropyl phosphorofluoridate unlike the aminopeptidase activity, and was only partially susceptible to inhibition by 1,10-phenanthroline under conditions which completely inhibited aminopeptidase activity. A variety of *N*-blocked dipeptides were not hydrolysed although similar unblocked peptides and peptide amides were readily hydrolysed. These observations suggest that preparation AP2 was contaminated by a benzoylarginine β-naphthylamidase. An L configuration at the α C atom seems to be required, since unlike the LL diastereoisomer, D-leucyl-L-leucine was not detectably hydrolysed.

Molecular-weight determinations by gel filtration

The molecular weights of the L-aminoacyl β-naphthylamidases AP1 and AP2 were determined by

Fig. 2. pH-activity profiles for protein AP1 with leucine β -naphthylamide (a) and protein AP2 with alanine β -naphthylamide (b)

The activities were measured in 0.05M-potassium phosphate buffers, containing 0.001M-EDTA and 0.01% (w/v) bovine serum albumin.

Table 4. Effects of activators and inhibitors on hydrolysis of β -naphthylamides of leucine and alanine by protein AP1 and protein AP2 respectively

A 15 min preincubation was used, followed by a 10 min assay, with 0.5 μ l (0.025 μ g) of 'preparation AP1' or 5 μ l (5 μ g) of 'preparation AP2' which in controls released 0.10 μ mol and 0.12 μ mol of β -naphthylamine respectively under the conditions of routine assay.

Substance added	Concn. of substance added (M)	Effect on aminopeptidase activity	
		AP1	AP2
EDTA	0.01	No effect	No effect
Dithiothreitol	0.002	No effect	<10% Inhibition
Cysteine	0.002	No effect	<10% Inhibition
<i>p</i> -Chloromercuribenzoate	0.0001	98% Inhibition	98% Inhibition
5,5'-Dithiobis-(2-nitrobenzoic acid)	0.001	35% Inhibition	40% Inhibition
1,10-Phenanthroline	0.002	No effect	98% Inhibition
	0.010	No effect	100% Inhibition
<i>N</i> -Ethylmaleimide	0.01	30% Inhibition	70% Inhibition
Iodoacetamide	0.001	<10% Inhibition	35% Inhibition
Di-isopropyl phosphorofluoridate	0.001	No effect	No effect
Iodosobenzoate	0.002	40% Inhibition	90% Inhibition

gel filtration on a column (150cm $\times$ 2.5cm) of Sephadex G-100, by using markers of myoglobin (mol.wt. 17800), carbonic anhydrase (mol.wt. 30000), ovalbumin (mol.wt. 44000) and bovine serum albumin (mol.wt. 68000). The protein with activity with leucine β -naphthylamide, AP1, was eluted at a position corresponding to a molecular weight of 58000, whereas the alanine β -naphthylamidase, AP2, was eluted at a position corresponding to a molecular weight of 74000.

Effects of pH and other additives

Initial studies had shown that the enzymes were stable during preparation and on storage, but the stability during assay was erratic. Studies with inhibitors indicated the presence of a free thiol group necessary for activity, but EDTA and thiol did not alleviate this erratic behaviour in assays. Incorpora-

tion of 0.01% bovine serum albumin into the assay mixture gave more consistent results. Optimal activity with β -naphthylamides was observed at neutral pH for both enzymes (Fig. 1). The effects of several additives on the enzymes during a 15min preincubation were determined (Table 4).

Both enzymes appear to contain an essential thiol group, but thiols were without effect in increasing activity, and often produced a small variable inhibition with protein AP2. This may be due to the reduction of a labile disulphide bond or to chelation of an essential metal ion, since 1,10-phenanthroline markedly inhibited protein AP2. Inhibition of both enzymes by *p*-chloromercuribenzoate was completely reversed after 15min incubation with 2mM-cysteine. Inhibition by 5,5'-dithiobis-(2-nitrobenzoic acid) was completely reversible with 2mM-cysteine in the case of protein AP1, but for protein AP2 it was largely reversed under these conditions.

Aminopeptidase activity during germination

The development of aminopeptidase activity during germination was examined by permitting dry peas to imbibe solutions of actinomycin D (20 µg/ml) or cycloheximide (1 mg/ml), inhibitors of RNA synthesis and protein synthesis respectively. Aminopeptidase activity with leucine β -naphthylamide and alanine β -naphthylamide in crude extracts of the treated peas showed no difference from normal controls, and gel electrophoresis showed the presence of both aminopeptidases and proline iminopeptidase.

Discussion

Two aminopeptidases and a proline iminopeptidase have been identified in pea. The aminopeptidases have been partially purified and characterized. They are small molecules by comparison with the better known aminopeptidases of mammalian origin, AP1 having a molecular weight of about 58000 and AP2 having a molecular weight of about 74000. Both are true aminopeptidases requiring a free L amino group and removing residues consecutively from the *N*-terminal end of peptides. Both are sensitive to inhibition by thiol antagonists, although protein AP2 is also sensitive to the metal chelator 1,10-phenanthroline, which probably reflects involvement of a metal ion in catalysis by this protein.

Protein AP1 has been most extensively studied in this work in view of its narrow specificity. Only peptide bonds formed by the carboxyl group of hydrophobic amino acid residues in aminoacyl β -naphthylamides and dipeptides are hydrolysed to any appreciable extent. The behaviour of the enzyme with oligopeptides indicates a slightly broader specificity which might indicate the involvement of several subsites in the binding of larger peptides. Although both leucine and phenylalanine in position 2 increased the rate of release of the *N*-terminal residue, only phenylalanine was effective in this respect in position 3.

Bonds formed by the α -carboxyl group of aspartic acid and glutamic acid proved highly refractory to cleavage by both enzymes. A lower rate of hydrolysis of dipeptides by protein AP1 was also found where the α -amino group of these amino acids contributed to the peptide bond; the proximity of the *C*-terminal carboxyl group also decreased hydrolysis rates by protein AP1.

The developmental origins of the aminopeptidases have been studied by using inhibitors of protein synthesis. Synthesis *de novo* during germination does not occur, the enzymes being present in an active form in crude extracts of pea seeds swollen in the

presence of inhibitors of protein synthesis. The general inability of aminopeptidases to attack native proteins or long chains of amino acids, which require an initial attack by an endopeptidase, suggests that the seed probably requires no special protection from the action of its own aminopeptidases.

Few peptidases or proteases of plants and plant seeds have been well characterized, except for the highly active mercaptoproteinases of the sap or fruit juices of some plants, e.g. papain, ficin, bromelain (Glazer & Smith, 1971), some non-specific peptide hydrolases, e.g. arachain (Cameron & Mazelis, 1971), and some carboxypeptidases. The carboxypeptidases surprisingly have an 'active serine'-type active site, e.g. seedlings of French beans (Shaw & Wells, 1972), cotton (Ihle & Dure, 1972), barley (Visuri *et al.*, 1969) and citrus peel (Zuber, 1968). This type of catalysis seems to be confined to the higher plants since carboxypeptidases of animal and bacterial origin utilize metal-ion or 'active-thiol' mechanisms of catalysis.

In terms of specificity, size and antagonism by thiol inhibitors the aminopeptidase (AP1) reported here closely resembles an aminopeptidase found in barley (Kolehmainen & Mikola, 1971) which also prefers hydrophobic residues in positions 1 and 2. These two plant aminopeptidases thus differ from the better known aminopeptidases of mammalian sources, which are large metallopeptidases.

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