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An aminopeptidase (AP1) from sprouts of sweet potato (*Ipomoea batatas* (L.) Lam. cv. Tainong 64)

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Abstract. A neutral aminopeptidase (AP1) was purified 139-fold from sprouts of sweet potato (*Ipomoea batatas* L. Lam. cv. Tainong 64) using a 4-step procedure comprising extraction from sprouts, DEAE-cellulose column, Sephacryl S-300 column, and hydroxyapatite column chromatography. The molecular weight of AP1 was estimated to be about 115,000 by gel filtration on Sepharose CL/4B, and the isoelectric point was 5.53. The pH optimum for L-alanine 4-nitroanilide was 7.0, and the enzyme was most stable from pH 4.0 to 6.6 when the temperature was below 37°C. AP1 hydrolyzed 4 -nitroanilides of both L-alanine and L-leucine, and dipeptide and oligopeptides of L-alanine. The enzyme was strongly inhibited by aprotinin, PMSF, leupeptin, *p*CMB, and 1, 10-phenanthroline, but was not affected by thiol compounds, suggesting that a serine residue may participate in the catalytic process.

Key words: Aminopeptidase; *Ipomoea batatas*; Purification; Sprouts.

Introduction

In plant tissues, most research on proteolysis has centered on the role of proteases in the mobilization of reserve proteins. However, considerable interest in the molecular basis of ontogenetical processes has led to the realization that proteases are intricately involved in plant metabolism.

Aminopeptidases (AP) have been studied in the following examples of plant tissues: (1) in monocots: resting seeds (Kolehmainen and Mikola, 1971) and germinating seeds (Sopanen and Mikola, 1975) of barley, developing seeds of maize (Vodkin and Scandalios, 1980), young leaves of wheat (Waters and Dalling, 1983; 1984); (2) in dicots: resting seeds of apricot (Ninomiya *et al.*, 1981; 1983) and cotyledons of imbibed seeds of pea (Elleman, 1974); (3) in gymnosperms: resting and germinating seeds of Scots pine (Salmia and Mikola, 1975).

In sweet potato (2n=6x=90), we have reported on the occurrence and some properties (Lin *et al.*, 1988), possible storage function (Lin and Chen, 1980), and drought-responsive function (Lin, 1989) of trypsin inhibitor molecules in roots and leaves. However, detailed information about how trypsin inhibitor molecules inhibit endogenous proteases, or how they themselves are metabolized is not available yet. Therefore, knowledge of proteases in sweet potato tissues is urgently needed. We have detected activities of endopeptidase (EC. 3. 4), aminopeptidase (EC. 3.4.11), carboxypeptidase (EC. 3.16.17), and dipeptidyl peptidase (EC.

Abbreviations: Ac, α-N-acetyl; AP, aminopeptidase; Bz, α-N -benzoyl; CP, carboxypeptidase; DEAE, diethyl amino ethyl; EDTA, ethylene diamine tetraacetic acid; EE, ethyl ester; EP, endopeptidase; NHNan, 4-nitroanilide; NHNap, β-naphthylamide; ONap, β-naphthylester; PAGE, polyacrylamide gel electrophoresis; PMSF, phenylmethane sulfonyl fluoride; SDS, sodium dodecyl sulphate; Su, α-N-succinyl; TI, trypsin inhibitor; 'T64', sweet potato cultivar Tainong 64.

3.4.14) in sprouts and resting roots. In this publication, we describe the purification and characterization of an aminopeptidase from sprouts of 'T64'.

Materials and Methods

Chemicals and Chromatographic Materials

Acrylamide, ammonium persulphate, Coomassie blue G-250, hydroxyapatite Bio-gel HT, and N,N'-methylene bisacrylamide were products of Bio-Rad (Richmond, CA, USA). Calibration kits for electrophoresis and Sepharose CL/4B gel-filtration, chromatofocusing kit (pH range 4-9), and Sephacryl S-300 were obtained from Pharmacia (Uppsala, Sweden). Ac-Tyr-EE, Ac-Tyr-NHNan, α-aminoacyl NHNan substrates, Bz-Arg-EE, Bz-Arg-NHNan, N-(3-carboxypropionyl)-Phe-NHNan, Cystine-bis-NHNan, DEAE-cellulose, Glu-Phe-NHNan, α-N-hippuryl-Arg, N-hippuryl-Phe, monobasic and dibasic sodium phosphates, PMSF, Su-Phe-NHNan, acetic acid, isopropanol, and methanol were purchased from E. Merck (Darmstadt, FRG); α-aminoacyl NHNap substrates, S-Bz-Cys-NHNan, dimethyl amino cinnamaldehyde, α-N-hippuryl-Lys, Fast Garnet GBC, hemoglobin, polyvinylpyrrolidone-40 (mol. wt. 40,000), TIs from soybean (T-9003) and turkey egg white (T-1886), tetrazotized o-dianisidine, and dimethyl formamide were obtained from the Sigma Chemical Company (St. Louis, Mo. USA). Diaflo ultrafiltration membranes PM10 (mol. wt. cutoff 10,000) ws obtained from Amicon Inc. (Danvers, MA. USA). All amino acid substrates were L-isomers except Bz-Arg-NHNan, which was a mixture of D- and L-isomers.

Plant Material

Roots of sweet potato (*Ipomoea batatas* (L.) Lam. cv. Tainong 64) were purchased from a local market. The roots were sprayed with 0.02% sodium azide solution and allowed to sprout in an incubator programmed at 32±1°C (12 h) and 25±1°C (12 h) with 75±2% relative humidity without light. It took about 3 months to obtain enough sprouts of first harvest for AP purification. Sprouts were frozen at -70°C until used.

Enzyme Extraction and Purification

All stages were carried out at 4°C. About 103 gm frozen sprouts were homogenized with 8 vol (v/w) of 10 mM sodium phosphate buffer, pH 7.0 (buffer A) containing 1% (w/v) PVP-40 in a Polytron homogenizer (Luzern, Switzerland) at high speed for 1 min 3 times at 4°C. The homogenate was filtered through 3 layers of cheesecloth and then centrifuged at 11,000 x g for 20 min. The supernatant, designated as crude extract, was directly applied to a buffer A equilibrated DEAE-cellulose column (3 × 55 cm) and eluted with buffer A to wash off unbound proteins. Bound proteins were eluted with a stepwise gradient of 0-0.15, 0.15-0.40 and 0.40-1.00 M NaCl in buffer A (total vol, 500 ml). Active fractions (5 ml per fraction) were pooled and concentrated by Diaflo ultrafiltration membranes PM 10 and chromatographed on a Sephacryl S-300 column (3 × 90 cm) which was pre-equilibrated and eluted with buffer A. Active fractions were pooled, concentrated as described above and applied to a hydroxyapatite Bio-Gel HT column (3 × 40 cm) pre-equilibrated with buffer A and eluted with a linear gradient of 10-100 mM sodium phosphate buffer, pH 7.0 (total vol, 500 ml). Active fractions were pooled and concentrated by a PM10 membrane.

Assays of Aminopeptidase Activity

The hydrolysis of Ala-4-nitroanilide was measured spectrophotometrically at 410 nm as reported (Erlanger *et al.*, 1961, 1966). The reaction mixture, which contained 0.3 ml of 1 mM substrate solution, 0.2 ml of 0.15 M citrate-phosphate buffer (pH 7.0, buffer B) and 0.2 ml of the enzyme solution, was incubated at 37°C for 6 h and terminated with 0.6 ml of 30% acetic acid. The absorbance of the reaction mixture was measured at 410 nm. One enzyme unit was defined as the amount of enzyme required to produce 1 μmol 4-nitroaniline/h under the assay conditions. This method was used during purification.

The aminopeptidase activity was also measured with Ala-β-naphthylamide as the substrate (Erlanger *et al.*, 1966). The reaction was performed as when amino acid derivatives of 4-nitroanilide were used as the substrates. The enzymatic reaction was terminated by adding 0.6 ml of 2% HCl in ethanol, and 0.6 ml of 0.06% dimethylamino cinnamaldehyde in ethanol was added finally. The absorbance at 540 nm was determined. One enzyme unit was defined as the amount of enzyme required to produce 1 μmol naphthylamine/h under the assay conditions.

The enzymatic hydrolysis of peptide substrates was followed by estimating liberated amino acids with

2, 4, 6-trinitrobenzenesulfonic acid in the presence of cupric ions (Waters and Dalling, 1983) and using leucine as the standard.

Assays of Other Proteolytic Activities)

The endopeptidase activity was determined by measuring spectrophotometrically the increase in absorbane at 254 nm using Nα-benzoyl-L-arginine ethyl ester (BAEE) or N-acetyl-L-tyrosine ethyl ester (ATEE) as the substrate (Schwert and Takenaka, 1955; Prescott and Wagner, 1976). The reaction was performed and terminated as assay of aminopeptidase activity with amino acid derivatives of 4-nitroanilide as the substrate. The A_{254} of the reaction mixture was measured.

The endopeptidase activity was also determined with casein as the substrate (Kunitz, 1946). The reaction mixture, which contained 0.2 ml of the enzyme solution and 0.6 ml of 2% casein in buffer A, was incubated at 37°C for 6 h. The reaction was terminated by adding 0.6 ml of 0.44 M trichloroacetic acid. After standing at room temperature for 1 h, the reaction mixture was centrifuged and A_{280} of the supernatant was measured.

The carboxypeptidase activity was determined by measuring absorbance at 254 nm using α-N-hippuryl -L-arginine as the substrate (Folk and Schirmer, 1963). The enzymatic reaction was performed and terminated as assays of aminopeptidase activity.

Boiled instead of native enzyme solutions were used in controls for the above enzymatic reactions.

Protein Estimation

The protein contents of enzyme preparations were determined by the modified Bradford procedure (Compton and Jones, 1985). The assay consisted of the addition of 5 ml of the standardized Coomassie blue G -250 dye solution to 0.1 ml of protein solution, immediate mixing, and determination of A_{595}. Bovine serum albumin was used as the standard. Protein contents during chromatographies were determined at 280 nm.

Estimations of Molecular Weight

The molecular weight of the enzyme was estimated by gel-filtration (Andrews, 1964) on a Sepharose CL/4B column (2 × 90 cm) with ribonuclease A (mol. wt. 13,700), chymotrypsinogen A (25,000), ovalbumin (43,000), bovine serum albumin (67,000), and aldolase (158,000) as standards.

Activity Staining of AP1 after PAGE

A vertical system of discontinuous slab gel electrophoresis (177 × 105 × 1.5 mm, or 95 × 55 × 0.75 mm) was used for AP separation. The gel system was prepared (Davis, 1964) with a stacking gel of 2.5 % (w/v) acrylamide (pH 6.8) and a separating gel of 7.5% (pH 8.8). Proteins were detected by both Coomassie blue and activity staining of AP. AP was detected on gels after electrophoresis by qualitatively measuring β-naphthylamine released from synthetic substrate (Toncsev, 1978). The gels were incubated in a solution of 1 mM Ala-NHNap at 37°C for 1 h and then transferred to a 0.1% solution of Fast Garnet GBC salt, which was dissolved in 10% N, N-dimethyl formamide. A dark red band indicated a positive result.

Isoelectric Point Determination

The isoelectric point of the purified AP was determined by chromatofocusing (Lampson and Tytell, 1965). The purified AP1 was desalted and applied to a poly-buffer exchanger (PBE 94) column (1.2 × 29 cm), which was equilibrated with 25 mM imidazole-HCl buffer (pH 7.4). The enzyme was eluted with 200 ml of Polybuffer 74, which was adjusted to pH 4.0 by HCl, at a flow rate of 22 ml/h. The protein content and pH value of each fraction (2 ml) were determined by Coomassie blue assay and pH meter, respectively.

Results and Discussion

Purification of Sweet Potato AP1

When a crude extract possessing AP activity was fractionated on a DEAE-cellulose column, three AP peaks and six protein peaks were found (Fig. 1). The first AP peak (AP1) emerged slightly ahead of the first protein peak; the second AP peak (AP2) coincided with the second protein peak; and the third AP peak (AP3) coincided with the fifth protein peak. The first AP peak which was eluted with ca. 0.13 M NaCl in buffer A was further purified successively by Sephacryl S-300 and hydroxyapatite columns to obtain 139-fold purification and 3.6% yield (Table 1). Five protein peaks but only one AP peak, which coincided with the fourth and smallest protein peak, were found on the Sephacryl S -300 column. When the pooled fraction containing the only AP peak was applied to a hydroxyapatite column,

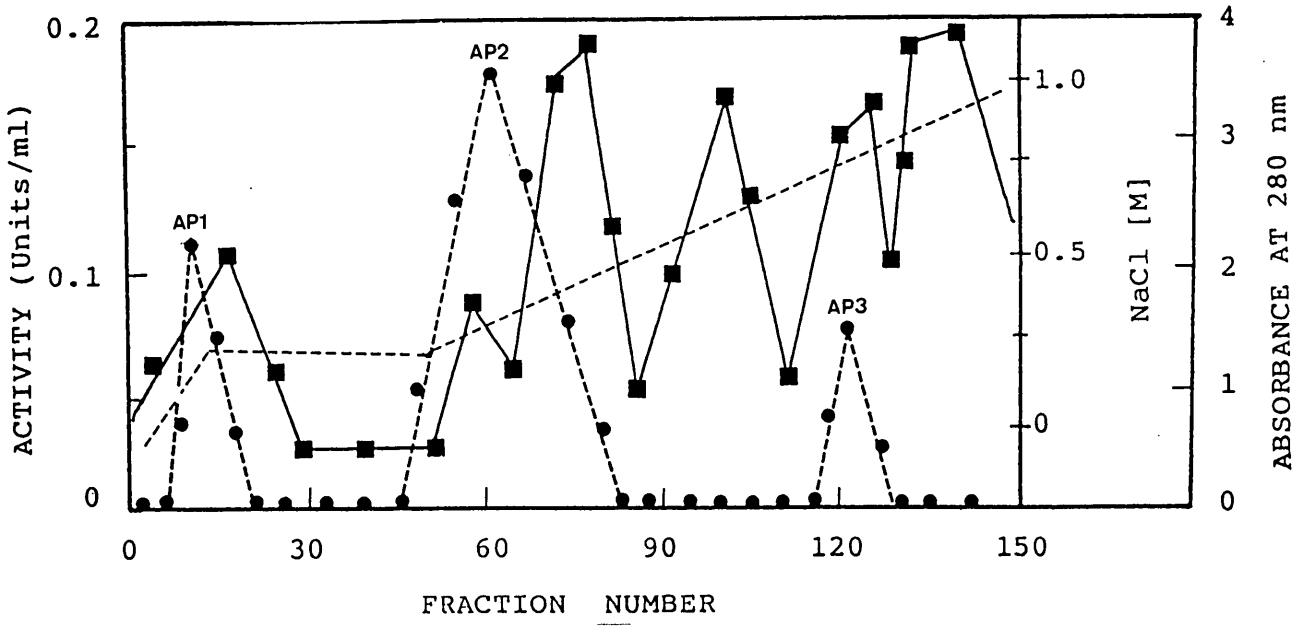

Fig. 1. DEAE-Cellulose column chromatography. The details were described in the text. (···●···) enzyme activity; (—■—) absorbance at 280 nm; (·····) NaCl gradient.

Table 1. *Purification of AP1 from sprouts of 'T64'*
The fresh weight of sprouts used was 103 g. One unit was defined as the amount of enzyme required to produce 1 μmol 4-nitroaniline/h under experimental conditions.

Procedure	Activity (units)	Protein (mg)	Spec. Act. (units/mg)	Recovery (%)	Purification (fold)
Crude extract	25.77	361	0.07	100	1
DEAE-cellulose	1.59	1.08	1.47	6.2	21.0
Sephacryl S-300	0.64	0.40	1.60	2.5	23.0
Hydroxyapatite	0.93	0.10	9.30	3.6	139

two protein peaks and only one AP peak, which coincided with the second and sharpest protein peak, were found. The isolated AP fraction was designated as AP1.

A neutral AP with mol wt of 65,000 from barley grains was eluted from a hydroxyapatite column with 50 mM sodium phosphate, pH 7.0, in a stepwise process (Kolehmainen and Mikola, 1971). AP2S of maize with mol wt 86,500 was eluted from the hydroxyapatite column with ca. 60 mM potassium phosphate and 1 mM $MgCl_2$, pH 6.6 (Vodkin and Scandalios, 1980). An AP from seeds of Japanese apricot with mol wt 56,000 was eluted from a DEAE-Sepharose column with 0.12 M NaCl in 10 mM sodium phosphate buffer (pH 6.0). During further purification on a hydroxyapatite column, the enzyme was eluted with ca. 60 mM sodium phosphate buffer pH 7.0 (Ninomiya *et al.*, 1983). The present AP1 preparation behaved in a similar way chromatographically to other plant APs, but the molecular size was strikingly different. It was homogeneous as examined by 7.5% Disc-PAGE (Fig. 2).

General Properties of AP1

The molecular weight of AP1 was estimated to be 115,000 by gel-filtration on Sepharose CL/4B. AP1 showed an electrophoretic mobility of 0.033 relative to bromophenol blue on 7.5% PAGE at pH 8.8 and an isoelectric point (pI) of 5.53.

Effect of Temperature on the Stability of AP1

AP1 solutions in 10 mM Na-phosphate buffer, pH 7.0, were incubated for 15 min at various temperatures,

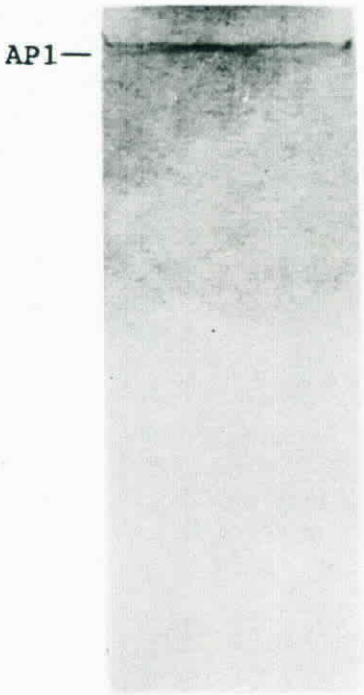

Fig. 2. The activity staining of AP1 on 7.5% polyacrylamide gel, pH 8.8. The anodic run with sample containing 5 μg protein.

and the residual activities were assayed under standard conditions (pH 7.0 for 6 h) after being cooled to 4°C. The enzyme activity was unstable to heat; only 50 and 15% of activities remained after preincubation at 42°C and 55°C, respectively.

Effect of pH on Enzyme Activity and Stability

AP1 activity was assayed at 37°C for 6 h in citrate-phosphate, phosphate, or Tris-HCl buffer at various pHs. The pH-activity profile (Fig. 3) indicated that the pH optimum was 7.0 in both citrate-phosphate and phosphate buffers. In this respect, the present enzyme resembles the group of enzymes, i.e. neutral aminopeptidases, which are characterized by their ability to hydrolyze peptide bonds between the α-carboxyl group of various amino acids and the amino group of some aromatic amines, for instance, β-naphthylamine, 4-nitroaniline, and 4-(phenylazo)-phenylamine, optimally at pH 7 to 8 (Elleman, 1974; Kolehmainen and Mikola, 1971; Ninomiya *et al.*, 1983; Vodkin and Scan-

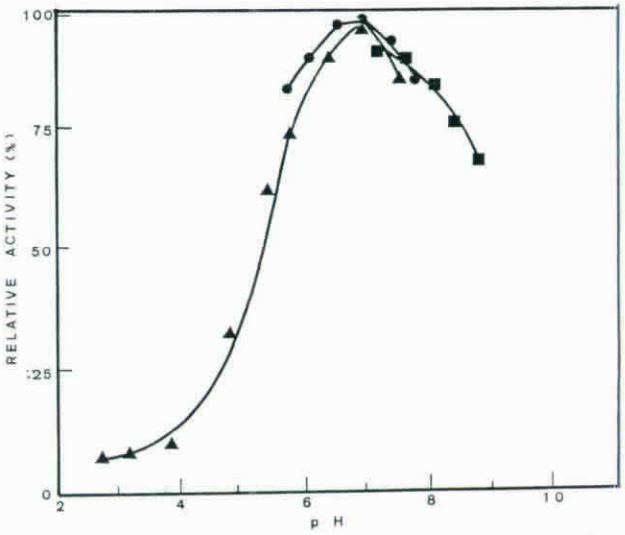

Fig. 3. The effect of pH on AP1 activity. The enzyme activity of AP1 containing 1.26 μg protein was assayed as described in the text in: (▲) 28.6 mM citrate-57.2 mM phosphate (pH 2.8-7.6); (●) 14.3 mM sodium phosphate (pH 5.8-7.8); or (■) 14.3 mM Tris-HCl buffer (pH 7.2-9.6). The maximal activity against Ala-NHNan was 4.79 μmol/h · mg.

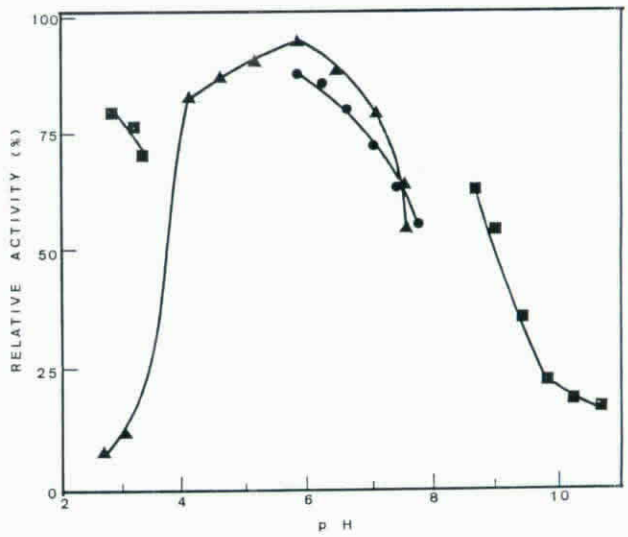

Fig. 4. The effect of pH on the stability of AP1. AP1 containing 1.26 μg protein was preincubated for 3 h at 37°C in : (■) 25 mM glycine-HCl (pH 2.8-3.4); (▲) 50 mM citrate-100 mM phosphate (pH 2.8-7.6); (●) 50 mM sodium phosphate (pH 5.8-7.8); or (■) 25 mM glycine-NaOH (pH 8.6-10.6). The residual activity was then assayed as described in the text. The maximal activity against Ala-NHNan was 4.79 μmol/h · mg.

dalios, 1980; Waters and Dalling, 1984).

In addition, AP1 was kept for 3 h at 37°C in citrate-phosphate, phosphate, glycine-HCl or glycine-NaOH buffer at various pHs. The residual activities were assayed under standard conditions. AP1 was stable between pH 4.0 and 6.6 in citrate-phosphate buffer (Fig. 4).

Substrate Specificity

(a) Synthetic substrates. AP1 *in vitro* effectively hydrolyzed Ala-NHNan and Leu-NHNan with rates of 4.79 and 1.79 μmol/h • mg, respectively, but AP1 had no detectable activity on cystine-bis-NHNan, Glu-NHNan, Ala-Ala-NHNan, or Ala-Ala-Ala-NHNan, or substrates for carboxypeptidase or endopeptidase (Table 2). With regard to its narrow specificity on L-aminoacyl NHNap or NHNan, the present enzyme resembles one AP from barley grains (Sopanen and Mikola, 1975) and AP1 from pea seeds (Elleman, 1974). However, the enzyme prefers Ala in the amino terminal residue to Leu while the reverse is true for the two APs mentioned above.

(b) Peptide substrates. The present enzyme effectively hydrolyzed dipeptides Ala-Ser and Ala-Ala, and oligopeptides of alanine (Table 3). The rates of hydrolysis against Ala-Ala, $(Ala-)_3$, $(Ala-)_4$, $(Ala-)_5$, and $(Ala-)_6$. were 90, 51, 17, 24 and 10%, respectively, of that against Ala-Ser (2.93 μmol/h • mg). On the other hand, AP1 did not hydrolyze Ala-Leu, Ala-Val, Ala-Met, Ala-Pro, Ala-Phe, Ala-Gly, Ala-Asn, Ala-Asp, Ala-Lys, or Leu-X where X stands for amino acid in general (Table 3).

As with some plant aminopeptidases (Kolehmainen and Mikola, 1971; Ninomiya *et al.*, 1981), the ability of AP1 of "T64" sprouts to hydrolyze peptide substrates with lower rates than the corresponding aminoacyl-β-naphthylamides or aminoacyl-4-nitroanilides is in contrast to other plant aminopeptidases which hydrolyze peptide substrates at much higher rates than aminoacyl-β-naphthylamide substrates (Waters and Dalling, 1983; 1984).

Table 2. *AP1 activity of 'T64' sprouts toward synthetic substrates*

In each assay, 1.26 μg of AP1 was used. The final concentration of synthetic substrates and casein were 0.429 mM and 0.429%, respectively. Activity toward Ala-NHNan was 4.79 μmol/h • mg protein.

Substrate	Activity (%)
Ala-NHNan	100
Leu-NHNan	37
Glu-NHNan	0
Cystine-bis-NHNan	0
Ala-Ala-NHNan	0
Ala-Ala-Ala-NHNan	0
Hippuryl-Lys	0
Hippuryl-Arg	0
Hippuryl-Phe	0
Ac-Tyr-NHNan	0
Ac-Tyr-EE	0
Bz-Arg-EE	0
Bz-Arg-NHNan	0
Bz-Cys-NHNan	0
3-carboxypropionyl-Phe-NHNan	0
Glutaryl-Phe-NHNan	0
Casein	0

Table 3. *AP1 activity of 'T64' sprouts toward peptide substrates*

All substrates were present in the reaction mixture at a concentration of 0.5 mM. In each assay, 0.60 μg of AP1 was used. The details were the same as those given in the text. Activity toward Ala-Ser was 2.93 μmol amino acid released/h • mg protein.

Substrate	Activity (%)
Ala-Ser	100
Ala-Ala	90
Ala-Leu	0
Ala-Met	0
Ala-Val	0
Ala-Phe	0
Ala-Pro	0
Ala-Gly	0
Ala-Asn	0
Ala-Asp	0
Ala-Lys	0
Leu-X[a]	0
Ala-Ala-Ala	51
Ala-Ala-Ala-Ala	17
Ala-Ala-Ala-Ala-Ala	24
Ala-Ala-Ala-Ala-Ala-Ala	10

[a]X stands for any of 20 amino acids found in proteins.

Kinetic Properties of AP1

AP1 activity was assayed under standard conditions at pH 7.0 with various amounts of enzyme preparation, ranging from 0.6 to 2.5 μg, and with a final concentration of 0.429 mM Ala-NHNan for different reaction times up to 16 h. There was a linear relationship between reaction velocity and enzyme concentration in the range used for reaction time up to 6 h.

The effect of substrate concentration on the rate of hydrolysis of Ala-NHNan, Leu-NHNan, and Ala-Ala in citrate-phosphate buffer, pH 7.0, was investigated at substrate concentrations from 0.05 to 0.45 mM when 0.6 μg of AP1 preparation was used. The apparent Michaelis-Menten constant (K_m) and maximum velocity (V_{max}) for the enzyme were calculated from the double-reciprocal plot of Lineweaver-Burk (Table 4). No significant inhibition was observed at high substrate concentrations.

Effects of Protease Inhibitors on AP1 Activity

The present enzyme was strongly inhibited by PMSF, aprotinin, leupeptin, *p*CMB, and 1, 10-phenanthroline; slightly inhibited by soybean trypsin inhibitor, and trypsin inhibitor from turkey egg white; but not significantly affected by 2-mercaptoethanol and DL-dithiothreitol (Table 5). These findings suggest that a serine residue may play a part in the catalytic process of the enzyme.

The present AP (AP1) is different from another AP (AP2) found in sprouts of 'T64' (Lin and Chan, 1992) in several aspects: 1). AP1 retains only 15% of activity while AP2 retains 95% of activity after being preincubated at 55°C for 15 min. 2). The mol. wt.,estimated by gel-filtration, and isoelectric point of AP1 are 115,000 and 5.53, while those of AP2 are 63,000 and 6.08, respectively. 3). The rates of hydrolysis of Ala

Table 4. *K_m and V_{max} values for the hydrolysis of some representative substrates by AP1 of 'T64' sprouts*

Substrate	K_m (mM)	V_{max} (μmol/h • mg)	V_{max}/K_m (μmol/h • mg mM)
Ala-NHNan	0.133	8.22	61.8
Leu-NHNan	0.059	1.42	24.1
Ala-Ala	0.650	4.47	6.88

Table 5. *Effects of some reagents on AP1 activity*

The mixtures each containing 0.1 ml of the AP1 solution (1.26 μg) of 'T64' sprouts and 0.1 ml of various reageants with the indicated final concentrations (mM), were incubated for 30 min at 25°C, and the enzyme activities of the mixtures were then assayed under standard conditions. The control activity using Ala-NHNan as substrate was 4.79 μmol/h • mg.

Addition	Concentration mM	Activity (%)	Type*
Control		100	
pCMB	0.2	0	C or S
1,10-Phenanthroline	2	16	M, C or S
	1	23	
PMSF	2	18	S or C
	1	45	
Aprotinin	1	19	S
Leupeptin	1	24	S or C
	0.1	58	
2-Mercaptoethaonl	5	98	C or M
	1	115	
DL-dithiothreitol	1	97	C or M
Soybean trypsin inhibitor	1[a]	88	S
Trypsin inhibitor form turkey egg white	1[a]	91	S
Trypsin inhibitors from sweet potato roots	1[a]	101	

*Mechanistic set of peptidase; C for cysteine type; S for serine type; M for metallo type; A for aspartic acid type (Storey and Wagner, 1986).

[a]mg/ml.

-NHNan by AP1 and AP2 are 4.79 and 2.09, respectively, while those of Ala-Ala are 2.60 and 1.68 μmol/h • mg protein, respectively. 4). Serine residue and cysteine residue participate in the catalytic process of AP1 and AP2, respectively.

Bachmair *et al.* (1986) proposed that the *in vivo* half-life of a yeast protein is a function of its amino-terminal residue. Thus AP1, which has quite specific substrate requirements, may play a key role in cellular regulation if their porposal can be extended from yeast to higher plants, and if removal of the amino-terminal residue of some proteins by aminopeptidases speeds up later hydrolysis by other proteases.

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臺農 64 號甘藷芽之一種胺肽酶 (AP1)

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經過粗抽處理、DEAE-cellulose、Sephacryl S-300，及 hydroxyapatite 管柱層析共四步驟從臺農 64 號甘藷芽可得一胺肽酶(AP1)，甚純化倍數爲 139。AP1 之分子量以 Sepharose CL/4B 膠體滲透法測定爲 115,000；而等電點爲 5.53。以 L-alanine-4-nitroanilide 爲基質在 citrate-phosphate 緩衝液測得之最適 pH 爲 7.0, AP1 在 pH 4.0 和 6.6 之間且在 37°C以下時是穩定的。AP1 水解 L-alanine -4-nitroanilide 及 L-leucine-4-nitroanilide，和由 L-alanine 構成之雙肽及寡肽。AP1 受 aprotinin, PMSF, leupeptin, pCMB，及 1, 10-phenanthroline 強烈抑制；但不受硫醇化合物所影響：顯示 serine 可能參預該酵素之催化過程。