Bot. Bull. Acad. Sin. (1994) **35:** 73-80

EJ100199400073

Activators of a trypsin-casein system isolated from old leaves of *Ipomoea batatas*

Yaw-Huei Lin[1] and Sin-Jong Huang

Institute of Botany, Academia Sinica, Taipei, Taiwan 115, Republic of China

(Received August 31, 1993; Accepted January 19, 1994)

Abstract. Trypsin in a casein substrate was activated by dialyzed water-extract of old leaves of sweet potato (*Ipomoea batatas* cv. Changhua). The extract was purified by ammonium sulfate precipitation and column chromatography on CM-Sephadex, DEAE-cellulose, and Sephadex G-100. Two activator fractions, SPTA I and SPTA II, were obtained. SPTA II was homogenous and SPTA I was nearly homogenous under urea polyacrylamide gel electrophoresis. Using gel filtration on a Sephadex G-100 column, the molecular weight of SPTA I was determined to be greater than 100,000 and that of SPTA Ii approx. 100,000. When 10 mg casein, 50 μg SPTA II, and 20 μg trypsin were present in 2.5 ml of solution, 170% activation was observed. Increasing the amount of SPTA II to 150 μg increased activation to1250%. The relative amount of activation decreased with increasing casein concentration. No similar activation of subtilisin or α-chymotrypsin was observed. When hemoglobin was used as a substrate, activation of trypsin could still be observed but was not as obvious as with casein. The observed activation of the trypsin-casein system is not due to the presence of Ca^{+2}, precursors of proteinase, or additional substrate. It may be abolished by pretreatment of activators with heat or with alkaline solution, suggesting that native conformation is essential for activation to occur. The activation can be counteracted by commercial Kunitz-type soybean trypsin inhibitor. A relationship between activation activity and leaf-and-stem producing potential of Changhua cultivar is proposed.

Keywords: Chemical properties; Enzyme assays; Mode of action; Protein activators; Purification; Soybean trypsin inhibitor.

Abbreviations: **EDTA**, ethylene diamine tetraacetic acid; **PAGE**, polyacrylamide gel electrophoresis; **SPTA**, sweet potato trypsin-casein activator; **SPTI**, sweet potato trypsin inhibitor; **TCA**, trichloro acetic acid; **TEMED**, N, N, N', N'-tetramethylethylenediamine; **TI**, trypsin inhibitor.

Introduction

Classical enzymatic experiments comprise two assays, E (experimental) and C (control), in one set. E consists of enzyme, substrate, buffer, and water (in some cases coenzymes are also required). C consists of the same components as in E except for the enzyme under study. The assay value is E minus C. When the influences of inhibitors or activators on proteases are studied, additional treatments, such as preincubation of inhibitor or activator with protease or substrate before proteolysis, are included (Bergmeyer, 1984; Erlanger, 1961; Kunitz, 1946). While studying the trypsin inhibitor activity of various plant tissues, we found that the classical two-assay system does not properly handle the complex situation in which the occurrence of inhibitor and/or activator is unknown in advance. On the basis of Kunitz's procedure (1946), we designed a modified version: a three-assay system comprising standard (S), experimental (E), and control (C) assays (Lin and Chen, 1980; for detail see the following text). Using this three-assay system, we were surprised to find activators of trypsin-casein in old leaves of sweet potato cultivar Changhua (Lin and Huang, 1979) and activator activity of trypsin-casein in root extracts of four sweet potato cultivars (Lin and Ho, 1986). In this work, we report the purification and the properties of SPTA from old leaves of cultivar Changhua.

[1]Corresponding author

Materials and Methods

Chemicals and Chromatographic Materials

Acrylamide, ammonium persulfate, N, N'-methylene bisacrylamide, RNA from baker's yeast (type III, R7125), TEMED, α-chymotrypsin (C4129), soybean (T9003), subtilisin, and trypsin from bovine pancreas (T8253) were purchased from The Sigma Chemical Company (St. Louis, USA). Blue dextran 2000, CM-Sephadex-C-

50, DEAE-cellulose, and Sephadex G-100 were obtained from Pharmacia (Uppsala, Sweden). Ammonium sulfate, disodium salt of EDTA, sodium salts of phosphoric acid, trichloroacetic acid, tris-hydroxy methyl amino methane, and urea were purchased from E. Merck (Darmstadt, Germany). Bovine serum albumin, casein, and phenol reagent for protein determination were purchased from Wako (Japan).

Plant Material

Fresh leaves of sweet potato (*Ipomoea batatas* (L.) Lam. cv. Changhua) were obtained from experimental fields or purchased from a local market.

Extraction and Purification of SPTA

All steps were carried out at 4–7 °C. Sweet potato leaves, cleaned and air-dried, were homogenized with 4 volumes (v/w) of double-distilled water in a Polytron homogenizer (Luzern, Swiss). The homogenate was filtered through two layers of cheesecloth and then centrifuged in a Sorvall RC-2B with a SS-34 rotor at 10,000 rpm for 10 min. The supernatant liquid, designated the crude SPTA, was slowly brought to 60% ammonium sulfate saturation, aged for 2 h, and then centrifuged as above. The precipitate was discarded. The supernatant liquid was brought to 80% ammonium sulfate saturation, aged for 2 h, and then centrifuged as above. The precipitate was collected, dissolved in double-distilled water, dialyzed, and lyophilyzed. Samples were dissolved with 50 mM tris-HCl buffer, pH 7.4, (buffer A) and passed through a batch of CM-Sephadex (C-50) pre-equilibrated with buffer A. SPTA did not bind to CM-Sephadex. The effluent was applied to a DEAE-cellulose column (2.5 x 10 cm) pre-equilibrated with 0.3 M NaCl in buffer A, and eluted with a linear gradient of 0.3–1.0 M NaCl in buffer A. Active fractions (2 ml each, 12 ml/h) were desalted by dialysis against double-distilled water. The dialyzed sample was concentrated by lyophilyzation and applied to a Sephadex G-100 column (2.5 x 78 cm, with a void volume of 130 ml determined with 0.2 g/l blue dextran 2000) and fractions (2 ml each, flow rate of 12 ml/h) were collected.

Assay of SPTA Activity

This followed a modification (Lin and Chen, 1980) of the procedure of Kunitz (1946).

Preparation of substrate for trypsin or α-chymotrypsin: one gram of casein was dissolved in 100 mM Na_2HPO_4-NaH_2PO_4 buffer, pH 7.6, to a final volume of 100 ml. The casein solution was heated at 100 °C for 15 min, then cooled in an ice-box. It was kept at room temperature (about 25 °C) before use.

Preparation of substrate for subtilisin: one gram of casein was dissolved in 250 mM Na_2HPO_4-NaOH buffer, pH 11.1, to a final volume of 100 ml, and then treated as above.

Preparation of enzymes: trypsin or α-chymotrypsin was dissolved in 0.25 mM HCl to a concentration of 200 μg/ml as a stock solution, then stored at 7 °C (it should be used within one week). Before use, trypsin and α-chymotrypsin were diluted to 20 and 10 μg/ml, respectively, whereas subtilisin was dissolved in double-distilled water immediately before use, to both 12 and 17 μg/ml.

Stop solution: Fifty-percent aqueous TCA solution was prepared as stock solution. It was diluted to 5% before use as a stop solution for enzyme reactions.

For each SPTA assay three different assays were undertaken – standard (S), experimental (E), and control (C). The standard assay was run by adding 0.5 ml double-distilled water and 1.0 ml trypsin solution (containing 20 μg trypsin in 0.25 mM HCl) to tubes containing 1.0 ml heated casein solution (10 g/l). Proteolytic reaction was allowed to proceed at 37 °C for 20 min. The solution was then poured into tubes containing 3.0 ml TCA (50 g/l). The precipitate formed was centrifuged after standing for at least 1 h at about 25 °C. The concentration of TCA-soluble peptides with aromatic amino acids in the supernatant solution was determined by measuring the absorbance of the solution at 280 nm. The experimental assay was run by preincubating 0.3 ml sample and 0.2 ml double-distilled water with 1.0 ml heated casein solution (10 g/l) at about 25 °C for 10 min. Finally 1.0 ml trypsin solution was added and the proteolytic reaction was carried out as for the standard assay. The control assay was run by preincubating 0.3 ml sample and 0.2 ml double-distilled water with 1.0 ml heated casein solution (10 g/l) at about 25 °C for 10 min. Subsequently 1.0 ml double-distilled water was added and the mixture was allowed to stand at 37 °C for a further 20 min before being poured into 3 ml TCA (50 grams per liter).

When the effect of SPTA on α-chymotrypsin or subtilisin was examined the same procedure was followed.

Determination of Water-Soluble Protein

Protein determinations were performed by the method of Lowry et al. (1951) with bovine serum albumin as the standard. During chromatography, protein contents were estimated from A_{280}.

Estimation of Molecular Weight

The molecular weight of the enzyme was estimated by gel-filtration (Andrews, 1964) on a Sephadex G-100 column (2.5 x 78 cm) with carbonic anhydrase (30,000), bovine serum albumin (67,000), phosphorylase b (94,000), and aldolase (158,000) as standards.

Detection of Aldoses, Ketoses, and Pentoses (Dawson et al., 1959)

Anthrone test: anthrone reagent was prepared by dissolving 0.2 g anthrone in 100 ml 18.3 M sulfuric acid and storing at 7 °C before use. Two milliliters of anthrone reagent was slowly added to a test tube containing the sample. The test tube was covered with a glass marble and then heated in a boiling water bath for 10 min. Sucrose was used as a positive control. Both aldo and keto sugars give green color.

Modified Bial test: modified Bial reagent was made by adding 10 ml of 11. 4 M HCl to freshly prepared 6% orcinol aqueous solution with addition of 25 drops of 10% $FeCl_3$ solution. Four milliliters of modified Bial's reagent was slowly added to a test tube containing the sample. The test tube was covered with a glass marble and then heated in a boiling water bath for 15 min. Glucose and baker's yeast RNA (type III, R7125) were used as positive controls.

Polyacrylamide Gel Electrophoresis

Rod gels with 48% urea (Bargress, 1968): Four kinds of solution were prepared. **a)** 9.05 g tris base, 12 ml of 1 N HCl, 135 μliter TEMED, and 36 g urea were dissolved in water to a final volume of 75 ml. **b)** 15 g acrylamide, 0.4 g bisacrylamide, and 48 g urea were dissolved in water to a final volume of 100 ml. **c)** 48 g urea was dissolved in water to a final volume of 100 ml., producing an 8 M urea solution which was also a 48% urea solution. **d)** 28 mg ammonium persulfate was dissolved in water to a final volume of 5 ml. To make 5% gels with 48% urea, solutions a, b, c, and d were combined in a ratio of 9:8:4:3. Electrode buffer was prepared by dissolving 3.03 g tris base and 14.4 g glycine in water to a final volume of 1000 ml, giving a pH of 8.3.

Rod gels without urea (Hames, 1981): 3 kinds of solution were needed for separating gels. **a)** 18.15 g tris base, 24 ml of 1 N HCl, and 200 μliter of TEMED were dissolved in water to a final volume of 100 ml with a pH value of 8.9. **b)** 20 g acrylamide and 0.68 g bisacrylamide were dissolved in water to a final volume of 100 ml. **c)** 0.40 g ammonium persulfate was dissolved in water to a final volume of 100 ml. To make 5% separating gels, solutions a, b, and c were combined in a ratio of 1:1:2. For stacking gels: **a)** 3 g tris base, 24 ml of 1 N HCl, and 200 μliter TEMED were dissolved in water to a final volume of 100 ml with a pH of 6.8. **b)** 14 g acrylamide and 0.25 g bisacrylamide were dissolved in water to a final volume of 100 ml. **c)** 1 mg riboflavin was dissolved in water to a final volume of 100 ml. To make 3.5% stacking gels, solutions a, b, and c were combined in a ratio of 1:1:2. Electrode buffer was prepared by dissolving 3.03 g tris base and 14.4 g glycine in water to a final volume of 1000 ml, giving a pH of 8.3.

Nature of Products of Trypsin-Casein in the Presence of SPTA

Supernatant liquid of assay E in the presence of SPTA eluted from DEAE-cellulose column was applied to Whatman #1 paper, developed with methanol: H_2O: pyridine (80:2:4) for 8 h, dried, and sprayed with 0.1% ninhydrin. Free arginine or lysine will give a blue color. The same supernatant was also analyzed by PAGE.

Results

Occurrence of SPTA

SPTA activity has been detected in leaves of four sweet potato cultivars (Lin and Ho, 1984). Old leaves of cultivar Changhua grown between July and October contain the highest SPTA activity.

Purification of SPTA

Due to the extreme instability of SPTA, an overall purification table could not be established, but some data on the yield of proteins were obtained. Five milligrams of SPTA I and 12 mg of SPTA II were obtained from 500 g of fresh leaves. Samples from fractions other than 60–80% saturation ammonium sulfate precipitate showed low SPTA activity when examined after the CM-Sephadex step. When active effluent from CM-Sephadex C-50 was applied to and eluted from a DEAE-cellulose column, a main protein peak was obtained which coincided with the highest activity. The protein yield in this single step was 19.7% (Fig. 1). When active fractions were applied to and eluted from a Sephadex G-100

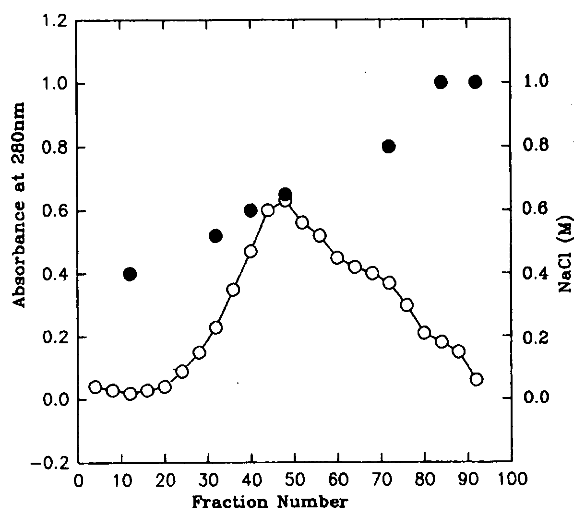

Fig. 1. Purification of SPTA in flow-through of CM-Sephadex C-50 on DEAE-cellulose column. Two-milliliter fractions and 12 ml/h flow rate. Closed circles, NaCl (M); open circles, A_{280}. Activity coincided with A_{280} and fractions 40 to 60 were pooled and concentrated for the next step.

column, two protein peaks and one shoulder were found. SPTA activity was found in the shoulder and the first protein peak, which were designated SPTA I and II, respectively (Fig. 2). The protein yield of SPTA I and II in this single step was 7.4% and 19.8%, respectively. PAGE shows that SPTA II is homogeneous and I is nearly homogeneous (Fig. 3).

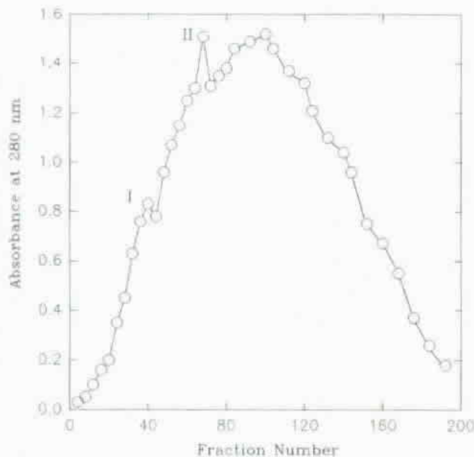

Fig. 2. Purification of SPTA fractions 40 to 60 (ref. Fig. 1.) on Sephadex G-100 column. **I**, SPTA I (fraction 44); **II**, SPTA II.

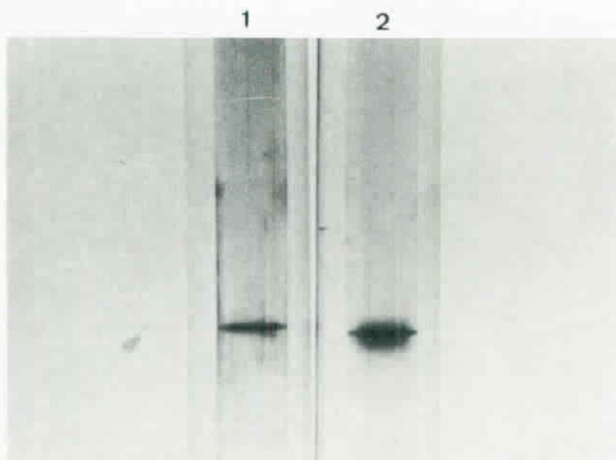

Fig. 3. Polyacrylamide gel electrophoresis. Five-percent urea gels were used with the positive electrode at the bottom. Lane 1, 15 μg SPTA I; lane 2, 15 μg SPTA II.

Properties of SPTA

According to the following observations the activity was not due to free small molecules: **1**) Activators could not pass through dialysis tubing. **2**) Both SPTA I and II were eluted early from the Sephadex G-100 column (Fig. 2). **3**) EDTA did not significantly affect activity. A 100-μg active sample eluted from a DEAE-cellulose column was preincubated in 25 mM EDTA (disodium salt) solution at 37 °C for 15 min and then dialyzed twice against 1000 volumes of double-distilled water before assaying. It was activated 58.7%, compared to 67.6% for a sample that had not been preincubated in EDTA, suggesting that neither Mg nor Ca caused the activation.

Using gel filtration on a Sephadex G-100 column, the molecular weight of SPTA I was determined to be greater than 100,000 and that of SPTA II approx. 100,000.

SPTA was more stable at lower temperatures than was sweet potato trypsin inhibitor (Lin and Ho, 1984), but SPTA eluted from DEAE-cellulose column was heat-labile, with about 8% activity remaining after being heated at 65 °C for 10 min (Fig. 4), a strong contrast with sweet potato TIs. SPTA eluted from DEAE-cellulose column lost all activity after being treated with pH 12.1 solution at 37 °C for 5 min and extensively dialyzed at 7 °C. Both SPTA I and II gave positive results in the anthrone test and in Bial's test (Fig. 5), suggesting that both are glycoproteins containing keto or aldo sugars.

Excluding some Possible Causes of E > (C+S)

There are some possible causes of E > (C + S) other than trypsin activation: **1**) SPTA is a protease which cooperates with trypsin. Table 1 shows that the A_{280} of all control assays were smaller than 0.070. This suggests that SPTA I and II are not proteases. **2**) Addition of the SPTA sample increased the quantity of substrate. Table 1 shows that the largest quantity of activator added was 0.151 mg, which was trivial compared with the 10 mg of casein present. **3**) The SPTA sample contained carboxypeptidase-B-like enzyme. Qualitative analysis of products of trypsin-casein in the presence or absence of SPTA showed similar protein patterns and no free arginine or lysine, indicating that SPTA is not a carboxypep-

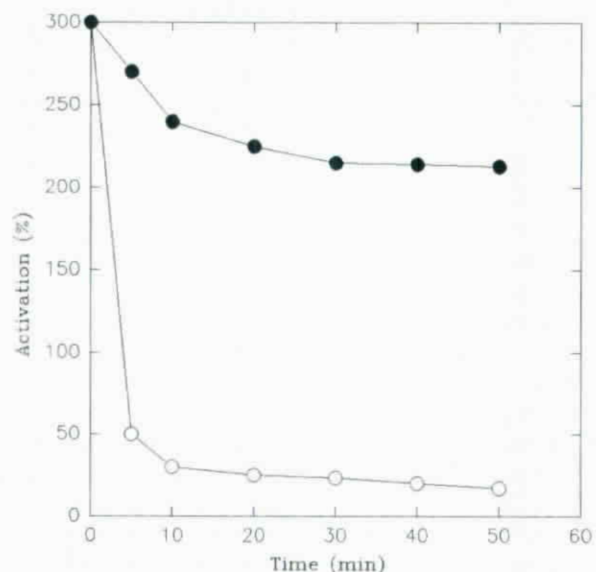

Fig. 4. Time course of heat denaturation of SPTA isolated from DEAE-cellulose column. Closed circles, 45 °C; open circles, 65 °C.

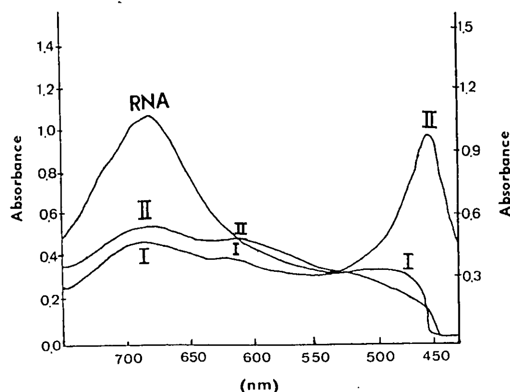

Fig. 5. Absorbance spectra of the solution after Bial's test. **I**, SPTA I; **II**, SPTA II.

tidase B-like enzyme. **4**) The SPTA sample contained some zymogen which was converted into active protease after combining with trypsin. If, instead of casein, SPTA eluted from a DEAE-cellulose column was incubated with 20 μg trypsin in the standard assay, there was no protease except trypsin in the final reaction mixture when examined by Sephadex G-100 column.

Activation of trypsin-casein by SPTA I and II

The is shown in Table 1. The highest activation (1834%) was observed when 45.6 μg of SPTA I was added.

Table 1. Activation of trypsin-casein by SPTA I and II. The final volume of the enzyme reaction mixture was 2.5 ml.

SPTA	Quantity (μg)	A_{280} casein +trypsin	A_{280} casein +SPTA	A_{280} casein +SPTA +trypsin	Activation (%)
I	9.0	0.454	0.017	0.794	71
	11.4	0.462	0.031	1.631	246
	15.0	0.460	0.031	1.843	294
	22.8	0.460	0.031	3.964[a]	755
	45.6	0.460	0.031	8.928[a]	1834
II	30.2	0.420	0.012	0.672	57
	37.8	0.424	0.019	0.783	80
	50.4	0.423	0.027	1.177	172
	75.6	0.420	0.021	2.355	456
	151.2	0.425	0.061	5.799[a]	1250

[a]Calculated from A_{280} of diluted supernatant liquid.

Specific Action of SPTA on Trypsin-Casein System

SPTA was specific to the trypsin-casein system, as shown in Table 2 and in previous work (Lin and Ho, 1984), although SPTA effluent from CM-Sephadex C-50 showed some activation of trypsin-hemoglobin system.

Table 2. Specific activation of trypsin-casein system by SPTA. As described for Table 1 in *Methods*, except that 130.5 μg SPTA, eluted from a DEAE-cellulose column, was used as the activator. The final volume of the enzyme reaction mixture was 2.5 ml.

Protease	Quantity (μg)	A_{280} casein +actvator	A_{280} casein +protease	A_{280} casein +protease +activator	Activation (%)
Trypsin	20	0.080	0.423	1.279	183
Chymotrypsin	10	0.080	0.475	0.585	5
Subtilisin	12	0.066	0.545	0.711	18
Subtilisin	17	0.053	0.794	1.001	19

SPTA Activity Could be Neutralized by Soybean Trypsin Inhibitor

SPTA activity could be neutralized by Kunitz-type Soybean TI, the final outcome being dependent on mixing sequence (Table 3). In all experiments, the molar ratio of activator to inhibitor was about 1.5:1. If activator and inhibitor were added at the same time (exp. 3 of Table 3), the inhibitor dominated. Whichever was added first to trypsin-casein system determined the final outcome (exp. 1 and 2 of Table 3).

Concentration Dependence of SPTA Action

Although percentage activation was linear with activator concentration when either SPTA I or II was used, it had a two-phase mode when other preparations were used (Fig. 6). The highest activation was 1700%.

Analysis of TCA-Soluble Hydrolysis Products by PAGE

Table 3. Influence of mixing sequence of activator (SPTA) and/or soybean trypsin inhibitor.

As described in *Methods*, except that 13 μg of soybean trypsin inhibitor in 0.2 ml of solution was used instead of double-distilled water. The activator was 100 μg of SPTA, eluted from a DEAE-cellulose column, in 0.3 ml of solution. In all cases the total time of trypsin action on casein was 20 min and the final volume of the enzyme reaction mixture was 2.5 ml. **C**, casein; **T**, trypsin; **A**, activator; **I**, inhibitor.

Experiment	Mixing sequence	Activation (%)
1	CT (5 min) + A (5 min) + I (10 min)[a]	70
2	CT (5 min) + I (5 min) + A (10 min)	- 8
3	CT (5 min) + AI (15 min)	-18
4	CA (10 min) + T (20 min)	320
5	CA (100 °C, 10 min) + T (20 min)	17
6	CI (10 min) + T (20 min)	-91

[a]This sequence means that casein and trypsin were mixed for 5 min, activator was added and mixed for 5 min, then inhibitor was added and proteolysis allowed to proceed for another 10 min before 3 ml 10% trichloroacetic acetic acid solution was added.

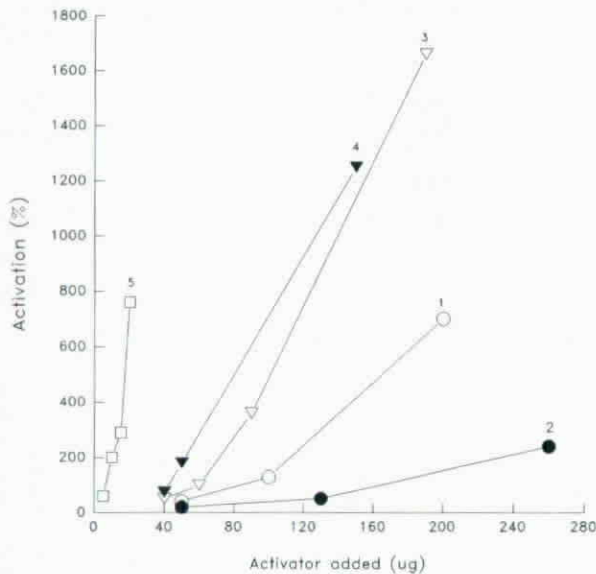

Fig. 6. Concentration dependence of SPTA action. Curve 1, sample precipitated from 60-80% ammonium sulfate saturation (AS-SPTA); curve 2, sample after CM-Sephadex C-50 chromatography (CM-SPTA); curve 3, sample after DEAE-cellulose column (DEAE-SPTA); 4, SPTA II; 5, SPTA I.

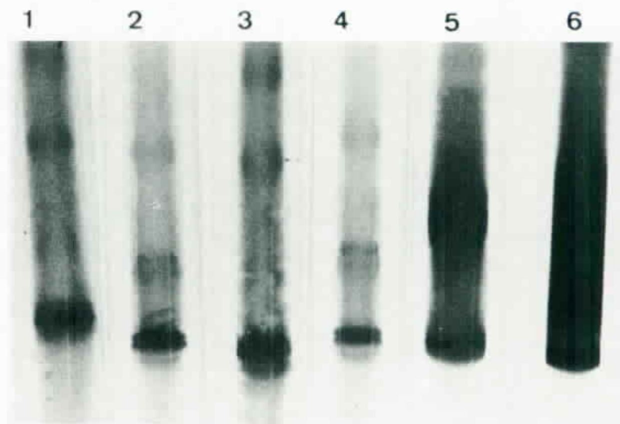

Fig. 7. Analysis of hydrolysis products of various assays by PAGE. Lanes with odd numbers were with 8 M urea; while those with even numbers were without. Lanes 1 and 2, experimental assay: casein + trypsin + SPTA after DEAE-cellulose column; lanes 3 and 4, standard assay: casein + trypsin; lanes 5 and 6, casein (10 g/l) only. Fifty microliters of trichloroacetic acid-soluble fraction from each assay was applied to each lane. Other conditions were the same as in Fig. 3.

On polyacrylamide gels (both with and without urea) the polypeptide patterns of the supernatant solution of the trypsin-casein system in the absence or presence of SPTA were similar (Fig. 7), suggesting a quantitative, rather than qualitative, change of casein hydrolysis by trypsin in the presence of SPTA.

Discussion

Figure 6 indicates that SPTA isolated at the ammonium sulfate precipitation step (AS-SPTA) showed higher activity than samples after CM-Sephadex C-50 chromatography (CM-SPTA) at the same concentration. This may be due to the removal of a special proteinase (which is the physiological target of SPTA action) during CM-Sephadex C-50 chromatography. The clue came from the observation that the control assay of AS-SPTA had high absorbance at 280 nm, indicating the presence of a proteinase.

The standard mechanism of protein inhibitors of proteinases has been reviewed by Laskowski and Kato (1980). Thus Kunitz-type soybean trypsin inhibitor binds with trypsin to form E-I complex as the first step. The mode of SPTA action, however, seems different from that of soybean trypsin inhibitor.

Considering the observations that **1**) the percentage activation of trypsin-casein by SPTA decreased with increasing casein concentration (data not shown) and **2**) SPTA did not activate trypsin-hemoglobin very much (Lin and Ho, 1984; this work), it was proposed that SPTA acts by rendering casein more susceptible to trypsin hydrolysis. The modification of substrate may be demonstrable by electrophoresis, spectrophotometry, or HPLC.

Although the exact mechanism of interaction between trypsin-casein and SPTA is unknown, experiment 3 of Table 3, in which the inhibitor determined the final outcome in spite of a disadvantageous molar ratio to the activator (about 1:1.5), suggests that soybean trypsin inhibitor binds more strongly to trypsin than does SPTA to casein or to trypsin-casein.

Changhua is well known in Taiwan as a leaf-producing cultivar, i.e. with a very high dry matter ratio of stems and leaves to roots. We have already observed that cultivars with higher apparent TI activities in roots also have higher apparent TI activities in leaves and stems (Lin and Ho, 1984). Stems and leaves of Changhua cultivar contain the highest SPTA activity and the lowest TI activity among four cultivars examined (Lin and Ho, 1984). The observation that old leaves of Changhua grown from July to October contain the highest SPTA activity is also significant when the physiological function of SPTA is considered. We propose that the high proteolysis activity (having a specific substrate range) and low TI activity in stems and leaves of Changhua cultivar may be one of the genetic factors which render this cultivar leaf-producing instead of root-producing. This proposal presumes the existence of a trypsin-like proteinase in stems and leaves of sweet potato cultivars which is regulated by both SPTA and SPTI. Some preliminary results have been published (Lin and Chu, 1988) or presented in this work (Fig. 6), but further work is needed to determine the exact mechanism.

Literature Cited

Andrews, P. 1964. Estimation of the molecular weights of proteins by Sephadex gel-filtration. Biochem. J. **91:** 222–233.

Bargress, R. R. 1968. Polyacrylamide gel electrophoresis instruction manual. Harvard University, Cambridge.

Bergmeyer, H. U. (ed.), 1984. Methods of enzymatic analysis, Vol. V, 3rd edn. p. 121. Verlag Chemie, Weinheim.

Dawson, R. M. C., D. C. Elliott, W. H. Elliott, and K. M. Jones (eds.). 1959. Data for biochemical research. Clarendon Press, London.

Erlanger, B. F., N. Kokowosky, and W. Cohen. 1961. The preparation and properties of two new chromogenic substrates of trypsin. Arch. Biochem. Biophys. **95:** 271–278.

Hames, B. D. 1981. An introduction to polyacrylamide gel electrophoresis. *In* B. D. Hames and D. Rickwood (eds.), Gel Electrophoresis of Proteins: a practical approach. IRL Press Limited, New York.

Kunitz, M. 1946. Crystalline soybean trypsin inhibitor. J. Gen. Physiol. **29:** 149–154.

Laskowski, M. Jr. and I. Kato. 1980. Protein inhibitors of proteinases. Ann. Rev. Biochem. **49:** 593–626.

Lin, Y. H. and S. J. Huang. 1979. Activation of trypsin-casein system by two protein fractions isolated from old leaves of *Ipomoea batatas*. *In* XI International Congress of Biochemistry. Toronto, Canada; July 8–13, 1979.

Lin, Y. H. and H. L. Chen. 1980. Level and heat stability of trypsin inhibitor activity among sweet potato (*Ipomoea batatas* Lam.) varieties. Bot. Bull. Acad. Sin. **21:** 1–13.

Lin, Y. H. and S. P. Ho. 1986. Soluble leaf proteins of sweet potato cultivars. Bot. Bull. Acad. Sin. **27:** 175–186.

Lin, Y. H. and H. H. Chu. 1988. Endopeptidases of sprouts and resting roots of sweet potato (*Ipomoea batatas* (L.) Lam. cv. Tainong 57). J. Chinese Biochem. Soc. **18:** 18–28.

Lowry, O. H., A. L. Farr, and J. J. Randally. 1951. Protein measurement with the folin phenol reagent. J. Biol. Chem. **193:** 265–275.

從彰化種甘薯老葉分離所得之胰蛋白酵素-牛奶蛋白系的致活因子

林耀輝　黃新榮

中央研究院植物研究所

胰蛋白酵素，當以牛奶蛋白爲基質時，可被透析過之彰化種甘薯老葉粗抽液所致活。粗抽液依次經硫酸銨沈澱法，CM-Sephadex 、 DEAE -纖維素、及 Sephadex G-100 管柱層析共四步驟可純化得兩種致活因子：Ⅰ和Ⅱ。試樣在含有尿素之聚丙烯醯胺膠體之電泳動情形顯示：致活因子Ⅰ近似均一、Ⅱ則是均一的。以 Sephadex G-100 管柱膠體滲濾法測得致活因子Ⅰ及Ⅱ之分子量分別爲大於十萬及等於十萬。當一萬 μg 牛奶蛋白、50 μg Ⅱ、及 20 μg 胰蛋白酵素均勻混合於 2.5 ml 溶液時，可檢驗到 170% 致活。當Ⅱ增爲 150 μg 時可得 1250% 致活。隨著牛奶蛋白之濃度遞增，百分致活遞減。對 α-胰凝乳蛋白酵素或枯草桿菌蛋白酵素並無致活現象。當以血紅蛋白爲基質時雖然仍可檢驗到胰蛋白酵素被致活，但不像以牛奶蛋白爲基質時那麼明顯。在此所檢驗到之胰蛋白酵素一牛奶蛋白系的致活並非來自 Ca^{+2}，蛋白酵素之前驅體，或額外基質的存在。致活活性可被加熱處理或鹼性溶液破壞，意味著致活因子之特殊三度空間立體構造是表現活性時必需的。又此致活活性可被市售之大豆胰蛋白酵素抑制因子所抵消。最後，對此致活活性和彰化種甘薯莖、葉之生產潛勢兩者間的關連提出工作假說。

關鍵詞：化學性質；酵素分析；作用模式；致活蛋白質；純化；大豆胰蛋白酶抑制因子。