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Purification and Properties of Phenylalanine Ammonia-lyase from Chinese Cabbage

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Phenylalanine ammonia-lyase (PAL; EC 4.3.1.5), the first enzyme in the phenylpropanoid biosynthesis, catalyzes the elimination reaction of ammonium ion from L-phenylalanine. PAL was purified from the cytosolic fraction of Chinese cabbage (*Brassica campestris* ssp. *napus* var. *pekinensis*) through ammonium sulfate fractionation, DEAE-cellulose chromatography, Sephadex G-200 chromatography, and Q-Sepharose chromatography. It consists of four identical subunits, the molecular mass of which was estimated to be about 38,000 daltons on SDS-PAGE. The optimal pH and temperature of the purified enzyme are 8~9 and 45°C, respectively. Its activity is greatly inhibited by Zn^{2+} ion, and strongly activated by caffeic acid. The purified PAL has some different characteristics compared to those obtained with other PALs.

Keywords: Characterization, Chinese cabbage (*Brassica campestris* ssp. *napus* var. *pekinensis*), Phenylalanine ammonia-lyase, Purification.

Introduction

Phenylalanine ammonia-lyase (PAL; EC 4.3.1.5) is one of the most extensively studied enzymes in higher plants. This enzyme is also present in some fungi but not in animals. Phenylalanine ammonia-lyase, usually identified as a tetrameric enzyme, catalyzes the conversion of L-phenylalanine to *trans*-cinnamic acid in the first step of the phenylpropanoid pathway, which supplies the precursors for flavonoid pigments, lignin, stilbenes, some alkaloids, and coumarins (Hahlbrock and Grisebach, 1979; Hahlbrock and Scheel, 1989). It also plays an important role in plant development and pathogen defense (Dixon and Lamb, 1990). Since PAL was first discovered in barley by Koukl and Conn (1961), it was identified and purified in various higher plants such as sweet potato root (Minamikawa and Uritani, 1965), spinach leaves (Nishizawa *et al.*, 1979), bean leaves (de Cunha, 1988), alfalfa (Jorrin and Dixon, 1990), suspension-cultured cells of French bean (Bolwell and Rodgers, 1991), loblolly pine (Whetten and Sederoff, 1992), pine cell cultures (Campbell and Ellis, 1992), and cotton hypocotyls (Dubery and Smit, 1994).

In higher plants, PAL activity varies greatly with the stage of development, with cell and tissue differentiation and upon exposure to various kinds of stress (Hahlbrock and Scheel, 1989). PAL activity is remarkably enhanced in higher plants by light (Zucker, 1965; Heller *et al.*, 1979), phytochrome (Brödenfeldt and Mohr, 1988), and fungal elicitor (Dalkin *et al.*, 1990). Chloroplast PAL of spinach leaves is regulated by light via the ferredoxin/thioredoxin system (Nishizawa *et al.*, 1979).

Some of the PAL genes were cloned and their modes of expression were analyzed. Four PAL genes were identified in parsley (Lois *et al.*, 1989), four to five in *Arabidopsis thaliana* (Ohl *et al.*, 1990), approximately 40–50 in potato haploid genome (Joos and Hahlbrock, 1992), two in peas (Yamada *et al.*, 1992), and two to four in tobacco (Pellegrini *et al.*, 1994). They indicate the existence of multiple forms of PALs in a single species of higher plants. Because PAL is important with respect to environmental stress, regulation of the PAL gene is being actively examined. For example, the expression of the PAL gene in parsley was found to be tissue-specific and stress-specific (Lois *et al.*, 1989).

The present study describes the purification and characterization of PAL from Chinese cabbage (*Brassica campestris* ssp. *napus* var. *pekinensis*), a species of the genus *Brassica*.

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Materials and Methods

Chemicals Bovine serum albumin (BSA), L-phenylalanine, L-tryptophan, L-tyrosine, L-cysteine, *t*-cinnamic acid, caffeic acid, dithiothreitol, apoferritin (horse spleen), β-amylase (sweet potato), alcohol dehydrogenase, acrylamide, N,N′-methylenebis-acrylamide, N,N,N′,N′-tetramethylethylenediamine (TEMED), ammonium persulfate, DEAE-cellulose, Sephadex G-200, Q-Sepharose, and Coomassie brilliant blue R-250 were purchased from Sigma Chemical Co. (St. Louis, USA). All other chemicals and reagents used were of the highest grade commercially available.

Plant material Fresh leaves of Chinese Cabbage (*Brassica campestris* ssp. *napus* var. *pekinensis*) were purchased from a commercial market in Chuncheon, Korea. The leaves were washed thoroughly prior to the purification procedure.

Enzyme assay Phenylalanine ammonia-lyase (PAL) activity was measured by a modification of spectrophotometric assay described previously (Whetten and Sederoff, 1992). The reaction mixture contained 50 mM Tris-HCl (pH 9.0), 2 mM L-phenylalanine and enzyme in a total volume of 1.5 ml. The assay was carried out at 30°C for 4 h, unless otherwise described, and the reaction was stopped by the addition of 1 ml 2 N HCl. The *t*-cinnamic acid formed was extracted into 2 ml of toluene by vortexing for 10 s and centrifuging at 1,500 × *g* for 10 min. The absorbance at 290 nm of *t*-cinnamic acid recovered in the toluene phase was measured using toluene as a blank.

To determine the effect of effectors, PAL was assayed in duplicate in a standard reaction mixture in the presence of the substance to be tested. *t*-Cinnamic acid production was determined as described above, using suitable blanks and controls in each case to determine the *t*-cinnamic acid formed due to enzyme action.

Protein concentration was determined according to the procedure of Lowry *et al.* (1951) using bovine serum albumin (BSA) as a standard.

Purification of phenylalanine ammonia-lyase PAL was purified from Chinese cabbage using a combination of ammonium sulfate fractionation, ion-exchange chromatography and gel filtration. All purification steps were carrid out in a cold room at 4°C with 50 mM Tris-HCl, pH 8.5 (Buffer A).

Step 1. Preparation of crude extract: The fresh leaves (1.2 kg) were ground up with 1.2 l of buffer A in a Waring Blender. The mixture was squeezed through 10 layers of cheesecloth (premoistened with buffer A). The crude extract was then clarified by centrifugation (5,000 × *g*, 10 min.) at 4°C and the supernatant (Fr 1, 2100 ml) was taken out to the next purification step.

Step 2. Ammonium sulfate fractionation: Solid ammonium sulfate was added to the crude extract (Fr 1) to achieve a concentration of 20% saturation, and the mixture was stirred on ice for 1 h. The supernatant was recovered after centrifugation for 20 min at 10,000 rpm using the Sorvall GSA rotor. Then, solid ammonium sulfate was added to the supernatant to a final concentration of 60% saturation. After 1 h on ice the resulting precipitate was collected by centrifugation as described above, and resuspended in buffer A (Fr 2, 260 ml). The suspension was dialyzed overnight against three changes of 3 l in buffer A.

Step 3. DEAE-cellulose chromatography: Fraction 2 was loaded onto a DEAE-cellulose column (2.5 × 17 cm) equilibrated with buffer A. The column was washed with 500 ml of buffer A until the protein content of the effluent returned to the baseline level (OD_{280} less than 0.01). Elution was carried out with a linear gradient of 0 to 0.5 M NaCl in buffer A at a flow rate of 30 ml/h. Fractions containing PAL activity were pooled (Fr 3, 160 ml), concentrated by precipitation at 60% ammonium sulfate saturation, and resuspended in 20 ml of buffer A.

Step 4. Sephadex G-200 gel filtration: The concentrated Fr 3 was then loaded onto a Sephadex G-200 gel filtration column (2.5 × 45 cm) equilibrated with buffer A. Elution was performed with buffer A at a flow rate of 30 ml/h. Fractions containing PAL activity were pooled (Fr 4, 60 ml).

Step 5. Q-Sepharose chromatography: Fraction 4 was loaded onto a Q-Sepharose column (1.5 × 6 cm) equilibrated with buffer A. The column was washed with buffer A, and elution was performed with a linear gradient of 0 to 1 M NaCl in buffer A at a flow rate of 40 ml/h. Fractions containing PAL activity were pooled (Fr 5, 33 ml) and desalted by dialysis. The purified enzyme preparation (Fr 5) was stored at −70°C and used for characterization.

SDS-PAGE Denaturing PAGE was performed as described by Laemmli (1970) with 12% acrylamide gels in the presence of 0.1% SDS. Polypeptides separated on the gel were stained with Coomassie brilliant blue R-250.

Isoelectric focusing Isoelectric focusing was performed in vertical slab gels using ampholines in the pH range of 3–10 (Robertson *et al.*, 1987). The isoelectric point was determined from a calibration curve using isoelectric point markers. The calibration proteins included: phycocyanin (pI, 4.45, 4.65, 4.75), β-lactoglobulin B (pI, 5.10), bovine carbonic anhydrase (pI, 6.00), human carbonic anhydrase (pI, 6.50), equine myoglobin (pI, 7.0), human hemoglobin A (pI, 7.10), human hemoglobin *c* (pI, 7.50), lentil lectin (pI, 7.80, 8.00, 8.20), and cytochrome *c* (pI, 9.60).

Molecular weight estimation by gel filtration Sephadex G-200 gel filtration was carried out to estimate the molecular weight of phenylalanine ammonia-lyase purified from Chinese cabbage. A calibration mixture of horse spleen apoferritin (440 kDa), sweet potato β-amylase (220 kDa), and alcohol dehydrogenase (150 kDa) was chromatographed on a column of Sephadex G-200. Then, the molecular weight of the Chinese cabbage PAL was determined from the calibration curve.

Results and Discussion

Purification The crude extract (Fr 1) was sequentially purified using a combination of differential ammonium sulfate fractionation, DEAE-cellulose chromatography, Sephadex G-200 gel filtration, and Q-Sepharose chromatography. Elution profiles during chromatographic steps are shown in Figs. 1–3. On the basis of the elution profile of Q-Sepharose chromatography as shown in Fig. 3,

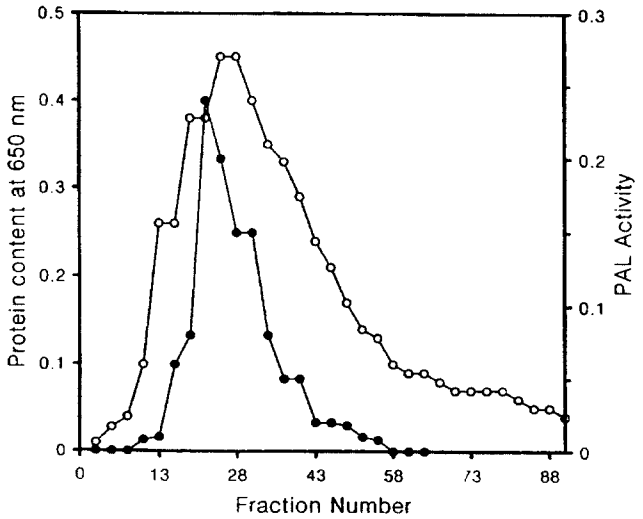

Fig. 1. The elution profile of DEAE-cellulose ion-exchange chromatography. Fraction 2 was applied to a DEAE-cellulose column (2.5 × 17 cm), and eluted with a linear gradient of 0 to 0.5 M NaCl at a flow rate of 30 ml/h. Fractions were collected and determined for PAL activity (-●-) as described in the section on Materials and Methods, and protein content (-○-) in the fractions was determined according to the Lowry method (1951).

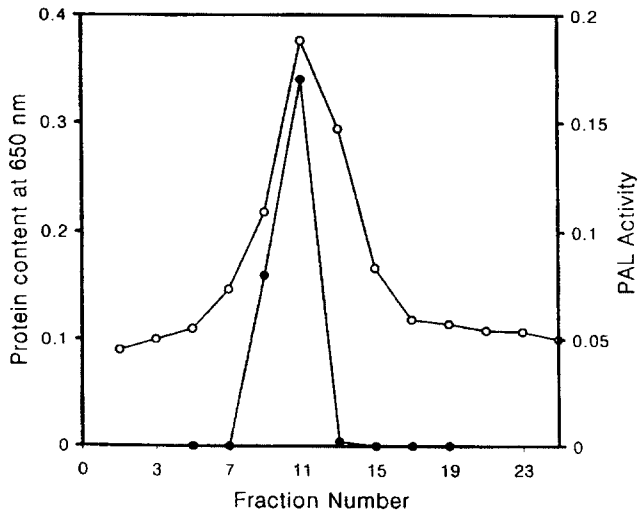

Fig. 3. The elution profile of Q-Sepharose ion-exchange chromatography. Fraction 4 was applied to a Q-Sepharose column (1.5 × 6 cm), and eluted with a linear gradient of 0 to 1.0 M NaCl at flow rate of 40 ml/h. Fractions were collected and determined for PAL activity (-●-) as described in the section on Materials and Methods, and protein content (-○-) in the fractions was determined according to the Lowry method (1951).

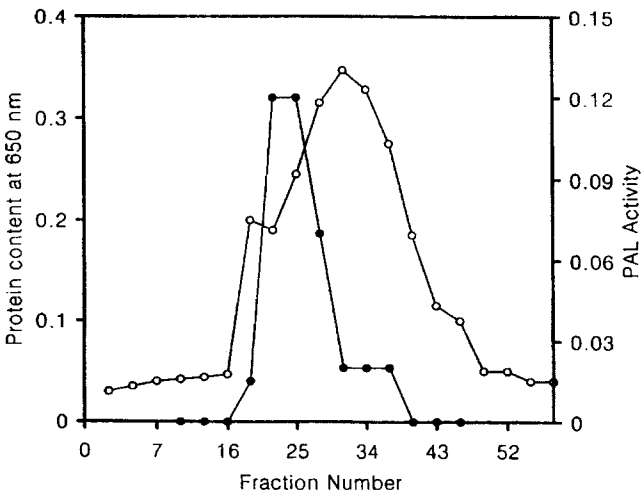

Fig. 2. The elution profile of Sephadex G-200 gel filtration. Fraction 3 was loaded onto a column of Sephadex G-200 (2.5 × 45 cm), and eluted with buffer A at a flow rate of 30 ml/h. Fractions were collected and determined for PAL activity (-●-) as described in the section on Materials and Methods, and protein content (-○-) in the fractions was determined according to the Lowry method (1951).

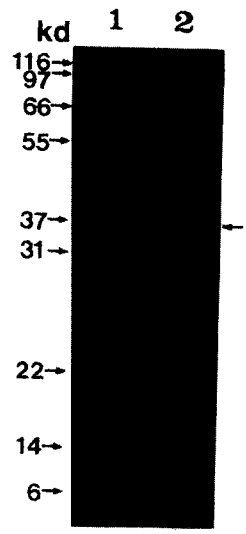

Fig. 4. SDS-polyacrylamide gel electrophoresis of PAL purified from Chinese cabbage. The proteins were analyzed on 12% SDS-polyacrylamide gel, and stained with Coomassie brilliant blue R-250. Lane 1, protein markers; Lane 2, Chinese cabbage PAL (Fraction 5).

a single activity peak coincided with a single peak of protein, indicating that PAL obtained from Chinese cabbage was near the homogeneous state. The purified protein (Fr 5) after Q-Sepharose chromatography showed a single protein band on SDS-PAGE, representing the purity of PAL from Chinese cabbage (Fig. 4, lane 2).

Molecular weight estimation The native molecular weight of Chinese cabbage PAL was estimated to be about 160,000 daltons by gel filtration on a Sephadex G-200 column (data not shown). However, the molecular weight of the Chinese cabbage PAL subunit was about 38,000 daltons, which was estimated from the mobility on SDS-PAGE, indicating that the native Chinese cabbage PAL

consists of four identical subunits with about 38,000 daltons. PALs, in all organisms examined to date, were identified as tetrameric enzymes. However, Chinese cabbage PAL appeared to be relatively small compared to other PALs, but has a very similar subunit size with leaf mustard PAL (Lim *et al.*, 1997). This shows that two different species of the genus *Brassica* contain a similar PAL size, which is an interesting characteristic in an evolutionary aspect. PAL from beans (*P. vulgaris*) was found to have a subunit molecular weight of 83,000 (Bolwell and Rodgers, 1991). The native and subunit molecular weights of PAL in loblolly pine were known to be 280,000 and 74,000, respectively (Whetten and Sederoff, 1992).

Effect of duration of reaction The time-course of the formation of *t*-cinnamic acid at 30°C catalyzed by Chinese cabbage PAL indicated the linearity for up to 12 h (data not shown). These results indicated that the enzyme was stable at the assayed temperature and *t*-cinnamic acid, one of the products of the reaction, might not have any inhibitory effects on the Chinese cabbage PAL. In some cases, *t*-cinnamic acid was reported to inhibit PAL activity.

pH and temperature optimum The assay of enzyme activity over a pH range is shown in Fig. 5, demonstrating a pH optimum of 8.0–9.0. This is similar to that reported for other phenylalanine ammonia-lyase (Hanson and Havir, 1981). The optimum temperature for the Chinese cabbage PAL was measured to be 45°C among temperature ranges tested (Fig. 6). This temperature was higher than 30°C for the optimum temperature of PAL from cotton hypocotyls (Dubery and Smit, 1994). However, it is similar to that for leaf mustard PAL (Lim *et al.*, 1997).

Isoelectric point The isoelectric point of the purified PAL was determined to be 5.6. This isoelectric point is comparable to those obtained for the enzymes from *Helianthus annuus* (pI = 4.8) (Jorrin *et al.*, 1988), *Z. mays* (pI = 4.95) (Havir *et al.*, 1971), and leaf mustard (pI = 5.7) (Lim *et al.*, 1997). No isoforms were found during isoelectric focusing.

Effect of substrate concentration The rate of *t*-cinnamic acid formation by the Chinese cabbage PAL was measured using 0.05–1 mM concentration of L-phenylalanine. As shown in Fig. 7, the data was plotted according to Lineweaver and Burk (1934). The K_m value for L-phenylalanine was found to be 2.1×10^{-4} M. This value is lower than that (1.7×10^{-3} M) for barley PAL (Koukol and Conn, 1961), and similar to that (2.7×10^{-5} M) for loblolly pine PAL (Whetten and Sederoff, 1992). The K_m and K_{cat} values for the purified PAL at the used concentration were found to be 0.052 ΔA_{290}/min and 0.25 ΔA_{290}/min mM, respectively.

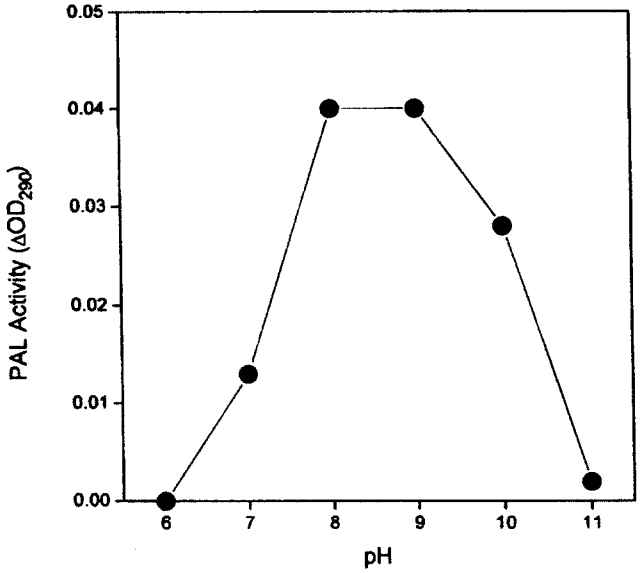

Fig. 5. The pH-dependence profile of the PAL activity purified from Chinese cabbage. PAL activity was determined as described in the section on Materials and Methods. Phosphate buffer (50 mM) was used for pH 6 and 7, whereas Tris buffer (50 mM) was used for pH 8–11.

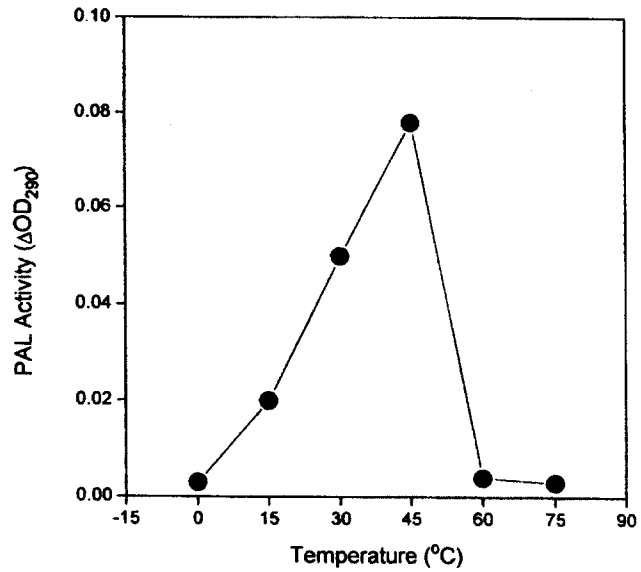

Fig. 6. The temperature-dependence profile of the PAL activity purified from Chinese cabbage. PAL activity was determined at various temperature as described in the section on Materials and Methods.

Effect of metal ions Effects of various metal ions on the activity of the Chinese cabbage PAL were examined (Table 1). Concentrations of metal ions tested were 1 mM in all cases. Li_2SO_4 and $MgCl_2$ have no effect on the Chinese cabbage PAL. However, $ZnSO_4$ had a significant inhibitory effect on the PAL activity. It is known that Cu^{2+},

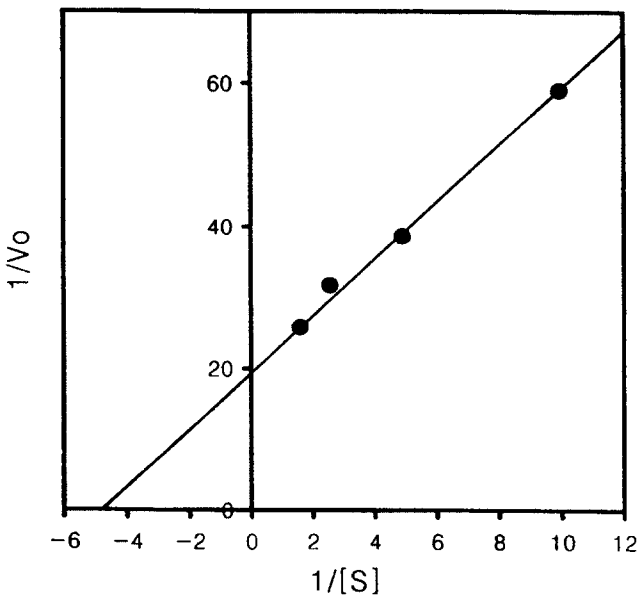

Fig. 7. The Lineweaver–Burk plot showing the rate of *t*-cinnamic acid by Chinese cabbage PAL as a function of substrate concentration. PAL activity was assayed as described in the section on Materials and Methods.

Table 1. Effects of various metal ions on the purified PAL activity from Chinese cabbage.

Matal ions[a]	Relative Activity (%)
None	100
Li_2SO_4	105
NaCl	99
$CuSO_4$	88
$ZnSO_4$	38
$MgCl_2$	93
$AlCl_3$	88

[a] Concentration of metal ions was 1 mM in all cases.

Zn^{2+}, and Ca^{2+} inhibit PAL activity obtained from barley (Koukol and Conn, 1961). In the case of barley PAL, Zn^{2+} was not the strongest one among the three metal ions. Moreover, Zn^{2+} at 1 mM could inhibit leaf mustard PAL completely (Lim *et al.*, 1997).

Effects of aromatic amino acids, phenolic and sulfhydryl compounds The effects of aromatic amino acids, phenolic and sulfhydryl compounds on the *in vitro* activity of the Chinese cabbage PAL were examined (Table 2). Aromatic amino acids such as L-tyrosine and L-tryptophan had no effect on the PAL activity. However, on the barley PAL (Koukol and Conn, 1961), L-tyrosine was reported to be a competitive inhibitor. Caffeic acid at 1 mM strongly activated the Chinese cabbage PAL. Caffeic acid was also found to be strong activator of the leaf mustard PAL activity (Lim *et al.*, 1997). *t*-Cinnamic acid had no effect on the Chinese cabbage PAL activity. These results were different from those obtained with cotton hypocotyl PAL (Dubery and Smit, 1994) and barley PAL (Koukol and Conn, 1961). Two sulfhydryl compouds, L-cysteine and dithiothreitol, had no effect on the Chinese cabbage PAL. However, L-cysteine had been known as an inhibitor of barley PAL.

Table 2. Effects of aromatic amino acids, phenolic, and sulfhydryl compounds on the activity of the purified PAL from Chinese cabbage.

Additions	Concentration	Relative Activity (%)
None	–	100
Aromatic Amino Acids		
L-Tyrosine	1×10^{-3} M	106
L-Tryptophan	1×10^{-3} M	102
Phenolic Compounds		
Caffeic acid	1×10^{-3} M	383
t-Cinnamic acid	5×10^{-3} M	106
Sulfhydryl Compounds		
L-Cysteine	1×10^{-3} M	100
Dithiothreitol	1×10^{-3} M	106

In conclusion, this article described the purification and the characterization of PAL from Chinese cabbage. The enzyme in its properties was similar to PAL purified from leaf mustard (Lim *et al.*, 1997). PAL from leaf mustard (*Brassica juncea* var. *integrifolia*) consists of four identical subunits and the molecular weight of the subunit is 40,000 daltons. Both PALs obtained from Chinese cabbage and leaf mustard were strongly activated by caffeic acid, and not inhibited by its product t-cinnamic acid. These characteristics are peculiar to Chinese cabbage and leaf mustard PALs. The similarities are interesting because Chinese cabbage and leaf mustard are classified into the same genus. However, further investigation is required to elucidate the functional and structural characteristics of the Chinese cabbage PAL.

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References

Bolwell, G. P. and Rodgers, M. W. (1991) L-Phenylalanine ammonia-lyase from French bean (*Phaseolus vulgaris* L.): characterization and differential expression of antigenic multiple Mr forms. *Biochem. J.* **279**, 231–236.

Brödenfeldt, R. and Mohr, H. (1988) Time courses for phytochrome-induced enzyme levels in phenylpropanoid metabolism (phenylalanine ammonia-lyase, maringenin-chalcone synthase) compared with time courses for phytochrome-mediated end-product accumulation (anthocyanin, quercetin). *Planta* **176**, 383–390.

Campbell, M. M. and Ellis, B. E. (1992) Fungal elicitor-mediated responses in pine cell cultures: III. Purification and characterization of phenylalanine ammonia-lyase. *Plant Physiol.* **98**, 62–70.

Da Cunha, A. (1988) Purification, characterization and induction of L-phenylalanine ammonia-lyase in *Phaseolus vulgaris*. *Eur. J. Biochem.* **178**, 243–248.

Dalkin, K., Edwards, R., Edington, B., and Dixon, R. A. (1990) Stress responses in alfalfa (*Medicago sativa* L.): I. Induction of phenylpropanoid biosynthesis and hydrolytic enzyme in elicitor-treated cell suspension cultures. *Plant Physiol.* **92**, 440–446.

Dixon, R. A. and Lamb, C. J. (1990) Molecular communication in interactions between plants and microbial pathogens. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **41**, 339–367.

Dubery, I. A. and Smit, F. (1994) Phenylalanine ammonia-lyase from cotton (*Gossypium hirsutum*) hypocotyls: Properties of the enzyme induced by a *Verticillium dahliae* phytotoxin. *Biochim. Biophys. Acta* **1207**, 24–30.

Hahlbrock, K. and Grisebach, H. (1979) Enzymic controls in the biosynthesis of lignin and flavonoids. *Annu. Rev. Plant Physiol.* **30**, 105–369.

Hahlbrock, K. and Scheel, D. (1989) Physiology and molecular biology of phenylpropanoid metabolism. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **40**, 347–369.

Hanson, K. R. and Havir, E. A. (1981) *The Biochemistry of Plants*, Conn, E. E. (ed.), Vol. 7, pp. 557–625, Academic Press, New York.

Havir, E. A., Reid, P. D., and Marsh, H. V. (1971) L-Phenylalanine ammonia-lyase (maize): Evidence for a common catalytic site for L-phenylalanine and L-tyrosine. *Plant Physiol.* **48**, 130–136.

Heller, W., Egin-Bühler, B., Gardiner, S. E., Knobloch, K.-H., Mattern, U., Ebel, J., and Hahlbrock, K. (1979) Enzymes of general phenylpropanoid metabolism and of flavonoid glycoside biosynthesis in parsley: differential inducibility by light during the growth of cell suspension cultures. *Plant Physiol.* **64**, 371–373.

Joos, H.-J. and Hahlbrock, K. (1992) Phenylalanine ammonia-lyase in potato (*Salanum tuberosum* L.). *Eur. J. Biochem.* **204**, 621–629.

Jorrin, J. and Dixon, R. A. (1990) Stress responses in alfalfa (*Medicago sativa* L.): II. Purification, characterization, and induction of phenylalanine ammonia-lyase isoforms from elicitor-treated cell suspension cultures. *Plant Physiol.* **92**, 447–455.

Jorrin, J., Lopex-Valbuena, R., and Tena, M. (1988) Purification and properties of phenylalanine ammonia-lyase from glutaredoxin (*Helianthus annuus* L.) hypocotyls. *Biochim. Biophys. Acta* **964**, 73–82.

Koukl, J. and Conn, E. E. (1961) The metabolism of aromatic compounds in higher plants: IV. Purification and properties of the phenylalanine deaminase of *Hordeum vulgare*. *J. Biol. Chem.* **236**, 2692–2698.

Laemmli, U. K. (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **227**, 680–685.

Lim, H.-W., Park, S.-S., and Lim, C.-J. (1997) Purification and properties of phenylalanine ammonia-lyase from leaf mustard. *Mol. Cells* **7**, 715–720.

Lois, R., Dietrich, A., Hahlbrock, K., and Schulz, W. (1989) A phenylalanine ammonia-lyase gene from parsley: structure, regulation and identification of elicitor and light responsive cis-elements. *EMBO J.* **8**, 1641–1648.

Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951) Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193**, 265–275.

Minamikawa, T. and Uritani, I. (1965) Phenylalanine ammonia-lyase in sliced sweet potato roots. *J. Biochem.* **57**, 678–688.

Nishizawa, A. N., Wolosiuk, R. A., and Buchanan, B. B. (1979) Chloroplast phenylalanine ammonia-lyase from spinach leaves. *Planta* **145**, 7–12.

Ohl, S., Hedrick, S. A., Chory, J., and Lamb, C. J. (1990) Funtional properties of a phenylalanine ammonia-lyase promoter from *Arabidopsis*. *Plant Cell* **2**, 837–848.

Pellegrini, L., Rohfritsch, O., Fritig, B., and Legrand, M. (1994) Phenylalanine ammonia-lyase in tobacco: Molecular cloning and gene expression during the hypersensitive reaction to tobacco mosaic virus and the response to a fungal elicitor. *Plant Physiol.* **106**, 877–881.

Robertson, E. F., Dannelly, K., Malloy, P. J., and Reeves, H. C. (1987) Rapid isoelectric focusing in a vertical polyacrylamide minigel system. *Anal. Biochem.* **167**, 290–294.

Whetten, R. W. and Sederoff, R. R. (1992) Phenylalanine ammonia-lyase from loblolly pine: Purification of the enzyme and isolation of complementary DNA clones. *Plant Physiol.* **98**, 380–386.

Yamada, T., Tanaka, Y., Sriprasertsak, P., Kato, H., Hashimoto, T., Kawamata, S., Ichinose, Y., Kato, H., Shiraishi, T., and Oku, H. (1992) Phenylalanine ammonia-lyase genes from *Pisum satium*: Structure, organ-specific expression and regulation by fungal elicitor and suppressor. *Plant Cell Physiol.* **33**, 715–725.

Zucker, M. (1965) Induction of phenylalanine deaminase by light and its relation to chlorogenic acid synthesis in potato tuber tissue. *Plant Physiol.* **40**, 779–784.