PERGAMON

Phytochemistry 51 (1999) 853–859

PHYTOCHEMISTRY

Rapid purification and characterization of cystine lyase b from broccoli inflorescence

Koji Ukai [1], Jiro Sekiya*

Division of Applied Life Sciences, Graduate School of Agriculture, Kyoto University, Kyoto, 606-8502, Japan

Received 6 January 1999; accepted 20 January 1999

Abstract

We found three isoforms (a, b, and c) of cystine lyase in broccoli (*Brassica oleracea* var. *italica*) inflorescence tissues. Cystine lyase b, the most abundant isoform, was rapidly purified to homogeneity. The native enzyme had a M_r of 160,000 and composed of four identical subunits with a M_r of 40,000. Thiocysteine and pyruvate were confirmed as reaction products. The purified cystine lyase b utilized L-cystine and *S*-alkyl L-cysteine sulfoxide as substrates. Other properties of cystine lyase b were almost similar to those of the isoform a as reported previously. Cystine lyase a and b were localized in cytosolic and/or vacuole fraction. A high activity of cystine lyase was detected in some *Allium* species in addition to Cruciferae plants.

Keywords: *Brassica oleracea* var. *italica*; Cruciferae; Broccoli; Purification; Cystine lyase; Thiocysteine

1. Introduction

Cystine lyase is an enzyme catalyzing β-elimination reaction of L-cystine to yield thiocysteine, pyruvate and ammonia (Hamamoto & Mazelis, 1986; Ukai & Sekiya, 1997a). The enzyme activity was first detected in several Cruciferae plants (Mazelis, Beimen & Creveling, 1967), but there was a confusion about the substrate specificity whether L-cysteine and cystathionine acted as a substrate with partially purified enzymes in earlier works (Anderson & Thompson, 1979; Hall & Smith, 1983). However, Mazelis, Scott and Gallie (1982) reported that cystine lyase was distinguished from cystathionine β-lyase (EC 4.4.1.8) and did not utilize L-cysteine as a substrate. This was confirmed by cystine lyase a completely purified from broccoli (*Brassica oleracea* var. *italica*) (Ukai & Sekiya, 1997a). Cystine lyase also utilized *S*-alkyl L-cysteine sulfoxides (Hamamoto & Mazelis, 1986; Ukai & Sekiya, 1997a) which occurred in Amaryllidaceae, Cruciferae and Leguminosae (Fowden, 1964; Virtanen, 1965). These compounds were also active as substrates for another plant C-S lyase, alliin lyase (EC 4.4.1.4), which occurs mostly in Amaryllidaceae (Tobkin & Mazelis, 1979). Alliin lyase is thought to be responsible for formation of the characteristic flavor from *S*-alkyl L-cysteine sulfoxides. However, methyl methanethiosulfinate having antibacterial activity was reported to be produced from *S*-methyl L-cysteine sulfoxide by cystine lyase reaction (Marks, Hilson, Leichtweis & Stoewsand, 1992; Kyung & Fleming, 1994). Cystine lyases in Cruciferae, thus, seem to be involved in formation of some interesting sulfur compounds.

Three isoforms (a, b and c) of cystine lyase were found in broccoli inflorescence tissues (Ukai & Sekiya, 1997a). We have attempted to purify and characterize three isoforms to understand cystine lyase. In our previous paper, purification and characterization of cystine lyase a were described (Ukai & Sekiya, 1997a). The present paper describes rapid purification and characterization of the most abundant isoform, cystine lyase b, from broccoli inflorescence tissues, subcellular

* Corresponding author. Fax: +81-75-753-6128.
E-mail address: jiro@kais.kyoto-u.ac.jp (J. Sekiya)
[1] Current address: Nakano Central Research Institute, Nakano Uinegar Co., Ltd., 2–6 Nakano-cho, Handa, Aichi 475-0873, Japan.

0031-9422/99/$ - see front matter
PII: S0031-9422(99)00067-9

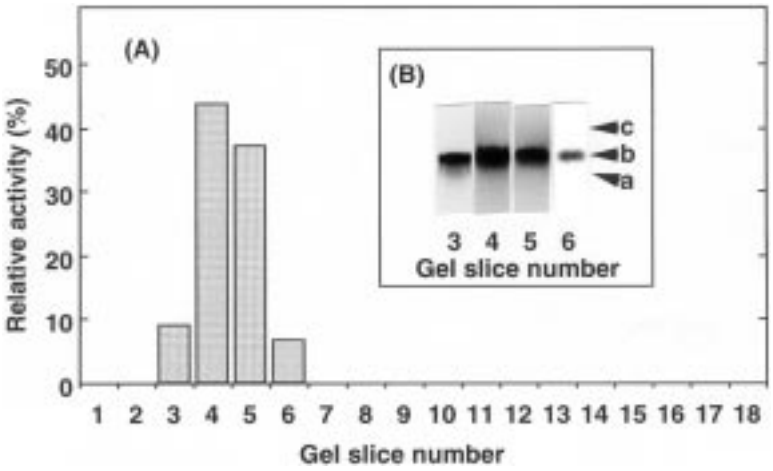

Fig. 1. Preparative PAGE of cystine lyase. (A) Cystine lyase activity after preparative PAGE. Cystine lyase was extracted from each gel slice after the preparative PAGE and the enzyme activity was measured by the method 1. (B) The extract from each gel slice was concentrated and subjected to analytical PAGE on an 8% gel. The gel was visualized by the activity staining.

localization of cystine lyase and distribution of cystine lyase in higher plants.

2. Results

2.1. Purification

Cystine lyase activity was followed by the activity staining after PAGE during the purification (Ukai & Sekiya, 1997b) in addition to the enzyme assay by the method 1. Most of the cystine lyase b activity occurred in the fraction precipitated by 50% saturation with $(NH_4)_2SO_4$, while cystine lyase a was precipitated between 50 and 70% saturation with $(NH_4)_2SO_4$ (Ukai & Sekiya, 1997a). $(NH_4)_2SO_4$ fractionation was, thus, effective to isolate cystine lyase b from the isoforms a and c. After the preparative polyacrylamide gel electrophoresis (PAGE), gel slices, 3–6, contained cystine lyase b (Fig. 1). The active fraction after Mono Q column chromatography showed a single protein band by analytical PAGE and the activity was detected at the same position by activity staining (Fig. 2). Summary of the purification of cystine lyase b is given in Table 1. Cystine lyase b was purified 729-fold with an activity yield of 10%. The purified cystine lyase b could be stored at -20° for more than 6 months without a significant loss of the activity. The purified enzyme retained its full activities on heating at 40° for 5 min, but lost 70% of the activity by heating at 50° for 5 min.

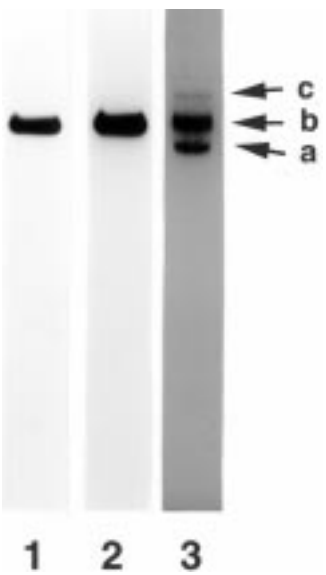

Fig. 2. PAGE of purified cystine lyase b. Lane 1, purified cystine lyase b stained by Coomassie brilliant blue; lane 2, purified cystine lyase b visualized by the activity staining; and lane 3, the mixture of isoforms a, b and c visualized by the activity staining.

2.2. Structural properties

M_r of cystine lyase b was estimated to be 160,000 by gel filtration with a Diol-300 column. Sodium dodecylsulfate (SDS)-PAGE showed a single protein band with a M_r of 40,000. These results indicate that cystine lyase b is composed of 4 identical subunits. Cystine lyase b was neither stained by the thymol-H_2SO_4 reagent (Racusen, 1979) after PAGE, nor adsorbed by concanavalin A Sepharose. These results suggest that cystine lyase b does not contain carbohydrate residues in its molecule. N-terminal amino acid was modified.

Carboxymethoxylamine and hydroxylamine completely inhibited the enzyme activity at 0.5 mM. Cystine lyase b shows an absorption maximum at 427 nm. These indicate that cystine lyase b is a pyridoxal 5′-phosphate dependent enzyme. Iodoacetate, *N*-ethylmaleimide and EDTA did not inhibit the activity.

Table 1
Summary of purification of cystine lyase b from broccoli inflorescence

Procedure	Protein (mg)	Total activity[a] (nKat)	Specific activity (nKat/mg protein)	Purification (fold)
Crude extract[b]	22,700	14,700	0.65	1
Heat-treatment	5020	13,400	2.67	4
Acid-treatment	2530	9630	3.81	6
$(NH_4)_2SO_4$ fractionation	1250	6810	5.45	8
Preparative PAGE (b)	117	1890	16.2	25
Mono Q anion exchange	3	1490	497	765

[a] Enzyme activity was measured by the method 1 based on detection of a thiol group.
[b] Purification was started from 1 kg of fresh broccoli inflorescence.

2.3. Reaction products

Peaks 1 and 2 were products of the cystine lyase b reaction (Fig. 3). Retention times of peaks 1 and 2 were consistent with those of cystine lyase a reaction, indicating that the peak 1 was a 3,7-dimethyl-4-bromomethyl-6-methyl-1,5-diazabicyclo[3,3,0]-octa-3,6,diene 2,8-dione (monobromobimane) derivate of L-cysteine and the peak 2 was that of thiocysteine. Mass numbers of the peak 1 and 2 were 312 $[M+H]^+$ and 344 $[M+H]^+$ by LC-MS analysis, respectively, supporting the above conclusion. The ratio of peak 1 to peak 2 was about 0.18 under the standard reaction condition. L-Cysteine may be a secondary product derived from thiocysteine, because thiocysteine is labile (Smith & Venkartraghavan, 1985). Pyruvate was also detected as a reaction product by the assay method 2. An amount of pyruvate formed was almost equal to that of the sum of thiocysteine and L-cysteine formed under the standard reaction condition.

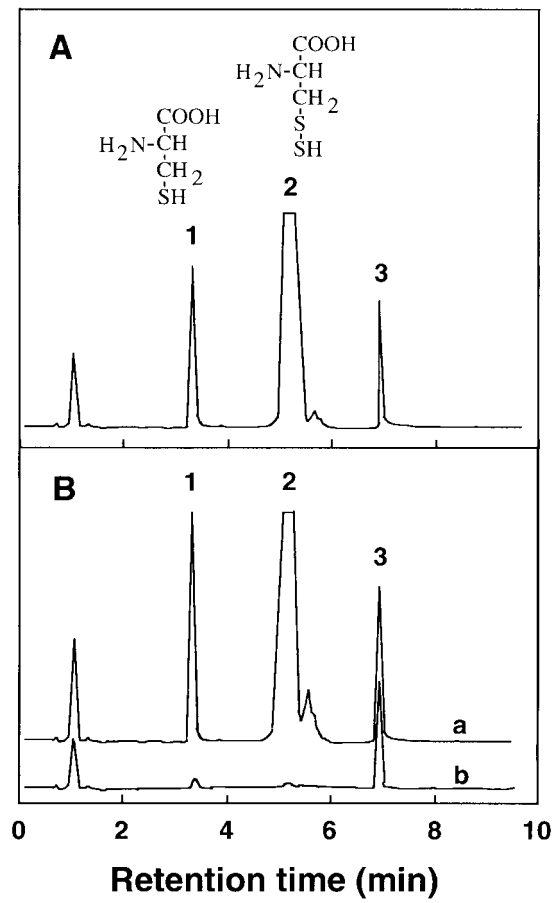

Fig. 3. HPLC elution profiles of reaction products. After the enzyme reaction was carried out at 30° for 15 min, reaction products were derivatized with monobromobimane and subjected to HPLC analysis as described in the text. (A) Enzyme reaction was done with purified cystine lyase a as a reference. (B) Reaction products with purified cystine lyase b (a) and with heat-denatured cystine lyase b (b).

2.4. Substrate specificity

L-Cystine was the best substrate for cystine lyase b among the compounds tested, while D-cystine was a poor substrate (Table 2). *S*-methyl L-cysteine sulfoxide

Table 2
Substrate specificity of cystine lyase b

Compound	Relative enzyme activity (%)[a]	
	(Method 1)	(Method 2)
L-Cystine	100	100
D-Cystine	18	12
L-Cysteine	1	0
D-Cysteine	0	0
L-Cystathionine	1[b]	0
L-Djenkolic acid	nd[c]	0
O-Acetylserine	nd[c]	0
S-Methyl L-cysteine	nd[c]	0
S-Ethyl L-cysteine	nd[c]	0
S-Methyl L-cysteine sulfoxide	nd[c]	46
S-Ethyl L-cysteine sulfoxide	nd[c]	64

[a] The enzyme activities were measured by the method 1 and the method 2 as described in the text with replacement of L-cystine with various compounds at the same concentration (6 mM). The enzyme activity is expressed as the values relative in percent to that measured with L-cystine.
[b] The activity was measured from homocysteine formation after derivatized with monobromobimane essentially by method 1.
[c] nd: not determined.

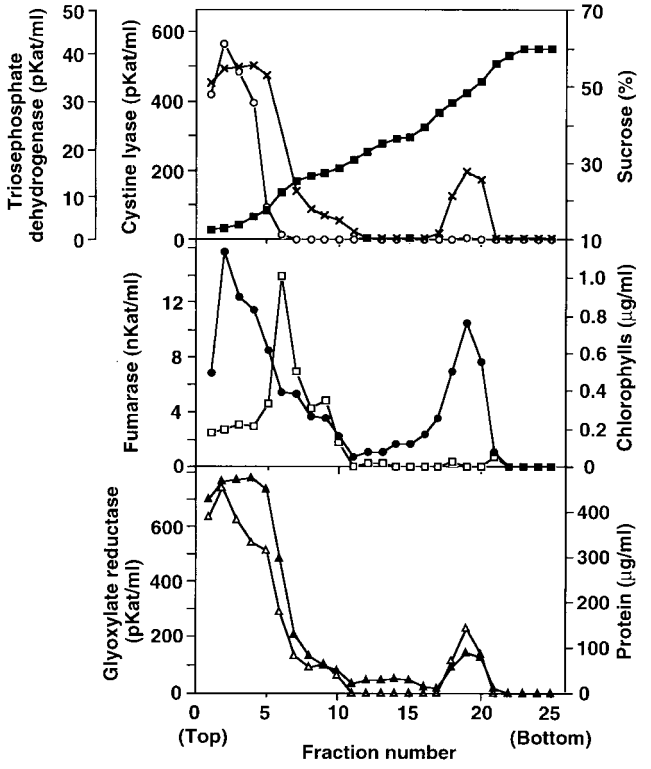

Fig. 4. Sucrose density gradient centrifugation of cystine lyase from broccoli inflorescence. Triosephosphate dehydrogenase (x); cystine lyase (○); sucrose concentration (■); fumarase (□); chlorophylls (●); glyoxylate reductase (△); and protein (▲).

and *S*-ethyl L-cysteine sulfoxide acted as a substrate, but to a lesser extent compared to L-cystine. L-Cystathionine, L- and D-cysteine, L-djenkolic acid, *O*-acetylserine, *S*-methyl L-cysteine and *S*-ethyl L-cysteine were not the substrate for cystine lyase b. K_m values were 0.9 mM for L-cystine, 9.8 mM for *S*-methyl L-cysteine sulfoxide and 11.9 mM for *S*-ethyl L-cysteine sulfoxide. These findings confirm that cystine lyase b catalyzes β-elimination of L-cystine. Optimal pH for the reaction with L-cystine was 8.5, and the enzyme showed more than 50% of the activity between pH 3 and 11.

2.5. Subcellular localization

Cystine lyase activity was found at the top layer of the sucrose gradient (Fig. 4). In addition, cystine lyase activity was not found in isolated chloroplasts nor mitochondria. These mean that cystine lyase localizes in the cytosol and/or vacuole fraction. Isoform of cystine lyase at the top layer was confirmed to be cystine lyases a and b by PAGE.

2.6. Distribution of cystine lyase in higher plants

As summarized in Table 3, cystine lyase activity was distributed in several plant species. The higher activity was found in leek (*A. tuberosum*) and garlic (*A. sativum*) in addition to Cruciferae plants. Pea (*P. sativum*), clover (*T. repens*) and sweet pepper (*C. annuum* var. *annuum*) also contained cystine lyase activity, but to a lesser extent. In broccoli, the enzyme activity was detected in all organs, inflorescence, leaf and root; the highest activity was found in inflorescence tissues (Fig. 3).

3. Discussion

We rapidly purified cystine lyase b, the most abundant isoform of cystine lyase, to homogeneity from broccoli inflorescence tissues. We used the activity staining on polyacrylamide gel in addition to the assay method 1 to follow the enzyme activity during the purification. Among the purification procedures, fractionation with $(NH_4)_2SO_4$ and preparative PAGE were effective to separate cystine lyase b from cystine lyase a (Ukai & Sekiya, 1997a). M_r of cystine lyase was a little larger than that of cystine lyase b. Even when proteinase inhibitors, EDTA and phenylmethanesulfonyl fluoride, were used to prepare the crude extract, these two isoforms, a and b, were detected. The ratio of cystine lyase a activity to b activity was constant during the purification, judged from darkness of the bands on the polyacrylamide gel stained for the activity. These findings indicate that one of the isoforms was not an artificial product derived from another one. We detected the third isoform (cystine lyase c) in broccoli inflorescence tissues. Therefore, at least 3 isoforms of cystine lyase are present in broccoli.

Hamamoto and Mazelis (1986) purified one (cystine lyase I) of 2 cystine lyase isoforms (I and II) from broccoli (*B. oleracea* var. *botrytis*) buds; cystine lyase I was eluted faster than cystine lyase II from a DEAE Fractogel column. When cystine lyase a and b were subjected to Mono Q column chromatography, cystine lyase b was eluted at 125 mM KCl and cystine lyase a at 155 mM KCl. We could not assign lyase a and b to cystine lyase I and II, because we found three isoforms. There is a discrepancy in the quaternary structure of the enzymes: cystine lyase I was reported to be a homotrimer with a M_r of 152,000, while cysteine lyases a and b were homotetramers. The enzymes we purified did not contain carbohydrate moieties, while cystine lyase I and II contained them.

Thiocysteine was confirmed as a reaction product from L-cystine by retention time of HPLC and LC-MS. An almost equal amount of pyruvate was also produced. These results indicate that cystine lyase b also catalyzes β-elimination of L-cystine. Substrate specificity was essentially the same as cystine lyase a (Ukai & Sekiya, 1997a) and cystine lyase I

Table 3
Distribution of cystine lyase activity in various plants

Family	Species	Tissue	Activity[a] (pKat/g fresh wt)	Specific activity[a] (pKat/mg protein)
Cruciferae	*Brassica oleracea* L var. *italica* Plen.	Inflorescence	879	174
		Leaf	273	36
		Root	23	9
	Brassica oleracea L. var. *botrytis* L.	Inflorescence	741	229
		Leaf	203	67
	Brassica napus L.	Leaf	88	9
	Raphanus sativus L.	Leaf	743	38
Chenopodiaceae	*Spinacia oleracea* L.	Leaf	ND[b]	ND[b]
Leguminosae	*Mimosa pudica* L.	Leaf	ND[b]	ND[b]
	Trifolium repens L.	Leaf	15	5
	Pisum sativum L.	Leaf	15	7
Umbelliferae	*Oenanthe javanica* DC.	Leaf	ND[b]	ND[b]
	Daucus carota L.	Leaf	ND[b]	ND[b]
Solanaceae	*Lycopersicon esculentum* Mill.	Leaf	ND[b]	ND[b]
	Capsicum annuum L. var. *angulosum* Mill.	Leaf	ND[b]	ND[b]
	Capsicum annuum L. var. *annuum*	Leaf	23	< 1
Compositae	*Chrysanthemum coronarium* L.	Leaf	ND[b]	ND[b]
Liliaceae	*Tulipa gesneriana* L.	Leaf	ND[b]	ND[b]
	Lilium longiflorum Thunb.	Leaf	ND[b]	ND[b]
	Allium tuberosum Rottl.	Leaf	1348	150
	Allium fistolosum L.	Leaf	3	< 1
	Allium sativum L.	Leaf	565	63
Gramineae	*Oryza sativa* L.	Leaf	ND[b]	ND[b]
	Zea mays L.	Leaf	ND[b]	ND[b]

[a] Crude enzyme solution was used as the enzyme source. The activity was assayed by the method 1.
[b] ND: not detected.

(Hamamoto & Mazelis, 1986). This means that cystine lyase b does not involve cystathionine β-lyase activity. L-cysteine was inert as the substrate for purified cystine lyase b. However, the crude enzyme solution utilized L-cysteine as the substrate apparently, probably because of oxidation of L-cysteine to L-cystine in the crude enzyme solution. Alliin lyase from *Allium* species utilized *S*-alkyl L-cysteine sulfoxides as substrates, but not L-cystine (Schwimmer, 1971). Thus, cystine lyase b is distinguished from alliin lyase.

Cystine lyase activity was found in cytosolic and/or vacuole fraction (Fig. 4), because the extraction method used here burst vacuoles. On the other hand, cystathionine β-lyase was reported to be associated with chloroplasts (Ravanel, Ruffet & Douce, 1995) and/or the cytosol (Droux, Ravanel & Douce, 1995). These results also support that cystine lyase is different from cystathionine β-lyase.

Occurrence of cystine lyase has been restricted to *Brassica* species in Cruciferae and some members in Leguminosae so far (Mazelis et al., 1967; Staton & Mazelis, 1991). We found a high cystine lyase activity in some *Allium* species as shown in Table 3. The activity detected here is different from alliin lyase, because this activity was followed by the formation of thiocysteine. In addition, alliin lyase did not utilize L-cystine as the substrate. Supposing that *S*-alkyl L-cysteine sulfoxides are involved or produced in the crude enzyme solution, the products by alliin lyase reaction of these compounds are not thiocysteine. Thus, our findings shown in Table 3 indicate that *Allium* species also have cystine lyase.

At present the physiological role of cystine lyase in higher plants is obscure. One possible role is a member in the biosynthetic pathway of methyl methanethiosulfinate (Marks et al., 1992). Another possible role is as a donor of a reduced sulfur, for example, in the proposed biosynthetic pathway of glucosinolates (Larsen, 1981). Recently, it was reported that cystine lyase was involved in [2Fe-2S] cluster formation of *Synechocystis* ferredoxin (Leibrecht & Kessler, 1997). However, because ferredoxin is widespread in all higher plants in which cystine lyase activity is not found, further investigation will be required.

4. Experimental

4.1. Materials

Sources of the compounds used were described previously (Ukai & Sekiya, 1997a). Fresh inflorescence tissues of broccoli (*B. oleracea* var. *italica* cv. Endeavor) were obtained from a commercial field of Mr Tarao in Uji, Kyoto, Japan. Other plant materials were harvested in the university experimental field.

4.2. Crude enzyme solution

All procedures were carried out at 4°. Fresh tissues were homogenized in 50 mM Tris-HCl buffer, pH 8.0, with 10% (w/w) insoluble polyvinylpyrrolidone and the homogenate was centrifuged for 10 min at 10,000 g. The supernatant obtained was made to 80% saturation with $(NH_4)_2SO_4$ and centrifuged for 10 min at 10,000 g. The precipitate was dissolved in 50 mM Tris-HCl buffer, pH 8.0, desalted with Sephadex G-25 and used as crude enzyme solution.

4.3. Enzyme purification

All procedures were carried out at 4° unless otherwise stated. Preparation of the crude extract, heat-treatment and acid-treatment were carried out as described previously (Ukai & Sekiya, 1997a). The supernatant after the acid-treatment was brought to 50% saturation with $(NH_4)_2SO_4$. After the centrifugation for 10 min at 15,000 g, the precipitate was dissolved in 50 mM Tris-HCl buffer, pH 7.5, containing 100 μM pyridoxal 5′-phosphate and desalted by dialysis against the same buffer. The desalted enzyme was applied on an 8% polyacrylamide gel for a preparative PAGE (PES NA-P, Nakano Central Research Institute, Japan). The preparative PAGE and the extraction from the gel were carried out by the procedures described previously (Ukai & Sekiya, 1997a). The enzyme in the active fraction from the gel was further purified by Mono Q column chromatography under the same condition used for cystine lyase a (Ukai & Sekiya, 1997a).

4.4. Enzyme assay

Two methods were used for measurement of cystine lyase activity; method 1 based on the detection of thiol compounds formed, and method 2 based on the detection of pyruvate formed (Ukai & Sekiya, 1997a). The assay mixture contained 50 mM Tris-HCl buffer, pH 8.0, 6 mM L-cystine, 20 μM PLP and the enzyme solution in a total volume of 0.9 ml. After the reaction for 8 min at 30°, reaction products were measured by method 1 or 2. Fumarase (Hatch, 1978), triosephosphate dehydrogenase (Heber, Pon & Hebar, 1963), and glyoxylate reductase (Zelitch & Gotto, 1962) were assayed as described elsewhere.

4.5. Measurements for proteins, sucrose and chlorophylls

Protein was assayed for the method of Bradford with bovine serum albumin as the standard (Bradford, 1976). Chlorophylls were measured by the method of Arnon (1949) and sucrose concentration was determined with a refractometer.

4.6. Polyacrylamide gel electrophoresis

Native PAGE on an 8% gel was performed by the method of Davis (1964). Cystine lyase activity was visualized on the gel by the activity staining method described previously (Ukai & Sekiya, 1997b). SDS-PAGE (4-20% polyacrylamide gradient gel) were performed by the procedure of Laemmli (1970). Proteins were stained with Coomassie brilliant blue.

4.7. LC-MS

For LC-MS, the fractions containing monobromobimane derivatives of the reaction products were collected repeatedly by HPLC and concentrated in vacuo. Hitachi M-1000 LC-MS was used for measurement (Ukai & Sekiya, 1997a).

4.8. Sucrose density gradient centrifugation

Broccoli inflorescence tissues (10 g) were homogenized for 20 s at 4° with 50 ml of 50 mM Tris-HCl buffer, pH 7.5, containing 0.35 M sucrose, 5 mM $MgCl_2$, 2 mM 2-mercaptoethanol and 1 mM EDTA in a Polytron homogenizer at a maximal speed. The homogenate was passed through four layers of gauze, and the filtrate was centrifuged for 5 min at 1000 g. The supernatant was applied onto a 20–60% (w/w) linear sucrose gradient in 50 mM Tris-HCl buffer, pH 7.5, containing 5 mM $MgCl_2$, 2 mM 2-mercaptoethanol and 1 mM EDTA, and centrifuged for 20 min at 23,000 rpm (Hitachi RPS40 T rotor). After centrifugation, 1 ml fraction was collected and the activities of cystine lyase and marker enzymes were assayed.

Acknowledgements

The authors are grateful to Drs K. Hashimoto (Kyoto Pharmaceutical University) and T. Ueno (Kyoto University) for performance of LC/MS. We would like to thank Mr Y. Tarao for his generous gift of broccoli plants.

References

Anderson, N. W., & Thompson, J. F. (1979). Cystine lyase: β-cystathionase from turnip roots. *Phytochemistry*, *18*, 1953.

Arnon, D. I. (1949). Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. *Plant Physiology*, *24*, 1.

Bradford, M. M. (1976). A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*, *72*, 248.

Davis, B. J. (1964). Disc electrophoresis II. Method and application to human serum proteins. *Annals of New York Academy of Science*, *121*, 404.

Droux, M., Ravanel, S., & Douce, R. (1995). Methionine biosynthesis in higher plants. II. Purification and characterization of cystathionine β-lyase from spinach chloroplasts. *Archives of Biochemistry and Biophysics*, *316*, 585.

Fowden, L. (1964). The chemistry and metabolism of recently isolated amino acids. *Annual Review of Biochemistry*, *33*, 173.

Hatch, M. D. (1978). A simple spectrophotometric assay for fumarate hydratase in crude tissue extracts. *Analytical Biochemistry*, *85*, 271.

Hall, D. I., & Smith, I. K. (1983). Partial purification and characterization of cystine lyase from cabbage (*Brassica oleracea* var *capitata*). *Plant Physiology*, *72*, 654.

Hamamoto, A., & Mazelis, M. (1986). The C-S lyase of higher plants. Isolation and properties if homogeneous cystine lyase from broccoli (*Brassica oleracea* var *botrytis*). *Plant Physiology*, *80*, 702.

Heber, U., Pon, N. G., & Hebar, M. (1963). Localization of carboxydismutase and triosephophate dehydrgenase in chloroplasts. *Plant Physiology*, *38*, 355.

Kyung, K. L., & Fleming, H. P. (1994). S-Methyl-L-cysteine sulfoxide as the precursor of methyl methasnethiosulfinate, the principal antibacterial compound in cabbage. *Journal of Food Science*, *59*, 350.

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

Larsen, P. O. (1981). Glucosinolates. In E. E. Conn, *Biochemistry of plants*, *vol. 7* (p. 501). New York: Academic Press.

Leibrecht, I., & Kessler, D. (1997). A novel L-cysteine/cystine lyase directing [2Fe-2S] cluster formation of *Synechocytis* ferredoxin. *Journal of Biological Chemistry*, *272*, 10442.

Marks, H. S., Hilson, J. A., Leichtweis, H. C., & Stoewsand, G. S. (1992). S-Methylcysteine sulfoxide in *Brassica* vegatablea and formation of methyl methanethiosulfinate from Brussels sprouts. *Journal of Agricultural and Food Chemistry*, *40*, 2098.

Mazelis, M., Beimer, N., & Creveling, R. K. (1967). Cleavage of L-cystine by soluble enzyme preparation from *Brassica* species. *Archives of Biochemistry and Biophysics*, *120*, 371.

Mazelis, M., Scott, K., & Gallie, D. (1982). Non-identity of cystine lyase with β-cystathionase in turnip roots. *Phytochemistry*, *21*, 991.

Racusen, D. (1979). Glycoprotein detection in polyacrylamide gel with Thymol and sulfuric acid. *Analytical Chemistry*, *99*, 474.

Ravanel, S., Ruffet, M. L., & Douce, R. (1995). Cloning of an *Arabidopsis thaliana* cDNA encoding cystathionine beta-lyase by functional complementation in *Escherichia coli*. *Plant Molecular Biology*, *29*, 875.

Staton, A. L., & Mazelis, M. (1991). The C-S lyases of higher plants: Homogeneous β-cystathionase of spinach leaves. *Archives of Biochemistry and Biophysics*, *290*, 46.

Schwimmer, S. (1971). S-Alkyl-L-cysteine sulfoxide lyase. In H. Tabor, & C. W. Tabor, *Methods in enzymology*, *vol. XVIIB* (p. 475). New York: Academic Press.

Smith, D. J., & Venkartraghavan, V. (1985). Synthesis and stability of thiocysteine. *Synthetic Communications*, *15*, 945.

Tobkin Jr, H. E., & Mazelis, M. (1979). Alliin lyase: preparation and characterization of the homogeneous enzyme from onion bulbs. *Archives of Biochemistry and Biophysics*, *193*, 150.

Ukai, K., & Sekiya, J. (1997a). Purification and characterization of cystine lyase a from broccoli inflorescence. *Bioscience, Biotechnology and Biochemistry*, *61*, 1890.

Ukai, K., & Sekiya, J. (1997b). A new staining method for lyase catalyzing cleavage of a C-S bond in sulfur-containing compounds after polyacrylamide gel electrophoresis. *Bioscience, Biotechnology and Biochemistry*, *61*, 124.

Virtanen, A. I. (1965). Studies on organic sulphur compounds and other labile substances in plants. *Phytochemistry*, *4*, 207.

Zelitch, I., & Gotto, A. M. (1962). Properties of a new glyoxylate reductase from leaves. *Biochemical Journal*, *84*, 541.