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# Purification and Characterization of Cystine Lyase a from Broccoli Inflorescence

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One of the three isoforms of an enzyme degrading L-cystine was purified to homogeneity from broccoli (*Brassica oleracea* var. *italica*) inflorescences, with use of a sensitive assay based on derivatization of a reaction product with monobromobimane. The reaction product with a thiol group was found to be thiocysteine from results of liquid chromatography-mass spectrometry and high-resolution mass spectrometry. Pyruvate was also a reaction product, formed in equimolar amounts. The purified enzyme catalyzed  $\beta$ -elimination of L-cystine to yield thiocysteine, pyruvate and possibly ammonia, so it was cystine lyase a. L-Cystine but not D-cystine was a substrate of the enzyme. S-Methyl L-cysteine sulfoxide and S-ethyl L-cysteine sulfoxide were substrates but were less suitable than L-cystine. L- and D-cysteine and also cystathionine were not substrates. The purified enzyme ( $M_r$  186,000) was composed of four identical subunits ( $M_r$  45,000) and was pyridoxal 5'-phosphate-dependent.

Key words: Brassica oleracea var. italica; broccoli; cystine lyase; thiocysteine; purification

Cystine lyase has been thought to be an enzyme that catalyzes the  $\beta$ -elimination reaction of L-cystine to yield thiocysteine, pyruvate, and ammonia.<sup>1)</sup> This enzyme has been found in several Cruciferae<sup>1)</sup> and partially purified from cabbage<sup>2)</sup> and turnip.<sup>3)</sup> One of the isoforms of cystine lyase has been purified to homogeneity from broccoli.<sup>4)</sup> Similar enzymes were purified from onion,<sup>5)</sup> garlic,<sup>6)</sup> and leek,<sup>7)</sup> all in the genus *Allium*, and from *Acacia furnesiana*,<sup>8)</sup> in the family Fabaceae. In most earlier studies of cystine lyase, the enzyme was assayed in terms of the amount of pyruvate formed, without direct proof that thiocysteine was actually formed.<sup>9)</sup>

This enzyme is not found in many higher plants, and may be the enzyme responsible for characteristic flavor of plants in which it is present, by degrading sulfur-containing amino acids. Such degradation occurs in many species in Amaryllidacea and Cruciferae.<sup>10,11)</sup> In addition, cystine lyase may be involved in the metabolism of *S*-methyl L-cysteine sulfoxide found in Cruciferae as a nonproteinaceous amino acid.<sup>12)</sup>

Cystine lyase from higher plants has isoforms.<sup>2,4)</sup> The physiological role of cystine lyase in *Brassica* may be important and interesting, but the isoforms have not been characterized in detail. We set out to purify and characterize the isoforms of cystine lyase from broccoli inflorescences. Here, we describe a new assay based on derivatization of the thiol group, and purification and properties of cystine lyase a.

# Materials and Methods

*Plant materials and chemicals.* Fresh inflorescence tissues of broccoli (*Brassica oleracea* L. var. *italica* Plen. cv. Endeavor, Takii Seeds Co., Ltd., Kyoto, Japan) were obtained from a commercial grower in Kyoto, Japan. L-Cysteine, D-cysteine, L-cystine, D-cystine, Coomassie brilliant blue (CBB), NADH, and pyridoxal 5'-phosphate (PLP) were purchased from Nacalai

Tesque (Kyoto). L-Cystathionine, S-methyl L-cysteine and S-ethyl L-cysteine were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). S-Methyl L-cysteine sulfoxide and S-ethyl L-cysteine sulfoxide were prepared by oxidation of the corresponding S-alkyl L-cysteine with acidified  $H_2O_2$  and crystallized from an ethanol-water solution.<sup>13)</sup> 3,7-Dimethyl-4-bromomethyl-6-methyl-1,5-diazabicyclo[3.3.0]octa-3,6-diene-2,8-dione (monobromobimane) was purchased from Calbiochem (Thiolyte MB; La Jolla, CA, U.S.A.). Lactate dehydrogenase from *Leuconostock mesenteroides* was obtained from Oriental Yeast Co., Ltd. (Tokyo, Japan). Other chemicals were obtained from Wako Pure Chemical Ind. (Osaka, Japan) and Nacalai Tesque.

*Enzyme purification.* All procedures were done at 4°C, unless otherwise stated.

Step 1. Crude extract. Fresh broccoli inflorescences (1 kg) were homogenized with a Polytron homogenizer in 5 volumes of 50 mM Tris–HCl buffer (pH 7.5) containing  $100 \,\mu$ M PLP (TP buffer) and 100 g insoluble polyvinylpyrrolidone. The homogenate was passed through four layers of gauze. The filtrate obtained was centrifuged at  $15,000 \times g$  for 10 min and the pellet was discarded. The supernatant obtained was used as the crude extract for further purification.

Step 2. Heat treatment. The crude extract was heated at  $45^{\circ}$ C for 5 min with stirring by a magnetic stirrer. The solution was then cooled quickly and centrifuged at  $15,000 \times g$  for 10 min. The pellet was discarded.

Step 3. Acid treatment. HCl (6 M) was added dropwise to the supernatant from step 2 with stirring until the pH reached 4.0,<sup>4)</sup> and the solution was stirred for 20 min more. The solution was centrifuged at  $15,000 \times g$  for 10 min and the pellet was discarded.

Step 4. Ammonium sulfate fractionation. The supernatant was brought to 50% saturation with ammonium sulfate and centrifuged at  $15,000 \times g$  for 10 min. To the supernatant, more ammonium sulfate was added to bring to 70% saturation. The precipitate collected by centrifugation was dissolved in a minimum volume of TP buffer and desalted by gel filtration with Sephadex G-25.

Step 5. Preparative gel electrophoresis. Large-scale slab gel electrophoresis was done by the method of Oshima *et al.*<sup>14)</sup> with a preparative gel electrophoresis apparatus (PES NA-P, Nakano Central Research Institute, Japan). The enzyme solution after step 4 was concentrated and put on 8% polyacrylamide gel with a Pasteur pipette. Electrophoresis was done at 4°C at a constant current of 40 mA. After about 12 h, the gel was first cut vertically at one edge and the enzyme was stained as described previously.<sup>15)</sup> The rest of the gel was then cut horizontally into slices 1 cm

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*Abbreviations*: CBB, Coomassie brilliant blue; LC-MS, liquid chromatography-mass spectrometry; monobromobimane, 3,7-dimethyl-4bromomethyl-6-methyl-1,5-diazabicyclo[3.3.0]octa-3,6-diene-2,8-dione; PLP, pyridoxal 5'-phosphate; TP buffer, 50 mM Tris-HCl buffer, pH 7.5, containing 100 μM PLP.

wide, and the parts containing the enzyme were homogenized in a small volume of TP buffer. The homogenate was centrifuged at  $15,000 \times g$  for 10 min and a clear supernatant was obtained.

Step 6. Mono Q column chromatography. The supernatant containing the enzyme was put on a Mono Q 10/10 column. After the column was washed with 30 ml of TP buffer, the enzyme was eluted with a linear gradient of 0 to 0.4 m KCl in 180 ml of TP buffer. The active fractions were pooled, concentrated, and examined by PAGE and SDS-PAGE for homogeneity.

*Enzyme assay*: Two methods were used for the enzyme assay; method 1 was generally used during purification and characterization of the enzyme, and method 2 was used during characterization.

Method 1 (detection of a thiol group). The standard 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. The reaction was started by the addition of L-cystine and the mixture was incubated at 30°C for 8 min. The reaction was stopped by the addition of  $100 \,\mu$ l of 20% trichloroacetic acid. The mixture was then centrifuged at  $15,000 \times g$  for 5 min and the precipitated proteins were removed. Then the supernatant was assayed for reaction product with a thiol group as described previously.<sup>16)</sup> A 20  $\mu$ l portion of the supernatant was pipetted into a mixture of 10 µl of 15 mM monobromobimane and 280 µl of 50 mM Tris-HCl buffer (pH 8.0) and the mixture was kept at 30°C for 15 min in the dark. To this mixture,  $190 \,\mu l$  of 10% acetic acid was added and the proteins that precipitated were removed by centrifugation at  $15,000 \times g$  for  $5 \min$ . Monobromobimane derivatives of thiol compounds were separated by HPLC with an Ultrasphere-ODS (C-18) column  $(4.6 \times 150 \text{ mm}, \text{Beckman}, \text{Beckman})$ Fullerton, CA, U.S.A.) and detected with a fluorescence spectrophotometer at an excitation wavelength of 380 nm and an emission wavelength of 480 nm. The mobile phase was ethanol-water-acetic acid (15:85:0.25 by volume) and the flow rate was 1.1 ml/min. The amount of each thiol compound formed was expressed as L-cysteine equivalents with reference to an L-cysteine calibration curve.

Method 2 (detection of pyruvate). The reaction mixture and the incubation conditions were the same as in method 1. After the reaction, portion of the reaction mixture was deproteinized and assayed for pyruvate as described elsewhere.<sup>9)</sup>

*Protein measurement.* Protein was assayed by the method of Bradford<sup>17</sup> with bovine serum albumin as the standard.

*Gel electrophoresis.* SDS PAGE was done on a 4-20% polyacrylamide gradient gel as described by Laemmli.<sup>18)</sup> Native PAGE was done on a 8% polyacrylamide gel by the method of Davis.<sup>19)</sup> After the electrophoresis, gels were stained with CBB for protein or by the method for staining of activity.<sup>15)</sup>

Identification of reaction products by LC-MS and high-resolution mass spectrometry. For LC-MS and high-resolution mass spectrometry, fractions containing the reaction products were collected repeatedly by HPLC and concentrated under reduced pressure. A Hitachi M-1000 LC-MS system was used for LC-MS. A JEOL JMS-HX/HX110A mass spectrometer was used for the mass spectrometry. A matrix of 3% glycerol in methanol was added to the sample as the internal standard.

# Results

#### Cystine-degrading enzyme activity in broccoli inflorescences

When a crude extract was incubated with L-cystine, a peak 2 at the retention time of 5.2 min was found by assay method 1 (Fig. 1). This peak disappeared when a heat-denatured crude extract was used instead. Without L-cystine, peak 2 did not appear. Thus, peak 2 was indicated to be an enzymatic product of L-cystine. The mono-bromobimane derivative of L-cysteine was coincident with peak 1. Peak 3 was a degradation product of mono-bromobimane. As described below, the compound of peak 2 was the monobromobimane derivative of thiocysteine. Pyruvate was also detected after the enzyme reaction by assay method 2. We tentatively concluded that the enzyme catalyzing formation of the product of peak 2 from L-cystine

was cystine lyase. In preliminary experiments, the enzyme activity producing thiocysteine was the highest in broccoli when 20 plant species were tested (not listed). Therefore, broccoli inflorescences were used for purification of cystine lyase.

#### Enzyme purification

The steps in purification of the enzyme and results are summarized in Table I. After each step, the activity was assayed by method 1. Of the procedures used, the combination of ammonium sulfate fractionation and preparative PAGE effectively purified the cystine lyase a. Cystine lyase a was found in the fraction precipitated by ammonium sulfate between 50% and 70% saturation, and most of cystine lyase b, an abundant isoform of this enzyme, was precipitated by 50% saturation (unpublished). When the extracts from different gel slices in step 5 were assayed, slices 3 to 7 contained cystine lyase activity (Fig. 2). Activity staining of the 8% polyacrylamide gel showed two strong bands (a and b) and one weak band (c). Cystine lyase a (gel slices 6 and 7) was separated from isoforms b and c by preparative PAGE. The enzyme extracted from slices 6 and 7 was further purified by Mono Q column chromatography. The active fraction obtained during this chromatography gave a single band when the gel of native PAGE was stained with CBB. The band stained with CBB corresponded to the



Fig. 1. HPLC Elution Profiles of Reaction Products.

After the enzyme reaction at  $30^{\circ}$ C for 15 min, reaction products were derivatized with monobromobimane and analyzed by HPLC as described in the text. The native crude extract (A) and a heat-denatured crude extract (B) were used as enzyme sources.

 
 Table I.
 Summary of Purification of Cystine Lyase a from Broccoli Inflorescences

Procedure		Proteins (mg)	Total activity <sup>a</sup> (nkat)	Specific activity (nkat/mg protein)	Purifica- tion (fold)
Crude extract <sup>b</sup>		22,700	14,700	0.65	1
Heat treatment		5,020	13,400	2.67	4
Acid treatment		2,530	9,630	3.81	6
$(NH_4)_2SO_4$ fractionation		172	1,220	7.09	11
Preparative electrophoresis					
	(a)	14.8	329	22.2	34
	(b)	17.7	277	15.6	24
	(c)	17.3	45	2.6	4
Mono Q amino exchange	(a)	0.75	243	324	498

" Enzyme activity was assayed by method 1, which was based on detection of a thiol group.

<sup>b</sup> Purification started with 1 kg of fresh broccoli inflorescences.

band shown by activity staining (Fig. 3). Thus, cystine lyase a had been purified to homogeneity.

#### Identification of reaction products

We tentatively referred to the enzyme catalyzing the formation of the compound of peak 2 in Fig. 1 as cystine lyase as mentioned above. Next, products formed with the purified enzyme were analyzed. Peaks 1 and 2 were detected as monobromobimane derivatives by HPLC, suggesting that the products contained a thiol group. The mass spectrum of peak 1 was the same as that of authentic L-cysteine derivatized with monobromobimane (data not shown), indicating that peak 1 was the monobromobimane derivative of L-cysteine. The mass spectrum of peak 2 gave m/z 344 (M+H)<sup>+</sup>, suggesting that peak 2 was a derivative of thiocysteine (Fig. 4). High-resolution mass spectrometry of peak 2 gave the value of  $344.0722 (M + H)^+$ , indicating peak 2 was  $C_{13}H_{18}O_4N_3S_2$  (calcd.; 344.0720). From these results, we concluded that peak 2 was the monobromobimane derivative of thiocysteine. The ratio of peak 1 to peak 2 was 0.17. However, when the incubation period was prolonged, the proportion of cysteine increased.

Pyruvate also was detected as a reaction product (Fig. 5). The ratio of thiocysteine to pyruvate was 0.83 : 1.00. This



**Fig. 2.** Cystine Lyase Activity after Preparative Gel Electrophoresis. (A) After the preparative gel electrophoresis, the gel was sliced. Slices were extracted with TP buffer. Cystine lyase activity of the extracts was assayed by method 1. The activity is expressed as a percentage of relative distribution. (B) The extract containing the activity was examined by analytical PAGE. After PAGE, activity in the gel was stained. The three isoforms are shown as a, b, and c.

finding means that the purified enzyme catalyzed the formation of almost equimolar amounts of thiocysteine and pyruvate. Ammonia may be formed by the enzyme reaction, although we did not assay it. We concluded that the enzyme purified here is cystine lyase catalyzing the following reaction:

L-cystine  $\longrightarrow$  thiocysteine + pyruvate + ammonia

#### Molecular weight

The molecular weight of cystine lyase a was estimated to be 186,000 by gel filtration (Fig. 6). SDS–PAGE gave a single protein band at the molecular weight of 45,000 (Fig. 6). These results indicate that cystine lyase a was composed of four identical subunits, *i.e.*, that it was a homotetrameric enzyme. Cystine lyase a after native PAGE was left unstained by thymol-H<sub>2</sub>SO<sub>4</sub> reagent<sup>20)</sup> and it was not adsorbed by a concanavalin A column, indicating that cystine lyase a did not contain carbohydrate. The amino acid at the *N*-terminal of cystine lyase a was modified, because no amino acid signal appeared when sequencing with a sequencer (Model 476A, Applied Biosystems) was tried.

## Substrate specificity and kinetic parameters

L-Cystine was the best substrate for cystine lyase a of the compounds tested, and D-cystine was not a substrate (Table II). This findings suggest that the reaction catalyzed by cystine lyase a is a stereospecific reaction. L- and D-Cysteine



Fig. 4. Mass Spectrum of the Reaction Product.

The compound of peak 2 formed by the reaction with the enzyme purified here was collected by HPLC and then examined by LC-MS.



Fig. 5. Changes with Time in Formation of Thiocysteine and Pyruvate. Thiocysteine  $(\bigcirc)$  and pyruvate (o) were measured by method 1 and method 2, respectively. The amount of thiocysteine was calculated from a standard curve drawn by the least square method from results when the monobromobimane derivative of L-cysteine was used.

Purified cystine lyase a was stained with CBB (lane 1) and by activity staining (lane 2).

were not substrates for the purified cystine lyase a, but thiocysteine was formed from L-cysteine when the crude extract was used as the enzyme source. These results suggest that L-cysteine was not a direct substrate of cystine lyase a although it was first oxidized to L-cystine in the crude extract and then used as a substrate. L-Cystathionine was not a substrate and L-djenkolic acid and O-acetylserine was poor substrates. Although S-methyl L-cysteine and S-ethyl L-cysteine were not substrates, their sulfoxides were substrates, but to a lesser extent than L-cystine.



Fig. 6. Estimation of Molecular Weight of Cystine Lyase a by Gel Filtration (A) and SDS-PAGE (B).

(A) Purified cystine lyase a  $(10 \,\mu\text{g} \text{ of protein})$  was treated by gel filtration on a Diol-300 column (600 × 7.5 mm). Elution was with 200 mM potassium phosphate buffer, pH 7.0, at a flow rate of 1 ml/min. Marker proteins: 1, apoferritin ( $M_r$ , 443,000); 2,  $\beta$ -amylase (200,000); 3, alcohol dehydrogenase (150,000); 4, bovine serum albumin (66,000); 5, carbonic anhydrase (29,000). The closed circle is for cystine lyase a.  $V_{\rm e}$ , elution volume.  $V_{\rm o}$ , void volume. (B) Purified cystine lyase a (1  $\mu$ g of protein) was treated by SDS-PAGE. Marker proteins: 1, phosphorylase b ( $M_r$ , 97,400); bovine serum albumin (66,000); 3, ovalbumin (45,000); 4, carbonic anhydrase (31,000); 5, trypsin inhibitor (21,500); 6, lysozyme (14,400). The closed circle is for cystine lyase a.

Table II. Substrate Specificity of Cystine Lyase a

Compound	Relative enzyme activity $(\%)^a$			
Compound -	(Method 1)	(Method 2)		
L-Cystine	100	100		
D-Cystine	6	2		
L-Cysteine	4	0		
D-Cysteine	0	0		
L-Cystathionine	$2^{b}$	2		
L-Djenkolic acid	nd <sup>c</sup>	12		
O-Acetylserine	nd	12		
S-Methyl L-cysteine	nd	0		
S-Ethyl L-cysteine	nd	0		
S-Methyl L-cysteine sulfoxide	nd	37		
S-Ethyl L-cysteine sulfoxide	nd	48		

<sup>*a*</sup> The enzyme activities were measured by method 1 or method 2 as described in the text with replacement of L-cystine with one of various compounds at the same concentration (6 mM). The enzyme activity is expressed as a percentage of that when L-cystine was used.

<sup>b</sup> Homocysteine formed was measured by method 1 after derivatization of it with monobromobimane.

<sup>c</sup> nd, not done.

Lineweaver-Burk plots gave  $K_{\rm m}$  values of 2.5 mM for L-cystine and 8.0 mM for S-methyl L-cysteine sulfoxide.

## Absorption spectrum and effects of some compounds on enzyme activity

The absorption maximum of the purified cystine lyase a was at 427 nm (Fig. 7), as is typical of a Schiff base formed between the enzyme and PLP. Carboxymethoxylamine and



**Fig. 7.** Absorption Spectrum of Purified Cystine Lyase a. Cystine lyase a (0.3 mg protein) was dissolved in 1 ml of 100 mM potassium phosphate buffer, pH 7.0.

Table III. Effects of Various Compounds on Cystine Lyase a Activity<sup>a</sup>

Compound (0.5 mм)	Relative activity (%)		
None	100		
Carboxymethoxylamine	2		
Hydroxylamine	15		
Iodoacetate	106		
N-Ethylmaleimide	102		
EDTA	103		

<sup>*a*</sup> Enzyme activity was assayed by method 2.



Fig. 8. Effects of Temperature and pH on the Activity (A and B) and Stability (C and D) of Cystine Lyase a.

(A) After reaction at the temperatures indicated, the thiocysteine that formed was measured by method 1. (B) After reaction at the pHs indicated, thiocysteine was measured by method 1. McIlvaine's buffer ( $\bigcirc$ ), Tris-HCl buffer ( $\bigcirc$ ), and glycine-NaOH buffer ( $\square$ ) were used. (C) After the enzyme solution in 50 mM Tris-HCl buffer, pH 8.5, was treated for 10 min at the temperatures indicated, the standard reaction mixture was prepared and kept at 30°C for 8 min. Thiocysteine formed was measured by method 1. (D) After the enzyme solution was treated for 24 h at 4°C in the buffers at different pHs as in (B), the standard reaction mixture was prepared and incubated for 8 min at 30°C. Thiocysteine was measured by method 1.

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hydroxylamine strongly inhibited cystine lyase a activity (Table III). When the reaction mixture did not contain PLP, the activity decreased to 60% of that with the complete mixture. These findings indicate that cystine lyase was a typical PLP-dependent enzyme. Iodoacetate, *N*-ethylmaleimide, and EDTA at the concentration of 0.5 mM did not inhibit the enzyme, suggesting that in the catalytic site, there was no cysteine residue or metal.

#### General properties

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The purified enzyme could be stored with little loss of activity at  $-20^{\circ}$ C for at least 6 months. The enzyme activity was maximum between 30 and 40°C (Fig. 8A). The pH optimum for the reaction was 8.5 (Fig. 8B). This result is also confirmed by assay method 2. Thermostability of the enzyme was examined by measuring the residual activities after 10 min incubation at various temperatures. The enzyme retained its full activities on heating at 40°C, but lost 60% of the activity by heating at 50°C (Fig. 8C). The enzyme was relatively stable regarding with pH changes during the storage. More than 50% of the activities was retained in the pH range of 3.0 to 11.0, although the highest activity was found around pH 8 (Fig. 8D).

## Discussion

We purified one of the isoforms of cystine lyase (cystine lyase a) from broccoli by two new method for detecting of the enzyme activity. Both methods we used here were useful in the purification of the enzyme. Method 1 is 100 times more sensitive than method 2 which was used earlier. The second advantage of method 1 was that measurement of the reaction product, thiocysteine, was specific to this enzyme reaction. Method 1 made it possible to monitor the enzyme activity throughout the various steps of purification. In addition, the method for staining for activity used made use of preparative PAGE during purification possible.

Other *Brassica* species have two isoforms,  $^{2,4)}$  but broccoli we used had three (a, b, and c). The cystine lyase a reported here was a minor isoform and cystine lyase b was the major one. Cystine lyase a and b had fairly constant, but the activity of cystine lyase c was dependent on harvest time and cultivar (data not shown). Preliminary results of an examination of cystine lyase b indicate that most properties were similar to those of cystine lyase a.

Thiocysteine has been believed to be one of the reaction products of cystine lyase. However, thiocysteine has been assayed only by cyanolysis so far.<sup>1.3.21)</sup> Cyanolysis is not specific to thiocysteine. Here we showed direct evidence of thiocysteine as a reaction product of cystine lyase by derivatization with monobromobimane followed by LC-MS and high-resolution mass spectrometry. Cysteine was also detected as one of reaction products and proportion of cysteine to thiocysteine increased with an increase in incubation time. Earlier study reported that thiocysteine is labile.<sup>22)</sup> Therefore, we concluded that thiocysteine is a primary product of the reaction and cysteine is a secondary one.

The name of 'cystine lyase' has been used as a second name for cystathionine  $\beta$ -lyase,<sup>23)</sup> since earlier reports showed that partially purified cystathionine  $\beta$ -lyase uses L-cystine as a substrate for some extent.<sup>9,24-26)</sup> Even the purified cystathionine  $\beta$ -lyase utilizes L-cystine as a substrate,  $^{27-29)}$  except for the spinach enzyme.<sup>30)</sup> In contrast, the plant enzyme completely purified here and earlier<sup>3,4)</sup> did not use cystathionine as a substrate, although earlier studies of partially purified plant cystine lyase showed that cystathionine is a substrate,  $^{2.9.26)}$  possibly as a result of contamination by other C–S lyases. From the evidence given above, cystine lyase from higher plants can be completely distinguished from cystathionine  $\beta$ -lyase by their substrate specificities. Thus, we propose that a new enzyme code number different from EC 4.4.1.8 should be given to plant cystine lyase.

There are some discrepancies between the cystine lyase a reported here and cystine lyase I reported by Hamamoto and Mazelis.<sup>4)</sup> The native protein ( $M_r$  186,000) of cystine lyase a was a homotetramer consisting of identical subunits  $(M_r 45,000)$ , but cystine lyase I  $(M_r 152,000)$  was a homotrimer of identical subunits ( $M_r$  49,000). Cystine lyase a was not adsorbed by a concanavalin A column and did not contain a carbohydrate residue, but cystine lyase I is a glycoprotein. The cystine lyase b we have obtained from broccoli inflorescences seemed not to be a glycoprotein (unpublished). We do not know the reasons for these discrepancies, but there was a difference between the purification procedures of these two enzymes: cystine lyase a was purified with an assay method specific for thiocysteine, but cystine lyase I was purified with an assay that detects pyruvate. Further investigation will be required to clarify discrepancies.

Unlike other C–S lyases such as alliin lyase<sup>5,7)</sup> and cystathionine  $\beta$ -lyase,<sup>30)</sup> the physiological function of cystine lyase in higher plants is unknown. One possible function is that of a donor of reduced sulfur (sulfide). S-Methyl L-cysteine sulfoxide, a substrate of this enzyme, is a precursor of methyl methanethiosulfinate, an antibacterial compound, in cabbage.<sup>31)</sup> Another possible function of cystine lyase is to help for methyl methanethio-sulfinate formation in Cruciferae.

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