Pergamon

PII: S0031-9422(97)00566-9

Phytochemistry, Vol. 47, No. 2, pp. 189–196, 1998

Printed in Great Britain
0031-9422/98 $19.00+0.00

CYSTATHIONINE β-LYASE FROM *ECHINOCHLOA COLONUM* TISSUE CULTURE

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(*Received in revised form* 2 *June* 1997)

Key Word Index—*Echinochloa colonum*; Gramineae; amino acid biosynthesis; sulphur metabolism.

Abstract—Cystathionine β-lyase (EC 4.4.1.8), the second enzyme unique to methionine biosynthesis, catalyses the α,β-elimination of cystathionine to yield homocysteine, pyruvate, and ammonia. The enzyme was purified to electrophoretic homogeneity from cell suspension cultures of *Echinochloa colonum*. Purification was achieved following ammonium sulphate fractionation and ion-exchange, hydrophobic-interaction, size-exclusion, and dye-affinity chromatography. The specific activity of cystathionine β-lyase in the final extract was 11 900 nmol min^{-1} mg^{-1} (protein). This represented a 5400-fold purification and a recovery of 50%. The substrate specificity was limited to cystathionine and djenkolate, and the β,γ-unsaturated amino acid aminoethoxyvinylglycine was a reversible competitive inhibitor with an apparent inhibition constant of 23.4 μM. The enzyme had a tetrameric quaternary structure with a subunit M_r of 43 k. Isoenzyme analysis of cystathionine β-lyase was performed by ion exchange chromatography. In extracts of *E. colomun* cell suspension cultures, leaves, and roots a single form of cystathionine β-lyase was eluted from the ion exchange column between 180 and 190 mM KCl.

INTRODUCTION

In higher plants and micro-organisms, methionine can be considered to be derived from aspartate and cysteine [1]. During biosynthesis, trans-sulphuration results in the sulphur moiety being transferred from the three carbon skeleton of cysteine to the four carbon skeleton of homoserine. This process is catalysed by two enzymes—cystathionine γ-synthase (EC 4.2.99.9) and cystathionine β-lyase (EC 4.4.1.8)—and proceeds via the intermediate cystathionine [Fig. 1(a and b)]. Reverse-trans-sulphuration, in which the sulphur moiety is transferred in the opposite direction, occurs in mammals and some filamentous fungi [2]. This is catalysed by cystathionine β-synthase (Ec 4.2.1.22) and cystathionine γ-lyase (EC 4.4.1.1) [Fig. 1 (c and d)].

Cystathionine γ-synthase [Fig. 1(a)], the first enzyme of trans-sulphuration, has been purified from barley seedlings [3], wheat leaves [4], and to apparent homogeneity from spinach leaves [5]. The enzyme has been reported to be a heterotetramer of 215 k and requires pyridoxal 5′-phosphate (PLP) for activity. A cDNA clone of cystathionine γ-synthase isolated from *Arabidopsis thaliana* shows a high degree of similarity with the enzyme from *Escherichia coli* [6].

Cystathionine β-lyase [Fig. 1(b)], the second enzyme of trans-sulphuration, cleaves cystathionine by α,β-elimination to yield homocysteine, pyruvate, and ammonia. The enzyme has been purified from spinach (*Spinacia oleracea*) leaves [7, 8] and spinach chloroplasts [9]. A preliminary report of purification from a

(a). γ-synthesis.
***O*-Phosphohomoserine + Cysteine → Cystathionine + Pi**

(b). β-elimination.
Cystathionine + H_2O → Homocysteine + Pyruvate + Ammonia

(c). β-synthesis.
Homocysteine + Serine → Cystathionine + H_2O

(d). γ-elimination.
Cystathionine + H_2O → Cysteine + α-ketobutyrate + Ammonia

Fig. 1. The metabolism of cystathionine: Trans-sulphuration catalysed by cystathionine γ-synthase (a) and cystathionine β-lyase (b). Reverse-trans-sulphuration catalysed by cystathionine β-synthase (c) and cystathionine γ-lyase (d).

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cell suspension culture of *Echinochloa colonum* has been presented [10]. A cDNA clone encoding cystathionine β-lyase, has been isolated from *A. thaliana* [11]. An *N*-terminal sequence, containing general features of a chloroplast transit peptide, indicates that the enzyme would be localized in the chloroplast. From the above sources, cystathionine β-lyase had a pH optimum in the range 8.3 to 8.9 and was dependent on PLP for activity. The enzyme had a low K_m (in the range 0.13 to 0.3 mM) for cystathionine and would also catalyse α,β-elimination from the dithioacetal djenkolate. In addition, cystine and some mixed disulphides of cysteine and homocysteine were cleaved by cystathionine β-lyase prepared from whole spinach leaves [7, 8]. The M_r of cystathionine β-lyase was 210 k in whole spinach leaf extracts [8] and in the range 160 to 170 k in spinach chloroplast and *E. colonum* extracts [9, 10].

Two cystathionine β-lyase isoenzymes, one associated with the cytosol and one with the chloroplast, have been demonstrated by ion-exchange chromatography in spinach leaves [9] and subcellular fractionation of barley (*Hordeum vulgare*) leaf protoplasts [12]. *In vivo* and *in vitro* the β,γ-unsaturated amino acid rhizobitoxine (Fig. 2) isolated from *Rhizobium japonicum* [13, 14] has been shown to inhibit cystathionine β-lyase [15, 16, 17]. Inhibition has also been observed with L-α-(2-aminoethoxyvinyl)glycine (AVG), a close structural analogue of rhizobitoxine (Fig. 2) [9, 18].

Microbial cystathionine β-lyase has been purified from *Salmonella typhimurium* [19] and from a cystathionine β-lyase over-producing strain of *E. coli* [20, 21]. The enzyme required PLP for activity, had a pH optimum of *ca* 8.5, and in addition to cystathionine, cleaved a wide range of sulphur containing compounds including djenkolate and cystine [19, 20]. The deduced amino acid sequence of cystathionine β-lyase (product of the *MetC* gene) and cystathionine γ-synthase (product of the *MetB* gene) show extensive homology (36% at the amino acid level), leading to the conclusion that the genes evolved from a common ancestor [21].

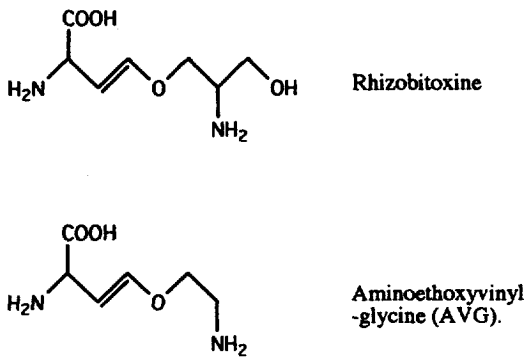


Fig. 2. Structures of the β,γ-unsaturated amino acids rhizobitoxine and L-α-(2-aminoethoxyvinyl)glycine.

This report describes purification to apparent homogeneity of cystathionine β-lyase from a cell suspension culture of *E. colonum*, a C_4 photosynthetic grass. In addition reaction and substrate specificity, physical properties and isoenzyme distribution are presented.

RESULTS

Cystathionine β-lyase purification

Cystathionine β-lyase was purified to electrophoretic homogeneity from 300 g of *E. colonum* cell suspension cultures (Table 1). The specific activity of the homogenous enzyme was 11 900 nmol min^{-1} mg^{-1} (protein). This represented a recovery of 50% and a purification of 5400-fold over the crude (clarified) extract. Following each stage of purification, cystathionine β-lyase was recovered as a single peak of activity. Respectively, for Mono Q, Octyl Sepharose, and Yellow-1 chromatography, activity eluted at 175 mM KCl, 19% saturation ammonium sulphate, and 90 mM KCl. Following each stage of purification aliquots of 200 μl were removed for electrophoretic analysis. Figs 3 and 4 show native and SDS polyacrylamide gels of each sample following silver staining. In each case a single protein band was observed in extracts following Yellow-1 chromatography. Storage of the final preparation in buffer B for at least 9 months at −80°, resulted in less than 5% loss of activity.

Kinetic characterization

Cystathionine β-lyase was assayed to determine whether it could also cleave cystathionine by γ-elim-

Table 1. The purification of cystathionine β-lyase from 300 g of *E. colonum* cell suspension cultures

Purification step	Total protein (mg)	Total activity (nmol min^{-1})	Specific activity (nmol min^{-1} mg^{-1} (protein))	Recovery (%)	Purification (fold)
Crude (clarified)	1830	4020	2.2	100	1
Sephadex G-25	462	5550	12	138	5
Q Sepharose	24.8	4260	172	106	78
Octyl Sepharose	5.2	2940	560	73	255
Sephacryl S-200HR	1.47	2610	1780	65	808
Yellow-1 agarose	0.17	2020	11900	50	5400

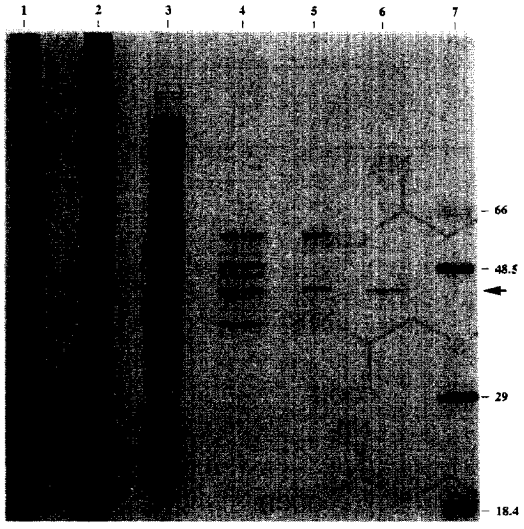


Fig. 3. SDS polyacrylamide gel (12% acrylamide) showing the progress of cystathionine β-lyase purification from *E. colonum* cell suspension cultures. Lanes: (1) Crude extract; (2) G.25 eluate; (3) Q-Sepharose eluate; (4) Octyl-Sepharose eluate; (5) Sephacryl S-200 eluate; (6) Yellow-1 agarose eluate; (7) M_r markers (kDa). Each lane was loaded with 75 μl of extract. Protein bands were visualized by silver staining. The arrow indicates the position of cystathionine β-lyase.

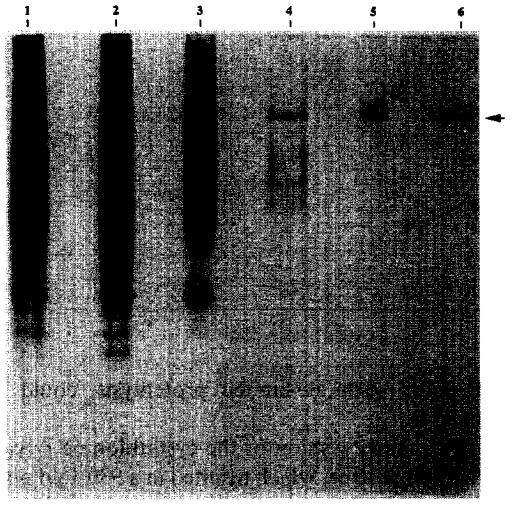


Fig. 4. Native polyacrylamide gel (10% acrylamide) showing the progress of cystathionine β-lyase purification from *E. colonum* cell suspension cultures. Lanes: (1) Crude extract; (2) G.25 eluate; (3) Q-Sepharose eluate; (4) Octyl-Sepharose eluate; (5) Sephacryl S-200HR eluate; (6) Yellow-1 agarose eluate. Each lane was loaded with 100 μl of extract. Protein bands were visualized by silver straining. The arrow indicates the position of cystathionine β-lyase.

ination, or synthesize cystathionine by β- or γ-synthesis.

γ-Synthesis [Fig. 1(a)]: It was anticipated that if γ-synthesis occurred, the cystathionine synthesized would have been immediately cleaved by β-elimination. Standard homocysteine-monobromobimane (mBBr) reaction mixtures (minus cystathionine) containing 5 mM *O*-succinylhomoserine and 1 mM cysteine were incubated for up to 160 min. Following analysis by HPLC the homocysteine-(mBBr) derivative was not observed at any time.

β-Synthesis [Fig. 1(c)]: β-synthesis of cystathionine from homocysteine and serine would be followed by β-elimination of cystathionine. The pyruvate coupled assay was used to determine whether this was the case. Standard assay mixtures, minus cystathionine and lactate dehydrogenase (LDH), and containing 5 mM homocysteine and 5 mM serine were incubated for 60 min. A decrease in absorption at 340 nm was not observed upon the addition of LDH, indicating pyruvate was not synthesized.

γ-Elimination [Fig. 1(d)]: Cysteine-mBBr, the putative derivative following γ-elimination, could be detected with a sensitivity similar to that of the homocysteine-mBBr derivative of β-elimination. Following incubation of standard homocysteine-mBBr reaction mixtures with cystathionine (0.5 to 10 mM) for up to 160 min the cysteine-mBBr derivative was not observed following analysis by HPLC.

Substrate specificity: The relative activity of cystathionine β-lyase was determine for a range of compounds using the pyruvate coupled assay. As shown in Table 2 the only two compounds that were cleaved were cystathionine and djenkolate.

AVG inhibitor characterization: The inhibition of cystathionine β-lyase activity was determined using the pyruvate coupled assay. AVG did not effect the LDH coupling reaction and was demonstrated to be reversible by dialysis. AVG increased the apparent K_m for cystathionine, while no effect on the V_{max} was observed (Fig. 5). Inhibition was therefore characterized as reversible-competitive [28]. A K_i of 23.4 μM was calculated from a secondary (Dixon) plot of K_m^{app} vs AVG concentration.

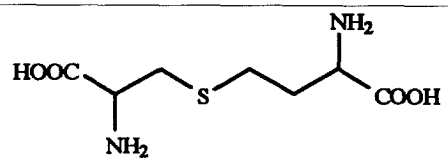
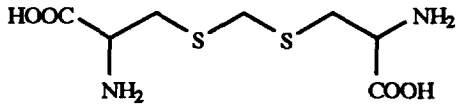
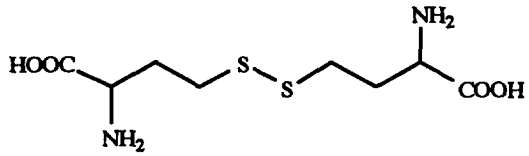
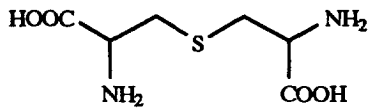
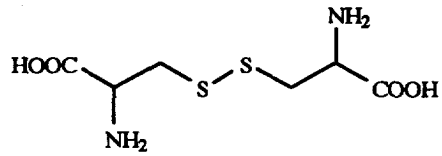
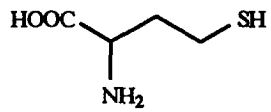
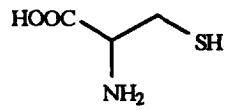
Subunit M_r *and tertiary structure*

The subunit M_r of cystathionine β-lyase was determined by SDS-PAGE to be 43 k (see Fig. 4). A single protein band was observed following silver staining, indicating that the native protein was composed of identical M_r subunits.

Cystathionine β-lyase isoenzymes

Following fractionation by Mono Q FPLC of crude desalted *E. colonum* cell suspension culture extracts (4, 7, and 11 days post-subculture), a single peak of cystathionine β-lyase activity was identified. This eluted between 180 and 185 mM KCl and the recovery of cystathionine β-lyase activity was always greater than 91% of the total column loading. The elution profile was not altered by addition of detergents (Triton X-100 (0.1%) or Tween-20 (0.1%)) or protease inhibitors (phenylmethanesulphonyl fluoride 0.5 mM and iodoacetate 10 μM) to the extraction buffer. Crude desalted extracts of *E. colonum* plant leaves (12 and 24 days post germination) and roots (24 days

Table 2. The substrate specificity of *E. colonum* cystathionine β-lyase. Each putative substrate was assayed at 5 mM in the pyruvate coupled assay

Compound	Structure	Relative activity (%)
L(+)-Cystathionine		100
L-Djenkolate		120
L-Homocystine		0
DL-Lanthionine		0
L-Cystine		0
DL-Homocysteine		0
L-Cysteine		0

post germination) were also analysed by ion-exchange chromatography. In each case a single peak of cystathionine β-lyase activity (>90% recovery) eluted at *ca* 190 mM KCl.

DISCUSSION

This paper presents the first purification to electrophoretic homogeneity of cystathionine β-lyase from tissue culture cells of *E. colonum* a C_4 photosynthetic grass. Previously, purification has only been attempted from spinach [8, 9].

The specific activity of the final cystathionine β-lyase preparation was 11 900 nmol min^{-1} mg^{-1} (protein), which compares favourably to that observed in cystathionine β-lyase purified from spinach chloroplast (12 900 nmol min^{-1} mg^{-1} (protein), [9]). However, both of these preparations had *ca* 10-fold higher specific activity than the preparation from whole spinach leaf extracts [8]. While it is not possible to determine why these figures disagree, artefacts of purification, such as limited proteolysis, could be responsible.

A preliminary version of the cystathionine β-lyase purification scheme, which resulted in a 930-fold purification, employed a heat denaturation step following ammonia sulphate precipitation [10]. This procedure was also used in cystathionine β-lyase purification from spinach [9]. During the current investigation it was observed that variations in the extract, such as volume or protein concentration, could affect the heat stability of cystathionine β-lyase leading to large variations in the final yield. Therefore, during development of the current protocol, heat denaturation was eliminated from the procedure.

In micro-organisms and plants, the enzymes of cystathionine metabolism (Fig. 1) have been shown in many instances to be flexible in substrate requirement and reaction specificity [29–31]. For example it has been suggested that due to the common ancestry of cystathionine β-lyase and cystathionine γ-synthase, sufficient activity may remain in each protein to allow

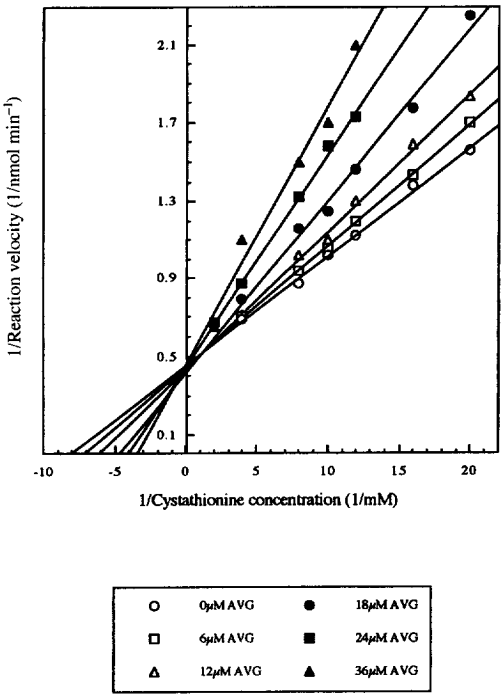


Fig. 5. AVG inhibition: double reciprocal plot of *E. colonum* cystathionine β-lyase initial velocity vs cystathionine concentration in the presence of AVG.

γ-synthesis and β-elimination [21]. In fact β-elimination has been observed by cystathionine γ-synthase of *Neurospora crassa* mutants that lack the cystathionine β-lyase enzyme [32]. In addition, overproduction of cystathionine β-lyase in *E. coli* mutants, has been shown to enable growth on serine as the sole carbon source [33]. It was proposed that cystathionine β-lyase acted as a β-synthase [Fig. 1(c)] catalysing the condensation of serine and homocysteine to form cystathionine, which would be cleaved to homocysteine, pyruvate, and ammonia. Therefore, to determine whether the enzymes from plants share the same flexibility, *E. colonum* cystathionine β-lyase was assayed for cystathionine γ-synthase, cystathionine β-synthase, and cystathionine γ-lyase activity. It is important to note that each assay was designed to be specific for the particular reaction under investigation. This has not been a feature of cystathionine β-lyase characterization in other reports, for example the pyruvate coupled assay (this work) and the dinitrophenol hydrazine colour reaction [8] would not have distinguished between β- or γ-elimination. Cystathionine β-lyase from *E. colonum* was only observed to cleave cystathionine by β-elimination; the enzyme was not observed, even under the most favourable conditions, to catalyse γ-elimination, or β- or γ-synthesis. Thus it would appear the cystathionine β-lyase from plants is more specific for β-elimination in trans-sulphuration than the equivalent enzyme from micro-organisms.

Catabolism of methionine, cysteine and other sulphur containing compounds has been shown to involve a broad class of enzymes—the C-S lyases—capable of cleaving carbon sulphur bonds [30]. The relationship of cystathionine β-lyase to these catabolic C-S lyases was investigated. In bacteria and fungi, the substrate specificity of cystathionine β-lyase was shown to be broad, including cystathionine, djenkolate, lanthionine, homolanthionine, cystine, and homocystine (see for example: [20, 34]). The substrate specificity of cystathionine β-lyase purified from spinach leaves was confined to cystathionine, djenkolate, and cystine [8]. However, the enzyme from spinach chloroplasts was not active toward cystine [9]. The activity of *E. colonum* cystathionine β-lyase, limited to cystathionine and djenkolate (Table 2), was in close agreement with the enzyme from spinach chloroplasts [9]. Therefore, the enzyme isolated in this work would appear to be specific for methionine biosynthesis—djenkolate has only been found in a limited number of plant tissues [35] and is not therefore physiologically relevant in *E. colonum* or spinach.

The β,γ-unsaturated amino acid AVG was used to inhibit *E. colonum* cystathionine β-lyase. Inhibition was not time dependent and was reversible by dialysis. The LDH coupling reaction was unaffected by AVG. While the apparent K_m of cystathionine β-lyase for cystathionine increased with AVG concentration, the V_{max} was unaltered (Fig. 5). Therefore, in accordance with kinetic models, inhibition was of the reversible competitive type [28]. A secondary plot of Fig. 5 showed the inhibition constant (K_i) to be 23.4 μM. Therefore cystathionine β-lyase from *E. colonum* was less sensitive to AVG inhibition than the *E. coli* enzyme (K_i = 3.1 μM, reversible competitive inhibitor). However, AVG caused a time-dependent irreversible inactivation of spinach chloroplast cystathionine β-lyase [9]. Inactivation proceeded via a reversible enzyme-inhibitor complex with a K_i^{app} of 110 μM. Both cystathionine β-lyase preparations have been shown to require PLP for activity [9, 10] and it has also been demonstrated that β,γ-unsaturated amino acids such as AVG are efficient inhibitors of PLP dependent enzymes [36]. It is therefore suggested that active site heterogeneity rather than mechanistic differences account for the difference in reactivity between the spinach and *E. colonum* enzyme.

Following SDS-PAGE, cystathionine β-lyase migrated as a single protein band, corresponding to subunit M_r of 43 k (Fig. 3). The native M_r of cystathionine β-lyase has previously been reported to be 160 k [10]. Therefore, as has been reported for the enzyme from spinach chloroplasts [9] the enzyme from *E. colonum* would appear to be a homotetramer. Cystathionine β-lyase isoenzymes were identified during subcellular fractionation of barley leaf protoplasts, one isoenzyme was located in the plastid (60% of the total activity) while the other was present in the non-plastid fractions [12]. In crude spinach leaf extracts two peaks of cystathionine β-lyase activity were resolved by Mono Q FPLC [9]. Fractionation of spinach leaf chloroplasts following the same

procedure, resulted in only the second peak of activity being detected. From this it was concluded that two isoenzymes were present, one of which was located within the chloroplast [9]. However, isoenzymes were not reported in a previous purification from spinach leaves [8]. Ion-exchange chromatography of crude desalted extracts of *E. colonum* cell suspension cultures, leaves and roots resolved a single form of cystathionine β-lyase eluting between 180 and 190 mM KCl. Modification to the methods of extraction, chromatography, assay conditions, and stabilization did not alter the elution profiles of cystathionine β-lyase. Therefore it was concluded the *E. colonum* cell suspension cultures or plants did not contain the isoenzymes observed in barley [12] and spinach leaves [9].

During a study of intracellular localization of cystathionine β-lyase in maize (*Zea mays*) mesophyll and bundle sheath cells, a cytosolic isoenzyme was not detected [37]. Thus it would appear that maize and *E. colonum* share the same isoenzyme complement.

EXPERIMENTAL

Reagents and plant material. Unless otherwise stated, reagents were obtained from Sigma. Chromatography resins were obtained from Pharmacia except Yellow-1 agarose which was purchased from Affinity Chromatography Limited (Isle of Man). Monobromobimane (mBBr) was obtained from Calbiochem. Seeds of *Echinochloa colonum* were planted in organic compost (Levington M3, Fisons, U.K.), watered daily, and incubated at 26° (±3°) with a 14 hr light period. Following harvest, plant samples were immediately frozen in liquid N_2. Friable callus tissue culture of *E. colonum* was obtained as a gift from Rhône-Poulenc Agriculture Ltd. (Ongar, UK). Growth was maintained on a high NH_4 salt medium [22], supplemented with sucrose (2%), 2,4-dichlorophenoxyacetic acid (2 mg ml^{-1}), and agar (0.8%). Cell suspension cultures were initiated (0.5 g callus in 20 ml medium minus agar) and stocks maintained by subculturing 8 ml of suspension into 50 ml of fresh medium every 7 days. All suspension cultures were maintained at 25° (±1°) in the light on an orbital shaker at 160 rpm (stroke 4 cm). Cells were harvested by filtration under a slight vacuum, washed with 4 vol. of Tris–HCl buffer (50 mM, pH 7.8), and frozen under liquid N_2.

Enzyme assays. Cystathionine β-lyase activity was detected by measuring either pyruvate (pyruvate coupled assay) or homocysteine (homocysteine–mBBr assay) production. Pyruvate coupled assay: Cystathionine β-lyase activity was measured at 340 nm by coupling pyruvate production to NADH oxidation using lactate dehydrogenase (LDH) [7]. Homocysteine–mBBr assay: The reaction of mBBr with low M_r thiols yields highly fluorescent derivatives (thioethers) that can be sepd by reversed-phase HPLC [23, 24]. Following incubation in a reaction mixt., homocysteine produced by cystathionine β-lyase was derivatized with mBBr and the derivative analysed by HPLC according to the method of ref [9]. In each assay, cystathionine β-lyase activity was measured at 25°. Variations to the standard assay procedures, employed during enzyme characterization, are given in results. Sp. act. is expressed as nmol (product) min^{-1} mg^{-1} (protein).

Cystathionine β-lyase purification. All buffers were equilibrated to 4°, adjusted to the indicated pH and vacuum filtered through 0.45 μm membranes. All procedures were carried out at 4°. *E. colonum* cell suspension cultures (300 g fr. wt) were pulverized under liquid N_2 using a pestle and mortar and extracted in a bottom driven blender in 900 ml of extraction buffer containing Tris–HCl (0.1 M, pH 7.8), KCl (50 mM), dithiothreitol (DTT) (5 mM), EDTA (2 mM), ethanediol (10%). The mixt. was strained through 6 layers of muslin and centrifuged at 22 000 *g* for 10 min. The clarified extract was gradually brought to 40% satn with solid $(NH_4)_2SO_4$ and stirred for 15 min. Pptd protein was pelleted by centrifugation at 22 000 *g* for 15 min. $(NH_4)_2SO_4$ was added to the supernatant to increase the satn to 55%. Following stirring for 15 min, pptd protein was pelleted by centrifugation for 25 min at 22 000 *g*. The protein pellet was resuspended in a minimum vol. (*ca* 15 ml) of buffer A containing Tris–HCl (50 mM, pH 7.8), KCl (50 mM), DTT (2 mM), EDTA (1 mM), and ethanediol (10%) and loaded on to a column of Sephadex G.25 (140 ml) equilibrated in the same buffer. The protein fraction from Sephadex G.25 was loaded (1 ml min^{-1}) onto a column of Q-Sepharose Fast Flow (42 ml) equilibrated in buffer A. Unbound protein was washed from the column (3 vol.) and bound protein eluted with a linear gradient (240 ml total vol.) of 50 to 230 mM KCl (1 ml min^{-1}). Frs of 3 ml were collected. Frs from Q-Sepharose containing cystathionine β-lyase activity were pooled and brought to 30% satn with solid $(NH_4)_2SO_4$. The mixt. was stirred for 10 min and clarified by centrifugation at 40 000 *g* for 15 min. The supernatant was loaded (1 ml min^{-1}) onto an Octyl-Sepharose CL-6B column (30 ml) equilibrated in buffer A containing 30% satn $(NH_4)_2SO_4$. After the unbound protein was washed from the column (1 vol.), bound protein was eluted with a linear gradient (160 ml total vol.) of 30 to 5% satn $(NH_4)_2SO_4$ (1 ml min^{-1}). Frs of 1 ml were collected. Pooled, active frs from Octyl-Sepharose were brought to 70% satn $(NH_4)_2SO_4$ and stirred for 30 min. Pptd protein was pelleted by centrifugation at 48 000 *g* for 40 min. The pellet was resuspended in a minimum vol. (*ca* 4 ml) of buffer B containing Tris–HCl (25 mM, pH 7.8), KCl (30 mM), DTT (1 mM), EDTA (0.5 mM), and ethanediol (5%). This was loaded (21 ml hr^{-1}) onto a column of Sephacryl S-200HR (500 ml) also equilibrated in buffer B. The column was developed overnight (21 ml hr^{-1}) and frs of 3 ml were collected. Active frs from Sephacryl S-200HR were pooled and loaded (0.4 ml min^{-1}) onto a column of Yellow-1 agarose (27 ml).

Unbound protein was collected and re-applied to the column to ensure maximum absorption of cystathionine β-lyase activity. Bound protein was eluted with a linear gradient (130 ml total vol.) of 30 to 200 mM KCl at a flow rate of 1 ml min^{-1}. Frs of 1 ml were collected; those containing cystathionine β-lyase activity were pooled, frozen in liquid N_2, and stored at −80°.

Isoenzyme analysis. Cell suspension cultures, or plant leaves or roots were extract in 5 vol. of Q-Sepharose extraction buffer containing Tris–HCl (100 mM, pH 7.8), KCl (50 mM), DTT (5 mM), EDTA (2 mM), ethanediol (10%), PLP (0.1 mM), and polyvinylpolypyrrolidone (4%) using a pestle and mortar. The extract was clarified by centrifugation (20 000 *g*, 10 min) and subjected to 80% satn $(NH_4)_2SO_4$ pptn. The soln was stirred at 4° for 20 min and pptd protein collected by centrifugation (20 000 *g*, 15 min). The pellet was resuspended and desalted through a column of Sephadex G.25 (6 ml) equilibrated in Tris–HCl (50 mM, pH 7.8), KCl (50 mM), DTT (1 mM), EDTA (1 mM). Protein, collected as a single fr., was immediately subjected to Q-Sepharose FPLC. Extracts from Sephadex G.25 (50 mg protein) were loaded (0.2 ml min^{-1}) onto a column of Q-Sepharose (4 ml, attached to a Pharmacia FPLC system) equilibrated in Q-Sepharose column buffer containing Tris–HCl (50 mM, pH 7.8), KCl (50 mM), DTT (1 mM), and EDTA (1 mM). Unbound protein was washed from the column in *ca* 3 column vol. Proteins were eluted (0.8 ml min^{-1}) with a linear gradient from 80 to 280 mM KCl (100 ml total vol.) and 280 to 600 mM KCl (4 ml total vol.). Frs of 2 ml were collected and immediately assayed for cystathionine β-lyase activity.

Miscellaneous procedures. Protein concn was determined according to the dye binding method of ref [25] using BSA as a reference. PAGE was performed using the discontinuous buffer design of ref [26]. Proteins were fractionated using the SE600 vertical slab gel unit (Hoefer U.S.A.) under non-denaturing conditions or denaturing conditions in the presence of 0.1% SDS. M_r standards contained: bovine serum albumin (66 k), porcine heart fumarase (48.5 k), bovine carbonic anhydrase (29 k), bovine β-lactoglobulin (18.4 k), and bovine α-lactalbumin (14.2 k). Protein bands were visualized by silver staining [27].

Acknowledgements—We thank Dr M. Droux of Rhône-Poulenc Agriculture (Lyon, France) for his instruction on the analysis of monobromobimane derivatives. This work was supported by the Science and Engineering Research Council (U.K.) and Rhône-Poulenc Agriculture Ltd (Ongar, U.K.).

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