A New Member of Plant CS-lyases

A CYSTINE LYASE FROM ARABIDOPSIS THALIANA*

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Cystine lyases catalyze the breakdown of L-cystine to thioceysteine, pyruvate, and ammonia. Until now there are no reports of the identification of a plant cystine lyase at a molecular level, and it is not clear what biological role this class of enzymes have in plants. A cystine lyase was isolated from Brassica oleracea (L.), and partial amino acid sequencing allowed the corresponding full-length cDNA (BOCL3) to be cloned. The deduced amino acid sequence of BOCL3 showed highest homology to the deduced amino acid sequences of several Arabidopsis thaliana genes annotated as tyrosine aminotransferase-like, including a coronatine, jasmonic acid, and salt stress-inducible gene, CORI3 (78.8% identity), and the unidentified rooty/superroot1 gene (44.8% identity). A full-length expressed sequence tag clone of CORI3 was obtained and recombinant CORI3 was synthesized in Escherichia coli. Isolated recombinant CORI3 catalyzed a cystine lyase reaction, but no aminotransferase reactions. The present study identifies, for the first time, a cystine lyase from plants at a molecular level and redefines the functional assignment of the only functionally identified member of a group of A. thaliana genes annotated as tyrosine aminotransferase-like.

Cystine lyases are grouped into a family of proteins (EC 4.4.1.-), including alalinases, cystathionases, and several other subfamilies, which cleave cysteine-containing compounds (CS-lyases) (1, 2). Plant CS-lyases are involved in a range of processes, including the synthesis and degradation of natural products, amino acid metabolism, and generation of precursors for the synthesis of ethylene, polyamines, and particular proteins (2). Although several plant pyridoxal 5-phosphate (PLP)1-dependent CS-lyases have been characterized comprehensively (3, 4), little is still known about the molecular character and biological role of cystine lyases in plant metabolism (1). Cystine lyases have previously been purified from broccoli and characterized with regard to substrate specificities and kinetics in the presence or absence of inhibitors (1, 5). However, there are no reports available that describe the identification of a plant cystine lyase at the molecular level. Plant cystine lyases generally catalyze the cleavage of the β-carbon-sulfur bond of L-cysteine, resulting in the liberation of thioceysteine, pyruvate, and ammonia (Fig. 1) (1). Thioceysteine can then be further metabolized into thioceysteine, thiocysteine, hydrogen sulfide, iron-sulfur clusters for protein assembly, or elemental sulfur (6).

Aminotransferases (EC 2.6.1.-) are similar to most CS-lyases in that they utilize PLP as a co-factor and that they are involved in amino acid metabolism (7). They may also be related phylogenetically (7, 8), and this is experimentally supported by the ability of porcine heart aspartate and alanine aminotransferases to cleave cysteine conjugates (9). It is therefore possible that some uncharacterized genes obtained from plant genomic sequencing projects and annotated as putative aminotransferases in reality encode CS-lyases. As a first example, this paper describes the redefinition of CORI3, incorrectly annotated as a tyrosine aminotransferase in the Arabidopsis thaliana genome, as the first identified plant cystine lyase. CORI3, a coronatine, jasmonic acid (10) and salt stress-inducible (11) A. thaliana transcript that encodes for a protein with tyrosine aminotransferase activity was reported last year (12). CORI3 belongs to a group of eight A. thaliana genes that are annotated as putative tyrosine aminotransferases in GenBankTM, including multiple alleles of rooty/superroot1 (13, 14). The identification of a new stress- and plant “signal”-inducible member of the CS-lyase family at the molecular level adds new impetus for an examination of the role of CS-lyases in such diverse areas as primary sulfur metabolism, hormone metabolism (14), and secondary plant metabolism (2).

EXPERIMENTAL PROCEDURES

Biochemicals and General Methods—All biochemicals were obtained from Sigma, Wako, or Kanto and were all of analytical or higher grade. Protein preparations and biochemical assays were concentrated and desalted using Amicon Ultrafree-MC 5000 nominal molecular weight filter devices from Millipore. SDS-PAGE was performed using high-Tris linear 10% SDS-polyacrylamide gels (15), and polypeptides were visualized by staining with Coomassie Brilliant Blue (R-250). Native PAGE was performed as described in Ref. 16. Protein concentrations were estimated using bovine serum albumin as a reference (17). Amino acid sequence alignments were performed using the ClustalX program (18). Primary amino acid sequence data were calculated using software available at ExPASy (Swiss Institute of Bioinformatics, www.expasy.org).

Isolation of a Cystine Lyase from Brassica oleracea (L.)—All purification procedures were carried out at 4 °C. The purification of a protein with cystine lyase activity from inflorescences of broccoli essentially followed the protocol developed by Ukai and Sekiya (16) with the following modification. Following ammonium sulfate fractionation, the 40–60% cut was dissolved in a minimum volume of TP buffer (20 mm

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1 The abbreviations used are: PLP, pyridoxal 5-phosphate; BOCL3, B. oleracea (L.) cystine lyase; BOCL3, cDNA encoding BOCL3; CORI3, complementary DNA of a coronatine-inducible A. thaliana mRNA; CORI3, protein encoded by CORI3; HPLC, high performance liquid chromatography.

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Tris-HCl (pH 6.4), 20 μM PLP, desalted by dialysis, and applied to a DEAE-Sepharose fast flow column equilibrated in TP buffer. Proteins were eluted with a linear gradient from 0 to 1 M NaCl in TP buffer, and fractions containing cystine lyase activity were pooled and diluted in TP buffer to achieve a lower salt content. The active diluted cystine lyase fraction was then applied to a Cibacron blue 3 G (Sigma) column equilibrated in TP buffer, and proteins were eluted with a linear gradient from 0 to 1 M NaCl. Active fractions were pooled and subjected to preparative native PAGE with activity staining and elution as described previously (16).

Amino Acid Sequencing and cDNA Cloning—Both of the isolated cystine lyase isoforms separated by SDS-PAGE were subjected to in-gel digestion by lysyl endopeptidase according to (19) and separated by HPLC using an ODS-80 Tm (Tosoh) column as described previously (20). Two common fractions were sequenced using Edman degradations as described previously (21). A degenerate 56-mer oligonucleotide probe BOCL-175 (5′-GGITGGAATGTICCIGGITGGMGIAICIGGITGTTIGGC-ITITCATGATTTIGATGG-3′, M = A + C, I = inseine) was designed based on peptide sequence obtained from fraction 75. The synthesized oligonucleotide was radiolabeled by 5′-phosphorylation using [γ-32P]ATP and T4 polynucleotide kinase (Toyobo) according to manufacturer’s instructions. Radiolabeled BOCL-175 was then used to screen a broccoli cDNA library prepared as described in Ref. 22 using RNA extracted from 6-month-old field-grown inflorescences. Approximately 280,000 individual unamplified plaques were screened as described in Ref. 22 and one full-length clone (BOCL-3), and several partial clones were isolated and sequenced in both directions, as described previously (11), after subcloning into pBlueScript II.

Heterologous Expression of BOCL3 and COR3—The full-length coding sequence of BOCL3 was amplified using pBOCL3 as a template and primers BOCLF (5′-GAAGATTCATGGCGACCACCCCCCTAGTGCG-3′) and BORM (5′-TTGTCGACTTCATCGGGACCTCT-TAGGTAGTTAGTTAGG-3′) for the pMALC-2 vector (New England Biolabs), BOSF2 (5′-AATTCGCACCGCGTGGGAGGTTTGC-3′) and BORR3 (5′-AAGTTCACCCCTAGGGATTTTCTCGGTTTGGTGAG-3′) for the pETBLUE2 vector (Novagen), and BOCYF (5′-TAATTCATGGCGACCACCCCCCTAGTGCG-3′) and BOCYR (5′-AATTCGCACCGCGTGGGAGGTTTGC-3′) for the pETBLUE2 vector (Novagen) and pGEX-6p2 vector (Amersham Biosciences), and pYES2 vector (Invitrogen). A full-length expressed sequence tag (AV442456) was obtained from the Kazusa DNA Research Institute (Kisarazu, Japan) and sequenced to confirm its identity as COR3 (12). The full-length coding sequence of COR3 was amplified using AV442456 as template and primers AV445F 5′-AATTCGAACCCCTAGTGCGAAGGTTTGC-3′ and AV445R 5′-AATTCGAACCCCTAGTGCGTTCGTTTGAGG-3′ for the pETBLUE2 vector. All PCR products and vectors were digested with appropriate restriction enzymes, purified using the GFX PCR DNA and gel band purification kit (Amersham Biosciences), and cloned into Nova Blue (Novagen) hosts according to each respective manufacturer’s protocols. Plasmid DNA isolated from positive colonies were verified by restriction analyses and PCR or DNA sequencing. Subsequently, they were used to transform expression hosts Tuner (DE3) pLacI (pETBLUE2) or AD494 (DE3) pLysS (pMALc2 and pGEX-6p2) for bacterial expressions, and INVSc1 (Invitrogen) for pYES2, as described by the manufacturer’s protocols. Bacterial expression was performed by inoculating a 50-mI LB culture containing appropriate antibiotics with 1 ml of an overnight culture (37 °C), incubation for 24 h at 16 °C, followed by an addition of isopropyl-1-thio-β-D-galactopyranoside to a final concentration of 0.2 mM and 24 h further incubation at 16 °C.

Extraction and Purification of Recombinant Protein—Soluble protein was harvested from bacteria using the method described in Ref. 20 and from yeast using the manufacturer’s protocols (Invitrogen). Only the buffer composition varied according to the method of purification. The initial bacterial lysate buffer contained 200 mM potassium phosphate buffer (pH 7.6), 200 mM NaCl, 6 mM dithiothreitol, 1 mM EDTA, and 0.1 mg/ml lysozyme (Sigma). Histidine tag purification was employed for recombinant protein expressed using the pETBLUE2 vector and EDTA, and DTT was omitted from the lysis buffer used in this case. Aliquots of total soluble protein were stored at −80 °C. Soluble yeast protein was extracted using acid-washed glass beads according to the manufacturer’s instructions (Invitrogen) using breaking buffer (100 mM potassium phosphate buffer (pH 7.6), 100 mM NaCl, 5% glycerol, 3 mM DTT, and 0.5 mM EDTA) and stored at −80 °C. Recombinant protein modified with a histidine tag was purified using chelating Sepharose fast flow (Amersham Biosciences) according to the manufacturer’s instructions. Recombinant glutathione S-transferase fusion proteins expressed using the pGEX-6p2 vector were purified using the MicroSpin glutathione S-transferase purification module (Amersham Biosciences) according to the manufacturer’s instructions. Purified recombinant protein was stored at −80 °C with or without the addition of glycerol up to a final concentration of 5%. Crude and purified proteins obtained from hosts harboring empty vectors were prepared in the same way as each respective construct and employed as negative controls for enzyme assays.

Cystine Lyase and Aminotransferase Activity Assays—All assays were conducted using crude or purified soluble protein obtained from plant extracts or cultures expressing vectors with inserts or without (negative control) as described above. Cystine lyase activity was measured by two different methods. Method 1 essentially followed that described in (23). Briefly, 0.1–1 μM of recombinant COR3, or 10–25 μg of crude protein, was added to 200 μl of reaction mixture (50 mM PIP, 50 mM potassium phosphate buffer (pH 7.6), 0.5–8 mM L-cystine (solutions as described in Ref. 23), and 0–4 mM L-tyrosine) and allowed to incubate at 30 °C for 10 min. 200 μl of 2,4-dinitrophenylhydrazine (0.1% w/v) was added to each reaction, mixed gently, and allowed to incubate at room temperature for 5 min. 1 ml of 2 N NaOH was then added, mixed, and incubated as above. The final mixture was diluted 1 in 4 using H2O and the absorbance at 520 nm measured using a U-2001 spectrophotometer (Hitachi). Method 2 was based on detecting the end product pyruvate (Fig. 1) by capillary electrophoresis using an Agilent Technologies HP3D system (fused silica capillary, 112.5 cm × 50 μm).

FIG. 1. Cystine lyase and tyrosine aminotransferase reaction schemes. A, cystine lyases cleave L-cystine and generate thiocysteine, ammonia, and pyruvate (11). B, tyrosine aminotransferases catalyze the reversible transfer of the amino group of L-tyrosine to 2-oxoglutarate and generate L-glutamic acid and 4-hydroxyphenylpyruvate (7).
2-mercaptoethanol, 0.012 M lithium chloride, 0.013 M citrate, 2.8% ethanol (pH 2.7 adjusted with citrate), elution buffer B (0.024 M trilithium citrate, 0.035 M lithium hydroxide, 4% ethanol) and allowed to incubate as described above and employed as a qualitative assay for tyrosine, H2O. All aminotransferase reactions will in theory generate L-glutamate using capillary electrophoresis.

Brieﬂy, 0.1–1 µg of recombinant CORI3, or 10–25 µg of crude protein, was added to 200 µl of reaction mixture (50 µM PLP, 50 mM potassium phosphate buffer (pH 7.6), 1–8 mM l-tyrosine) and allowed to incubate at 30 °C for 10–30 min. 66 µl of 2 N NaOH was then added and the entire reaction mixture mixed followed by a measurement of the absorbance at 331 nm as above with or without a 4-fold dilution using H2O. All aminotransferase reactions will in theory generate L-glutamate from 2-oxoglutarate (Fig. 1). Method 2 was therefore based on detecting the end product L-glutamate using capillary electrophoresis as described above and employed as a qualitative assay for tyrosine, aspartate, alanine, and tryptophan aminotransferase activities. Anion-exchange HPLC was also used in the detection of L-glutamate for increased sensitivity. An Hitachi L-7000 series HPLC with ﬂuorescent detector (excitation 340 nm, emission 450 nm) and ion-exchange resin (2619F, 4 × 150 mm, Hitachi) was employed. The following elution proﬁle was employed using elution buffer A (0.024 M trilithium citrate, 0.035 M lithium chloride, 0.013 M citrate, 2.8% ethanol (pH 7.7 adjusted with citrate), elution buffer B (0.024 M trilithium citrate, 0.035 M lithium chloride), elution buffer C (0.2 M lithium hydroxide, 4% ethanol), and reaction buffer (0.4 M borate, 0.4 M potassium hydrate, 0.22% 2-mercaptoethanol, 0.012% o-phthalaldehyde): 0 min, 100% buffer A; 16 min, 100% buffer A; 41 min, 90% buffer A, 10% buffer B; 50 min, 90% buffer A, 10% buffer B, 100 min, 100% buffer B; 125 min, 100% buffer B; 125.1 min, 100% buffer C; 140 min, 100% buffer C; 140.1 min, 100% buffer A; 155 min, 100% buffer A) with linear gradients in between the time points. The elution buffer flow rate was 0.4 ml/min while the reaction buffer flow rate was 0.6 ml/min.

All assays were calibrated using commercial standards of pyruvate and L-glutamate, respectively. Kinetic assay velocities, determined using crude preparations containing recombinant CORI3, were calculated using an estimate of the equivalent concentration of recombinant CORI3 in crude preparations, based on the amount of CORI3 that was recovered after purification.

RESULTS

Isolation of Broccoli Cystine Lyases—A cystine-lyase activity was isolated from broccoli using a combination of previously established procedures (16) and additional Cibacron blue afﬁnity chromatography. Two polypeptides migrating around 43 kDa by SDS-PAGE correlated with cystine lyase activity after elution from the Cibacron blue 3GA column with a linear salt gradient (data not shown). The active fractions were subjected to preparative native PAGE, and in-gel cystine lyase activity staining conﬁrmed that the major polypeptide(s), as visualized by Coomassie staining, co-migrated with cystine lyase activity staining (Fig. 2A). The approximate region displaying cystine lyase activity staining was eluted and subjected to SDS-PAGE (Fig. 2B), which revealed two major closely migrating bands. Both 43-kDa polypeptides were subjected to in-gel digestion, and peptide mapping by HPLC revealed more than 20 peaks exhibiting similar retention times in both polypeptide digests. Only two peaks, in the HPLC chromatogram of the two digests, displayed a different elution pattern. This supports that the two 43-kDa polypeptides are two closely related isoforms, as suggested previously (5, 25). Two peaks that appeared in the chromatograms of both digests (fraction 63 and 75, Fig. 3) were subjected to amino acid sequencing.

Cloning of BOCL3—A synthetic degerenize oligonucleotide 56-mer probe, BOCL-175, was designed based on the amino acid sequence of fraction 75. Approximately 280,000 plaques of an unampliﬁed broccoli inﬂorescence cDNA phage library were screened using radioactively labeled BOCL-175. One positive full-length clone, BOCL-3, with a 1284-base pair open reading frame was isolated (GenBank™ accession number AY187682). The deduced translation product comprises 423 amino acids and has a predicted molecular mass of 47.2 kDa and a theoretical pl of 8.3 (Fig. 3). The two amino acid sequences obtained from peptide mapping were both contained within the deduced amino acid sequence of BOCL-3 with the exception of Trp252, which was predicted to be a Tyr residue, and the two peptide sequences are underlined in Fig. 3. A search for homologous sequences in protein sequence data banks revealed highest homology to a group of eight A. thaliana putative plant tyrosine aminotransferases, which includes the functionally characterized CORI3 protein (Ref. 12; 78.8% positional amino acid identity) and the deduced amino acid sequence of multiple alleles of the root>superroot1 gene locus (Refs. 13 and 14; 44.8% positional amino acid identity) (Fig. 3). An alignment of the deduced amino acid sequence of BOCL3 and the group of eight putative A. thaliana tyrosine aminotransferases, other known plant CS-lyases (A. thaliana cystathionine lyase (3); Allium cepa alliinase (4)), an A. thaliana aspartate aminotransferase (ASP2 (26)), a Rattus norvegicus tyrosine aminotransferase (27), and a Synechoechistis cystine/cysteine lyase (28) was attempted. Although an alignment could be made, there was less than 12% overall positional amino acid identity between the A. thaliana aspartate aminotransferase, the A. thaliana cystathionine lyase, the Allium alliinase, and the Synechoechistis cystine/cysteine lyase and any other sequence. These were therefore eliminated from the final alignment that is summarized in Table I.

The strong inducibility of the CORI3 transcript in response to high salt conditions (11) and jasmonic acid application (10, 12), and the high degree of positional amino acid identity between the deduced amino acid sequences of CORI3 and BOCL3 warranted further examination of the protein encoded by CORI3. A full-length expressed sequence tag of CORI3 (AV442456) was therefore obtained and sequenced in both directions to verify the content.

Heterologous Expression of BOCL3 and CORI3—A range of bacterial and yeast expression vectors, empty or containing BOCL3 and CORI3, were prepared to examine the in vitro activity of the recombinant protein products. Recombinant CORI3 was synthesized in E. coli and puriﬁed using His tag puriﬁcation (Fig. 4, lanes 1–4). Likewise, expression of recombinant BOCL3 was attempted using three different bacterial expression vectors and one yeast expression vector.

Crude E. coli extracts containing recombinant CORI3 showed evidence of both cystine lyase and tyrosine aminotrans-
ferase activity (Fig. 4, lane 2) when assayed using two different assay methods (assay methods 1 and 2 described under “Experimental Procedures”). However, purified recombinant CORI3 only displayed cystine lyase activity (Fig. 4, lane 3), while crude protein extracted from E. coli harboring empty pETBLUE2 only catalyzed a tyrosine aminotransferase reaction (Fig. 4, lane 1), and after purification displayed neither cystine lyase nor aminotransferase activities (Fig. 4, lane 4). Crude protein extracted from E. coli harboring all three tested expression vectors fused with BOCL3, and yeast harboring pYES2-BOCL3 showed no sign of cystine lyase activity and no more tyrosine aminotransferase activity than that displayed by protein prepared from the respective microorganisms harboring empty vectors.

Characterization of Recombinant CORI3—Kinetic analyses of recombinant CORI3 was next pursued with the aim of uncovering the possible role of CORI3 in its native environment. Replicate assays were conducted at varying concentrations of L-cystine in the presence or absence of L-tyrosine. A plot of activity versus substrate concentration at different concentrations of L-tyrosine revealed little difference in the cystine lyase activity of CORI3 in the presence or absence of L-tyrosine at similar levels of concentration, except for 4 mM L-cystine and 4 mM L-tyrosine (Fig. 5). A theoretical V_{max} of 3571 nanokatals × mg^{-1} CORI3, and a theoretical K_{m} value of 2.32 mM L-cystine was calculated from the Lineweaver-Burk plot (data not shown). The specific cystine lyase activity of crude E. coli protein preparations containing recombinant CORI3 (Fig. 4, lane 2) and purified recombinant CORI3 (Fig. 4, lane 3) was 42 nanokatals × mg^{-1} total protein (2142 nanokatals × mg^{-1} CORI3 equivalent (see “Experimental Procedures”)) and 1306 nanokatals × mg^{-1} CORI3, respectively, using 4 mM L-cystine as substrate. This should be compared with the maximum specific activity previously reported with tyrosine aminotransferase activity assays for recombinant CORI3; 55.2 nanokatals × mg^{-1} CORI3 (12).

Alanine and aspartate aminotransferase activity assays were also conducted, since porcine heart aspartate and alanineaminotransferases with cystine-conjugate cleaving capability had been reported previously (9). Likewise, due to the high positional amino acid identity in the deduced amino acid sequences of CORI3 and rooty/superroot1 (42.8–45.0%), a possible involvement of CORI3 in tryptophan metabolism (29) was also investigated by assaying for tryptophan aminotransferase activity. All aminotransferase activity assays were conducted by monitoring for L-glutamate production (assay method 2 described under “Experimental Procedures”). However, no aminotransferase activity could be detected with any of the potential substrates.

![Table 1](https://example.com/image.png)

**Table 1**

Sequence identity between CORI3 and a group of six different A. thaliana putative tyrosine aminotransferase encoding sequences, a broccoli putative cystine lyase-encoding cDNA (BOCL3), and a functionally verified R. norvegicus tyrosine aminotransferase-encoding cDNA.

| Accession number | Species       | Annotation in GenBank | % identity with CORI3 |
|------------------|---------------|-----------------------|-----------------------|
| AF288090.1       | A. thaliana   | Tyrosine aminotransferase | 78.8                  |
| CAB7391.1        | A. thaliana   | Putative cystine lyase  | 73.4                  |
| AF301898         | A. thaliana   | Putative tyrosine aminotransferase | 45.0             |
| AF301900         | A. thaliana   | Putative tyrosine aminotransferase | 42.8             |
| BAB10727.1       | A. thaliana   | Putative tyrosine aminotransferase | 38.9             |
| BAA96894.1       | A. thaliana   | Putative tyrosine aminotransferase | 38.4             |
| T04612           | A. thaliana   | Putative tyrosine aminotransferase | 36.7             |
| M18340           | R. norvegicus | Tyrosine aminotransferase | 26.5             |

**Fig. 3.** Multiple sequence alignment with the deduced amino acid sequences of CORI3, BOCL3, and one of the rooty/superroot1-encoding alleles (accession number AF301900-1). The amino acid sequences of peptide fractions 63 and 75, derived from peptide mapping of native BOCL3, are indicated with bars. The predicted PLP-binding domain is indicated by a black box. Gray regions indicate two or more similar amino acids, and black regions indicate two or more identical amino acids.
DISCUSSION

The present paper presents several lines of evidence which suggest that CORI3 more properly should be regarded as a cystine lyase rather than as a tyrosine aminotransferase, although it cannot be excluded that it may also catalyze other until now untested reactions. (a) We were not able to detect any tyrosine aminotransferase activity with purified recombinant CORI3 preparations using two different methods of enzyme analysis, despite earlier contrary reports (12). E. coli, however, is capable of such activity, and residual native tyrosine aminotransferase-catalyzing proteins present in the purified preparations employed by Loupokhina et al. (12) may explain why such an activity could be detected, although the same method of purification as that reported herewith was employed. (b) The specific cystine lyase activity of purified recombinant CORI3 was more than 20 times higher than that previously reported for the specific tyrosine aminotransferase activity (12). (c) Only a slight inhibition of cystine lyase activity could be detected at the highest concentration of both L-tyrosine (4 mM) and L-cystine (4 mM) (Fig. 5). These concentrations are unrealistically high compared with physiological levels (30), and at lower levels there was no inhibition. For example, 2 mM L-cystine and 4 mM L-tyrosine produced equal amounts of pyruvate compared with assays excluding L-tyrosine (Fig. 5).

The purification of a plant cystine lyase is not novel (1, 16, 25); however, the identification of a plant-derived nucleic acid sequence that encodes a cystine lyase, CORI3, certainly is. The isolation of BOCL3 and its corresponding cDNA clone by classical biochemical methods proved essential for the identification of the true function of the protein encoded by CORI3. The annotation of CORI3 as a tyrosine aminotransferase-encoding cDNA was originally based on similarity to mammalian aminotransferases and not on the isolation of such a protein, although apparently confirmed, subsequently, by analyzing the aminotransferase activity of recombinant CORI3 (12).

Nevertheless, a high amino acid sequence similarity between plant cystine lyases and aminotransferases is not surprising given that both enzyme classes are PLP-dependent and involved in amino acid metabolism and that aminotransferases with cystine lyase activity have been reported previously (9). Further BLAST searches that only considered functionally characterized genes indicated that BOCL3 and CORI3 display highest amino acid sequence identity to the deduced amino acid sequence of two Hordeum vulgare nicotianamine aminotransferase encoding cDNAs (42% positional amino acid identity (31)) and several rat tyrosine aminotransferase encoding cDNAs. The present result suggests that also plant cystine lyases may be phylogenetically related with aminotransferases as suggested previously for other CS-lyase family members (7).

Confirmation of the in vitro activity of recombinant BOCL3 remains elusive, however, and even surprising given the high positional amino acid identity between BOCL3 and CORI3 and the fact that the same expression method that was used successfully for CORI3 also was tried for BOCL3 expression. The absence of native glycosylation when BOCL3 is expressed in heterologous hosts such as E. coli and S. cerevisiae may also play a role in impairing activity directly or by hindering proper folding of BOCL3 into a functionally active state. Expression of BOCL3 in A. thaliana may therefore provide a successful alternative, and such trials are currently in progress.

The redefinition of the probable catalytic function of CORI3, as the first identified plant cystine lyase, calls for a reassessment of the probable catalytic function of the proteins encoded by the other seven members of the group of highly homologous A. thaliana genes annotated as putative tyrosine aminotransferases (Table 1). The rooty/superroot1 gene is the only other investigated member of this group apart from CORI3. A. thaliana mutants with mutations in alleles of this gene locus were isolated based on an altered phenotype, and further analysis showed that these mutants contained elevated levels of indole acetic acid (32, 33). The putative gene sequence of several
alleles of the locus in question was obtained (13), but the catalytic function of the protein encoded by rooty/superroot1 (rooty/superroot1) remains unknown despite a multitude of approaches (13, 14). If rooty/superroot1 acts as a CS-lyase in planta, as suggested by the sequence similarity of rooty/superroot1 and CORI3, one possible role could be as the proposed CS-lyase that catalyzes the desulfhydration of S-alkyl thioldroximate to afford thioldroximate in the glucosinolate biosynthetic pathway (2, 35), with an indirect effect upon indole acetic acid accumulation in a manner exemplified by CYP83B1 (34).

Alternative functional roles for CORI3 and cystine lyases also need to be considered. Cystine lyases may be involved in sulfur metabolism, for example, by maintaining thiocysteine availability for cyanide degradation by rhodanese (36, 37) or for reduced sulfur compound generation. It has also been suggested that plant cystine lyases may be identical with cystathionine lyases (23, 38), but such a concept has later been found to be improper after analysis of highly purified cystine lyase protein preparations (16). It is still possible, nevertheless, that cystine lyases such as CORI3 can complement cystathionine lyases in planta with regards to thiocysteine production.

CONCLUSIONS

The identification of a new plant CS-lyase member, the first plant cystine lyase identified at a molecular level, adds impetus to a number of potential investigations such as the possible involvement of CS-lyases in glucosinolate biosynthesis (2, 35), indole acetic acid metabolism, and sulfur metabolism. The inducible nature of CORI3 also makes it highly desirable to find out more about the biological role of cystine lyases in plants and CORI3 in particular. Further in depth investigations into the metabolic role of cystine lyases and L-cystine are now also opened. To achieve an insight into the true role of CORI3 in planta, our primary strategy is to create transgenic Arabidopsis with up-regulated and down-regulated expression of CORI3.

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