Proc. Natl. Acad. Sci. USA
Vol. 87, pp. 9519–9523, December 1990
Biochemistry

Structure and expression of cytosolic cyclophilin/peptidyl-prolyl cis–trans isomerase of higher plants and production of active tomato cyclophilin in *Escherichia coli*

(maize/*Brassica napus*/cyclosporin A/enzyme evolution/rotamase)

CHARLES S. GASSER*†‡, DORIAN A. GUNNING†§, KIM A. BUDELIER†¶, AND SHERRI M. BROWN†

*Department of Biochemistry and Biophysics, University of California, Davis, CA 95616; and †Plant Gene Expression, Monsanto Company, 700 Chesterfield Village Parkway, St. Louis, MO 63198

Communicated by Eric E. Conn, September 7, 1990 (received for review August 22, 1990)

ABSTRACT **cDNA clones encoding proteins of ≈18 kDa in which 83% of the amino acids are conserved relative to the published sequences of mammalian cyclophilin/rotamase (CyP) have been isolated from tomato, maize, and *Brassica napus*. In correspondence with the mammalian genes, but in contrast with the *Neurospora* gene and one yeast CyP gene, the plant CyP genes encode only mature proteins lacking transit peptides. RNA blot analyses demonstrate that CyP genes are expressed in all plant organs tested. Southern blots of genomic DNA indicate that there are small families (two to eight members) of CyP-related genes in maize and *B. napus*. A vector was constructed for expression of the tomato cDNA in *E. coli*. SDS/polyacrylamide gels show that extracts of appropriately induced cells harboring this vector contain nearly 40% of the protein as a single ≈18-kDa band. While the majority of this protein is sequestered in insoluble inclusion bodies, the soluble extracts have higher levels of peptidyl-prolyl cis–trans isomerase (rotamase) activity than extracts of wild-type cells. This additional activity is sensitive to inhibition by the cyclic undecapeptide cyclosporin A.**

Cyclophilin (CyP) was first identified in 1984 by Handschumacher *et al.* (1) as a protein from mammalian thymocytes that specifically binds the immunosupressive cyclic undecapeptide cyclosporin A (CsA). Substantial evidence indicates that CyP is the *in vivo* target for CsA in suppression of tissue rejection (1–4) by inhibition of the activation of specific subsets of T lymphocytes (5). Recently, it was discovered that CyP is identical to a previously described enzyme, peptidyl-prolyl cis–trans isomerase ("rotamase"), that catalyzes rotation of the peptide bond on the amino side of proline residues (6, 7) and facilitates the folding of proteins *in vitro* (8–10). Binding of CsA effectively eliminates the rotamase activity of mammalian CyP (6, 7). The theory that interference with rotamase activity represents the mode of action of CsA is now supported by the observation that a second immunosupressive drug, FK506, binds to and inhibits the activity of a different protein with rotamase activity (11–13).

CyP/rotamase has also been identified in *Saccharomyces cerevisiae* (14) and *Neurospora crassa* (15) and has been shown to be the target for CsA toxicity in these species (16). In addition, the *ninaA* gene of *Drosophila*, which is important in the development of normal eyes, is also a homolog of mammalian CyP (17, 18). These findings indicate that rotamases may play significant roles in a range of critical processes, including signal processing during development.

Genes homologous to CyP have now been isolated from humans (19), rats (20), Chinese hamsters (21), mice (22), yeast (14, 23, 24), *N. crassa* (15, 25), *Echinococcus granulosus* (26), *Escherichia coli* (27, 28), and *Drosophila* (17, 18). Structural (29, 30), enzymological (10, 13, 31, 32), and site-directed mutagenesis (33) studies have been initiated to characterize the mode of action and mechanism of inhibition of rotamases. Despite these efforts, the mechanism of prolyl isomerization has not been unambiguously determined, and the mode of action of CsA in inhibiting the enzyme is still unclear.

Prior to this report, the only information concerning CyP in plants was the detection of a CsA-binding activity from zucchini, which was eluted at 17–18 kDa from gel filtration columns (34). No information on the relationship between this binding activity and rotamase activity was available. We now report the isolation, from three species of higher plants, of cDNA cones encoding proteins homologous to mammalian CyP.‖ We have examined the pattern of expression of the corresponding genes and found that CyP appears to be expressed in all organs of higher plants. Analysis of tomato CyP produced in *E. coli* demonstrates that the protein has rotamase activity that is inhibited by CsA.

MATERIALS AND METHODS

DNA and RNA Isolation and Characterization. DNA was isolated from plant material and Southern blots were prepared and hybridized as described (35, 36). Total and poly(A)$^+$ RNA was isolated and Northern blots were prepared on nylon membranes and hybridized (35).

Library Construction. cDNA libraries were constructed from mature maize embryos (*Zea mays* cv. Mo17) and tomato (*Lycopersicon esculentum* cv. VF36) shoot tips (containing the shoot apex, and leaves and floral buds <2 mm in length) in λgt10 (37) and λZAP (Stratagene), respectively, as described (35). A cDNA library was prepared from *Brassica napus* (cv. Westar) immature flower buds, <4 mm in length, in the plasmid vector pTZ18R-B by Invitrogen (San Diego, CA). A library of *B. napus* cv. Westar genomic DNA was constructed in λ phage EMBL3 (38) as described (36).

Isolation of CyP Homologous Clones. The tomato cDNA library was screened for cDNA clones of genes that are expressed at high levels in young buds but are present in low copy number in the genome. ^{32}P-labeled single-stranded cDNA was synthesized from poly(A)$^+$ RNA isolated from tomato buds <4 mm long and used to screen filter replicas of

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Abbreviations: CyP, cyclophilin; CsA, cyclosporin A; sAAPFn, *N*-succinyl-Ala-Ala-Pro-Phe *p*-nitroanilide; IPTG, isopropyl β-D-thiogalactopyranoside.

‡To whom reprint requests should be addressed.
§Present address: American Cyanamid Co., P.O. Box 400, Princeton, NJ 08543.
¶Present address: Enimont America Inc., 2000 Cornwall Road, Monmouth Jct., NJ 08852.

‖The sequences reported in this paper have been deposited in the GenBank data base (accession nos. M55018, M55019, and M55021).

20,000 tomato cDNA clones as described (35). A duplicate set of filters was hybridized with tomato nuclear DNA that had been ^{32}P-labeled by random oligonucleotide priming (39). Plaques that hybridized strongly with the bud cDNA probe and showed no hybridization with the genomic DNA probe were isolated as putative clones from highly expressed, low-copy-number genes.

Plasmid subclones were made from the isolated phage by coinfection with the helper phage M13K07 (40) according to instructions supplied with λZAP by Stratagene. The resulting plasmids carry the cDNA inserts in the vector pBluescript SK(−) (Stratagene). The sequence of one of the clones, pMON9976, was found to be homologous to the cDNA for human CyP.

The cDNA insert from pMON9976 was used as a probe to isolate homologous clones from the *B. napus* cDNA library by standard colony-hybridization procedures (41); thus, the *B. napus* clones (e.g., pMON8612) are in the vector pTZ18R-B (Invitrogen). Several clones of putative CyP genes were isolated from the *B. napus* genomic library by screening with the *B. napus* cDNA clones. All of the genomic clones isolated contained similar hybridizing restriction fragments (data not shown) and one was chosen for further characterization. The 3.4-kilobase (kb) *Bgl* II fragment of this clone was subcloned into pBluescript KS(+) (Stratagene), forming pMON8635.

The *B. napus* clone was used as a probe to isolate homologous clones from the maize cDNA library by standard plaque hybridizations (41). The insert of one maize clone was subcloned into pBluescript KS(+), resulting in pMON8712.

DNA Manipulation and Sequencing. Expression-vector construction and subcloning for sequencing were done by standard techniques (42). Single-stranded replicas were produced (40) from plasmid subclones and were sequenced by the dideoxy chain-termination method (43) using ^{35}S-labeled nucleotides and modified T7 polymerase (United States Biochemical).

Construction of an Expression Vector for Tomato CyP. An *Nde* I site was engineered at the translation start codon in pMON9976 by the method of Kunkel (44), using the oligonucleotide 5′-AATTCCTCTAGAGACATATGGCAAA-TCCA-3′, resulting in pCG17. The modified cDNA insert was removed from pCG17 on a *Pst* I–Sal I fragment and inserted into pUC118 (digested with the same enzymes), resulting in pCG18. The insert was then transferred as an *Nde* I–*Bam*HI fragment into these same sites of pET3c (45), resulting in pCG19. In pCG19 the start of translation of tomato CyP is situated correctly relative to the φ10 T7 RNA polymerase promoter and the gene *10* translation start site for efficient expression in bacteria containing T7 RNA polymerase.

Production and Characterization of Recombinant Tomato CyP. pCG19 and pET3c were introduced into *E. coli* BL21(DE3) (45), which carries the T7 RNA polymerase coding region under the control of the β-galactosidase promoter/operator combination (45). Cultures (40 ml) of these two strains were grown to an OD_{600} of 0.4–0.5 in LB medium containing ampicillin (100 μg/ml). Then isopropyl β-D-thiogalactopyranoside (IPTG, 0.5 mM) was added, the incubation was continued for an additional 3 hr, and the cells were chilled to 0°C and harvested by centrifugation. The cells were washed with 20 ml of 20 mM Tris·HCl (pH 7.8) and resuspended in 1.5 ml of the same buffer. Cells were lysed by two passages through a French pressure cell at 18,000 psi (1 psi = 6.9 kPa). The lysates were centrifuged at 12,000 × *g* for 10 min and the pellets and supernatants were separated and stored at 4°C.

Extracts were analyzed by SDS/PAGE and protein concentrations were determined by the Lowry method (46). Extraction of total proteins from *E. coli* and dissolution of inclusion bodies were accomplished by the addition of 2× SDS gel loading buffer (125 mM Tris·HCl, pH 6.8/4% SDS/20% glycerol/10% 2-mercaptoethanol/0.02% bromophenol blue) and incubation at 100°C for 3 min, followed by a 30-sec centrifugation at 15,000 × *g*.

Rotamase activity was assayed by a modification (33) of the method of Fischer *et al.* (47), except that the final concentration of chymotrypsin (Sigma) in the assay mixture was 10 μg/ml, and the assays were performed at 20°C. The succinyl-Ala-Ala-Pro-Phe *p*-nitroanilide (sAAPFn) for the assays was from Sigma and CsA (Sandimmune) was from Sandoz Pharmaceutical. Progress of chymotryptic cleavage of sAAPFn in the activity assays was monitored by measuring A_{390} at 0.1-sec intervals on a Shimadzu UV-160 recording spectrophotometer.

RESULTS

Isolation and Sequence Analysis of cDNA Clones for Higher Plant CyP. In performing a general screen for tomato cDNA clones derived from highly expressed, low-copy-number genes, we discovered that one of the isolated clones shared significant sequence homology with the human CyP cDNA (see below). This cDNA clone was subsequently used as a probe to directly isolate homologous cDNAs from *B. napus* and maize libraries. Sequence analysis of several independent clones indicated that all clones from a given species were derived from a single gene or from genes with identical sequences (data not shown). A representative clone was chosen from each species for complete nucleotide sequencing. The longest clone isolated from the *B. napus* cDNA library was missing 9 nucleotides of coding region at the 5′ end (by comparison with the other plant sequences). The sequence of this region was determined from a *B. napus* genomic clone that was identical to the cDNA in the regions of overlap. The restriction/hybridization pattern of this clone indicated that the gene did not contain an intron in the coding sequence (data not shown).

The tomato, *B. napus*, and maize clones contained open reading frames that would encode proteins of 171, 171, and 172 amino acids, respectively. The sequences of the predicted proteins, aligned with the sequences of human CyP (19) and the most similar form of yeast CyP (14) are shown in Fig. 1*A*. The plant proteins are very similar to each other, with ≈78% amino acid identity and ≈87% amino acid conservation among the three species. The maize sequence is not significantly more diverged from either of the two dicot species than the two dicot sequences are from each other. Human CyP is 74% identical and 83% conserved relative to all the plant sequences. The yeast sequence is slightly more diverged, having 66% amino acid identity and 78% amino acid conservation relative to the plant sequences. The same degree of similarity was seen for the cytoplasmic form of *Neurospora* CyP (15) (data not shown).

Fig. 1*B* shows the sequences of the regions 5′ of the putative translation initiation codons. In all three sequences in-frame termination codons are present just upstream of the translation initiation codons, indicating that these clones cannot encode larger preproteins.

Hybridizations to Southern blots containing genomic DNA isolated from *B. napus* and maize were performed with the appropriate cDNA clones to obtain an estimate of the copy number of CyP-related genes in these species. In *B. napus* the strongest band in each lane hybridized with an intensity similar to the single-copy control (Fig. 2*A*, lane 1) and corresponded with the gene in the *B. napus* genomic clone mentioned above. In addition to this band there were two to six additional hybridizing bands of varying intensity visible in the different digestions. In maize DNA up to eight bands were visible in the lane containing *Sac* I-digested DNA (Fig. 2*B*, lane 2). These experiments indicate the presence of small

A

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     1                                                  50
TOM  MANPKVFFDL TIGGAPAGRV VMELYADTTP KTAENFRALC TGEKGVGKMG
B.n. .V.......M .V.DKA...I ........V. E......... ...R.I..S.
MAI  ....R....M .V.......I ......NEV. ......... ........S.
HUM  .V..T....I AVD.E.L... SF.....KV. .........S .....F.---
YEA  .--SQ....V EAD.Q.I... .FK..N.IV. ......... .....F.---
CON  M    VFFD          GR     LY    P  TAENFRAL  TGE G G

     51                                                 100
TOM  KPLHYKGSTF HRVIPGFMCQ GGDFTAGNGT GGESIYGAKF NDENFVKKHT
B.n. ........A. .....K.... .......... .......M.. K.........
MAI  .......... .....E.... .....R.... .......E.. P..K..R.QP
HUM  ----....C. ..I....... .....RH... ..K....E.. E....IL...
YEA  ----.A..P. .....D..L. .......... ..K....G.. P....K.H.D
CON      Y GS F HR IP FM Q GGDFT  NGT GG SIYG KF  DE F

     101                                                150
TOM  GPGILSMANA GPGTNGSQFF ICTAKTEWLN GKHVVFGQVV EGMDVIKKAE
B.n. .......R.. .SN....... ...E..S..D .......... .....VRDI.
MAI  A..V...... ..N....... ...VA.P..D .......... .....V.AI
HUM  .......... ..N....... .........D .......K.K ...NIVEAM.
YEA  R..L...... ..N....... .T.VPCP..D .......E.. D.Y.IV..V.
CON   PG LSM NA G  TNGSQFF I T    WL  GKHVVFG V   G       E

     151                  173
TOM  AVGSSSGRCS KPVVIADCGQ L*-
B.n. K...D...T. .K..TC.... .*-
MAI  K..TRN.ST. .V.KV..... .S*
HUM  RF..RN.KT. .KITI..... .E*
YEA  SL..P..ATK ARI.V.KS.E .*-
CON    G   G           G  L
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B

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BRASSICA   TAATCTCTCAAACCTTCTCTCAAACACAAAATG
TOMATO                          TAGAGAGAAATG
MAIZE                        TAGCTCCCGATCTCGATG
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FIG. 1. (*A*) Alignment of CyP sequences. The amino acid sequences of tomato (TOM), maize (MAI), and *B. napus* (B.n.) CyP predicted from the nucleotide sequences of the cDNA and genomic clones are shown aligned with the published sequences of human (HUM; ref. 19) and yeast (YEA; ref. 14) CyP. The entire tomato sequence is shown, but for the other sequences only the residues that differ from the tomato sequence are shown. Dots indicate residues that are identical with the tomato sequence and dashes indicate gaps introduced to allow for optimal alignment of the sequences. The consensus (CON) sequence shows those positions where all five sequences are identical. Single-letter codes for amino acid residues are used and stars indicate translation termination codons. (*B*) Nucleotide sequences of regions 5′ of the predicted translation start codons in genomic (*Brassica napus*) and cDNA (tomato and maize) clones encoding plant CyP. The translation start codons are shown in boldface. Underlined sequences indicate termination codons that are in the same reading frame as the translation start codons.

families of genes with homology to CyP in allotetraploid *B. napus* and diploid maize.

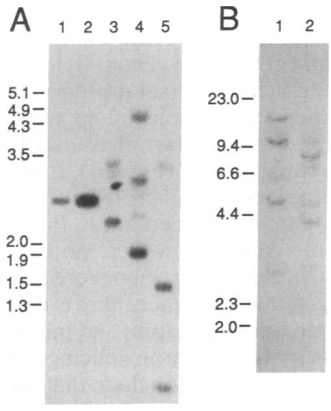

FIG. 2. Autoradiographs of Southern blots of *B. napus* and maize genomic DNA. (*A*) Control plasmid DNA and total DNA from *B. napus* hybridized with ^{32}P-labeled pMON8612. Lanes: 1 and 2, *Eco*RI-digested pUC18, 5 pg (single-copy reconstruction) and 25 pg (five-copy reconstruction), respectively; 3–5, 10 μg of *B. napus* DNA digested with *Bam*HI, *Eco*RI, or *Hin*dIII, respectively. (*B*) Maize DNA (10 μg) digested with *Hin*dIII (lane 1) or *Sac* I (lane 2) and hybridized with the maize CyP cDNA insert from pMON8712. The final wash for each filter was in 3 mM sodium phosphate/45 mM NaCl at 65°C. Numbers at left, sizes of marker DNA in kilobase pairs.

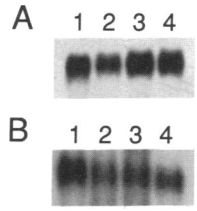

FIG. 3. RNA blots of various tissues hybridized with plant CyP cDNAs. (*A*) Total RNA (10 μg per lane) from the indicated organs of tomato, hybridized with the insert from the tomato CyP cDNA clone, pMON9976. Lanes: 1, young leaves; 2, floral buds <2 mm long; 3, the upper 5 mm of actively growing shoots; 4, stamens at anthesis. (*B*) RNA from the indicated organs of *B. napus*, hybridized with the insert from the *B. napus* CyP cDNA clone, pMON8612. Lanes: 1, floral buds <4 mm long; 2, immature stamens from buds 4–8 mm long; 3, young leaves; 4, roots from germinated seeds. Exposure time for each blot was 22 hr.

CyP Genes Are Expressed in Disparate Plant Organs. CyP has been shown to be present in a broad range of organs of mammals (34). Northern blotting experiments on tomato and *B. napus* (Fig. 3) showed that CyP genes are expressed at significant levels in a number of plant organs. Additional experiments showed similar levels of CyP mRNA in sepals, immature stamens, mature ovaries, and seedlings of tomato (R. Beale, personal communication). The transcript in both tomato and *B. napus* was ≈750 bases long.

Expression of Recombinant Tomato CyP in *E. coli*. To confirm that the plant cDNAs encoded proteins with rotamase activity, the tomato enzyme was produced in *E. coli*. A transcriptional fusion was made between the tomato coding sequence and a combination of the φ10 RNA polymerase promoter and the gene *10* translational leader of bacteriophage T7 in the vector pET3c (45). This plasmid, pCG19, was introduced into *E. coli* BL21(DE3) (45) and expression was induced by the addition of IPTG. This induction led to the accumulation of large amounts of a protein of ≈18 kDa (Fig. 4, lanes 2–5), consistent with the size of tomato CyP predicted from the cDNA sequence. The same *E. coli* strain containing the pET3c vector without the cDNA insert failed to accumulate this protein (Fig. 4, lane 8).

Insoluble inclusion bodies were present in French-press lysates of induced *E. coli* cells that contained pCG19, and were absent from similar lysates of cells containing pET3c (data not shown). When these bodies were separated from the lysate by centrifugation, the recombinant tomato CyP was preferentially partitioned in the pellet (Fig. 4, lanes 5 and 6). This indicates that the majority of the tomato CyP protein produced is sequestered in relatively insoluble inclusion bodies, as has been observed for many other recombinant proteins overexpressed in *E. coli* (48).

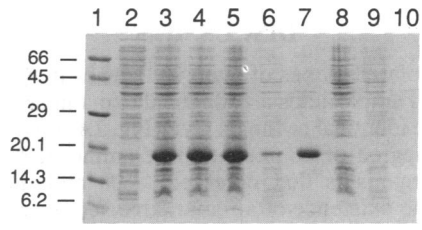

FIG. 4. SDS/15% PAGE of proteins isolated from *E. coli*. Lanes: 1, size standards (molecular masses in kilodaltons at left); 2–5, total protein from *E. coli* containing pCG19 prior to induction with IPTG and after 1, 2, or 3 hr of induction, respectively; 6, 10 μg of soluble extract from 3-hr induced pCG19-containing cells; 7, an equivalent portion of the pellet from the extraction run in lane 6; 8, total protein from *E. coli* containing pET3c that had been induced with IPTG for 3 hr; 9, soluble extracts from cells as in lane 8; 10, equivalent portion of pellet from extraction in lane 9.

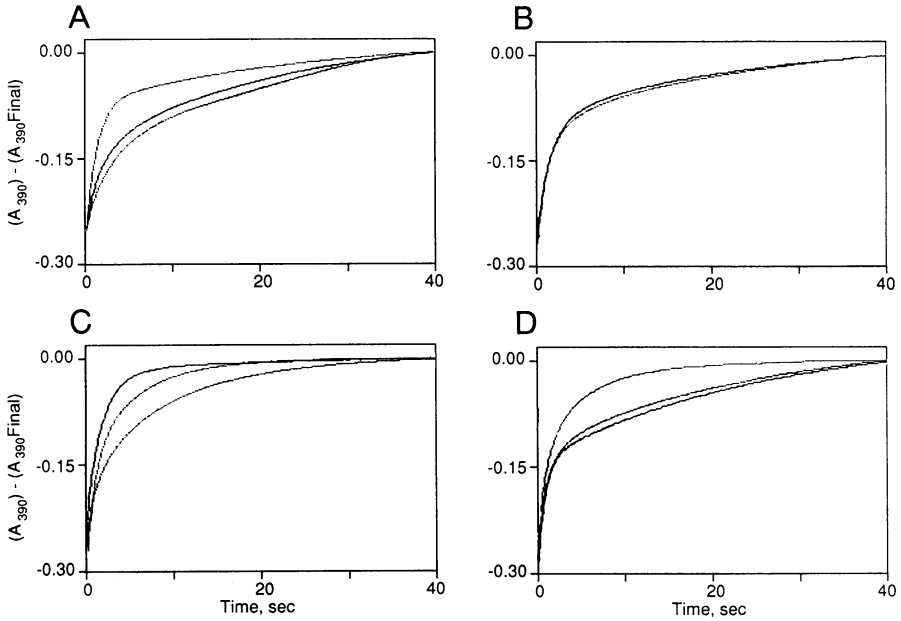

FIG. 5. Rotamase assays of extracts of *E. coli* expressing tomato CyP. Cells harboring pCG19 (vector with insert) or pET3c (no insert) were grown to logarithmic phase and induced for 3 hr with 0.5 mM IPTG. Soluble extracts of the cells were isolated and assayed for protein and rotamase. (*A*) Assays of extract from *E. coli* harboring pET3c; upper curve, 4 μg of protein; middle curve, 1 μg; lower curve, 0.5 μg. (*B*) Nearly coincident curves produced by assays of 5 μg of extract from pET3c-containing cells done in the presence (lower curve) or absence (upper curve) of 1 μM CsA. (*C*) As in *A* except extracts from cells harboring pCG19. (*D*) Assays on extract containing 1 μg of protein from cells containing pCG19 (upper curve), 1 μg of protein from cells containing pET3c (middle curve), or 1 μg of protein from cells containing pCG19 with the assay performed in the presence of 1 μM CsA (lower curve).

Tomato CyP Has Rotamase Activity That Is Inhibited by CsA. The soluble portion of the extracts of *E. coli* containing either pET3C or pCG19 were assayed for rotamase activity (47). In this assay chymotrypsin is used to cleave a chromogenic peptide, sAAPFn. The peptide can be cleaved only when it is in the trans Ala-Pro conformation (47). More than 80% of the sAAPFn is in this conformation at equilibrium and is cleaved in the first few seconds of the reaction, usually before recording can begin. The remainder must undergo the relatively slow isomerization from the cis form before it can be cleaved. Rotamase activity is detected by the acceleration of the rate of the second phase of the reaction by facilitation of the cis–trans isomerization. Addition of increasing amounts of the extract from cells containing pET3c led to detectable increases in the rate of cleavage of the sAAPFn (Fig. 5*A*), consistent with the report of Liu and Walsh (27) of an endogenous rotamase activity in *E. coli*. Fig. 5*B* confirms their result that this endogenous activity is insensitive to CsA inhibition.

Similar assays of extracts from cells containing pCG19 showed higher levels of rotamase activity (Fig. 5 *C* and *D*). The addition of 1 μM CsA to these extracts decreased this activity to a level that coincided with the level seen in the extracts from cells containing pET3c (Fig. 5*D*). This additional rotamase activity must derive from the tomato CyP expression vector. We conclude that tomato CyP encoded by our cDNA clone has rotamase activity that is inhibited by CsA.

DISCUSSION

We have isolated clones of plant CyP genes in which 74% of the encoded amino acids are identical to mammalian CyP and 55–66% are identical to fungal CyP (Fig. 1*A*; refs. 14, 15, and 19). This degree of conservation of amino acid sequences between organisms that diverged >600 million years ago indicates the existence of a remarkable set of constraints on the structure of this protein. The *ninaA* genes of *Drosophila melanogaster* appear to encode a distinct family of related proteins since they share only ≈45% sequence identity with mammalian, fungal, and plant CyP and additionally include a putative transmembrane domain (17, 18). The degree of difference between the *ninaA* protein and the other members of the CyP family suggests divergence from the other members prior to the divergence of plants, animals, and fungi. It may be that additional genes which are more closely related to the *ninaA* genes exist in these other species. It is possible that the additional CyP-related sequences detected in genomic Southern blots of *B. napus* and maize DNA (Fig. 2) represent such additional plant CyP genes. Finally, a new family of rotamases, the FK506-binding proteins, has been identified in mammals, and these proteins show no sequence similarity to the CyP family members over the regions of protein that have been sequenced (11–13). There are, therefore, at least three divergent families of rotamase or rotamase-related proteins in higher organisms.

The plant CyP proteins contain a 7-amino acid insertion relative to the mammalian and fungal sequences following residue Gly-47 (Fig. 1*A*). This region would carry a positive charge due to the presence of two lysine residues. The conservation of this sequence between the distantly related monocotyledonous and dicotolydenous plants (Fig. 1*A*) indicates a strong selective pressure for maintenance of the sequence of this region.

The *E. coli* rotamase (27), the *ninaA* gene product (17, 18), a subset of the CyP of *Neurospora* (15), and the product of one CyP gene identified in yeast (24) are synthesized as precursor proteins that include amino-terminal extensions for targeting the enzymes to specific subcellular locales. We examined the plant cDNA and genomic clones to see whether they could encode such precursor proteins. In all three cases, in-frame translation termination codons were present a short distance 5′ of the putative start of translation identified by homology to mammalian CyP (Fig. 1*B*). It is conceivable that an exon encoding additional protein sequence is present further 5′ and can be spliced onto the existing mature sequence in some cases. However, no cDNA clones with alternative sequences in this region were found. In addition, in the *B. napus* genomic sequence there is no AG dinucleotide between the upstream termination and initial start codons as would be necessary for an intron splicing acceptor site (Fig. 1*B*). On the basis of this we conclude that the genes we have identified encode only mature, presumably cytosolic CyP.

As observed in humans (34), CyP genes appear to be expressed in all organs of plants (Fig. 3; R. Beale, personal communication). While quantitative measurements of CyP messenger levels were not made, we can conclude that the message is relatively abundant due to the ease with which we detected CyP mRNA in total RNA. The general expression of CyP genes is consistent with the hypothesis that rotamase plays an important role in the folding of many proteins *in vivo* (8–10, 31).

Final confirmation of the identity of the cDNA clones we had isolated from plants as authentic rotamase/CyP was achieved through the characterization of tomato CyP produced in *E. coli*. Under appropriate induction, bacteria harboring the expression plasmid, pCG19, accumulated a large amount of an 18-kDa protein that was primarily sequestered in insoluble inclusion bodies (Fig. 4). We were, however, able to detect rotamase activity in the soluble extracts from these cells that was significantly in excess of the endogenous *E. coli* activity (Fig. 5*D*). In addition, this activity was inhibited by 1 μM CsA, a concentration that has no effect on the bacterial enzyme (Fig. 5*D*; ref. 27). This demonstrates that a portion of the protein produced by our vector does fold into active rotamase. The inhibition of the activity by CsA is indicative of binding of the inhibitor by the enzyme, so that the tomato rotamase is an authentic CyP. Since we do not know what portion of the tomato CyP present in the soluble fraction is in the active form, we were unable to more fully characterize the kinetic properties of this enzyme. The availability of this ready source of plant CyP should greatly accelerate our studies on the activity and specificity of this enzyme. An understanding of the properties of this enzyme may aid in further studies to determine whether rotamases have a role in the regulation of plant developmental processes similar to those seen in animal systems.

We thank Judy Callis for helpful comments on the manuscript, Veronica Lippuner for her assistance in vector construction, Rachanee Beale for providing us with her unpublished data, Jun Lui and Chih-ming Chen for advice on rotamase assays, and Dilip Shah, Steve Rogers, Rob Fraley, and Ernest Jaworski for their support of this work. This work was supported by funds from the Monsanto Company and from the College of Agricultural and Environmental Sciences at the University of California, Davis.

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