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Molecular cloning and analysis of cDNA encoding a plant tryptophan decarboxylase: Comparison with animal dopa decarboxylases

(*Catharanthus roseus*/vinblastine/mRNA hybridization)

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ABSTRACT **The sequence of a cDNA clone that includes the complete coding region of tryptophan decarboxylase (EC 4.1.1.28, formerly EC 4.1.1.27) from periwinkle (*Catharanthus roseus*) is reported. The cDNA clone (1747 base pairs) was isolated by antibody screening of a cDNA expression library produced from poly(A)$^+$ RNA found in developing seedlings of *C. roseus*. The clone hybridized to a 1.8-kilobase mRNA from developing seedlings and from young leaves of mature plants. The identity of the clone was confirmed when extracts of transformed *Escherichia coli* expressed a protein containing tryptophan decarboxylase enzyme activity. The tryptophan decarboxylase cDNA clone encodes a protein of 500 amino acids with a calculated molecular mass of 56,142 Da. The amino acid sequence shows a high degree of similarity with the aromatic L-amino acid decarboxylase (dopa decarboxylase) and the α-methyldopa-hypersensitive protein of *Drosophila melanogaster*. The tryptophan decarboxylase sequence also showed significant similarity to feline glutamate decarboxylase and mouse ornithine decarboxylase, suggesting a possible evolutionary link between these amino acid decarboxylases.**

Tryptophan decarboxylase (TDC; EC 4.1.1.28, formerly EC 4.1.1.27) catalyzes the conversion of L-tryptophan to tryptamine. This enzyme has been detected in numerous plant systems, and it has been suggested that its primary role is to supply possible precursors for auxin biosynthesis (1–3). In the Gramineae, TDC catalyzes the synthesis of precursors for the protoalkaloids, which have considerable physiological activity in higher animals (4). Tryptophan-derived tryptamines are also precursors of the tricyclic β-carboline alkaloids formed by condensation with a one- or two-carbon moiety (5). In periwinkle (*Catharanthus roseus*), TDC produces tryptamine for biosynthesis of two commercially important antineoplastic monoterpenoid indole alkaloids, vinblastine and vincristine (6).

The TDC from *C. roseus* has been purified to homogeneity (ref. 7; J. Alvarez, T. Owen, W. Kurz, and V.D.L., unpublished work). It occurs as a dimer consisting of two identical subunits of M_r 54,000, and it requires pyridoxal phosphate for activity (7). The enzyme is characteristic of plant aromatic decarboxylases, which usually exhibit high substrate specificity. For example, TDC will decarboxylate L-tryptophan and 5-hydroxy-L-tryptophan but is inactive toward L-phenylalanine and L-tyrosine (7), whereas the tyrosine decarboxylases from *Syringa vulgaris* (8), *Thalictrum rugosum* (9), and *Escholtzia californica* (9) accept L-tyrosine and L-dopa (3,4-dihydroxy-L-phenylalanine) as substrates but not L-tryptophan or 5-hydroxy-L-tryptophan. The aromatic L-amino acid decarboxylases [dopa decarboxylase (DDC), EC 4.1.1.28] of *Drosophila melanogaster* (10–12) and mammals (13) have a broader substrate specificity, with L-dopa, tyrosine, phenylalanine, and possibly histidine also serving as substrates (14). In animals, the role of aromatic L-amino acid decarboxylase is to produce the major neurotransmitters dopamine and serotonin (14–16), and in *D. melanogaster*, the DDC enzyme serves a second, inducible role in the sclerotization of the insect cuticle (17).

In this paper, we describe the isolation and identification of TDC cDNA clones by antibody screening of a cDNA expression library. We have characterized a full-length cDNA clone whose protein sequence, predicted from the nucleotide sequence,§ shows significant similarity to that of DDC from *D. melanogaster* (11, 12) and to other amino acid decarboxylases from diverse animal origins. Furthermore, we demonstrate that the protein encoded by the cDNA clone is active *in vitro*.

MATERIALS AND METHODS

cDNA Synthesis and DNA Sequencing. Seedlings of *C. roseus* (L.) G. Don cv. Little Delicata were germinated and grown for 5 days in the dark as described (18). Seedlings were harvested after 18 hr of light treatment and total RNA was isolated as described (19).

Poly(A)$^+$ RNA was isolated by chromatography on oligo(dT)-cellulose (20) and double-stranded cDNAs were prepared according to the procedure of Gubler and Hoffman (21). After ligation with *Eco*RI linkers the cDNA was inserted into the *Eco*RI site of the expression vector λZAP (Stratagene; ref. 22). A library containing 3.1×10^5 recombinant phages was obtained and after amplification, 2×10^5 plaques were screened with specific polyclonal antiserum raised against TDC (V.D.L., unpublished work). Plasmids (pBluescript, Stratagene) containing a TDC cDNA insert were rescued by using the R408 f1 helper phage (22), and the nucleotide sequence of a full-length cDNA clone (pTDC5) was determined on both strands by the dideoxy chain-termination method (23). The sequencing strategy included subcloning of restriction fragments and the use of oligonucleotide primers. The sequence for each restriction site used for the subcloning was determined on at least one strand. Comparisons of the pTDC5 cDNA nucleotide sequence and of the deduced amino acid sequence with GenBank (Release 55) and National Biomedical Research Foundation (NBRF; Release 18) sequence libraries were performed by using the FASTA program package (24).

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Abbreviations: TDC, tryptophan decarboxylase; DDC, dopa decarboxylase; AMD, α-methyldopa-hypersensitive gene product.

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§The sequence reported in this paper is being deposited in the EMBL/GenBank data base (accession no. J04521).

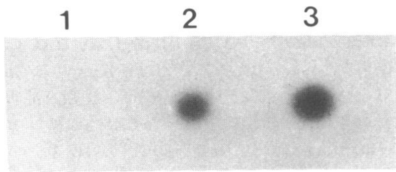

FIG. 1. TDC enzyme activity in extracts of pTDC5-transformed *E. coli*. The conversion of [^{14}C]tryptophan to [^{14}C]tryptamine was monitored in extracts of *E. coli* and of *C. roseus* leaves. Extracts (30 μl) were incubated in the presence of 0.1 μCi of [^{14}C]tryptophan (specific activity, 59 mCi/mmol) for 30 min. After addition of base, [^{14}C]tryptamine was extracted from the reaction mixture with ethyl acetate and reaction products were analyzed by thin-layer chromatography on silica gel [solvent: $CHCl_3/CH_3OH$/25% NH_3, 5:4:1 (vol/vol)] and autoradiography. Lane 1, *E. coli* transformed with the pBluescript vector; lane 2, *E. coli* transformed with pTDC5; lane 3, *C. roseus* leaves.

RNA Blot Hybridization. Poly(A)$^+$ RNA was isolated from 6-day-old developing seedlings and from young leaves of mature plants as described above. These tissues were chosen as a likely source of TDC poly(A)$^+$ RNA based on the presence of high levels of TDC enzyme activity (18). RNA was denatured, fractionated by electrophoresis in formaldehyde/agarose gels, and then transferred to nitrocellulose filters (25). Blotted RNA was hybridized to ^{32}P-labeled pTDC5 DNA and autoradiography was performed using Kodak XAR-5 films.

TDC Activity in Extracts of *E. coli*. A culture (100 ml) of the *E. coli* strain XL1-blue containing pTDC5 or pBluescript was incubated at 37°C for 2 hr before addition of the inducer (isopropyl β-D-thiogalactopyranoside) at a final concentration of 1 mM. Incubation was continued for an additional 2 hr. Cells were harvested, washed in 10 mM Tris·HCl, pH 7.5/1 mM EDTA, resuspended, and lysed in 3 ml of 0.1 M Hepes,

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                                                                                     met gly ser ile asp ser thr
1     CTCTCTCTCTCTCTCTCTAAGACTTTCTCTCTCTACACATACACCTACACCAGAAAAAAGAAAAAAATA   ATG GGC AGC ATT GAT TCA ACA

      asn val ala met ser asn ser pro val gly glu phe lys pro leu glu ala glu glu phe arg lys gln ala his
91    AAT GTA GCC ATG TCC AAT TCT CCA GTT GGA GAA TTT AAG CCA CTT GAA GCT GAG GAA TTC CGA AAA CAA GCC CAT

      arg met val asp phe ile ala asp tyr tyr lys asn val glu thr tyr pro val leu ser glu val glu pro gly
166   CGT ATG GTA GAT TTC ATA GCC GAT TAT TAC AAA AAT GTG GAA ACA TAT CCG GTC CTT AGC GAA GTC GAA CCT GGA

      tyr leu arg lys arg ile pro glu thr ala pro tyr leu pro glu pro leu asp asp ile met lys asp ile gln
241   TAT CTC CGA AAA CGT ATC CCC GAA ACC GCT CCT TAC CTC CCC GAA CCA CTT GAC GAC ATC ATG AAA GAT ATT CAG

      lys asp ile ile pro gly met thr asn trp met ser pro asn phe tyr ala phe phe pro ala thr val ser ser
316   AAG GAT ATT ATC CCA GGA ATG ACA AAT TGG ATG AGC CCT AAT TTT TAT GCA TTT TTT CCT GCC ACT GTT AGT TCA

      ala ala phe leu gly glu met leu ser thr ala leu asn ser val gly phe thr trp val ser ser pro ala ala
391   GCT GCC TTT TTA GGA GAA ATG TTG TCT ACT GCC CTA AAT TCA GTA GGC TTT ACT TGG GTT TCT TCA CCA GCC GCC

      thr glu leu glu met ile val met asp trp leu ala gln ile leu lys leu pro lys ser phe met phe ser gly
466   ACC GAA TTA GAA ATG ATT GTT ATG GAT TGG TTG GCT CAG ATC CTT AAA CTC CCC AAA TCT TTC ATG TTT TCA GGT

      thr gly gly gly val ile gln asn thr thr ser glu ser ile leu cys thr ile ile ala ala arg glu arg ala
541   ACC GGT GGC GGC GTC ATC CAA AAC ACC ACT AGC GAG TCC ATT CTT TGT ACA ATC ATT GCC GCC CGG GAA AGG GCC

      leu glu lys leu gly pro asp ser ile gly lys leu val cys tyr gly ser asp gln thr his thr met phe pro
616   CTG GAG AAG CTC GGT CCC GAT AGT ATT GGA AAA CTT GTC TGT TAC GGA TCC GAT CAA ACC CAT ACC ATG TTC CCC

      lys thr cys lys leu ala gly ile tyr pro asn asn ile arg leu ile pro thr thr val glu thr asp phe gly
691   AAA ACT TGC AAA TTG GCG GGA ATT TAT CCG AAT AAT ATT AGG TTA ATA CCT ACG ACC GTC GAA ACG GAT TTC GGC

      ile ser pro gln val leu arg lys met val glu asp asp val ala ala gly tyr val pro leu phe leu cys ala
766   ATC TCA CCT CAA GTT CTA CGA AAA ATG GTC GAG GAT GAC GTG GCG GCC GGA TAT GTA CCG CTG TTC TTA TGC GCT

      thr leu gly thr thr ser thr thr ala thr asp pro val asp ser leu ser glu ile ala asn glu phe gly ile
841   ACC CTG GGT ACC ACC TCG ACC ACG GCT ACC GAT CCT GTG GAC TCA CTT TCT GAA ATC GCT AAC GAG TTT GGT ATT

      trp ile his val asp ala ala tyr ala gly ser ala cys ile cys pro glu phe arg his tyr leu asp gly ile
916   TGG ATC CAC GTG GAT GCT GCT TAT GCG GGA AGC GCC TGT ATA TGT CCC GAG TTT AGA CAT TAC TTG GAT GGA ATC

      glu arg val asp ser leu ser leu ser pro his lys trp leu leu ala tyr leu asp cys thr cys leu trp val
991   GAA CGA GTT GAC TCA CTG AGT CTG AGT CCA CAC AAA TGG CTA CTC GCT TAC TTA GAT TGC ACT TGC TTG TGG GTC

      lys gln pro his leu leu leu arg ala leu thr thr asn pro glu tyr leu lys asn lys gln ser asp leu asp
1066  AAG CAA CCA CAT TTG TTA CTA AGG GCA CTC ACT ACG AAT CCT GAG TAT TTA AAA AAT AAA CAG AGT GAT TTA GAC

      lys val val asp phe lys asn trp gln ile ala thr gly arg lys phe arg ser leu lys leu trp leu ile leu
1141  AAA GTT GTG GAC TTC AAA AAT TGG CAA ATC GCA ACG GGA CGA AAA TTT CGG TCG CTG AAA CTT TGG CTC ATT TTA

      arg ser tyr gly val val asn leu gln ser his ile arg ser asp val ala met gly lys met phe glu glu trp
1216  CGT AGC TAT GGA GTT GTT AAT TTA CAG AGT CAT ATT CGT TCT GAC GTC GCA ATG GGC AAA ATG TTC GAA GAA TGG

      val arg ser asp ser arg phe glu ile val val pro arg asn phe ser leu val cys phe arg leu lys pro asp
1291  GTT AGA TCA GAC TCC AGA TTC GAA ATT GTG GTA CCG AGA AAC TTT TCT CTT GTT TGT TTT AGA TTA AAA CCT GAC

      val ser ser leu his val glu glu val asn lys lys leu leu asp met leu asn ser thr gly arg val tyr met
1366  GTT TCG AGT TTA CAT GTA GAA GAA GTG AAT AAG AAA CTT TTG GAC ATG CTT AAC TCG ACG GGA CGA GTT TAT ATG

      thr his thr ile val gly gly ile tyr met leu arg leu ala val gly ser ser leu thr glu glu his his val
1441  ACT CAT ACT ATT GTG GGA GGC ATA TAC ATG CTA AGA CTG GCT GTT GGC TCA TCG CTA ACT GAA GAA CAT CAT GTA

      arg arg val trp asp leu ile gln lys leu thr asp asp leu leu lys glu ala ter
1516  CGC CGT GTT TGG GAT TTG ATT CAA AAA TTA ACC GAT GAT TTG CTC AAA GAA GCT TGA TGAATAAGTAAGGGTTTTTTTTA

1597  ATTTTTTTTTAAATTTTATATTTGCTGATTGTTTGAAGAGTTTAAAAATAAAGTGATTTGTAAAGGTTTATTGTACTCAAACAATCATGCAATTAATTAT

1697  ATGTATTAATTATGACATGAGAATAAAATAGAATTTGTGTGTGCAAAAAAA
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FIG. 2. Nucleotide sequence of the pTDC5 cDNA clone and its deduced amino acid sequence. The putative polyadenylylation signal is underlined.

pH 7.5/1 mM dithiothreitol. Debris was removed by centrifugation and the supernatant was desalted by passage through a Sephadex G-25 column. TDC enzyme activity in bacterial supernatants was determined by monitoring the conversion of L-[*methylene*-^{14}C]tryptophan to [^{14}C]tryptamine (18). Supernatants (30 μl) were incubated in the presence of 0.1 μCi of [^{14}C]tryptophan (specific activity, 59 mCi/mmol; 1 Ci = 37 GBq) for 30 min and reactions were stopped with 100 μl of 0.1 M NaOH. Radioactive tryptamine was extracted from the reaction mixture with ethyl acetate and was analyzed by silica gel thin-layer chromatography and autoradiography. TDC enzyme activity in leaves was determined as described (18).

RESULTS

TDC Enzyme Activity in *E. coli*. When the original cDNA library was screened with the anti-TDC antibody, 27 clones were identified. Six clones were selected for further analysis. Partial sequence analysis revealed no differences among these clones, except for their lengths. Therefore the clone having the longest cDNA insert (pTDC5) was selected for further characterization. To confirm that this cDNA clone corresponded to TDC, enzyme activity was measured in extracts from *E. coli* transformants. Fig. 1 shows that [^{14}C]tryptamine was produced with extracts from cells carrying pTDC5 (lane 2) and with extracts from *C. roseus* leaves (lane 3) but not with extracts from cells containing only the vector (lane 1). This result indicated that TDC activity was retained by the protein produced from a TDC cDNA clone under the control of the *lac* promoter of the pBluescript vector. No attempts were made to quantify the level of activity of TDC in *E. coli*.

Sequence Analysis of a TDC cDNA Clone. DNA sequence analysis of pTDC5 revealed the presence of an open reading frame coding for a protein of 500 amino acids, which corresponded to a molecular mass of 56,142 Da (Fig. 2). The 5′ nontranslated region of pTDC5 contained 69 nucleotides and included, near its beginning, a long stretch of alternating pyrimidines. Sequence around the methionine initiation codon (AAUAAUGGG) matched closely the consensus sequence for plant gene initiation codons (AACAAUGGC)

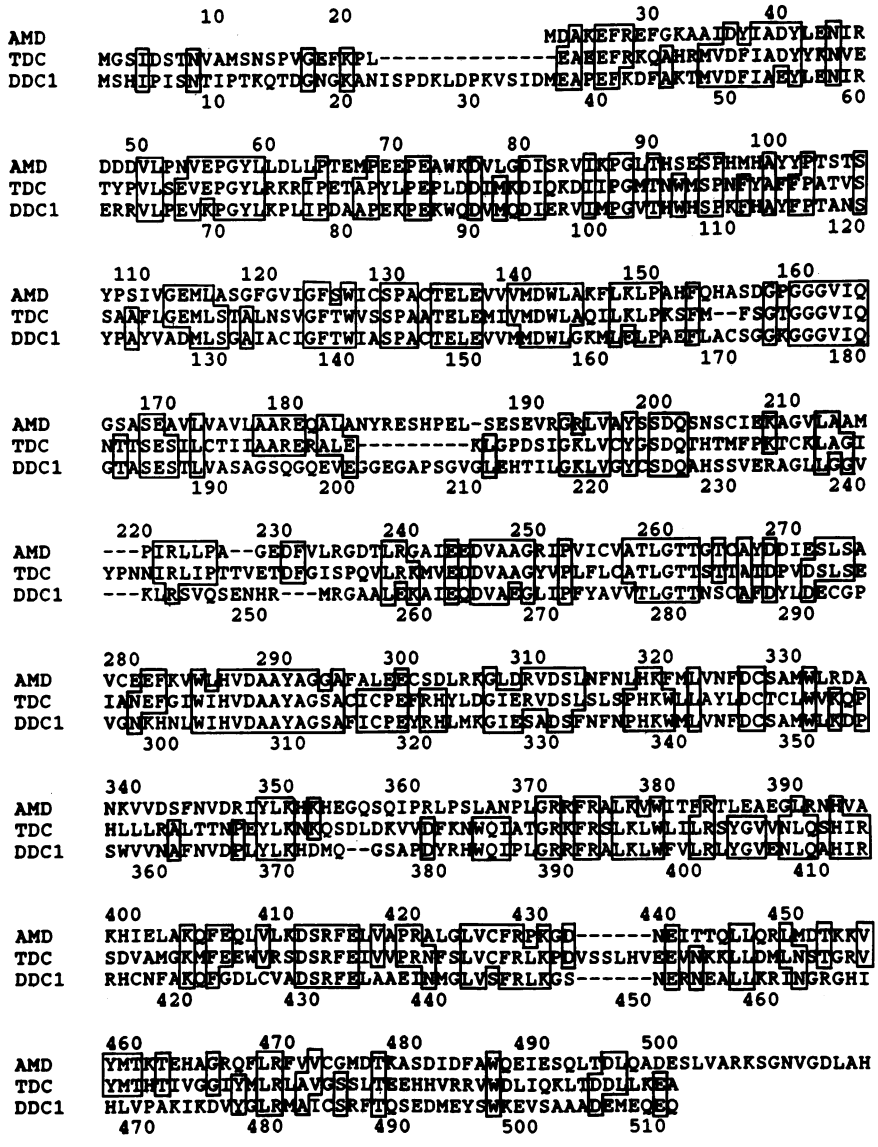

FIG. 3. Amino acid sequence alignment of *D. melanogaster* AMD protein (28), *C. roseus* TDC, and *D. melanogaster* DDC1 (11, 12). Boxes show TDC residues present in AMD and/or DDC1 sequences. Amino acids (standard one-letter symbols) are numbered for TDC (top) and DDC1 (bottom).

(26). The 3′ nontranslated region consisted of 168 nucleotides up to the poly(A) tail and contained an AAUAAA putative poly(A)$^+$-addition signal 17 nucleotides upstream from the start of the poly(A) tail. Examination of the predicted amino acid sequence did not reveal a signal sequence (27), which is consistent with the proposed cytoplasmic location of TDC (18).

Comparison of TDC cDNA nucleotide and deduced amino acid sequences with nucleotide sequences in the GenBank DNA sequence data base and with amino acid sequences in the NBRF protein sequence data base revealed 39% amino acid identity with the DDC isoenzyme 1 (DDC1) from *D. melanogaster* (11, 12) and 35% identity with the protein corresponding to the *D. melanogaster* α-methyldopa-hypersensitive gene (AMD; ref. 28) (Fig. 3). The areas of amino acid similarity extended throughout the protein and were not restricted to a particular portion of either structure. Limited proteolysis of pig kidney DDC and amino acid sequencing of a tryptic fragment produced a sequence for 50 amino acid residues one-third of the distance from the carboxyl terminus of this protein (29). Comparison of this 50-amino acid sequence with periwinkle TDC and *D. melanogaster* DDC1 gave 20 and 32 identical amino acids, respectively. Furthermore, comparison of *C. roseus* TDC to feline glutamate decarboxylase (30) showed that 19% of the amino acid residues were identical between these two proteins; the similarity increased to 25% on a 396-amino acid stretch. Mouse ornithine decarboxylase (31) showed a statistically significant (24) 12% amino acid sequence similarity to the plant TDC, and this similarity also extended throughout the protein sequence. We found that the sequence Pro-His-Lys, beginning at position 317 in TDC, was identical to the sequence at the pyridoxal phosphate binding sites of *D. melanogaster* DDC (9, 10), feline glutamate decarboxylase (30), and pig DDC (32). In contrast, the AMD protein, whose enzymatic function is unknown, contained the sequence Leu-His-Lys at the pyridoxal phosphate binding domain.

Structural similarities between TDC and *D. melanogaster* DDC1 proteins were further revealed by comparing their hydropathy profiles (Fig. 4). Close examination of the alignment of hydrophobic and hydrophilic regions for the two proteins showed a striking match between them, except for the area near the amino terminus and the region around TDC residue 225.

TDC mRNA Accumulation. When total poly(A)$^+$ RNA isolated from 6-day-old seedlings was probed with a 1.6-kilobase (kb) cDNA fragment isolated from pTDC5, a 1.8-kb mRNA was detected (Fig. 5, lane 1). Young leaves from the mature plant also contained a 1.8-kb mRNA (lane 2). A fainter signal corresponding to a transcript of 3.2 kb was also present in both lanes. This signal could represent either a precursor form of the TDC mRNA or an unrelated transcript having some sequence similarity to TDC.

DISCUSSION

A TDC cDNA clone of *C. roseus* was isolated by antibody screening of an expression library. The antigenicity and enzyme activity (Fig. 1) of the encoded protein established the identity of the TDC cDNA. This cDNA contained an open reading frame coding for a 56-kDa polypeptide, which is consistent with the subunit molecular mass of 54 kDa (7) and 56 kDa (V.D.L., unpublished work) observed for TDC isolated from *C. roseus* cell suspension cultures and developing seedlings, respectively. Blot hydridization of RNA extracted from 6-day-old seedlings and from young leaves of mature plants identified a 1.8-kb mRNA (Fig. 5), which coincided in size with the insert of clone pTDC5 (1747 nucleotides).

The amino acid sequence deduced from the *C. roseus* clone showed surprising similarity to DDC from *D. melanogaster* (Fig. 3). The 39% amino acid sequence identity was present throughout the two proteins and could be extended to the predicted distribution of potential α-helices and β-sheets. This indicates that the amino acid differences between the two proteins do not significantly alter their secondary structures and suggests the importance of such conserved domains to mediate subunit assembly as well as catalytic function and substrate specificity. These observations also lead to the hypothesis that the two decarboxylases evolved from a common ancestor. In this view it is not surprising to find a high level of similarity between *C. roseus* TDC and the AMD protein, since it has been proposed that the *D. melanogaster* proteins, which also share extensive sequence similarity (35%), evolved by gene duplication (28). The sequence similarity observed between TDC, feline glutamate decarboxylase, and mouse ornithine decarboxylase also suggests an evolutionary link between these three amino acid decarboxylases.

Most decarboxylases require for their activity a pyridoxal phosphate cofactor linked to the ε-amino group of a lysine residue. The observed similarities around the pyridoxal

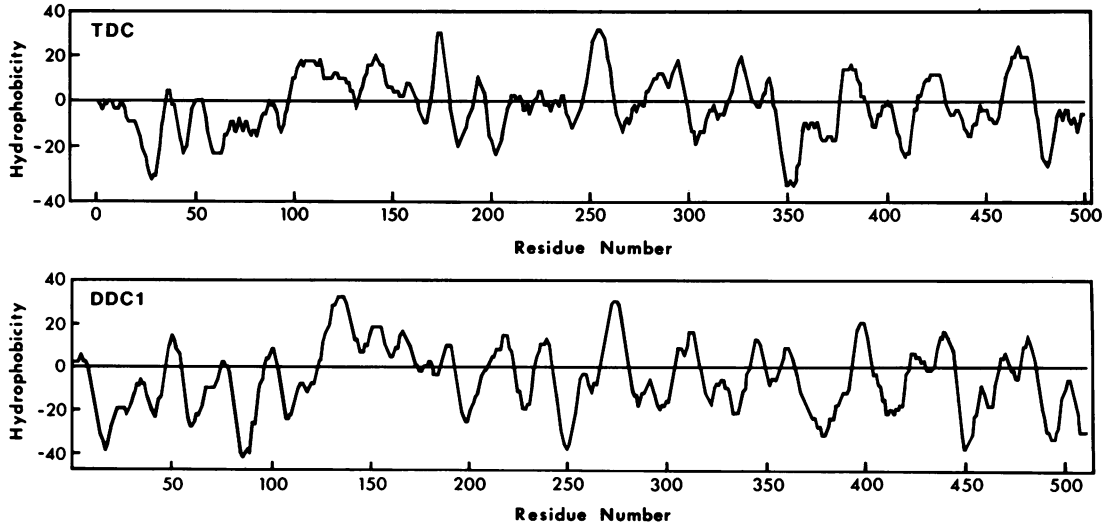

FIG. 4. Hydropathy profile of TDC and DDC1. Each value was calculated as the average hydropathic index of a sequence of nine amino acids and plotted to the middle residue of each sequence. Positive and negative values indicate hydrophobic and hydrophilic regions of the proteins, respectively (33).

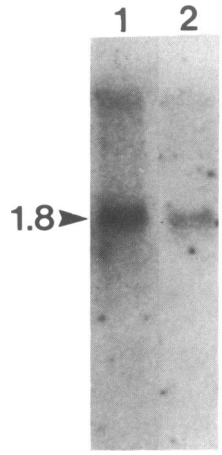

FIG. 5. TDC mRNA accumulation. Total poly(A)$^+$ RNA (1 μg per lane) from 6-day-old *C. roseus* seedlings and from young leaves of mature plants were electrophoresed in an agarose/formaldehyde gel and transferred to nitrocellulose paper. The hybridization probe was ^{32}P-labeled pTDC5 insert (specific activity, 1.2×10^8 cpm/μg). Lane 1, 6-day-old etiolated seedlings; lane 2, young leaves of mature plants. Size of the major hybridizing RNA (1.8 kb) is indicated.

phosphate binding site of pig kidney DDC, *D. melanogaster* DDC, and feline glutamate decarboxylase with that of periwinkle TDC strongly suggests that lysine-319 of TDC binds pyridoxal phosphate.

The aromatic amino acid decarboxylases of plants, insects, and mammals are remarkably similar in subunit structure, molecular mass, and kinetic properties (34). Plant aromatic amino acid decarboxylases (7–9), in contrast to those from animals, display high substrate specificity for indole or phenol substrates but not for both. The strong similarity observed between periwinkle TDC and DDC1 of *D. melanogaster* suggests that plant aromatic amino acid decarboxylases specific for tyrosine, phenylalanine, or dihydroxyphenylalanine (dopa) may be structurally similar to TDC and may, therefore, also be evolutionarily related. The recent purification of specific L-tyrosine decarboxylases (9) to homogeneity should allow the cloning of these genes and a direct test of this hypothesis.

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