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# Molecular cloning and sequencing of a cDNA of rat dopa decarboxylase: Partial amino acid homologies with other enzymes synthesizing catecholamines

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ABSTRACT **Dopa decarboxylase (DDC; aromatic-L-amino-acid decarboxylase; aromatic-L-amino-acid carboxy-lyase, EC 4.1.1.28) was purified from rat liver and its partial sequence was determined. Synthetic oligonucleotides were used to construct and screen rat liver cDNA libraries, and three clones were isolated and sequenced. The 2 kilobases of DDC cDNA cloned consisted of a 5′-noncoding segment of 78 nucleotides, a coding region of 1440 nucleotides, and a 3′-noncoding region of 438 nucleotides. The encoded protein of 480 amino acid residues had a molecular weight of 54,000. A special feature of the primary structure of rat DDC was a repeating structure consisting of 29 amino acid residues. A sequence of 58 amino acid residues, including this repeating structure of rat DDC, was found to show homologies with those of rat tyrosine hydroxylase, human dopamine β-hydroxylase, and bovine phenylethanolamine *N*-methyltransferase, other mammalian enzymes that synthesize catecholamines. These results indicate that catecholamine biosynthetic enzymes are structurally related and suggest that their homologous domains are important for catechol–protein interactions.**

The biosynthetic pathway of catecholamines has been well established and four enzymes—tyrosine hydroxylase [TH; tyrosine 3-monooxygenase; L-tyrosine, tetrahydropteridine: oxygen oxidoreductase (3-hydroxylating), EC 1.14.16.2], dopa decarboxylase (DDC; aromatic-L-amino-acid decarboxylase; aromatic-L-amino-acid carboxy-lyase, EC 4.1.1.28), dopamine β-hydroxylase [DBH; dopamine β-monooxygenase; 3,4-dihydroxyphenethylamine, ascorbate:oxygen oxidoreductase (β-hydroxylating), EC 1.14.17.1], and phenylethanolamine *N*-methyltransferase (PNMT; *S*-adenosyl-L-methionine:phenylethanolamine *N*-methyltransferase, EC 2.1.1.28)—are known to be involved in biosynthesis of epinephrine from tyrosine. Recently, three of these enzymes (all but DDC) were cloned (1–3) and it became possible to investigate the regulation of these steps of catecholamine synthesis at the molecular level. DDC catalyzes decarboxylation not only of dopa to dopamine but also of 5-hydroxytryptophan to serotonin (4–7), and it is the sole enzyme necessary in both catecholamine and indoleamine biosynthesis. The enzyme is found in neural tissues and also in peripheral organs, especially liver and kidney. But there are no reports concerning the regulation of DDC expression in neural tissue, nor is it known why high activity of DDC is expressed in liver and kidney. Recently, the DDC gene of *Drosophila* was cloned and sequenced (8), but in *Drosophila* >90% of the DDC is concerned with cuticle sclerotization (9). We have purified rat liver DDC, obtained polyclonal and monoclonal antibodies to it, and determined some of its enzymological properties (10). We have also studied the difference between rat DDC and rat histidine decarboxylase (L-histidine carboxy-lyase, EC 4.1.1.22), which synthesizes histamine (11). Molecular cloning and sequencing of rat DDC cDNA should be useful in understanding the regulation of the mammalian DDC gene, the relationship between DDC and catecholamine biosynthetic enzymes, and the mechanism of decarboxylation. However, there are no reliable data on the amino acid sequence of mammalian DDC. Accordingly, we determined the partial amino acid sequence of rat DDC and synthesized oligodeoxyribonucleotides to screen and construct rat liver cDNA libraries.

Here, we report the sequence of the entire coding region of rat DDC mRNA and a comparison of the deduced amino acid sequence with those of rat TH, human DBH, and bovine PNMT.¶

## MATERIALS AND METHODS

**Partial Sequence Analysis of Purified Rat DDC.** Rat DDC was purified from rat liver as described (10). DDC (7.12 mg) was carboxymethylated with 0.1 M iodoacetate (12) and digested with trypsin for 24 hr. L-1-tosylamido-2-phenylethyl chloromethyl ketone-treated trypsin was added twice at a DDC/trypsin ratio of 100. The tryptic digest was fractionated by gel-permeation chromatography (Sephadex G-50, 0.6 × 150 cm) in 0.1 M ammonium bicarbonate. Three fractions (I–III) were collected and then loaded onto a reverse-phase $C_{18}$ column (8 × 150 mm; Nakarai Chemical, Kyoto, Japan) equilibrated with 0.1% trifluoroacetic acid. A Toyo Soda (Tokyo) HLC803 system was used and a linear gradient of 0–60% acetonitrile over a period of 60 min was applied at a flow rate of 1 ml/min. Seven fractions were collected and purified further by reverse-phase HPLC and sequenced with a gas-phase sequencer (model 470A; Applied Biosystems).

**Synthesis of Oligodeoxyribonucleotides.** A 41-base oligodeoxyribonucleotide (3′-TACCAGCTGATATATCGGCT-GATAGACCTGCCGTATCTCCC-5′) was synthesized according to the sequence of rat DDC (peptide II-8, Met-Val-Asp-Tyr-Ile-Ala-Asp-Tyr-Leu-Asp-Gly-Ile-Glu-Gly). A mixture of 32 oligodeoxyribonucleotides (17 bases long) was synthesized according to the amino acid sequence of Ala-Phe-Asn-Met-Asp-Pro (peptide II-7).

**Isolation of RNA.** Total RNA was extracted from rat organs by the guanidine thiocyanate cesium trifluoride procedure

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Abbreviations: DDC, dopa decarboxylase; TH, tyrosine hydroxylase; DBH, dopamine β-hydroxylase; PNMT, phenylethanolamine *N*-methyltransferase.

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¶The sequence reported in this paper has been deposited in the GenBank data base (accession no. M27716).

essentially as described by Chirgwin *et al.* (13). Poly(A) RNA was isolated on poly(U) paper (Takara Shuzo, Kyoto, Japan).

**Construction and Screening of cDNA Libraries.** An oligo(dT)-primed cDNA library was constructed by the method of Okayama and Berg (14) as described (15). Primer-extension cDNA of DDC-specific mRNA was sythesized by using a 10 $\mu$g of rat liver poly(A) RNA. Double-stranded cDNA was ligated to *Eco*RI-cut λgt11 and the cDNA library was introduced into *Escherichia coli* Y1090 (16). The 41-base-long oligodeoxyribonucleotide was labeled with T4 polynucleotide kinase and [$\gamma$-$^{32}$P]ATP. cDNA libraries were screened as described (15) except that the filters were hybridized at 50°C with 5'-end-labeled oligodeoxyribonucleotides ($2.6 \times 10^6$ cpm/pmol) in 4× SSC (1× SSC = 0.15 M NaCl/0.015 M sodium citrate)/10× Denhardt's solution (1× Denhardt's solution = 0.02% bovine serum albumin/0.02% Ficoll/0.02% polyvinylpyrrolidone)/0.1 mg of denatured *E. coli* DNA per ml for 48 hr. The filters were washed twice for 30 min with 4× SSC/0.1% $NaDodSO_4$ at room temperature and then twice with the same solution at 42°C. Candidate clones were isolated and repurified.

**DNA Sequencing.** DNA was sequenced by the M13 chain-termination method of Sanger *et al.* (17). M13 phages were introduced into *E. coli* JM109.

**Northern Blot Analysis.** Total RNA was electrophoresed in 1% agarose gel containing 2.2 M formaldehyde (18). Blot hybridization was carried out as described (15). The blots were probed with the *Eco*RI insert from clone p-12 labeled to a specific activity of $1 \times 10^8$ cpm/$\mu$g by nick-translation.

## RESULTS

**Isolation and Sequence Analysis of DDC Clones.** Nine tryptic peptides of rat DDC were purified and sequenced. The amino acid sequences of these peptides were compared with that of *Drosophila* DDC. As one peptide, II-8, showed 63% homology with the $NH_2$-terminal region of *Drosophila* DDC, a single 41-base-pair (bp) oligodeoxyribonucleotide was synthesized wherein the homology with the nucleotide sequence encoding *Drosophila* DDC was maximized. This oligonucleotide was used as a hybridization probe. We first screened $3 \times 10^5$ transformants of a rat liver cDNA library constructed with oligo(dT) primer but obtained no positive clones. So we constructed a cDNA library with primers of 17-base oligodeoxyribonucleotides deduced from the sequence of peptide II-7 (Ala-Phe-Asn-Met-Asp-Pro). On screening 60,000 plaques of this cDNA library, we obtained one positive clone (P-12) with a cDNA insert of ≈1.0 kbp. Nucleotide sequence analysis revealed that clone P-12 contained a 984-bp cDNA insert, including the coding sequence for the amino acid sequence of four tryptic peptides (II-7, II-8, III-6, I-2).

For cloning of the 3' region of DDC cDNA, this insert was nick-translated and used to screen a rat cDNA library constructed with oligo(dT) primer. About $1 \times 10^5$ transformants were rescreened, two positive clones were isolated, and one of these (O-91) was found to have a cDNA insert of 1.4 kilobases (kb). The restriction maps and strategies for sequencing the cDNA inserts of P-12 and O-91 cDNAs are shown in Fig. 1. The P-12 cDNA covered 78 nucleotides of the 5' untranslated region and 984 nucleotides of the coding region, while the O-91 cDNA contained 896 nucleotides of the coding region, 438 nucleotides of the 3' untranslated region, and 47 nucleotides of poly(A). The cDNA insert of O-91 contained a 442-nucleotide sequence in common with P-12. The $NH_2$-terminal amino acid of purified rat DDC was not determined, but the translational initiation site was assigned to the methionine codon at nucleotide residues 1–3. This is the first ATG triplet that appears downstream of the nonsense codon TAG or TGA found in the frame indicated by the asterisks in Fig. 2. This assignment is supported by the fact that the sequence surrounding the ATG triplet is consistent with the favored sequence flanking a functional initiation codon (RNAUGG) (R = purine) (19). The sequence of peptide II-7 showed that the second ATG triplet was not the initiation methionine. There is a possible polyadenylylation signal AATAAA (20) at nucleotide 1933. We have clone 2003 nucleotides of cDNA, a length that is consistent with that of DDC mRNA (2100 nucleotides; Fig. 3). From the cDNA

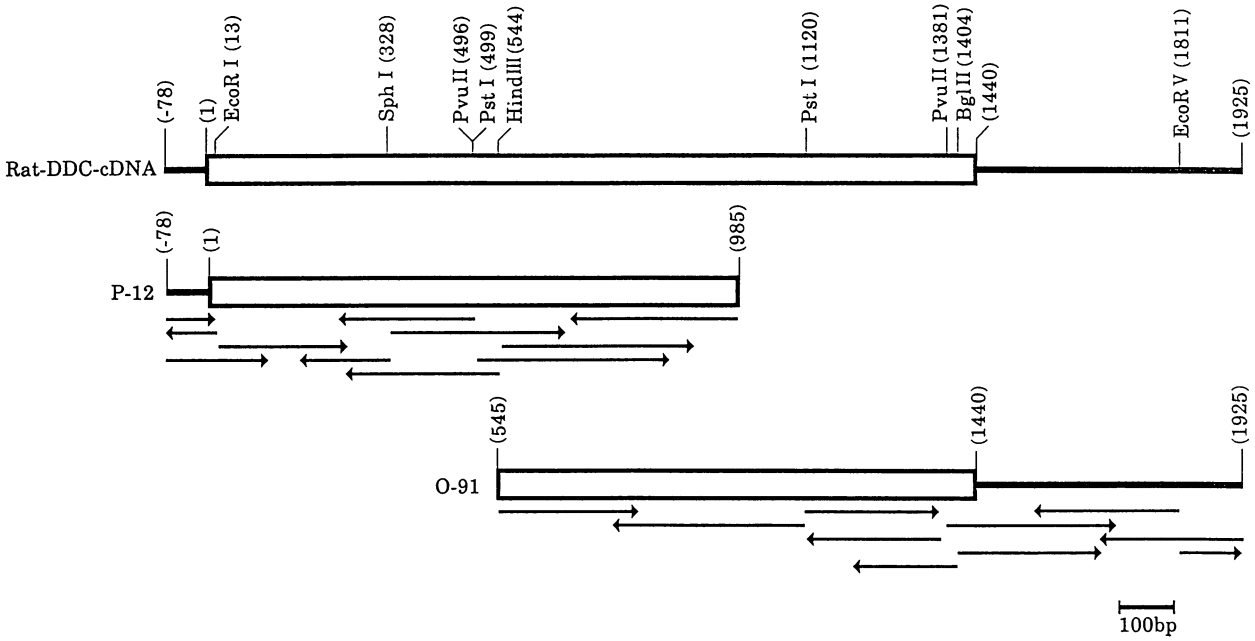


FIG. 1. Restriction maps and sequencing strategy of P-12 and O-91 inserts. Open boxes represent the protein coding region and lines represent the 5' and 3' untranslated regions. The sequencing strategy is summarized below the maps. Nucleotide residues are numbered in the 5' to 3' direction, beginning with the first residue of the ATG triplet encoding the initiating methionine. Nucleotides on the 5' side of residue 1 are indicated by negative numbers. The restriction endonuclease sites are identified by numbers indicating the 5'-terminal nucleotide generated by cleavage.

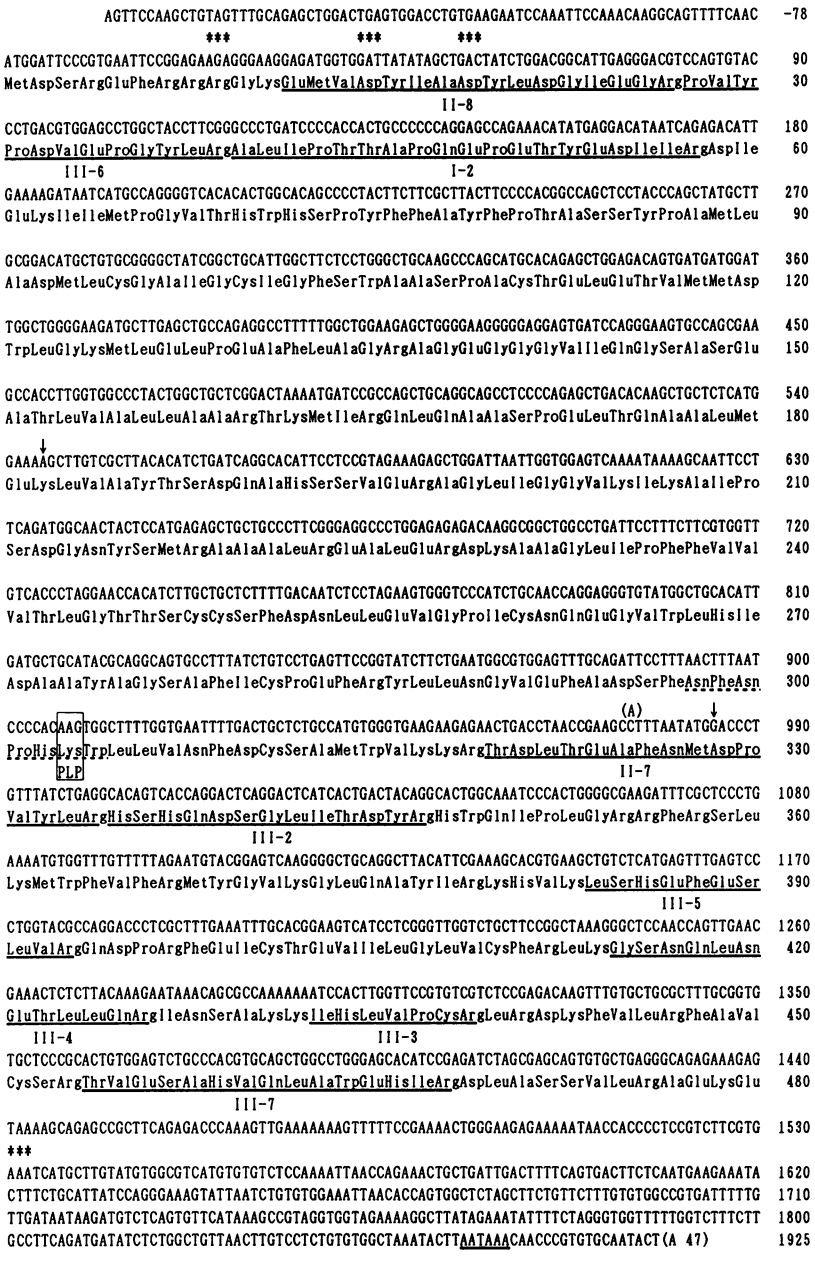

FIG. 2. Nucleotide sequence of the DDC cDNA. The nucleotide sequences of P-12 (nucleotides −78 to 985) and O-91 (nucleotides 545–1925) are combined. The 5′-end nucleotide 545 of the O-91 cDNA insert and the 3′-end nucleotide 985 of the P-12 cDNA insert are indicated by arrows. The predicted amino acid sequence is shown below the nucleotide sequence. Amino acid sequences confirmed with a peptide sequencer are underlined. The asterisks in the 5′-noncoding region are the nonsense codons described in *Results*. The polyadenylation signal is underlined. The dotted line (amino acids 298–304) indicates the pyridoxal 5′-phosphate binding region, which is completely consistent with that of pig DDC. The pyridoxal 5′-phosphate binding lysine (PLP) (residue 303) is boxed.

sequence, we conclude that the rat DDC protein is composed of 480 amino acid residues, with a calculated molecular weight of 54,049.7. This value is slightly larger than the reported molecular weight of the protein moiety of DDC (10). The partial amino acid sequence of the pyridoxal peptide of pig DDC (21) is completely consistent with that of amino acid residues 298–304 of rat DDC, indicating that Lys-303 is the pyridoxal 5′ phosphate binding site.

**Amino Acid Sequence Homology.** We searched for a specific feature in the primary structure of rat DDC and found that amino acid residues 85–112 have 34.5% homology with amino acid residues 147–175. The nucleotide sequences of the corresponding regions showed 40.2% homology. Thus, these two regions might be formed by gene duplication during evolution of the DDC gene but are not formed independently. The significance of this repeated structure in the rat DDC is unknown, but these regions seem to be essential elements of the enzyme. Therefore, we compared the amino acid sequence around these regions with those of three other mammalian enzymes synthesizing catecholamines. Surprisingly, we found some homologies in these regions of rat DDC with those of rat TH (1), DBH (2), and bovine PNMT (3) (Fig. 4). Gaps were inserted to obtain maximal alignments of the amino acid residues of rat DDC with those of these three enzymes. With this alignment, the amino acid residues 58–115 of rat DDC show 24.1% identity with those of residues 118–178. Moreover, amino acid residues 58–115 of rat DDC match residues 310–363 of rat TH in 16 of 54 positions (27.6%), and residues 58–115 of rat DDC show identities in 14 of 54 positions (24.1%) with both residues 345–402 of human DBH and residues 44–101 of bovine PNMT. When the conservative amino acids substituted (22) are included (hatched boxes in Fig. 4), the homologies increase to 45%, 38%, 40%, and 40% between rat DDC 58–115 and the other four sequences, respectively.

## DISCUSSION

Using an oligodeoxyribonucleotide probe and primers, in this work we cloned and sequenced a cDNA that encodes DDC from rat liver. The $NH_2$-terminal amino acid sequence of purified DDC has not been determined and its $NH_2$ terminus may be blocked. But we found a codon for the initiation methionine at nucleotides 1–3. The DDCs of *Drosophila* and

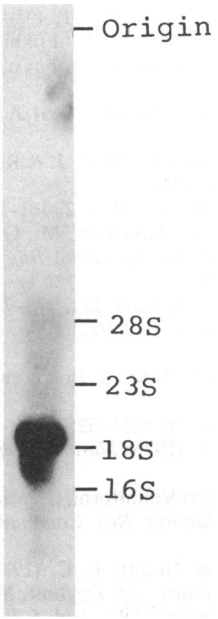


FIG. 3. Autoradiogram obtained by Northern blot analysis of rat liver RNA with a rat DDC cDNA probe. Fifty micrograms of total rat liver RNA was analyzed as described in *Materials and Methods*.

mammals are remarkably similar in subunit structure, molecular weight, kinetic properties, and substrate specificities (23). The observation that *Drosophila* DDC gave only a single precipitin line in the double-diffusion immunoprecipitation test has been considered evidence for the existence of a single molecular form of *Drosophila* DDC (24), but, recently, two distinct *Drosophila* DDC protein isoforms formed by the differential splicing have been predicted from results on cloning of its gene (5). The two *Drosophila* DDCs differ only in their $NH_2$-terminal regions, with other portions being identical. The $NH_2$-terminal region of rat DDC has no homology with those of *Drosophila* DDCs, but the rest of rat DDC shares 58% homology with the common regions of *Drosophila* DDCs (Fig. 5). So we suggest that the translational initiation site of *Drosophila* DDC exists in the second AUG triplet, located in the $NH_2$-terminal part of the common region. Moreover, the sequence around the first AUG triplet of *Drosophila* DDC is not consistent with that of a favored sequence flanking a functional initiation codon (16), whereas that of the second AUG triplet is consistent with that of a favored sequence. If this is so, *Drosophila* DDC is also a one gene/one protein enzyme like mammalian DDCs (25). We also compared our result with other pyridoxal 5′-phosphate-dependent enzymes, including mammalian ornithine decarboxylase (26) and bacterial histidine decarboxylase (27), but we could not find any significant homology; it should be noted, however, that rat DDC also contained a glycine-rich

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R:MDSREFRRRGKEMVDYIADYLDGIEGRPVYPDVEPGYLRALIPTT  45
D: EAP  KDFA T   F  E  EN RE R L E K    KP   DA

 APQEPETYEDIIRDIEKIIMPGVTHWHSPYFFAYFPTASSYPAML  90
   EK  KWQ VMQ   RV           K H      N    YV

 ADMLCGAIGCIGFSWAASPACTELETVMMDWLGKMLELPEAFLAG 135
    S   A   T I         V             AE   C

 RAGEGGGVIQGSASEATLVALLAARTKMIRQLQAASPELTQAALM-180
 SG K       T   S    SAGSQGQEVEGGEG PSGVGLEHTIL

 EKLVAYTSDQAHSSVERAGLIGGVKIKAIPSDGNYSMRAAALREA 225
 G   G C             L    LRSVQ -E HR  G   EK

 LERDKAAGLIPFFVVVTLGTTSCCSFDNLLEVGPICNQEGVWLHI 270
 I Q V E     YA       NS A  Y D C  VG KHNL I V

 DAAYAGSAFICPEFRYLLNGVEFADSFNFNPHKWLLVNFDCSAMW 315
              Y H MK I S           M

 VKKRTDLTEAFNMDPVYLRHSHQDSGLITDYRHWQIPLGRRFRSL 360
 L DPSWVVN   V  L  K DM G AP--               A

 KMWFVFRMYGVKGLQAYIRKHVKLSHEFESLVRQDPRFEICTEVI 405
  L   L L   EN   H  R CNFAKQ GD CVA S   LAA -

 L-GLVCFRLKGSNQLNETLLQRINSAKKIHLVPCRLRDKFVLRFA 450
 NM   S       ER  A  K   GRGH     AKIK VYG  M

 VCSRTVESAHVQLAWEHIRDLASSVLRAEKE-               480
 I   FTQ EDMEYS KEVS-- AADEM- Q Q
```

FIG. 5. Comparison of amino acid sequence (single-letter code) of rat DDC and that of *Drosophila* DDC. Only unmatched amino acid residues are shown in *Drosophila*. To obtain the highest homology, some gaps (–) were inserted in *Drosophila* DDC.

region, which is suggested by Marceau *et al.* (28) to interact with cofactor in pyridoxal 5′-phosphate enzymes and nucleotide binding enzymes.

Rat DDC has repeating regions in its primary structure. Amino acid residues 58–115 match residues 118–178 in 14 of 61 positions (24.1%) and regions including these 61 amino acid residues show homologies with the sequences of catecholamine-synthesizing enzymes (Fig. 4). *Drosophila* DDC also has a repeating structure, and the amino acid sequence of residues 154–177 shows matches of 6 of 24 amino acids (25.0%) with the corresponding sequence of residues 58–115 of rat DDC. Moreover, amino acid residues 58–115 of rat DDC show 79.3% homology with the corresponding amino acid residues of *Drosophila* DDC. This degree of homology is higher than that of the overall homology between rat and *Drosophila* DDCs (58%), suggesting that residues 58–115 are essential for enzyme function. Some homologies between bovine PNMT and rat TH have been reported (20), but we examined the homologies of different regions from those

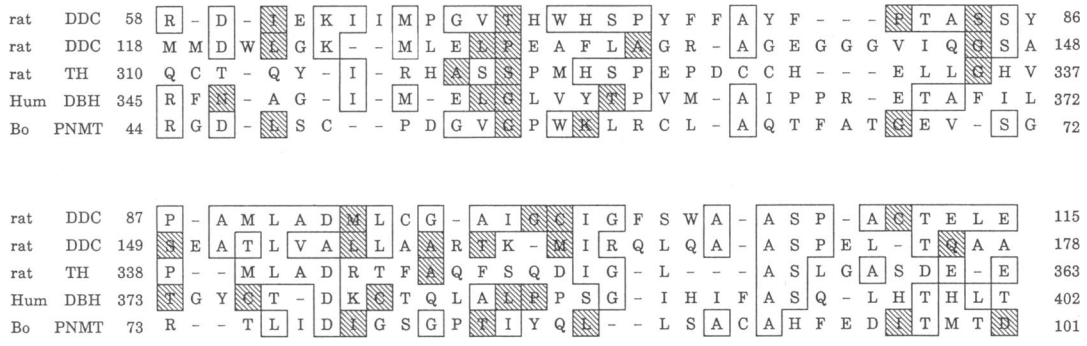


FIG. 4. Comparison of amino acid sequences of catecholamine-synthesizing enzymes. Amino acid residues 58–115 of rat DDC are compared with residues 118–178 of rat DDC, residues 310–363 of rat TH, residues 345–402 of human DBH, and residues 44–101 of bovine PNMT. Amino acids are indicated by the single-letter code. Gaps (–) have been inserted to achieve maximum homology. Identical residues and conserved residues with residues 58–115 of rat DDC are shown in open boxes and hatched boxes, respectively.

reported previously. Residues 310–363 of rat TH, which show 27.6% homology with those of rat DDC, are completely conserved in human TH (29). The complete conservation of this region in THs suggests that this region is essential for enzyme activity. When TH is treated with chymotrypsin, the resulting protein with a molecular weight of 34,000 possesses TH activity (30) and is reported to contain residues 310–363 (19). This observation is consistent with the view that this region is concerned with TH activity. These results suggest that the regions of the four catecholamine-synthesizing enzymes that show homology may be important for catechol–protein interaction. It has been suggested that all genes of catecholamine-synthesizing enzymes originated from a common ancestral gene (31). Our results clearly show the common origins of at least restricted regions of catecholamine-synthesizing enzymes.

The significance of the repeating structure in rat DDC is not known. Studies on the expression and site-directed mutagenesis of a cloned rat DDC cDNA should provide information on the function of the repeating structure.

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