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Functionally Important Residues of Aromatic L-Amino Acid Decarboxylase Probed by Sequence Alignment and Site-Directed Mutagenesis

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To identify functional residues of rat liver L-aromatic amino acid decarboxylase (AADC), we aligned the sequences of 13 group II amino acid decarboxylases and performed mutational analysis on the residues that were invariant or conservatively substituted. Replacements of His192, Asp252, Asp271, Ser296, Lys303, Tyr332, and Arg355 with alanine residues decreased the AADC activity (k_{cat}/K_m) by more than 10^4-fold. Conservative replacements of [Asp252→Glu], [Lys303→Arg], and [Tyr332→Phe] also resulted in decreases in activity by more than 10^4-fold, indicating that both the chemical properties and the shape of these residues are essential for catalysis. The presence of a Schiff base between the amino group of Lys303 and the coenzyme pyridoxal 5′-phosphate is important for catalysis, probably at the transaldimination step. The enzyme activity was essentially unaffected by conservative mutation of [Arg355→Lys], showing that the presence of a basic group at position 355 is necessary and sufficient for the catalysis. Replacement of [Thr246 →Ala], [His269→Ala], and [Trp363→Leu] yielded mutant enzymes that were 1-8% as active as the wild-type enzyme; these residues are not essential for the catalysis but are considered to contribute to the activity through conformational or other effects. The roles of the catalytically important residues of the group II amino acid decarboxylases probed in this study were discussed in the light of their relationship with the residues of other pyridoxal enzymes.

Key words: active site, amino acid decarboxylase, evolution, pyridoxal 5′-phosphate, site-directed mutagenesis.

Pyridoxal 5′-phosphate (PLP)-dependent enzymes have important roles in amino acid metabolism (*1*). PLP itself is a versatile catalyst that can catalyze almost all of the reactions of PLP enzymes, such as transamination, decarboxylation, β- and γ-elimination, racemization, aldol cleavage *etc.* (*1*). In the presence of an enzyme protein, however, PLP usually catalyzes only one type of reaction and shows substrate specificity. It is thus of great interest to know how the active site architecture of a group of PLP enzymes can provide a microenvironment suitable for a specific type of reaction. The mechanism and action of aminotransferases have been well documented (*2, 3*). Decarboxylases form a large group of comparable size to that of aminotransferases. The critical action of decarboxylases is to cleave the C-C bond between the α-carbon atom and the carboxyl carbon atom, and in this respect decarboxylases differ from most other PLP enzymes, which act primarily on the C(α)-H bond. Despite this intriguing feature of their catalytic action, the reaction mechanism of decarboxylases is poorly understood, mainly because the amounts of decarboxylases that can be obtained are too small to be used for detailed physicochemical characterization, including X-ray crystallography. Recent advances in genetic manipulation, however, have allowed the high-level expression of both prokaryotic (*4*) and eukaryotic (*5*) decarboxylase proteins in bacterial cells. This in turn has allowed the determination of a large number of amino acid sequences of decarboxylases, most of which belong to group II in the classification of Sandmeier *et al.* (*6*, for a list of the amino acid sequences, see the references therein). Multiple alignment of the sequences of the group II decarboxylases has revealed that a limited number of residues are invariant (*7*, this study). Although the three-dimensional structures of the group II decarboxylases are yet to be determined, we consider that these invariant residues have important roles in the structure/function of the decarboxylases.

Aromatic L-amino acid decarboxylase (AADC) is the best characterized of the amino acid decarboxylases (*5, 8*). An expression system of the rat liver enzyme in *Escherichia coli* has already been constructed (*5*), and this enzyme is

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Abbreviations: AADC, aromatic L-amino acid decarboxylase (aromatic L-amino acid carboxy-lyase, EC 4.1.1.28); GluDC, glutamate decarboxylase (L-glutamate 1-carboxy-lyase, EC 4.1.1.15); HisDC, histidine decarboxylase (L-histidine carboxy-lyase, EC 4.1.1.22); K303A AADC, AADC in which the residue Lys303 is replaced by an Ala residue (other mutant AADCs are expressed in the same way); PIPES, 1,4-piperazine-bis(ethanesulfonic acid); PLP, pyridoxal 5′-phosphate; WT AADC, wild-type AADC.

thus suitable for mutational analysis on the function of the group II decarboxylases. In this study, we multiply aligned the sequences of the group II decarboxylases with known sequences, performed systematic mutation on the residues of rat liver AADC which are invariant or conservatively substituted among these enzymes, and studied the effects of these mutations on the catalytic function of AADC.

MATERIALS AND METHODS

Materials—Enzymes for DNA manipulations and pTV118N were obtained from Takara Shuzo (Kyoto). PIPES and HEPES were from Dojindo Laboratories (Kumamoto); L-dopa was from Nacalai Tesque (Kyoto). All other chemicals were of the highest grade commercially available. *E. coli* strain MD55, which lacks tryptophanase [L-tryptophan indole-lyase (deaminating), EC 4.1.99.1], was a generous gift from Dr. Yasushi Kawata, Tottori University. The medium used for the bacterial growth contained 0.5% yeast extract (Oriental Yeast, Tokyo), 1% Polypepton (Nihon Pharmaceutical, Tokyo), and 0.5% NaCl at pH 7.4.

Preparation of the Wild-Type and Mutant AADCs—Plasmid pKKAADCII (*5*) was used to express the wild-type AADC in *E. coli* cells. pKKAADCII comprises the prokaryotic expression vector pKK233-2 containing in its *Nco*I-*Hin*dIII site the AADC cDNA with the nucleotide sequence just downstream of the initiation codon being modified for increased expression level without changing the N-terminal amino acid sequence. The *Nco*I-*Hin*dIII fragment of pKKAADCII, containing the entire coding frame of AADC, was inserted into the *Nco*I-*Hin*dIII site of pTV118N. The resultant plasmid, denoted pTVAADCII, was used to transform *E. coli* JM103 cells. Single-stranded pTVAADCII DNA was recovered from the transformed JM103 cells in the presence of helper phage M13KO7. The single-stranded pTVAADCII DNA was used for site-directed mutagenesis of the AADC cDNA. Mutagenesis reactions were performed with oligonucleotides designed to induce single amino acid substitutions at target residues using the Sculptor™ *in vitro* mutagenesis system (Amersham, Buckinghamshire, UK). The resultant double-stranded mutant pTVAADCII DNAs were cut with *Apa*I and *Xba*I, and the *Apa*I-*Xba*I fragments, which contain almost all of the coding frame of AADC, were ligated back to pKKAADCII. The individual mutant pKKAADCII plasmids were verified by sequencing the entire coding frame on an Applied Biosystems DNA sequencer model 470A.

Measurement of Enzyme Activity in E. coli Cell Lysates—*E. coli* MD55 cells were transformed with the wild-type and mutant AADC expression vectors. We changed the host *E. coli* strain from JM109 (*5*) to MD55, because the expression level of AADC in MD55 cells was 3-5-fold higher than that in JM109 (data not shown), although the relation of the lack of tryptophanase to the enhanced expression level is unclear at present. A single colony of transformed cells was inoculated into 3 ml of growth medium in the presence of 50 μg/ml ampicillin and incubated for 16 h at 37°C. The cells were transferred into 3 ml of the same medium and incubated for 6.5 h at 37°C. The cells were recovered by centrifugation and the resultant pellets were suspended in 100 μl of 50 mM Tris-HCl buffer, pH 8.0, containing 25% sucrose and 10 mM EDTA. Then 2.5 μl of 10% lysozyme and 25 μl of 0.5 M EDTA, pH 8.0, were added. The mixture was placed on ice for 30 min, then 125 μl of 50 mM Tris-HCl, pH 8.0, containing 1% polyoxyethylene (*20*) cetyl ether, 0.4% sodium deoxycholate, and 62.5 mM EDTA was added. The resultant mixture was placed on ice for 30 min and centrifuged at 10,000 $\times g$ for 10 min to remove precipitant. The AADC activities in the supernatant solutions were measured as follows. The reaction mixture (1 ml) contained 50 mM PIPES-NaOH, pH 7.0, 10 μM Na-EDTA, 5 μM PLP, 1 mM L-dopa as a substrate, 0.1 mM pargyline hydrochloride as a monoamine-oxidase inhibitor, and the enzyme. The reaction was started by adding 25 μl of *E. coli* lysate, allowed to proceed at 25°C for 30 min, then stopped by addition of 0.2 ml of 25% (w/v) perchloric acid. Then 0.5 ml of 0.5 M potassium phosphate buffer, pH 7.5, was added, and the mixture was placed on ice for 5 min to precipitate potassium perchlorate. The mixture was centrifuged, and aliquots of the supernatant were analyzed for the amount of dopamine on an HPLC system LC-6A (Shimadzu, Kyoto) equipped with an electrochemical detector model CB-100 (Eicom, Kyoto) according to the method of Miwa *et al.* (*9*) with minor modifications. Liquid chromatography was performed on a Cosmosil C_{18} Econopak column (4.6 mm $\times$ 150 mm, Nacalai Tesque) with a mobile phase of 7% (v/v) methanol, 0.1 M potassium phosphate, 0.1 mM Na-EDTA, and 0.03% (w/v) sodium heptanesulfonate, pH 3.1. The detector potential was set to 0.7 V relative to the Ag/AgCl reference electrode. The peak area was measured and the amount of dopamine was calculated by comparing it with the peak area of the standard compound.

Measurement of the Activity of the Purified AADC—The enzyme was purified as described previously (*5*). The assay conditions were the same as described above for the activity measurement of the *E. coli* cell lysates. The concentration of the enzymes in the reaction mixture was 10 nM (wild type, K317A, K317R, R355A, and W363F) or 200 nM (T246A). The rate of dopamine formation was determined for substrate concentrations between 0.01 and 1 mM.

Protein Concentration—The concentration of the purified AADCs was determined spectrophotometrically using a molar extinction coefficient of $\varepsilon_M = 79{,}000\ M^{-1} \cdot cm^{-1}$ at 280 nm (*5*). H269A and D271E AADCs could not be purified to homogeneity. Therefore, the partially purified enzymes were subjected to 10% SDS-PAGE and transferred to a Immobilon™-P^{SQ} membrane (Millipore, Bedford, Massachusetts, USA) using a Saltblot® II (Saltorius, Göttingen, Germany) semi-dry blotting apparatus. Blots were probed with rabbit anti-rat AADC antibody, followed by alkaline phosphatase-conjugated goat anti-rabbit IgG antibody (Cappel). Band intensity was analyzed using NIH Image (ver. 1.59), and the amounts of H269A and D271E AADCs were estimated by comparison with the bands of known amounts of WT AADC.

Identification of the Lysine Residue That Binds Pyridoxal 5′-Phosphate—Two hundred micrograms of the wild-type (WT) AADC (holoenzyme) was reduced with 3 mg of sodium borohydride in 0.5 ml of 50 mM PIPES-NaOH buffer, pH 7.0, for 30 min at 25°C. The solution was desalted by passing through a Sephadex G-25 (medium) column (15 $\times$ 50 mm) equilibrated with H_2O, and was concentrated to 0.2 ml. To the solution was added 1.8 ml of

0.5 M Tris-HCl, pH 8.5, containing 7 M guanidine hydrochloride, 20 μl of 1 M DTT, and 41.4 μl of 1 M iodoacetate (neutralized with NaOH). The mixture was incubated for 1 h at 37°C, then dialyzed against 500 ml of water (6 h×3), and lyophilized. The lyophilized material was suspended in 0.5 ml of 0.1 M Tris-HCl, pH 8.0, containing 1 mM $CaCl_2$. Then 10 μl of 0.2 mg/ml TPCK-treated trypsin was added, and the mixture was incubated at 37°C for 1 h. The mixture became clear at the end of the digestion. The digestion was stopped by adding 50 μl of 1 M HCl, and the solution was applied to a Cosmosil C_{18} Econopak column (4.6 mm×150 mm, Nacalai Tesque, Kyoto) equilibrated with 0.1% trifluoroacetic acid. Peptides were eluted by increasing the acetonitrile concentration in the mobile phase from 0 to 40% in 40 min, then from 40 to 80% in 10 min at a flow rate of 1 ml/min. Peptide peaks were monitored at 210-400 nm with 2-nm intervals using a Beckman 168 diode array detector. The WT AADC protein (200 μg) was also treated and analyzed in the same way, without the reduction by sodium borohydride. Comparison of the elution pattern of the tryptic peptides of the $NaBH_4$-reduced WT AADC and the nonreduced WT AADC allowed identification of the tryptic peptide containing the phosphopyridoxyllysine residue.

RESULTS

Multiple Sequence Alignment of the Group II Decarboxylases—Sequence alignments of the group II PLP-dependent amino acid decarboxylases gave many deletions and insertions (7). This is partly because the sequences of the group II decarboxylases vary in size ranging from as little as 377 residues of the *Morganella morganii* histidine decarboxylase (HisDC) to as much as 593 residues of rat glutamate decarboxylase (GluDC). We surveyed amino acid sequences from 13 species of group II amino acid decarboxylases and found that the region between Ser188 and Phe411 is highly conserved among the decarboxylases with the exceptions of *E. coli* and petunia GluDCs. This region, which we named the "core region," starts with the sequence of S[D/E/K][Q/D][A/T/S]H[S/Y/T]X[I/L/V] and ends with the sequence of V[C/V]F. Based on this, we performed multiple sequence alignment on the core region sequences of the decarboxylases (Fig. 1). A rooted dendrogram derived from this alignment using the UPGMA method (*10*) is shown in Fig. 2. Figure 2 also shows the schematic diagram of the entire primary structures of the enzyme proteins, which were adjusted to coincide at the core region. The length of the C-terminal region did not vary greatly among the group II decarboxylases (usually 50-100 residues). The length of the N-terminal region was more variable and was related to the phylogeny of the enzyme. Decarboxylases acting on aromatic amino acids (AADCs, tryptophan decarboxylase, tyrosine decarboxylases), animal HisDCs, and *Drosophila melanogaster* GluDC have 187-222 residues. Rat GluDC has a larger N-terminal region (295 residues). On the other hand, HisDCs and GluDCs from plants and bacteria had apparently shorter N-terminal residues (114-123 for HisDCs and 156 for GluDCs). Figure 2 indicates the following phylogenic relationship between the group II decarboxylases. Aromatic amino acid decarboxylases and animal HisDCs are closely related to each other and form one subgroup; animal GluDCs are distant from this subgroup but are closer to the latter than are the plant and bacterial HisDCs and GluDCs. The plant and bacterial HisDCs are closer to the former decarboxylases than are the plant and bacterial GluDCs.

Alignment of the core region (Fig. 1) clearly showed the presence of a number of invariant or conservatively substituted residues. Among them, residues with functional groups in their side chain are of special interest because these functional groups may be involved in the catalytic reaction of the group II decarboxylases. These residues are Thr246, His269, Asp271, Ser296, Lys303 (invariant residues), and Asp252, Lys317, Tyr332, Arg355, Trp363 (conservatively substituted residues). To analyze the structural requirement of the group II decarboxylases for catalyzing decarboxylation of amino acid substrates, the roles of these residues were investigated by site-directed mutagenesis on the residues.

Site-Directed Mutagenesis Studies—The above amino acid residues were replaced by alanine residues, except for Trp363, which was replaced by a leucine residue. For Asp252, Asp271, Lys303, Lys317, Tyr332, Arg355, and Trp363, conservative replacements (Glu for Asp, Arg for Lys, Phe for Tyr, Lys for Arg, and Phe for Trp) were additionally performed. The H192A mutant enzyme was also constructed (for the catalytic significance of the residue His192, see "DISCUSSION"). The resultant mutant enzymes were expressed in *E. coli* cells. The levels of expression were measured by immunoblotting analysis, and were similar to that of the wild-type enzyme (data not shown). Ten mutant enzymes, H192A, D252E, D252A, D271A, S296A, K303R, K303A, Y332F, Y332A, and R355A, did not show measurable activities (Table I), even in the presence of excess concentrations of substrate (10 mM L-dopa) or coenzyme (500 μM PLP) (data not shown). Mutant enzymes which showed activity in the *E. coli* cell lysates were purified and their kinetic parameters were determined (Table I). K317A, K317R, R355K, and W363F showed similar kinetic properties to the wild-type enzyme. T246A, H269A, and D271E had significantly lower k_{cat} values, being 0.7% (T246A), 2.8% (H269A), and 0.1% (D271E) of that of the wild-type enzyme, but had K_m values similar to that of the wild-type enzyme. The W363L mutant enzyme could not be purified to homogeneity, probably due to its low stability. The activity of W363L in *E. coli* cell lysate was 1% of the wild-type enzyme, while their expression levels were almost the same. Therefore, the substitution of Leu for Trp363 is considered to decrease significantly but not abolish the catalytic ability of AADC.

Lys303 Is the Residue That Binds PLP through an Azomethine Linkage—Tryptic digests of the $NaBH_4$-reduced and nonreduced WT AADCs were subjected to reversed-phase HPLC analysis (Fig. 3). A peak with a retention time of 44.3 min (peak 3) was found in the digest of the $NaBH_4$-reduced AADC, but not in the digest of the nonreduced AADC (Fig. 3A). This peak showed absorption at wavelengths higher than 300 nm (Fig. 3B), reflecting the presence of the coenzyme covalently attached to the peptide by $NaBH_4$ reduction. Two peaks with retention times of 37.4 min (peak 1) and 43.0 min (peak 2) were found in the digest of the nonreduced AADC, but these peaks exist in small amounts in the digest of the $NaBH_4$-reduced AADC. N-terminal sequence analysis showed that

the peak 1 and peak 3 peptides start with the amino acid sequence YLLNG-, and the peak 2 peptide starts with WLLVN-. In the primary structure of rat AADC protein, the residue preceding the sequence WLLVN- is Lys303 (*11*). These results clearly show that PLP is firmly bound to Lys303 by $NaBH_4$-reduction and the Lys303(PLP)-Trp304 peptide bond becomes resistant to tryptic digestion, whereas PLP is weakly bound to Lys303 unless it is reduced with $NaBH_4$ and trypsin digestion yielded two peptides (peaks 1 and 2) by the cleavage of the Lys303-Trp304 peptide bond. Therefore, we can conclude that PLP is attached to Lys303 *via* an azomethine linkage formed between the aldehyde group of PLP and the ε-amino group of Lys303. Comparison of the peak height of the peak 1 and 2 peptides of the $NaBH_4$-reduced AADC tryptic digest with those of the nonreduced one shows that 80–86% of Lys303 binds PLP.

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                 190        200        210        220        230        240
Aro Rat          SDQAHSSVERAGLIGGV---KIKAIPS--DGNYSMRAAALREALERDKAAGLIPFFVVVTL
Aro Drosophila   SDQAHSSVERAGLLGGV---KLRSVQS--E-NHRMRGAALEKAIEQDVAEGLIPFYAVVTL
His Rat          SDQAHSSVEKAGLISLV---KIKFLPV--DDNFSLRGEALQKAIEEDKQQGLVPVFVCATL
His Drosophila   SDQAHSSVEKAALIGLV---RMRYIEA--DEDLAMRGKLLREAIEDDIKQGLVPFWVCATL
Trp Perwinkle    SDQTHTMFPKTCKLAGIYPNNIRLIPTTVETDFGISPQVLRKMVEDDVAAGYVPLFLCATL
Tyr Parsley      SDQTHSALQKAAKIAGIDPKNFRAIETTKSSNFQLCPKRLESAILHDLQNGLIPLYLCATV
Tyr Poppy        SDQTHCALQKAAQVAGINPKNFRAIKTFKENSFGLSAATLREVILEDIEAGLIPLFVCPTV
Glu Rat          SEHSHYSIKKAGAALGFGTDNVILIKCNERGK--IIPADLEAKILDAKQKGFVPLYVNATA
Glu Drosophila   SDQCHYSIKSCAAVCGLGTDHCIVVPSDEHGK--MITSELERLILERKAKGDIPFFVNATA
His Tomato       SKDSHYSIFKAARMYRM---ELQTINTLVNGEIDY--EDLQSKLLVNKNKPAI---ININI
His Morganella   SKDTHYSVAKIVKLLRI---KSQVVESQPNGEIDY--DDLMKKIADDKEAHPI---IFANI
Glu Petunia      IVTGANVQVCWEKFARY--FEVELKEVKLSEGYYV--MDPEKAVEMVDE-NTIC--VAAIL
Glu E. coli      LVCGP-VQICWHKFARY--WDVELREIPMRPGQLF--MDPKRMIEACDE-NTIG--VVPTF
                                                      +          +

                      250        260        270        280        290
Aro Rat          GTTSCCSFDNLLEVGPICNQEGV------WLHIDAAYAGS--AFICPEFRY-LL-NGVEFA
Aro Drosophila   GTTNSCAFDYLDECGPVGNKHNL------WIHVDAAYAGS--AFICPEYRH-LM-KGIESA
His Rat          GTTGVCAFDKLSELGPICAREGL------WLHVDAAYAGT--AFLRPELRG-FL-KGIEYA
His Drosophila   GTTGSCSFDNLEEIGIVCAEHHL------WLHVDAAYAGS--AFICPEFRT-WL-RGIERA
Trp Perwinkle    GTTSTTATDPVDSLSEIANEFGI------WIHVDAAYAGS--ACICPEFRH-YL-DGIERV
Tyr Parsley      GTTSSTTVDPLPALTEVAKKYDL------WVHVDAAYAGS--ACICPEFRQ-YL-DGVENA
Tyr Poppy        GTTSSTAVDPISPICEVAKEYEM------WVHVDAAYAGS--ACICPEFRH-FI-DGVEEA
Glu Rat          GTTVYGAFDPIQEIADICEKYNL------WLHVDAAWGGG--LLMSRKHRH-KL-SGIERA
Glu Drosophila   GTTVLGAFDDINTIADICQKYNC------WMHIDAAWGGG--LLMSRTHRHPRF-TGVERA
His Tomato       GTTFKGAIDDLDFVIQTLENCGYSND-NYYIHCDRALCGLILPFIKHAKKITFK-KPI---
His Morganella   GTTVRGAIDDIAEIQKRLKAAGIKRE-DYYLHADAALSGMILPFVDDAQPFTFA-DGI---
Glu Petunia      GSTLNGEFEDVKRLNDLLVEKNKETGWDTPIHVDAASGGFIAPFIYPELEWDFRLPLV---
Glu E. coli      GVTYTGNYEFPQPLHDALDKFQADTGIDIDMHIDAASGGFLAPFVAPDIVWDFRLPRV---
                 • •      +                    +• • •  •     +          +

                    300        310        320        330        340        350
Aro Rat          DSFNFNPHKWLLVNFDCSAMWVKKRTDLTEAFNMDPVYLRHSHQDSGLITDYRHWQIPLGR
Aro Drosophila   DSFNFNPHKWMLVNFDCSAMWLKDPSWVVNAFNVDPLYLKHDMQGSA--PDYRHWQIPLGR
His Rat          DSFTFNPSKWMMVHFDCTGFWVKDKYKLQQTFSVNPIYLRHA--NSGVATDFMHWQIPLSR
His Drosophila   DSIAFNPSKWLMVHFDATALWVRDSTAVHRTFNVEPLYLQHE--NSGVAVDFMHWQIPLSR
Trp Perwinkle    DSLSLSPHKWLLAYLDCTCLWVKQPHLLLRALTTNPEYLKNKQSDLDKVVDFKNWQIATGR
Tyr Parsley      DSFSLNAHKWFLTTLDCCCLWVRNPSALIKSLSTYPEFLKNNASETNKVVDYKDWQIMLSR
Tyr Poppy        DSFSLNAHKWFFTTLDCCCLWVKDPSALVKALSTNPEYLRNKATDSRQVVDYKDWQIALSR
Glu Rat          NSVTWNPHKMMGVLLQCSAILVKEKGILQGCNQMCAGYLFQPDKQYDVSYDTGDKAIQCGR
Glu Drosophila   DSVTWNPHKLMGALLQCSTIHFKEDGLLISCNQMSAEYLFMTDKQYDISYDTGDKVIQCGR
His Tomato       GSISISGHKFLGCPMSCGVQITRRSY--VSTLS-KIEYINSADAT-----------ISGSR
His Morganella   DSIGVSGHKMIGSPIPCGIVVAKKEN--VDRISVEIDYISAHDKT-----------ITGSR
Glu Petunia      KSINVSGHKYGLVYAGIGWVVWRNKDDLPDELIFHINYLGADQPT---------FTLNFSK
Glu E. coli      KSISASGHKFGLAPLGCGWVIWRDEEALPQELVFNVDYLGGQIGT---------FAINFSR
                  •   +  •             +              ++                +  ++

                    360        370        380        390        400        410
Aro Rat          R-FRSLKMWFVFRMYGVKGLQAYIRKHVKLSHE-FESLVRQDPRFEIC--TEVILGL-VCF
Aro Drosophila   R-FRALKLWFVLRLYGVENLQAHIRRHCNFAKQ-FGDLCVADSRFELA--AEINMGL-VCF
His Rat          R-FRSIKLWFVIRSFGVKNLQAHVRHGTDMAKY-FESLVRSDPVFEIP--AERHLGL-VVF
His Drosophila   R-FRALKVWFVLRSYGIKGLQRHIREGVRLAQK-FEALVLADHRFELP--AKRHLGL-VVF
Trp Perwinkle    K-FRSLKLWLILRSYGVVNLQSHIRSDVAMGKM-FEEWVRSDSRFEIV--VPRNFSL-VCF
Tyr Parsley      R-FRALKLWFVLRSYGVGQLREFIRGHVGMAKY-FEGLVNMDKRFFVV--APRLFSM-VCF
Tyr Poppy        R-FRSLKLWMVLRSYGVTNLRNFLRSHVKMAKT-FEGLICMDGRFEIT--VPRTFAM-VCF
Glu Rat          H-VDIFKFWLMWKAKGTVGFENQINKCLELAEYLYAKIKNREE-FEMVFNGEPEHTN-VCF
Glu Drosophila   H-NDIFKLWLQWRAKGTEGFEQQQDRLMELVQYQLKRIREQSDRFHLIL--EPECVN-VSF
His Tomato       NGFTPIFLWYCLSKKGHARLQQDSITCIENARYLKDRLLEAG----ISVMLNDFSIT-VVF
His Morganella   NGHTPLMLWEAIRSHSTEEWKRRITRSLDMAQYAVDRMQKAG----INAWRNKNSIT-VVF
Glu Petunia      GSSQVIAQYYQLIRLCYEGYKNVMENCQENASVLREGLEKTGRFNIISK---EIGVPLVAF
Glu E. coli      PAGQVIAQYYEFLRLGREGYTKVQNASYQVAAYLADEIAKLGPYEFICTGRPDEGIPAVCF
                      + +                                    +          • •
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Fig. 1. Multiple sequence alignment of the core region sequences of the group II decarboxylases. The amino acid sequences are taken from the literature as follows: poppy AADC (*32*); tomato HisDC (*33*); petunia GluDC (*34*); and other enzymes (references in Ref. *6*). Gaps are indicated by dashes. Completely conserved amino acid positions found among all group II decarboxylases are marked by closed circles. Highly conserved amino acids, which showed conservative changes, are marked by plus signs.

DISCUSSION

PLP-dependent amino acid decarboxylases are divided into four groups according to the primary-structure-based classification of Sandmeier *et al.* (*6*). Group I includes glycine decarboxylase; group II, decarboxy ases acting on aromatic amino acids, HisDC, and GluDC; group III, procaryotic ornithine, lysine, and arginine decarboxylases (biodegradative type); group IV, eucaryotic ornithine and arginine decarboxylases, procaryotic arginine decarboxylase (biosynthetic type), and diaminopimelate decarboxylase. The group II decarboxylases form the largest group in the amino acid decarboxylases. The primary structures of the group II decarboxylases have been determined for enzymes of various phylogenic origins. The most phylogenically distant enzyme pair, parsley tyrosine decarboxylase and petunia GluDC, shows only 10% homology in the core region. Multiple sequence alignment of the group II decarboxylases (Fig. 1) showed that, of the residues which have functional groups in their side chains, only five residues are invariant and five residues are conservatively substituted. That the plant and bacterial GluDCs are by far the most distant from other decarboxylases is reflected in

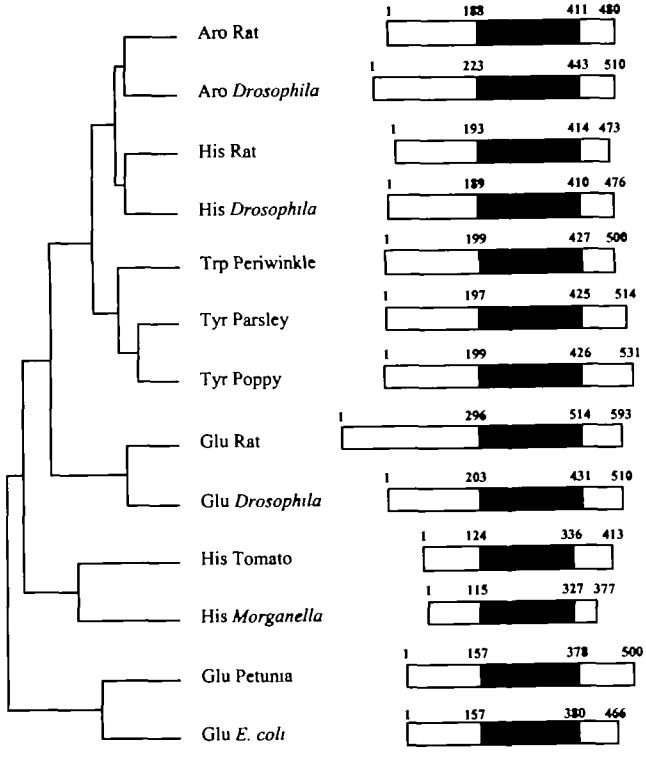

Fig. 2. **Evolutionary pedigrees of the group II decarboxylases.** A rooted dendrogram derived from the sequence alignment shown in Fig. 1 using the UPGMA method (left), and a schematic diagram of the structures of the enzyme proteins, which were adjusted to coincide at the core region (right). The core regions are indicated by closed columns, and other regions by open columns. Numbers indicate the amino acid residue numbers of each enzyme. In the case of rat HisDC, only the region that is homologous with AADC and is supposed to be the mature product is shown (*35*). The following abbreviations are used: Aro, aromatic L-amino acid decarboxylase; His, histidine decarboxylase; Tyr, tyrosine decarboxylase; Trp, tryptophan decarboxylase; Glu, glutamate decarboxylase.

TABLE I. **Steady-state kinetic parameters of rat AADC mutants expressed in *E. coli* for L-dopa (pH 7.0, 25°C).**

Enzyme	k_{cat} (s^{-1})	K_m (mM)	k_{cat}/K_m ($mM^{-1} \cdot s^{-1}$)
Wild type	5.0	0.086	58
H192A	nd[a]	nd	
T246A	0.035	0.056	0.63
D252A	nd	nd	
D252E	nd	nd	
H269A	0.14[b]	0.032	4.4
D271E	0.0050[b]	0.028	0.18
D271A	nd	nd	
S296A	nd	nd	
K303R	nd	nd	
K303A	nd	nd	
K317R	1.9	0.032	59
K317A	2.7	0.044	61
Y332F	nd	nd	
Y332A	nd	nd	
R355K	1.4	0.023	59
R355A	nd	nd	
W363F	2.4	0.033	73
W363L	0.05[c]	0.023	2.2

[a]nd: Activity was not detected in *E. coli* lysate. Assuming that these enzymes are expressed to the same extent as WT AADC, the lower detection limit of activity (k_{cat}) is 0.0005 s^{-1}. [b]Protein concentration was estimated by immunoblotting. [c]Estimated from the activity in the *E. coli* cell lysate. See "RESULTS" for details.

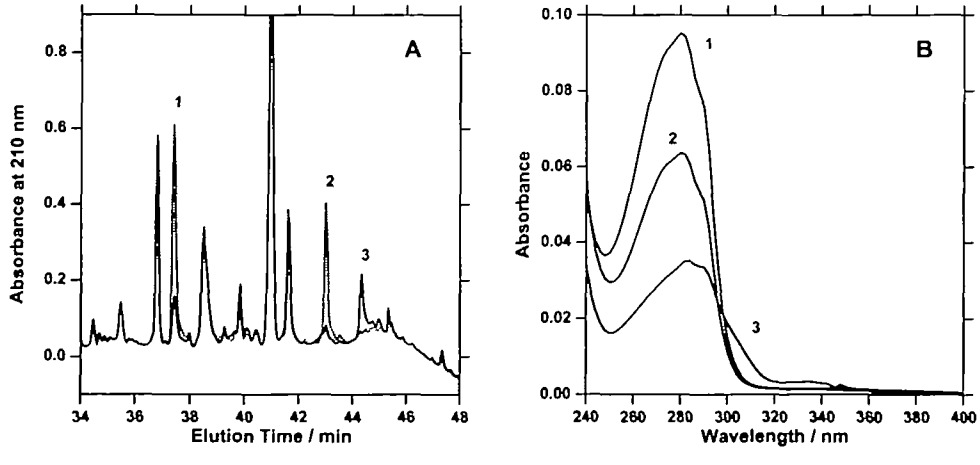

Fig. 3. **Identification of the peptide that binds the coenzyme PLP.** (A) Elution profiles of the tryptic digests of $NaBH_4$-reduced (solid line) and nonreduced (dotted line) WT AADCs on a reversed-phase HPLC. The profiles between the elution time of 34 and 48 min are shown. The two profiles showed essentially identical patterns to each other before 34 min and after 48 min. (B) Absorption spectra of the peak 1, 2, and 3 peptides. Absorption spectrum between 240 and 400 nm at the peak of the elution of each peptide was taken from the Beckman 168 diode array detector.

the sequences of the N-terminal part of the core region of these decarboxylases. As described above, the sequence S[D/E/K][Q/D][A/T/S]H[S/Y/T]X[I/L/V] that starts with the core region is not found in these GluDCs. This suggests that the core regions of these two enzymes are structurally and functionally somewhat different from those of the other group II decarboxylases.

Lys303 has been shown in pig AADC to bind the coenzyme PLP (*12*), but only one of the two subunits contains the coenzyme (*8*). The present study showed, however, that Lys303 in both subunits of rat AADC forms a Schiff base with PLP (Fig. 3), which is in accordance with the previous spectrophotometric analysis (*5*). As two PLP molecules in rat AADC have been shown to react uniformly with the substrate analog L-dopa methyl ester (*5*), it is considered that the two subunits of rat AADC are structurally and functionally symmetrical and each residue of a symmetrical pair has the same role in catalysis. In *Morganella* HisDC, Lys232 is the PLP-binding lysine (*13*). This corresponds to Lys303 of AADC (Fig. 1), which again supports the validity of the alignment of the two distantly related group II decarboxylases.

In PLP enzymes, the PLP-lysine Schiff bases undergo transaldimination reaction to form Schiff bases with added substrate amino acids. Afterwards, the ε-amino group of lysine exists as a free base. In aminotransferases, it is generally accepted that this amino group is a general base catalyst that abstracts the α-proton from the substrate amino acid-PLP Schiff base (*1, 2, 3, 14*). A similar role for this residue is considered in the other PLP enzymes, where removal of the α-proton is a prerequisite step for subsequent catalytic reactions (*1*). However, decarboxylases do not require removal of the α-proton for decarboxylation. One piece of evidence for this is the observation that α-methyldopa undergoes decarboxylation when it is reacted with pig AADC (*15*). We can thus expect that decarboxylation of the dopa-PLP Schiff base can occur in the absence of Lys303. The result of mutagenesis on this residue, however, showed clearly that Lys303 has an important role in catalysis. Therefore, Lys303 must function at steps other than the decarboxylation of the PLP-substrate Schiff base. In PLP enzymes, another role considered for the PLP-binding lysine is that it accelerates formation of the PLP-substrate Schiff base, or displacement of the product from the PLP-product Schiff base (*14, 16*). As a protonated imine is more susceptible to nucleophilic attack than a free aldehyde group, the ε-amino group of lysine in the Schiff base is readily replaced by the substrate (product) amino group. Therefore, we consider that the greatly decreased catalytic activity of K303A mutant enzyme is due to the impairment of the transaldimination process between the Lys303-PLP Schiff base and the substrate (product)-PLP Schiff base. Further studies on the mutant enzymes of this residue are now under way to elucidate the detailed mechanism of Lys303-mediated transaldimination in AADC.

Evidence has been accumulating which indicates that histidine participates in the catalytic reaction of decarboxylases. Akhtar *et al.* (*17*) suggested from analysis of kinetic isotope effects that a histidine residue operates as a proton donor at C^{α} of the quinonoid intermediate of fern methionine decarboxylase. Dominici *et al.* (*18*) showed that modification of a single histidine residue with diethylpyrocarbonate completely abolishes the activity of pig AADC. We describe here replacement of His269 and His192 with Ala. His269 is strictly conserved among the group II decarboxylases (Fig. 1). However, as H269A mutant enzyme retained significant activity (3%) as compared with WT AADC, His269 is not catalytically essential. On the other hand, His192, although it has no corresponding residue in petunia and *E. coli* GluDCs, was found to be catalytically essential (Table I). Several roles in catalysis are expected for this histidine residue in AADC. One is as a catalytic acid that donates a proton to the quinonoid intermediate. Also, this residue may participate in other reaction steps involving transfer of protons, such as the transaldimination process. The catalytic efficiency of rat AADC decreases at low pH (*5*). Therefore, the enzyme form with unprotonated histidine is considered to be the active form. This rules out the possibility that the histidine residue provides a positive charge that accepts the carboxylate group of substrates. That *E. coli* GluDC has no histidine residue corresponding to His192 of AADC may be related to the unique pH dependence of the kinetic parameter of the enzyme, which has an acidic pH optimum (*19*).

Tancini *et al.* (*20*) noted that an arginine residue is involved in the binding of substrates to pig AADC, from the result of chemical modification studies using phenylglyoxal. In a number of enzymes, arginine residues have been shown to be involved in the binding of carboxylate groups of substrates to enzymes (*21-23*). In aspartate aminotransferase, Arg292 and Arg386 have been shown to be the residues that bind the $\beta(\gamma)$- and α-carboxylate group of substrates, respectively (*2, 3, 22, 23*). Arg355 is highly conserved among the group II decarboxylases and a candidate for one of the residues that recognize the α-carboxylate group of substrates. Replacement of this residue with Lys showed essentially no effect on the activity of the enzyme, whereas replacement with alanine completely abolished the activity. This indicates that a positive charge is required at position 355. Relating Arg355 with the arginine residue modified by phenylglyoxal described above, we can consider that the positive charge is essential, but the specific arginine-carboxylate hydrogen bond as observed in aspartate aminotransferase (*2, 3*) is not required, for the recognition of the α-carboxylate group of substrate by AADC.

In PLP enzymes, PLP acts as an electron sink to stabilize the carbanion formed at C^{α} of substrates. The catalytic action of PLP is therefore enhanced by protonation at pyridine N. In AspAT, it is well documented that the negative charge of Asp222 stabilizes the N-protonated structure of the coenzyme pyridine ring (*24-26*). In the X-ray crystallographic structures of the other PLP enzymes whose primary structures are related with AspAT (*27*), an aspartate residue is found beside the pyridine N: Asp223 in tryptophanase (*28*), and Asp214 in tyrosine phenol-lyase (*29*). These aspartate residues are located at positions -36 to -43 relative to the PLP-binding lysine residue. The group II decarboxylases are distantly related in the primary structure with aminotransferases and lyases including AspAT, tryptophanase, and tyrosine phenol-lyase (*27*). Asp271 is the residue that is completely conserved among the group II decarboxylases at positions -32 to -33 relative to the PLP-binding lysine, and corresponds to the above aspartate residues interacting

with the pyridine N of PLP. Mutation of Asp271 to Ala completely diminished the activity of AADC (Table I). This indicates that a negative charge is essential at position 271 for the catalytic action of AADC, and supports the idea that the carboxylate group of Asp271 stabilizes the N-protonated form of the pyridine ring of the coenzyme, enhancing the catalytic ability of AADC. Mutation to Glu greatly decreased the k_{cat} value by 10^3-fold, without changing the K_m value (Table I). Therefore, accurate spatial arrangement of the negative charge of residue 271 is important for catalysis. This is in contrast to the case of Asp222 of AspAT, where mutation to a glutamate residue did not greatly alter the catalytic and other enzymological properties of AspAT (*24*). The cause of this difference, together with the validity of the Asp271–PLP interaction described above, will be made clear by crystallographic analyses of this enzyme.

The sequence alignment shown in Fig. 1 indicates the presence of a number of residues which undergo conservative substitutions. These residues are Asp252, Lys317, Tyr332, Arg355, and Trp363. The conservative substitutions suggest that the chemical properties of these side chains, such as charge and aromaticity, are important for the structure/function of the group II decarboxylases. The importance of a positive charge provided by Arg355, which can be substituted by a lysine residue, has been discussed above. A positive charge at position 317 is not required at all, and aromaticity of the position 363 side chain is not necessary (although it contributes significantly to the activity) for the catalytic action of AADC. On the other hand, aspartate and tyrosine side chains are strictly required for AADC at position 252 and 332, and cannot be substituted for by glutamate and phenylalanine side chains, respectively. These results show the ambiguity of the significance of "conservative substitutions" in protein functions, and indicate the limitations of the method to identify the catalytically essential residues based solely on the molecular evolution of a group of enzymes.

Recently, the X-ray crystallographic structure of ornithine decarboxylase (OrnDC) from *Lactobacillus* 30a has been determined (*30*). This group III decarboxylase had been thought to be evolutionarily unrelated to the group II decarboxylases (*6, 27*). However, Momany *et al.* (*31*) noted that a limited part of the primary structures of the group II and the group III decarboxylases show common structural motifs for PLP binding. In their alignment, His192 and Asp271 of AADC correspond to His223 and Asp316 of OrnDC. The crystal structure of OrnDC showed that the carboxylate group of Asp316 interacts with the pyridine N of PLP (*30*). This again supports the idea that Asp271 provides a negative charge to stabilize the N-protonated pyridine ring of PLP, thereby enhancing the electron-withdrawing ability of the coenzyme and the catalytic efficiency of AADC. Furthermore, His223 of OrnDC locates at the *re* face of the PLP-Lys355 Schiff base and undergoes stacking interaction with the pyridine ring of PLP (*30*). If this spatial relationship between the histidine residue and PLP applies to AADC, His192 is expected to locate at the *re* face of the substrate-PLP Schiff base. Therefore, the histidine residue cannot be the base that donates a proton to C^{α} of the quinonoid intermediate, because this protonation occurs at the *si* face of the Schiff base. The observation that the K_m values for substrates increases sharply as the pH of the solution decreases from 7.0 (*5*) suggests the presence of a protonated histidine residue which undergoes unfavorable interaction with the substrates. From this, we can consider the possibility that the imidazole group of His192 interact with the α-amino group of substrates in the enzyme-substrate complexes.

Much needs to be known about Thr246 and Ser296 before we discuss the role of these invariant residues bearing a hydroxy group in their side chains. The results of replacement of these residues with alanine showed that Ser296 is catalytically essential, while Thr246 contributes greatly to but is not essential for the function of AADC. Ser296 lies between the PLP-binding Lys303 and Asp271, the residue proposed to interact with the pyridine N of PLP. The three-dimensional structure of this region is well conserved among the three kinds of PLP enzymes, aspartate aminotransferase (*2, 3*) tyrosine phenol-lyase (*29*), and ornithine decarboxylase (*30*). Assuming that AADC also has this three-dimensional motif, we can expect that the side chain of Ser296 resides far from the coenzyme- and substrate-binding sites and that Ser296 should be considered to be involved in maintaining the structural integrity of the AADC (and the group II decarboxylase) protein, rather than to directly participate in the catalysis.

Some of the mutant enzymes were successfully purified from *E. coli* cells in sufficient amounts to be used for detailed characterization. Spectroscopic studies on the reactions of these mutant enzymes with substrates and inhibitors are now being carried out to unravel the roles of the possible active site residues. The precise mechanism of the catalytic reactions of AADC and the involvement of the active site residues must await elucidation of the crystal structure of the AADC protein. This is now under way in our laboratory and we hope to extend further our studies on this enzyme on the basis of the three-dimensional structure.

REFERENCES

1. Snell, E.E. (1985) Pyridoxal phosphate in nonenzymatic and enzymatic reactions in *Transaminases* (Christen, P. and Metzler, D.E., eds.) pp. 19-35, John Wiley & Sons, New York
2. Arnone, A., Rogers, P.H., Hyde, C.C., Briley, P.D., Metzler, C.M., and Metzler, D.E. (1985) Pig cytosolic aspartate aminotransferase: The structures of the internal aldimine, external aldimine, and ketimine and of the β subform in *Transaminases* (Christen, P. and Metzler, D.E., eds.) pp. 138-154, John Wiley & Sons, New York
3. Jansonius, J.N., Eichele, G., Ford, G.C., Picot, D., Thaller, C., and Vincent, M. (1985) Spatial structure of mitochondrial aspartate aminotransferase in *Transaminases* (Christen, P. and Metzler, D.E., eds.) pp. 109-137, John Wiley & Sons, New York
4. Vaaler, G.L. and Snell, E.E. (1989) Pyridoxal 5′-phosphate dependent histidine decarboxylase: Overproduction, purification, biosynthesis of soluble site-directed mutant proteins, and replacement of conserved residues. *Biochemistry* **28**, 7306-7313
5. Hayashi, H., Mizuguchi, H., and Kagamiyama, H. (1993) Rat liver aromatic L-amino acid decarboxylase: Spectroscopic and kinetic analysis of the coenzyme and reaction intermediates. *Biochemistry* **32**, 812-818
6. Sandmeier, E., Hale, T.I., and Christen, P. (1994) Multiple evolutionary origin of pyridoxal-5′-phosphate-dependent amino acid decarboxylases. *Eur. J. Biochem.* **221**, 997-1002
7. Jackson, F.R. (1990) Prokaryotic and eukaryotic pyridoxal-dependent decarboxylases are homologous. *J. Mol. Evol.* **31**, 325-329

8. Voltattorni, C.B., Giartosio, A., and Turano, C. (1987) Aromatic L-amino acid decarboxylase from pig kidney in *Methods in Enzymology* (Kaufman, S., ed.) Vol. 142, pp. 179-187, Academic Press, New York
9. Miwa, S., Fujiwara, M., Inoue, M., and Fujiwara, M. (1986) Effects of hypoxia on the activities of noradrenergic and dopaminergic neurons in the rat brain. *J. Neurochem.* **47**, 63-69
10. Saitou, N. and Nei, M. (1987) The neighbor-joining method: A new method for reconstructing phylogenetic trees. *Mol. Biol. Evol.* **4**, 406-425
11. Tanaka, T., Horio, Y., Taketoshi, M., Imamura, I., Ando-Yamamoto, M., Kangawa, K., Matsuo, H., Kuroda, M., and Wada, H. (1989) Molecular cloning and sequencing of a cDNA of rat dopa decarboxylase: Partial amino acid homologies with other enzymes synthesizing catecholamines. *Proc. Natl. Acad. Sci. USA* **86**, 8142-8146
12. Bossa, F., Martini, F., Barra, D., Voltattorni, C.B., Minelli, A., and Turano, C. (1977) The chymotryptic phosphopyridoxyl peptide of DOPA decarboxylase from pig kidney. *Biochem. Biophys. Res. Commun.* **78**, 177-184
13. Hayashi, H., Tanase, S., and Snell, E.E. (1986) Pyridoxal 5′-phosphate-dependent histidine decarboxylase. Inactivation by α-fluoromethylhistidine and comparative sequences of the inhibitor- and coenzyme-binding sites. *J. Biol. Chem.* **261**, 11003-11009
14. Toney, M.D. and Kirsch, J.F. (1993) Lysine 258 in aspartate aminotransferase: Enforcer of the Circe effect for amino acid substrates and general-base catalyst for the 1,3-prototropic shift. *Biochemistry* **32**, 1471-1479
15. O'Leary, M.H. and Baughn, R.L. (1977) Decarboxylation-dependent transamination catalyzed by mammalian 3,4-dihydroxyphenylalanine decarboxylase. *J. Biol. Chem.* **252**, 7168-7173
16. Lu, Z., Nagata, S., McPhie, P., and Miles, E.W. (1993) Lysine 87 in the beta subunit of tryptophan synthase that forms an internal aldimine with pyridoxal phosphate serves critical roles in transimination, catalysis, and product release. *J. Biol. Chem.* **268**, 8727-8734
17. Akhtar, M., Stevenson, D.E., and Gani, D. (1990) Fern L-methionine decarboxylase: Kinetic and mechanism of decarboxylation and abortive transamination. *Biochemistry* **29**, 7648-7660
18. Dominici, P., Tancini, B., and Voltattorni, C.B. (1985) Chemical modification of pig kidney 3,4-dihydroxyphenylalanine decarboxylase with diethyl pyrocarbonate. Evidence for an essential histidyl residue. *J. Biol. Chem.* **260**, 10583-10589
19. Fonda, M.L. (1985) L-Glutamate decarboxylase from bacteria in *Methods in Enzymology* (Meister, A., ed.) Vol. 113, pp. 11-16, Academic Press, New York
20. Tancini, B., Dominici, P., Barra, D., and Voltattorni, C.B. (1985) An essential arginine residue at the binding site of pig kidney 3,4-dihydroxyphenylalanine decarboxylase. *Arch. Biochem. Biophys.* **238**, 565-573
21. Hart, K.W., Clarke, A.R., Wigley, D.B., Waldman, A.D., Chia, W.N., Barstow, D.A., Atkinson, T., Jones, J.B., and Holbrook, J.J. (1987) A strong carboxylate-arginine interaction is important in substrate orientation and recognition in lactate dehydrogenase. *Biochim. Biophys. Acta* **914**, 294-298
22. Inoue, Y., Kuramitsu, S., Inoue, K., Kagamiyama, H., Hiromi, K., Tanase, S., and Morino, Y. (1989) Substitution of a lysyl residue for arginine 386 of *Escherichia coli* aspartate aminotransferase. *J. Biol. Chem.* **264**, 9673-9681
23. Cronin, C.N. and Kirsch, J.F. (1988) Role of arginine-292 in the substrate specificity of aspartate aminotransferase as examined by site-directed mutagenesis. *Biochemistry* **27**, 4572-4579
24. Yano, T., Kuramitsu, S., Tanase, S., Morino, Y., and Kagamiyama, H. (1992) Role of Asp222 in the catalytic mechanism of *Escherichia coli* aspartate aminotransferase: The amino acid residue which enhances the function of the enzyme-bound coenzyme pyridoxal 5′-phosphate. *Biochemistry* **31**, 5878-5887
25. Yano, T., Hinoue, Y., Chen, V.J., Metzler, D.E., Miyahara, I., Hirotsu, K., and Kagamiyama, H. (1993) Role of an active site residue analyzed by combination of mutagenesis and coenzyme analog. *J. Mol. Biol.* **234**, 1218-1229
26. Onuffer, J.J. and Kirsch, J.F. (1994) Characterization of the apparent negative cooperativity induced in *Escherichia coli* aspartate aminotransferase by the replacement of Asp222 with alanine. Evidence for an extremely slow conformational change. *Protein Eng.* **7**, 413-424
27. Alexander, F.W., Sandmeier, E., Mehta, P.K., and Christen, P. (1994) Evolutionary relationships among pyridoxal-5′-phosphate-dependent enzymes. Regio-specific α, β and γ families. *Eur. J. Biochem.* **219**, 953-960
28. Isupov, M., Dementieva, I., Zakomirdina, L., Wilson, K.S., Dauter, Z., Antson, A.A., Dodson, G.G., and Harutyunyan, E.G. (1994) X-ray study of tryptophanase at 2.1 Å resolution in *Biochemistry of Vitamin B_6 and PQQ* (Marino, G., Sannia, G., and Bossa, F., eds.) pp. 183-185, Birkhäuser Verlag, Basel
29. Antson, A.A., Demidkina, T.V., Gollnick, P., Dauter, Z., von Tersh, R.L., Long, J., Berezhnoy, S.N., Phillips, R.S., Harutyunyan, E.H., and Wilson, K.S. (1993) Three-dimensional structure of tyrosine phenol-lyase. *Biochemistry* **32**, 4195-4206
30. Momany, C., Ernst, S., Ghosh, R., Chang, N.L., and Hackert, M.L. (1995) Crystallographic structure of a PLP-dependent ornithine decarboxylase from *Lactobacillus* 30a at 3.0 Å resolution. *J. Mol. Biol.* **252**, 643-655
31. Momany, C., Ghosh, R., and Hackert, M.L. (1995) Structural motifs for pyridoxal-5′-phosphate binding in decarboxylases: An analysis based on the crystal structure of the *Lactobacillus* 30a ornithine decarboxylase. *Protein Sci.* **4**, 849-854
32. Facchini, P.J. and De, L.V. (1994) Differential and tissue-specific expression of a gene family for tyrosine/dopa decarboxylase in opium poppy. *J. Biol. Chem.* **269**, 26684-26690
33. Picton, S., Gray, J.E., Payton, S., Barton, S.L., Lowe, A., and Grierson, D. (1993) A histidine decarboxylase-like mRNA is involved in tomato fruit ripening. *Plant Mol. Biol.* **23**, 627-631
34. Baum, G., Chen, Y., Arazi, T., Takatsuji, H., and Fromm, H. (1993) A plant glutamate decarboxylase containing a calmodulin binding domain. Cloning, sequence, and functional analysis. *J. Biol. Chem.* **268**, 19610-19617
35. Joseph, D.R., Sullivan, P.M., Wang, Y.M., Kozak, C., Fenstermacher, D.A., Behrendsen, M.E., and Zahnow, C.A. (1990) Characterization and expression of the complementary DNA encoding rat histidine decarboxylase. *Proc. Natl. Acad. Sci. USA* **87**, 733-737 [published erratum appears in *Proc. Natl. Acad. Sci. USA* (1990) **87**, 7346]