Mutation of Tyrosine 332 to Phenylalanine Converts Dopa Decarboxylase into a Decarboxylation-dependent Oxidative Deaminase*

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A flexible loop (residues 328–339), presumably covering the active site upon substrate binding, has been revealed in 3,4-dihydroxyphenylalanine decarboxylase by means of kinetic and structural studies. The function of tyrosine 332 has been investigated by substituting it with phenylalanine. Y332F displays coenzyme content and spectroscopic features identical to those of the wild type. Unlike wild type, during reactions with L-aromatic amino acids under both aerobic and anaerobic conditions, Y332F does not catalyze the formation of aromatic amines. However, analysis of the products shows that in anaerobiosis, L-aromatic amino acids are converted into the corresponding aromatic aldehydes, ammonia, and CO2 with concomitant O2 consumption. Therefore, substitution of Tyr-332 with phenylalanine results in the suppression of the original activity and in the generation of a decarboxylation-dependent oxidative deaminase activity. In anaerobiosis, Y332F catalyzes exclusively a decarboxylation-dependent transamination of L-aromatic amino acids. A role of Tyr-332 in the Cα protonation step that catalyzes the formation of physiological products has been proposed. Furthermore, Y332F catalyzes oxidative deamination of aromatic amines and half-transamination of D-aromatic amino acids with Kcat values comparable with those of the wild type. However, for all the mutant-catalyzed reactions, an increase in Km values is observed, suggesting that Y™F replacement also affects substrate binding.

Dopa† decarboxylase (DDC; EC 4.1.1.28) is a homodimeric pyridoxal 5′-phosphate (PLP) enzyme that catalyzes as the main reaction the decarboxylation of L-aromatic amino acids into the corresponding aromatic amines shown in Reaction 1.

L-aromatic amino acid → aromatic amine + CO2

REACTION 1

Side reactions with turnover times measured in minutes are also catalyzed by the enzyme. In particular, DDC exhibits half-transaminase activity toward D-aromatic amino acids (1) and oxidative deaminase activity toward aromatic amines (2, 3), as shown in Reactions 2 and 3.

D-aromatic amino acid + PLP → aromatic ketoacid + pyridoxamine 5′-phosphate (PMP)

REACTION 2

Aromatic amine + 1/2 O2 → aromatic aldehyde + NH3

REACTION 3

Studies on the effect exerted by O2 on reaction specificity of the enzyme have shown that under anaerobic conditions, Reaction 1 takes place with a Kcat value approximately half that occurring in the presence of O2 and is accompanied by a decarboxylation-dependent transamination (4), and Reaction 2 occurs at the same extent either in the presence or absence of O2 (4). Reaction 3 does not occur in anaerobiosis and is replaced by half-transamination (1, 5).

Tancini et al. (6) reported the presence of an exposed and flexible region in the native pig kidney DDC molecule susceptible to tryptic digestion by which two fragments cleaved at the Lys-334–His-335 bond were formed. Although the nicked enzymatic species does not exhibit either decarboxylase or oxidative deaminating activities, it retains a large percentage of the native transaminase activity toward D-aromatic amino acids and displays a slow transaminase activity toward aromatic amines (1). Steady-state kinetic studies of native and nicked enzymatic species together with protection experiments against limited proteolysis of DDC by various substrates have suggested that ligand conformational changes occur at or near the tryptic cleavage region (1). The finding that recombinant rat liver DDC lacking this loop is incompetent for decarboxylation supports this view (7).

The spatial structure of ligand-free DDC and its complex with the anti-Parkinson drug carbidopa has been recently solved, but the flexible loop between residues 328 and 339 is invisible in the electron density map (8). A model based on the coordinates of the enzyme with the flexible loop in its hypothetical open form was built. Although in this modeled structure the loop is located at the dimer interface, far away from the active site, it is expected to extend toward the active site of the other monomer in a closed conformation upon substrate binding. Such loop closure could be an essential step in the catalytic mechanism of the enzyme, and it is also reasonable to suppose that some loop residues could even take part in the catalytic reactions.
Fig. 1. Active site view of the energy-minimized model zooming in on Tyr-332 of the flexible loop of DDC in complex with carbiDopa. Several residues around the α-carbon of the ligand, which is depicted in red, are represented in wire-frame mode. PLP is colored in yellow. Tyr-332, depicted in ball-and-stick mode, and residues colored in gray belong to the neighboring subunit. The flexible loop was modeled using the coordinates 1JSS deposited in the Protein Data Bank (8). Energy computations were done with the GROMOS96 (27) implementation of Swiss-Pdb Viewer (28).

EXPERIMENTAL PROCEDURES

Chemicals—L-Dopa, L- and D-5-hydroxytryptophan (5-HTP), 5-hydroxytryptamine (5-HT, serotonin), dopamine, PLP, pyridoxamine 5′-phosphate (PMP), NADH, bovine liver t-glutamate dehydrogenase, and Hepes were Sigma products. The liquid chromatography solvents (HPLC grade) were from Labscan. Ingredients for bacterial growth were from Difco. Oligonucleotides were from Invitrogen. PCR amplifications were performed using the Expand high fidelity PCR system commercialized by Stratagene. The restriction enzymes used for cloning were from Biolabs. D, L-[1-14C]Dopa (55 mCi/mmol) was a product of ICN Pharmaceuticals. All other chemicals were of the highest purity available.

Site-directed Mutagenesis—Site-directed mutagenesis was performed by overlap extension PCR (10). This method uses four oligonucleotide primers in three separate PCR reactions to introduce a mutation into the target DNA sequence. Two separate PCR reactions are run, one using primers 1 and 3 to amplify a portion of the target sequence and the other using primers 2 and 4 to amplify the other portion. A short section of DNA has identical sequence in both PCR products and corresponds to the sequence of primers 2 and 3 (“internal primers”), which are complementary to each other and carry the “mismatch,” i.e. the mutation. In the third PCR, the purified products of the previous two reactions are employed as template. Following denaturation, a small fraction of the template DNA will anneal to form heteroduplexes and will be extended at its recessed 3′ ends by the polymerase used in the reaction. The full-length sequence containing the mutation is then amplified using primers 1 and 4 (“external primers”). This mutant was produced using as external primers 5′-ATCGGCTCGTAT-AATTGTGGG-3′ and 5′-GTTGTGTTATTAATCTGTAGC-3′. Internal primers were 5′-GACCGGCTGTTCTTAAGCACAC-3′ and 5′-GTC- GCTTTAAGAACACCGGGGTC-3′. The newly inserted part of the expression construct, pKDDC-mutant, was sequenced to confirm mutation, and the plasmid was used to transform *Escherichia coli* strain SVS370.

Expression and Purification of Y332F Mutant—The conditions used for expression and purification of the mutant protein in *E. coli* (SVS370) were as described for the wild-type enzyme (2, 11). Since the mutant enzyme does not show detectable decarboxylase activity in the standard spectrophotometric assay, which measures production of aromatic amines, screening with antibodies to native DDC was therefore necessary to monitor the purification procedure. The purified mutant was homogenous as indicated by a single band on SDS-PAGE. The enzyme concentration was determined by using an ε420 of 3 × 10^5 M^−1 cm^−1. PLP content of holoDDC enzymes was determined by releasing the coenzyme in 0.1 M NaOH and by using ε420 = 6600 M^−1 cm^−1 at 388 nm.

Western Blotting—A sample of 20 μg of protein was subjected to SDS-polyacrylamide gel electrophoresis using a 12.5% acrylamide gel. The proteins were electroblotted to Immobilon-P membranes (Millipore), and Western blot analysis was performed according to Gallagher et al. (12).

Enzyme Assays—DDC mutant Y332F (2–5 μM) was incubated with 0.1–5 mM L-Dopa or L-5-HTP in 50 mM Hepes, pH 7.5, at 25 °C in the presence or absence of O2. Production of dopamine or 5-HT was determined with a spectrophotometric assay outlined by Sherald et al. (13) and modified by Charteris and John (14). Alternatively, production of aromatic amines as well as consumption of L-aromatic amino acids were measured by HPLC analysis. Aliquots were removed at time intervals, and trichloroacetic acid was added to a final concentration of 5% (v/v). The quenched solutions were centrifuged to remove protein, and the supernatants were analyzed using a Discovery (Supelco) C18 column (4.6 × 250 mm). The eluent was methanol:acetic acid:H2O, 24:1:75 with a flow rate of 0.6 mL/min. Detection was set at 280 nm. The concentration of L-aromatic amino acids and aromatic amines in the analyzed samples was determined from a standard curve generated from known concentrations of the compounds with respect to the internal standard. The amounts of ammonia and aromatic aldehyde (produced during the reaction of the Y332F mutant with L-aromatic amino acids or aromatic amines) were determined using the coupled assays with glutamate dehydrogenase and alcohol dehydrogenase, respectively, as described (2). The amount of aldehyde or ammonia was
measured by the decrease in absorbance at 340 nm due to the conversion of NADH to NAD⁺. The rate of production of 1⁴CO₂ during the reaction of mutant with [1⁴C]Dopa was determined as described (4). Radioactivity was determined with a Beckman Instruments LS 1801 liquid scintillation counter. H₂O₂ production and O₂ consumption were measured according to Refs 1 and 3, respectively. The detection and quantification of PLP and PMP content were performed using the HPLC procedure described previously (1, 4). The apparent pseudo-first-order rate constants, kₚ, of the reaction of the Y332F mutant at varying concentrations of L-5-HTP were obtained by measuring the decrease of the 425-nm absorbance band as described (1). The dependence of kₚ on L-5-HTP concentrations exhibits a saturation behavior, and a hyperbolic fit gives the value of apparent dissociation constant, KD, and a hyperbolic fit gives the value of apparent dissociation constant, KD, and a hyperbolic fit gives the value of apparent dissociation constant, KD, and a hyperbolic fit gives the value of apparent dissociation constant, KD, and a hyperbolic fit gives the value of apparent dissociation constant, KD, and a hyperbolic fit gives the value of apparent dissociation constant, KD, and a hyperbolic fit gives the value of apparent dissociation constant, KD, and a hyperbolic fit gives the value of apparent dissociation constant, KD, and a hyperbolic fit gives the value of apparent dissociation constant, KD, and a hyperbolic fit gives the value of apparent dissociation constant, KD.

**Spectral Measurements**—Absorption spectra were recorded in a Jasco V-550 spectrophotometer. CD measurements were carried out in a Jasco J-710 spectropolarimeter at a scan speed of 50 nm/min with a bandwidth of 2 nm.

**RESULTS**

**Reaction of Y332F Mutant with L-Aromatic Amino Acids under Aerobic and Anaerobic Conditions**—Like the wild type, the mutant binds 2 mol of PLP per dimer. Absorption and CD spectra of the Y332F mutant in the UV-visible and far UV region are essentially identical to those of the wild-type enzyme (data not shown). When the Y332F mutant was incubated at 25 °C under aerobic conditions with l-Dopa, no dopamine formation was detected either by the spectrophotometric or the HPLC assays. However, l-Dopa level decreases, and its decrease parallels the production of 3,4-dihydroxyphenylacetaldehyde and ammonia accompanied by O₂ consumption in a 1:2 molar ratio with respect to the products (Fig. 2). When the reaction was performed under the same experimental conditions in the presence of [1⁴C]Dopa, ¹⁴CO₂ release was observed. The initial velocities of these catalytic events are reported in Table I. Likewise, although no 5-HT production could be detected during the reaction of Y332F with l-5-HTP, conversion of l-5-HTP into 5-hydroxyindolacetaldehyde and ammonia as well as consumption of O₂ in a 1:2 molar ratio with respect to the products take place with the initial velocity values reported in Table I. During the reaction of Y332F, either with l-Dopa or with l-5-HTP, no detectable H₂O₂ was found. Initial velocities of oxidative activity, measured as l-aromatic amino acid consumption at varying concentrations of l-Dopa or l-5-HTP, have been determined. The kcat and Kₘ values are reported in Table II. As for the wild type, upon addition of l-Dopa to Y332F, an increased absorption centered at 425 nm immediately appears. This absorbance band, attributed to the external aldime, decreases with time, and after the time required for consumption of substrate, the original 425-nm absorbance of the holoenzyme reappears (Fig. 3). Qualitatively identical spectral changes are observed upon addition of l-5-HTP to the mutant. When the reaction of Y332F with l-Dopa or l-5-HTP was performed under anaerobic conditions, the concentration of the substrates remained almost unchanged with time, and neither dopamine nor 5-HT were produced. Instead, a conversion of PLP into PMP takes place very quickly, at 30 s, 89% (for l-Dopa) and 55% (for l-5-HTP) of the original coenzyme content is transformed into PMP.

**DISCUSSION**

Flexible loops that occlude active sites during catalysis are recognized as structural elements common to many enzymes. Loop closure induced by substrate binding has great importance in catalysis and specificity by recruiting functional groups into the active site (15–17), stabilizing reactive intermediates (18–20), and preventing the formation of stable abortive complexes (21). Kinetic and structural data have indicated that an 11-residue loop in DDC is conformationally dynamic, suggesting that this loop closes over the active site after substrate binding. This study was prompted to gain insights into the function of the mobile residue of this loop, Tyr-332.

Replacement of Tyr-332 with phenylalanine results in a protein that is still capable of selectively cleaving the correct bond between Cα and COOH of L-aromatic amino acids. This is supported by the finding that during the reaction of the mutant with l-Dopa in aerobicosis, CO₂ is released, and by the occurrence in anaerobiosis of a decarboxylation-dependent transamination of both l-Dopa and l-5-HTP. In fact, PMP formation would be promoted along the reaction pathway if the decarboxylated substrate (quinoid) intermediate is protonated at C4' instead of Cα. On the basis of these data, it can be anticipated that DDC does not require Tyr-332 for the decarboxylation step. Nevertheless, the Y332F mutant is unable to generate aromatic amines to any discernible extent. Since reprotamination at Cα after decarboxylation is necessary for the generation of aromatic amines (Scheme 1(a)), a role for Tyr-332 residue as a proton donor in this reprotamination step can be advanced. However, no accumulation of quinoid intermediate during the reaction of the Y332F mutant with L-aromatic amino acids can be observed. This would be explained by the fact that, although the original overall decarboxylation of the wild-type enzyme is completely suppressed, a new catalytic activity is generated in the presence of O₂. It consists of a decarboxylation-dependent oxidative deamination converting L-aromatic amino acids into CO₂, aromatic aldehydes, and ammonia occurring with a con-

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**Table I**

| Substrate or product | l-Dopa | l-5-HTP |
|----------------------|--------|---------|
| Aromatic amine       | Not detected | Not detected |
| l-Aromatic amino acid | 0.53 ± 0.04 | 0.063 ± 0.004 |
| Aromatic aldehyde   | 0.72 ± 0.03 | 0.053 ± 0.003 |
| Ammonia              | 0.75 ± 0.04 | 0.055 ± 0.002 |
| O₂                   | 0.39 ± 0.01 | 0.031 ± 0.002 |
| L⁴CO₂                | 0.82 ± 0.03 | Not determined |
Oxidative deaminase activity was determined by measuring either production of aromatic aldehyde or ammonia or consumption of L-aromatic amino acids (see Experimental Procedures). Half-transaminase activity was determined by measuring the rate constants of the 425-nm absorbance decrease at varying D-5-HTP concentrations, as described under "Experimental Procedures." Decarboxylase activity was determined by measuring production of aromatic amines, as described under "Experimental Procedures."

Errors reported are the standard error values derived by the curve fitting program. ND, not detected. \( k_{cat} \) and \( K_m \) were obtained from nonlinear regression fit to the Michaelis-Menten equation using SigmaPlot2000 (SPSS). The kinetic parameters for the decarboxylation-dependent oxidative deamination of aromatic amines catalyzed by the Y332F mutant, similar to those catalyzed by the wild-type DDC, are about 150-fold lower than those catalyzed by the mutant toward the corresponding L-aromatic amino acids (Table II). Moreover, reaction of the Y332F mutant with L-aromatic amino acids under anaerobic conditions is characterized by no accumulation of aromatic amine. In an \( O_2 \)-free atmosphere, if the amine were produced, it would accumulate since it would undergo a very slow transamination.

Under anaerobic conditions, the C4 of the quinonoid intermediate cannot be oxygenated, but it could be protonated, giving rise to a ketimine substrate intermediate that would yield by hydrolysis the PMP enzyme and the carbonyl compound (Scheme 1 (c)). This is consistent with our data in the absence of \( O_2 \). It should be noted that whereas in anaerobiosis the 100% of the catalytic events, for wild-type DDC, it takes place at a ratio of about once per 5 \( \times \) 10\(^3\) and 6.5 \( \times \) 10\(^3\) times for decarboxylation for L-Dopa and L-5-HTP, respectively (4). All together, these results indicate that since the quinonoid intermediate in Y332F cannot undergo protonation on C4, its only possible fate is oxygenation or protonation at C4' in the presence or absence, respectively, of \( O_2 \).

Since Tyr-332 likely plays a role as proton donor to C4 along the reaction pathway of decarboxylation, it is not surprising that the \( k_{cat} \) value for the oxidative deamination and half-transamination reactions catalyzed by the Y332F mutant toward aromatic amines and D-5-HTP, respectively, remains largely unchanged with respect to the corresponding reactions catalyzed by the wild type. However, the mutant exhibits an \( \sim \) 10-fold increase in the \( K_m \) values for amines and in the \( K_D \) values for D-5-HTP, as compared with those of wild type. The kinetic parameters for the decarboxylation-dependent oxidative deamination indicate that, although replacement of Tyr-332 with phenylalanine led to 10-fold increase in \( K_m \) value for L-Dopa, the \( k_{cat} \) value is similar to that for the overall decarboxylation catalyzed by the wild type for the same substrate. More substantial changes were found when L-5-HTP was used as substrate (40-fold increase in \( K_m \) value and \( \sim \)2-fold decrease
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Scheme 1. Proposed pathways for reactions of Y332F mutant with l-aromatic amino acids (b) in the presence or (c) in the absence of \( \text{O}_2 \); (a) represents the pathway for the overall decarboxylation.

The substitution of phenylalanine for tyrosine 332 has changed the catalytic properties of DDC toward l-aromatic amino acids: the original overall decarboxylation is completely abolished, and a new catalytic activity not inherent in the wild type is generated. Few site-directed mutagenesis experiments altering the reaction specificity of PLP enzymes have been reported, even if a complete suppression of the original activity has never been achieved (22–25). It is of interest that replacement of Cys-360 by Ala or Ser in eukaryotic ornithine decarboxylase greatly reduces the rate of decarboxylation and increases the rate of the abortive transamination. On this basis, a role for Cys-360 in facilitating decarboxylation has been proposed (24). Likewise, mutation of residues in the coenzyme binding pocket of DDC alters the nature of catalysis by enhancing decarboxylation-dependent transamination activity and reducing original decarboxylation activity (25). To the best of our knowledge, such a clear change in reaction specificity of Y332F DDC with a remarkably high new activity (\( k_{\text{cat}} \) values of 4.6 s\(^{-1}\) and 1.0 s\(^{-1}\) for l-Dopa and l-5HTP, respectively) has as of yet only been reported for papain that was converted into a peptide-nitrite hydratase by a single amino acid substitution at the...
active site (26). Strong evidence is provided that loop movement is critical for a correct positioning of Tyr-332 to allow its catalytic role in reprotonation of the quinonoid at Cα. This is the first PLP-α decarboxylase for which the residue responsible for the protonation of the decarboxylated reaction intermediate to form physiological amines has been identified.

REFERENCES
1. Bertoldi, M., Frigeri, P., Paci, M., and Borri Voltattorni, C. (1999) J. Biol. Chem. 274, 5514–5521
2. Bertoldi, M., Moore, P. S., Maras, B., Dominici, P., and Borri Voltattorni, C. (1996) J. Biol. Chem. 271, 23864–23859
3. Bertoldi, M., Dominici, P., Moore, P. S., Maras, B., and Borri Voltattorni, C. (1998) Biochemistry 37, 6552–6561
4. Bertoldi, M., and Borri Voltattorni, C. (2000) Biochem. J. 352, 533–538
5. Bertoldi, M., and Borri Voltattorni, C. (2001) Protein Sci. 10, 1178–1186
6. Tancini, B., Dominici, P., Simmaco, M., Schinini, M. E., Barra, D., and Borri Voltattorni, C. (1988) Arch. Biochem. Biophys. 260, 569–576
7. Ishii, S., Hayashi, H., Okamoto, A., and Kagamiyama, H. (1998) Protein Sci. 7, 1802–1810
8. Burkhard, P., Dominici, P., Borri Voltattorni, C., Jansonius, J. N., and Malashkevich, V. N. (2001) Nat. Struct. Biol. 8, 963–967
9. Ishii, S., Mizuguchi, H., Nishino, J., Hayashi, H., and Kagamiyama, H. (1996) J. Biochem. 120, 369–376
10. Higuchi, R. Innis, M. A., Gelfand, D. H., Snisky, J. J., and White, T. J. (1990) PCR Protocols: A Guide to Methods and Applications, pp. 177–183, Academic Press, Inc., San Diego, CA
11. Moore, P. S., Dominici, P., and Borri Voltattorni, C. (1996) Biochem. J. 315, 249–256
12. Gallagher, S., Winston, S. E., Fuller, S. A., and Hurrel, J. G. R. (1993) Current Protocols in Molecular Biology, pp. 10.8.1–10.8.17, Greene Publishing and Wiley-Interscience, New York
13. Sherald, A. F., Sparrow, J. C., and Wright, T. R. F. (1973) Anal. Biochem. 56, 300–305
14. Charteris, A., and John, R. A. (1975) Anal. Biochem. 66, 365–371
15. Clarke, A., Wigley, D. B., Chia, W. N., Barstow, D., Atkinson, T., and Holbrook, J. J. (1980) Nature 324, 699–702
16. Krell, T., MacLean, J., Boam, D. J., Cooper, A., Resmini, M., Brocklehurst, K., Kelly, S. M., Price, N. C., Lapthorn, A. J., and Coggins, J. R. (2001) Protein Sci. 10, 1137–1149
17. Zgiby, S., Piater, A. R., Bates, M. A., Thomson, G. J., and Berry, A. (2002) J. Mol. Biol. 315, 131–140
18. Pompliano, D. L., Peyman, A., and Knewles, J. R. (1990) Biochemistry 29, 3186–3194
19. Larsson, E. M., Larimer, F. W., and Hartman, F. C. (1995) Biochemistry 34, 4531–4537
20. Kato, H., Tanaka, T., Yamaguchi, H., Hara, T., Nishioka, T., Katsube, Y., and Oda, J. (1994) Biochemistry 33, 4995–4999
21. Contestable, R., Angelaccio, S., Mayrum, R., Bossa, F., and John, R. A. (2000) J. Biol. Chem. 275, 3879–3886
22. Vacca, R., Christen, P., Malashkevich, V. N., Jansonius, J. N., and Sandmeier, E. (1995) Eur. J. Biochem. 227, 481–487
23. Gruber, R., Kasper, P., Malashkevich, V. N., Strop, P., Gehring, H., Jansonius, J. J., and Christen, P. (1999) J. Biol. Chem. 274, 31203–31208
24. Jackson, J. N., Brooks, H. B., Osterman, A. L., Goldsmith, E. J., and Phillips, M. A. (2000) Biochemistry 39, 11247–11257
25. Bertoldi, M., Castellani, S., and Borri Voltattorni, C. (2001) Eur. J. Biochem. 268, 2975–2981
26. Dufour, E., Storer, A. C., and Ménard, R. (1995) Biochemistry 34, 16382–16388
27. Guex, N., and Peitsch, M. C. (1977) Electrophoresis 18, 2714–2723
28. Guex, N., Diemand, A., and Peitsch, M. C. (1999) Trends Biochem. Sci. 24, 364–367
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