Reaction of dopa decarboxylase with L-aromatic amino acids under aerobic and anaerobic conditions

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Analysis of the reaction of dopa decarboxylase (DDC) with L-dopa reveals that loss of decarboxylase activity with time is observed at enzyme concentrations approximately equal to the binding constant, K_d, of the enzyme for pyridoxal 5′-phosphate (PLP). Instead, at enzyme concentrations higher than K_d the course of product formation proceeds linearly until complete consumption of the substrate. Evidence is provided that under both experimental conditions no pyridoxamine 5′-phosphate (PMP) is formed during the reaction and that dissociation of coenzyme occurs at low enzyme concentration, leading to the formation of a PLP-L-dopa Pictet–Spengler cyclic adduct. Taken together, these results indicate that decarboxylation-dependent transamination does not accompany the decarboxylation of L-dopa proposed previously [O'Leary and Baughn (1977) J. Biol. Chem. **252**, 7168–7173]. Nevertheless, when the reaction of DDC with L-dopa is studied under anaerobic conditions at an enzyme concentration higher than K_d, we observe that (1) the enzyme is gradually inactivated and inactivation is associated with PMP formation and (2) the initial velocity of decarboxylation is approximately half of that in the presence of O_2. Similar behaviour is observed by comparing the reaction with L-5-hydroxytryptophan occurring in aerobiosis or in anaerobiosis. Therefore the reaction of DDC with L-aromatic amino acids seems to be under O_2 control. In contrast, the reactivity of the enzyme with D-aromatic amino acids does not change in the presence or absence of O_2. These and other results, together with previous results on the effect exerted by O_2 on reaction specificity of DDC towards aromatic amines [Bertoldi, Frigeri, Paci and Borri Voltattorni (1999) J. Biol. Chem. **274**, 5514–5521], suggest a productive effect of O_2 on an intermediate complex of the reaction of the enzyme with L-aromatic amino acids or aromatic amines.

Key words: decarboxylation-dependent transamination, dopa, oxygen, pyridoxal 5′-phosphate.

INTRODUCTION

Recombinant pig kidney dopa decarboxylase (DDC; EC 4.1.1.28) is a homodimeric enzyme that contains 1 mol of pyridoxal 5′-phosphate (PLP) per mol of monomer [1]. The enzyme is a stereospecific α-decarboxylase that catalyses the conversion of L-aromatic amino acids into their corresponding amines [2]. As claimed previously [3], DDC also catalyses a minor side reaction of L-aromatic amino acids, referred to as decarboxylation-dependent transamination, leading to reversible inactivation. In addition, the enzyme catalyses the half-transamination of D-aromatic amino acids accompanied by a Pictet–Spengler reaction [4]. A notable feature of DDC is its reaction specificity for aromatic amines. Under aerobic conditions the enzyme is able to catalyse an oxidative deamination of aromatic amines, producing equivalent amounts of aromatic aldehyde or ketone and ammonia, and consuming O_2 in a 1:2 molar ratio with regard to the products [5,6]. Nevertheless, half-transamination and Pictet–Spengler reactions take place when the enzyme reacts with aromatic amines under anaerobic conditions [4]. Thus the processing of aromatic amines by DDC seems to be under O_2 influence. To learn whether molecular oxygen exerts effects on the other reactions catalysed by DDC, the reactions of the enzyme with aromatic amino acids in L and D forms under anaerobic conditions were studied and compared with those occurring under aerobic conditions.

Here we report results showing that, whereas with D-aromatic amino acids the reaction specificity of DDC does not change in the presence or absence of O_2, when the enzyme reacts with L-aromatic amino acids it does seem to be affected by O_2. In the course of the investigation of this latter reaction it emerged that (1) the loss of decarboxylase activity following the decarboxylation of L-dopa occurs only at an enzyme concentration approximately equal to the binding constant, K_d, of the enzyme for PLP and results in the conversion of coenzyme into a PLP-L-dopa Pictet–Spengler adduct, and (2) whereas decarboxylation-dependent transamination does not occur during the decarboxylation of L-aromatic amino acids in air, it does take place in anaerobiosis. Moreover, we show that the k_{cat} of decarboxylation of L-dopa and L-5-hydroxytryptophan (L-5-HTP) in the presence of O_2 is approximately double that in its absence.

MATERIALS AND METHODS

Materials

L-Dopa and D-dopa, L-5HTP and D-5-HTP, α-methyldopa, α-methylornithine, α-methylglutamate, PLP and pyridoxamine 5′-phosphate (PMP) were purchased from Sigma. D,L-[1-^{14}C]Dopa (55 mCi/mmol) was a product of ICN Pharmaceuticals. All other chemicals were of the highest purity available.

Enzyme purification and assays

DDC was purified to homogeneity from *Escherichia coli* expressing pKKDDCΔ4Δ3′ as described [1,5] and was used throughout. The enzyme concentration was determined by using a molar absorption coefficient of $1.3 \times 10^5\ M^{-1} \cdot cm^{-1}$ [7]. DDC activity

Abbreviations used: DDC, dopa decarboxylase; GAD, glutamate decarboxylase; 5-HTP, 5-hydroxytryptophan; ODC, ornithine decarboxylase; PLP, pyridoxal 5′-phosphate; PMP, pyridoxamine 5′-phosphate.

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was determined by measuring amine production with the spectrophotometric assay outlined by Sherald et al. [8] and modified by Charteris and John [9]. The concentrations of L-dopa and L-5-HTP was determined at 280 nm by using molar absorption coefficients of 2630 and 5500 $M^{-1} \cdot cm^{-1}$ respectively. For the determination of kinetic parameters, the L-dopa and L-5-HTP concentrations were varied from 32 μM to 1.3 mM and from 40 μM to 1 mM respectively. In selected experiments, aimed at comparing the rate of production of CO_2 from L-dopa by DDC under aerobic or anaerobic conditions, the enzyme activity was measured by a radiochemical assay essentially as described by Stevenson et al. [10]. Assay incubations contained L-dopa (0.064 mM containing 358000 c.p.m. of D,L-[1-^{14}C]dopa) and enzyme in a final volume of 500 μl of 50 mM Hepes, pH 7.5. Reactions were started by the addition of the enzyme. Aliquots (100 μl) were withdrawn at zero time and after suitable time intervals, and added to 70 μl of 8 M sulphuric acid in a scintillation vial. After 30 min at room temperature to ensure the complete evolution of CO_2, scintillant was added and the residual radioactivity was determined with a Beckman Instruments LS 1801 liquid-scintillation counter. Spectroscopic measurements and enzymic assays were performed under anaerobic conditions with a cuvette for anaerobiosis and 1 ml Reacti-Vials (Aldrich) respectively. For spectroscopic measurements, nitrogen was flushed into the cuvette containing the enzyme dissolved in 50 mM Hepes, pH 7.5. The side arm of the cuvette contained the substrate. After 30–40 min, enzyme and substrate were mixed by tipping the substrate into the enzymic solution, and spectra were recorded. For enzymic assays two separate vials, one containing the enzyme in 50 mM Hepes, pH 7.5, and the other the substrate, were flushed with nitrogen for 30–40 min and thereafter the substrate was added to the enzyme with a Hamilton Gastight syringe inserted through the Teflon silicone septum. A positive pressure of nitrogen was maintained over the solution throughout the reaction [6].

O_2 consumption during the reaction of DDC with L-dopa or L-5-HTP was recorded with a Yellow Springs electrode (Yellow Springs Instruments, RDP Corporation, Dayton, OH, U.S.A.).

Ornithine decarboxylase (ODC) from *Lactobacillus* 30a and glutamate decarboxylase (GAD) from *E. coli* were purified as described previously [11,12].

HPLC detection of PLP, PMP and Pictet–Spengler adducts

Characterization of the reaction of PLP with L-dopa or L-5-HTP, the chemical synthesis of PLP-L-dopa and PLP-L-5-HTP Pictet–Spengler adducts and also their isolation by HPLC were performed as described previously for the corresponding D-aromatic amino acids [4]. The detection and quantification of PLP, PMP, PLP-aromatic amino acid Pictet–Spengler adducts during the reaction of DDC with L- and D-aromatic amino acids and α-methyldopa and also during the reaction of ODC or GAD with α-methylornithine or α-methylglutamate respectively were performed with the HPLC procedure described previously [4]. When the reaction with L-dopa was performed with 50 nM DDC, the detection and quantification of PLP and coenzyme derivatives were performed on a Jasco HPLC system equipped with a UV-1570 detector. Standard curves of peak area as a function of coenzyme or coenzyme adducts were prepared with commercially available PLP and PMP or coenzyme adducts obtained by synthesis.

Protection studies against limited tryptic proteolysis

Limited tryptic proteolysis was performed as described [13]. In the enzyme protection experiments, substrates at saturating concentration were included during the preincubation period before the addition of trypsin. At various times, aliquots were removed for electrophoresis analysis. Samples were boiled for 2 min in electrophoresis sample buffer and subjected to SDS/PAGE [10 % (w/v) gel].

Spectrophotometric measurements

Absorption spectra were measured with a Jasco V550 spectrophotometer at 25 °C. CD spectra were obtained with a Jasco 710 spectropolarimeter with a thermostatically controlled cell at 25 °C at a protein concentration of 10 μM.

Data analysis

The rate constant of decrease of PLP was obtained by fitting the PLP concentration–time curve to either eqn (1) or eqn (2) with one or two exponentials respectively:

$$[PLP]_t = ([PLP]_i - [PLP]_u)e^{-kt} + [PLP]_u \quad (1)$$

$$[PLP]_t = A_1 e^{-k_1 t} + A_2 e^{-k_2 t} + [PLP]_u \quad (2)$$

The rate constant of increase of coenzyme derivative (PMP or Pictet–Spengler adduct) was obtained by fitting the coenzyme derivative concentration–time curve to either eqn (3) or eqn (4) with one or two exponentials respectively:

$$[PLP_d]_t = ([PLP]_i - [PLP]_u)(1 - e^{-kt}) \quad (3)$$

$$[PLP_d]_t = A_1(1 - e^{-k_1 t}) + A_2(1 - e^{-k_2 t}) \quad (4)$$

Here $[PLP]_t$ and $[PLP_d]_t$ are the measured coenzyme concentrations, $[PLP]_i$ and $[PLP]_u$ are the concentrations of the initial PLP content and of the unreacted coenzyme respectively, and A_1 and A_2 are changes in coenzyme concentration associated with k_1 and k_2 respectively.

These results and those for the determination of K_m and k_{cat} were fitted to lines with the program Microcal Origin (Microsoft).

RESULTS

Reaction of DDC with L-dopa and L-5-HTP under aerobic and anaerobic conditions

As already reported for DDC purified from pig kidney [3], the incubation of recombinant enzyme at a concentration of 50 nM with L-dopa (2.6 mM) showed essentially no change in decarboxylase activity over a period of 15 min. Thereafter, despite the fact that substrate concentration was high, a gradual inactivation was observed (Figure 1). Activity was restored by adding PLP to the inactivated enzyme (results not shown). A loss of activity of DDC has previously been associated with the conversion of PLP bound to enzyme into PMP, suggesting that an abortive decarboxylation–transamination reaction accompanies the normal decarboxylation [3]. To verify whether the inactivation of DDC was due to the conversion of PLP into PMP or other coenzyme forms, aliquots were withdrawn at various times from a reaction mixture containing 50 nM DDC and 2.6 mM L-dopa in 50 mM Hepes, pH 7.5, and subjected to analysis by HPLC after total denaturation. The analyses revealed (1) a decrease in PLP level and a concomitant increase in PLP-L-dopa Pictet–Spengler adduct (Figure 1, curves a and c) and (2) no PMP formation. The best fit for both the decrease in PLP concentration and the increase in cyclic adduct was to two-exponential processes; the rate constants for the two phases were 0.058 ± 0.017 and 0.012 ± 0.001 min^{-1} (means $\pm$ S.D.). In contrast, the reaction of free PLP (100 nM) with L-dopa (2.6 mM) gave rise to a PLP-L-dopa Pictet–Spengler adduct following pseudo-first-order kinetics with a rate constant of 0.162 ± 0.024 min^{-1}. Moreover,

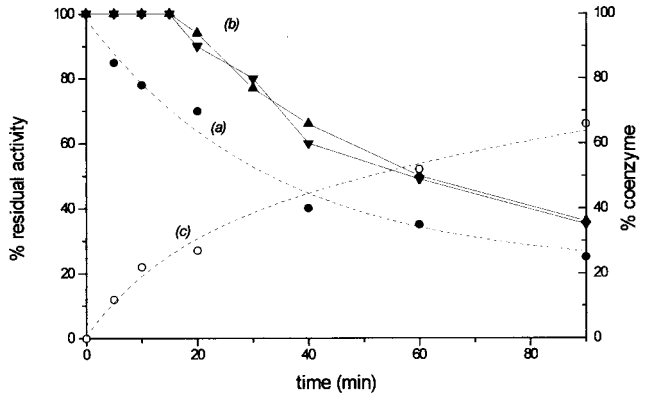

Figure 1 Coenzyme content and decarboxylase activity during the reaction of DDC with L-dopa under aerobic conditions

DDC (50 nM) was incubated at atmospheric oxygen concentration with 2.6 mM L-dopa in 50 mM Hepes, pH 7.5, at 25 °C. At the indicated times aliquots were removed and denatured. After removal of the precipitated protein by centrifugation, the supernatants were subjected to HPLC analysis as described in the Materials and methods section. Symbols: ● (curve a), original PLP; ○ (curve c), PLP-L-dopa Pictet–Spengler adduct; ▼ (curve b), undissociated PLP; ▲, decarboxylase activity at the indicated times expressed as a percentage of that measured during the initial linear phase. Results are means for three independent experiments; the S.E.M. in each case was less that 10% of the mean. Curve b was drawn from the experimental points of curve a after correction by taking the 70% undissociated PLP as 100%. Curves a and c (where 100% of coenzyme corresponds to 100 nM PLP) were theoretical from a fit to eqns (2) and (4) respectively. Fitting was also attemped with eqns (1) and (3) respectively but resulted in curves incompatible with the experimental data.

during the time (15 min) for which the decarboxylation proceeded linearly, there was a decrease (approx. 30%) in PLP concentration and a concomitant increase in that of the cyclic adduct. Although these results, in contrast with those previously reported [3], would rule out the idea that during the decarboxylation of L-dopa a decarboxylation-dependent transamination occurs, they do not explain the inactivation process.

Because the binding constant, K_d, of recombinant DDC for PLP has been estimated to be 44 nM [14], at the enzyme concentration used by us and by O'Leary and Baughn to assay decarboxylase activity we would expect dissociation of the coenzyme from the enzyme. The following experiment confirmed this idea. Aliquots, withdrawn from a stock solution of DDC, were diluted (approx. 1:200) to 50 nM and were transferred, either immediately or after 15, 30, 45 or 70 min, to Centricon-30 tubes. In all cases, after centrifugation at 5000 ***g*** for 10 min, analysis of the filtrates by HPLC revealed an amount of PLP ranging from 30% to 35% of the original content. This result implies that only approx. 70% of DDC was in the holoenzyme form when dopa was added to a 50 nM enzymic solution; thus nearly 30% of the initial coenzyme was already dissociated. Therefore, the 30% decrease in PLP level with the concomitant increase the PLP-L-dopa cyclic adduct mentioned above, occurring in the first 15 min of the reaction, could be ascribed to conversion of the dissociated PLP, via a Schiff base with L-dopa, into PLP-L-dopa cyclic adduct (Figure 1). The remaining 70% undissociated PLP has to be considered as the actual 100% of DDC-bound active coenzyme. Considering that decarboxylation proceeded linearly until 15 min, it can be assumed that it remained almost unchanged over this period. Therefore the real behaviour of the active enzyme-bound PLP during the reaction would be described by Figure 1 (curve b). This was drawn from the experimental points of Figure 1 (curve a) corrected by taking the 70% undissociated PLP as 100%. As can be seen, the time-dependent inactivation curve of DDC by L-dopa was strictly correlated with that describing the decrease in the active PLP concentration. At the same time the decrease in PLP level can be related to its conversion to the cyclic adduct. Taken together, these results indicate that under these experimental conditions, a fast dilution-induced dissociation of a fraction of the cofactor occurs before the addition of L-dopa. The irreversible reaction of this fraction with the substrate producing the Pictet–Spengler compound should displace the equilibrium of the coenzyme binding to the enzyme. This triggers the further dissociation of PLP from DDC, leading to a progressive loss of decarboxylase activity. On the basis of the experiments described above, we would expect, at an enzyme concentrations greater than K_d, that (1) the rate of decarboxylation would not gradually decrease with time and (2) the initial rate of decarboxylation should be higher than that measured at an enzyme concentration equal to K_d.

To verify this point, the reaction of DDC with L-dopa was studied at enzyme concentrations higher than K_d.

When 2.35 μM DDC was incubated with 30 mM L-dopa in 50 mM Hepes, pH 7.5, at 25 °C, a plot of dopamine formed against time was nearly linear until the complete decarboxylation of substrate, whether or not free PLP was added to the reaction mixture. By HPLC we observed that during the reaction the PLP content of the enzymic solution remained nearly unchanged, no PMP was produced and the L-dopa level decreased gradually with time. Only a very small amount (approx. 1% of the original content) of PLP-L-dopa cyclic adduct was detected at 30 min of reaction (Figure 2A) and the peak corresponding to L-dopa disappeared at 40 min.

All experiments described above were performed at atmospheric oxygen concentration. When the latter experiment was performed under anaerobic conditions, it was observed that (1) whereas the incubation containing no added coenzyme showed a gradual decrease in decarboxylase activity, the incubation containing added coenzyme showed essentially no change in activity over a period of 60 min and (2) the time-dependent loss of decarboxylase activity was accompanied by a decrease in PLP concentration and a concomitant increase in PMP and in PLP-L-dopa adduct, although to a smaller extent (Figure 2B). Furthermore, whereas under aerobic conditions the initial rate of decarboxylation of L-dopa was approx. 348 nmol of dopamine/min per nmol of enzyme, it was approx. 180 nmol of dopamine/min per nmol of enzyme under anaerobic conditions (Figure 2). The initial velocity of decarboxylation of 30 mM L-dopa by 50 nM DDC was estimated to be 230 and 119 nmol of dopamine/min per nmol of enzyme in the presence and in the absence of O_2 respectively. These results are consistent with the different extent of PLP dissociation depending on the enzyme concentration. When decarboxylation was measured as the percentage of [^{14}C]dopa converted, the ratio between the initial rates in the presence and in the absence of O_2 was 1.5–2 (results not shown). Similarly, the initial velocity of decarboxylation of L-5-HTP under aerobic conditions was nearly double that under anaerobic conditions. Additionally, during the decarboxylation of L-5-HTP, whereas the production of PLP-L-5-HTP Pictet–Spengler adduct in very small amounts was revealed only in the presence of O_2, PMP formation was detected only in the absence of O_2 (results not shown). The conversion of PLP into PMP occurred under anaerobic conditions with rate constants of 0.031 ± 0.008 and 0.009 ± 0.001 min^{-1} for L-dopa and L-5-HTP respectively. When we measured the initial velocity of decarboxylation of L-dopa or L-5-HTP in the presence of O_2, during which a small quantity of aromatic amines was produced, there was no detectable O_2 consumption.

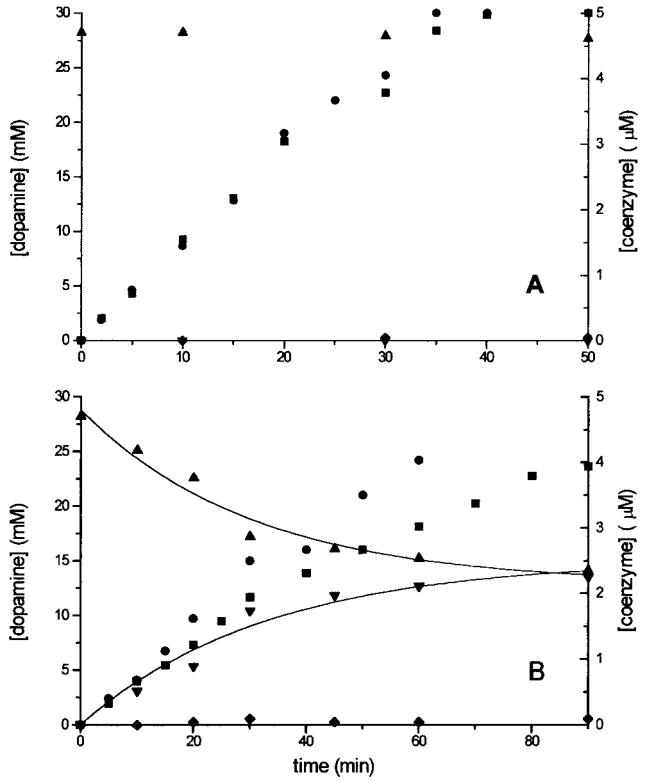

Figure 2 Time course of decarboxylase activity of L-dopa and coenzyme content under aerobic and anaerobic conditions

DDC (2.35 μM) was incubated in 50 mM Hepes, pH 7.5, with 30 mM L-dopa under aerobic (**A**) or anaerobic (**B**) conditions. Aliquots were removed at the indicated times and denatured. Dopamine production in the absence (■) or presence (●) of added PLP (100 μM) was determined by the spectrophotometric assay described in the Materials and methods section. PLP (▲), PMP (▼) and PLP-L-dopa adduct concentrations following reaction without added PLP were determined by HPLC, as described in the Materials and methods section. Results are means for three independent experiments; the S.E.M. in each case was less than 5% of the mean. The curves for PLP and PMP content in (**B**) were theoretical from a fit to eqns (1) and (3) respectively. The parameters were as follows: $[PLP]_i = 4.79 \pm 0.19$ (mean $\pm$ S.D.) μM, $[PLP]_u = 2.14 \pm 0.27$ μM and $k = 0.033 \pm 0.009$ min^{-1} in eqn (1); $[PLP]_i = 4.72 \pm 0.1$ μM, $[PLP]_u = 2.21 \pm 0.06$ μM and $k = 0.031 \pm 0.007$ min^{-1} in eqn (2).

Table 1 Kinetic parameters for L-dopa and L-5-HTP in 50 mM Hepes, pH 7.5, under aerobic and anaerobic conditions

The enzyme concentration was 74 nM for L-dopa and 112 nM for L-5-HTP.

	Aerobiosis		Anaerobiosis	
Substrate	K_m (μM)	k_{cat} (s^{-1})	K_m (μM)	k_{cat} (s^{-1})
L-Dopa	70.3 $\pm$ 5.2	4.26 $\pm$ 0.21	35.0 $\pm$ 11.0	2.57 $\pm$ 0.17
L-5-HTP	155 $\pm$ 14.0	1.99 $\pm$ 0.31	38.0 $\pm$ 7.0	0.99 $\pm$ 0.06

The initial velocity of the reaction catalysed by DDC in the presence or absence of O_2 was dependent on L-dopa or L-5-HTP concentrations as determined by saturation kinetics. The kinetic parameters for these substrates were determined and their values are reported in Table 1. The presence of molecular oxygen increased both k_{cat} and K_m values of the enzyme for both substrates.

Reaction of DDC, ODC and GAD with their α-methyl substrates under anaerobic conditions

It has been demonstrated that, under aerobic conditions, α-methyldopa undergoes decarboxylation by DDC followed by an oxidative deamination [6]. The occurrence of this reaction has also been demonstrated for GAD from *E. coli* and ODC from *Lactobacillus* 30a in the presence of α-methylglutamate or α-methylornithine respectively [15]. Under anaerobic conditions these α-decarboxylases do not catalyse this reaction to a significant extent [6,15]. Moreover, it has recently been reported that in the presence of O_2 a slow decarboxylation-dependent transamination also takes place, converting PLP into PMP with rate constants of 0.0315, 0.092 and 0.023 min^{-1} for DDC, ODC and GAD respectively [15]. Now we have observed that under anaerobic conditions this reaction occurs with rate constants 20-fold, 2-fold and 6-fold higher than in aerobiosis for DDC, ODC and GAD respectively.

Reaction of DDC with D-dopa or D-5-HTP under anaerobic conditions

When DDC was incubated with 5 mM D-dopa or D-5-HTP under anaerobic conditions, half-transamination and Pictet–Spengler reactions occurred with a kinetic behaviour identical with that observed under aerobic conditions [4].

Properties of DDC-L- and DDC-D-aromatic amino acids or aromatic amine intermediate complexes

Partial trypsinolysis of DDC leads to an exclusive cleavage of the Lys^{334}-His^{335} peptide bond [13]. It has recently been pointed out that, whereas the accessibility of trypsin to this cleavage site decreases significantly on the binding of L-aromatic amino acids or aromatic amines, it does not change appreciably on the binding of D-aromatic amino acids [4]. When these experiments were performed under the same experimental conditions but in an oxygen-free atmosphere, the results were identical with those under aerobiosis. Furthermore, although the absorption and CD spectral features of the intermediate L- and D-aromatic amino acids or the complexes of aromatic amines and enzyme were different from each other, they were identical with the corresponding features observed in the absence of O_2 (results not shown).

Taken together, these findings suggest that the binding of these ligands to DDC causes similar changes in the microenvironment of PLP under both aerobic and anaerobic conditions.

DISCUSSION

In 1977 it was observed that the decarboxylation of L-dopa by DDC in the absence of free PLP proceeded for only a few minutes and stopped thereafter, and that the addition of PLP permitted the resumption of decarboxylation. These events have been ascribed to a decarboxylation-dependent transamination that converts PLP into PMP [3]. However, although PMP was identified as a product of the reaction of DDC with α-methyl substrates by chromatography, electrophoresis and through an enzymic assay, the presence of PMP after decarboxylation of L-dopa was demonstrated only by measuring the ability of the spent decarboxylation solution to reconstitute apo-(aspartate aminotransferase) (which gives a measure of PLP plus PMP) and apo-(tyrosine decarboxylase) (which measures only PLP). The production of 3,4-dihydroxyphenylacetaldehyde in this reaction

mixture was considered as further evidence for the occurrence of the abortive decarboxylation. However, in the light of the recent finding that aromatic amines undergo oxidative deamination [5,6], the possibility cannot be excluded that the aromatic aldehyde is the product of the latter reaction. It must be taken into account that O'Leary and Baughn used an enzyme preparation with a specific activity approx. 20% lower than that used by us and no quantitative measurements of either PMP or 3,4-dihydroxyphenylacetaldehyde were reported. Our results indicate that during the decarboxylation of L-dopa by DDC no decarboxylation-dependent transamination occurred because no PMP could be detected. Moreover, the finding that the loss of decarboxylase activity with time accompanied by the complete conversion of PLP into PLP-L-dopa cyclic adduct was observed only at a DDC concentration equal to K_d was consistent with the explanation that the loss of activity could be due to the dissociation of the coenzyme and its irreversible combination with substrate. Nevertheless, this proposal would not explain the reactivation of apo-(aspartate aminotransferase) quoted in [3]. However, it seems that the experimental conditions used by O'Leary and Baughn for the identification of transamination products were not definitely aerobic. In fact, the enzymic solution to which L-dopa was added was flushed with N_2 to remove oxygen. In the light of our results obtained in anaerobiosis (see below), this could be a possible explanation of the discrepancy between our results and those in [3].

In addition, the results reported here indicate that, in an oxygen-free atmosphere, (1) the k_{cat} of decarboxylation of L-dopa or L-5-HTP is approximately half of the corresponding value determined in the presence of O_2, and (2) decarboxylation is accompanied by a decarboxylation-dependent transamination. The latter result is in line with the finding that the decarboxylation of α-methyldopa is accompanied by a decarboxylation-dependent transamination occurring in the absence of O_2 with a rate constant 20-fold that determined in the presence of O_2 [15]. Therefore, as has already been observed for aromatic amines [4], the reaction specificity for L-aromatic amino acids seems to be under O_2 influence. However, whereas O_2 behaves as a substrate in oxidative deamination, it does not seem to be a substrate in the decarboxylation reaction. Furthermore, considering that the presence of O_2 changed the k_{cat}/K_m values for the decarboxylation of L-dopa and L-5HTP by only factors of 1.2 and 2 respectively, it is hard to argue that this small difference could have a physiological significance. Conversely, molecular oxygen does not influence the reaction specificity of DDC for D-aromatic amino acids.

Molecular oxygen seems to exert a similar effect on other PLP-α-decarboxylases, suggesting that these enzymes share common active-site features. The following points are relevant: (1) although *Lactobacillus* 30a ODC and *E. coli* GAD catalyse the oxidative deamination of their α-methyl substrates in the presence of O_2, this reaction does not proceed to a significant extent in the absence of O_2 [15], and (2) under anaerobiosis the rate constant of decarboxylation-dependent transamination of their α-methyl substrates by *Lactobacillus* 30a ODC and *E. coli* GAD is 2-fold and 6-fold higher than under aerobiosis respectively.

It should be noted that external aldimines formed between DDC and L- and D-aromatic amino acids or aromatic amines present the same features (absorbance and CD spectra, protection behaviour against limited tryptic digestion) both in the presence and in the absence of O_2. The control of the reaction specificity by O_2 could therefore be determined by the reaction pathway after the formation of external aldimine intermediates. According to the Dunathan proposal [16] and our findings, the binding of L-aromatic amino acids to the active site of DDC requires placement of the Cα-COO^- perpendicular to the plane of the PLP ring under either aerobic or anaerobic conditions. On this basis it can be suggested that, whereas under anaerobic conditions the nascent CO_2 does not rapidly leave the active site, thus sterically preventing protonation at Cα, under aerobic conditions O_2 could favour CO_2 release, thus allowing protonation at Cα. Considering that the rate-limiting step of decarboxylation of L-dopa has been assigned to the decarboxylation step [17], this view could explain why the initial rate of L-aromatic amino acid decarboxylation (measured either as the production of aromatic amines or as the release of $^{14}CO_2$ from L-dopa) in anaerobiosis is lower than in aerobiosis. Thereafter, the fate of the quinonoid intermediate produced by Cα-COO^- bond scission seems to be different depending on the presence or absence of O_2. Support for this proposal is provided by the fact that k_{cat}/K_m values for both substrates are not greatly affected by the presence of O_2. This implies that the effect of O_2 takes place after the first irreversible step, i.e. the release of CO_2. It is of interest that Abell and Schloss [18] have demonstrated that several enzymes mediating carbanion chemistry are capable of reacting with molecular oxygen, thus catalysing oxygen-consuming side reactions. Among these enzymes, a PLP-dependent enzyme, GAD, has been shown to exhibit oxygenase activity. A delocalized carbanion, the quinonoid intermediate, could be generated during each reaction catalysed by DDC [5,6] and should in principle be capable of reacting with molecular oxygen. Taking into account our results and those reported previously [18], we might envisage that DDC contains a hydrophobic channel for small gaseous molecules such as CO_2 or O_2. However, at present it is not possible to understand why molecular oxygen has a different role and determines different effects on the reaction specificity of DDC depending on the nature of the substrate. The chemical structure of the quinonoid intermediate formed during the course of the reactions, its reactivity with O_2 and/or the substrate-induced conformational state of the enzyme could be relevant factors in this phenomenon.

In conclusion, whatever the role played by O_2 on the reactions catalysed by DDC, the main finding of this study is that, whereas during the decarboxylation of L-aromatic amino acids decarboxylation-dependent transamination does not occur in air, it takes place in anaerobiosis.

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