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Behavior of fluorinated analogs of L-(3,4-dihydroxyphenyl)alanine and L-*threo*-(3,4-dihydroxyphenyl)serine as substrates for Dopa decarboxylase☆

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Abstract

We have determined the kinetic parameters for Dopa decarboxylase (DDC) of three ring-fluorinated analogs of 3,4-dihydroxyphenylalanine (Dopa). The rank order of catalytic efficiency of decarboxylation (k_{cat}/K_m) is Dopa > 6-F-Dopa > 2-F-Dopa > 5-F-Dopa. This rank is consistent with previous in vivo and in vitro studies which indicate that, of the fluorinated analogs, 6-F-Dopa has pharmacokinetics that are most suited for positron emission tomographic (PET) evaluation of dopamine function. The effectiveness of PET as a diagnostic tool, the convenient half-life of ^{18}F (110 min) and the favorable pharmacokinetics of 6-[^{18}F]FDOPA have combined to make this an extremely valuable reagent to study dopaminergic activity. The reactions of the related fluorinated DOPS analogs show that, while 6-F-*threo*-3,4-(dihydroxyphenyl)serine (DOPS) is decarboxylated at approximately the same rate as the non-fluorinated substrate, 2-F-*threo*-DOPS is not converted into the corresponding amine. In both cases a Pictet–Spengler condensation with the pyridoxal 5′-phosphate (PLP) cofactor occurs to produce tetrahydroisoquinolines. Condensation of fluorinated catecholamines and catechol amino acids with endogenous aldehydes will be investigated as an approach to study possible mechanisms of L-Dopa-linked neurotoxicity. Published by Elsevier Science (USA).

Keywords: Dopa decarboxylase; Fluorinated L-Dopa analogs; Fluorinated *threo*-DOPS; Pyridoxal 5′-phosphate

In this report we describe the behavior of fluorinated analogs of L-Dopa (**1**) and of L-*threo*-DOPS (**2**) as substrates for dopa decarboxylase (DDC). Regarding the first series, fluorinated L-Dopas (**1b–d**) can function as biological precursors of fluorinated analogs of the biogenic amines, dopamine, DA (**3**), norepinephrine (NE) (**4**), and epinephrine (EPI) (**5**) [1]. These fluorinated amines, in turn, have proven to be valuable biochemical and diagnostic tools. For example, 2-F-NE (**4b**) and 2-F-EPI (**5b**) are selective β-adrenergic agonists while 6-F-NE (**4d**) and 6-F-EPI (**5d**) are selective α-adrenergic agonists [2,3]. Other studies have revealed that, in many other aspects such as uptake, storage, and metabolism, the fluorinated amines have behavior similar to that of the non-fluorinated parent [1]. This similarity has proven useful in other applications, for example, in the development of 6-[^{18}F]F-L-Dopa as a PET scanning agent to measure in vivo regional dopaminergic activity in the brain [4,5]. Likewise, 6-[^{18}F]F-DA has been used to image cardiac adrenergic activity, functioning as an in vivo precursor of 6-[^{18}F]F-NE [6,7].

The 2- and 5-fluorinated analogs of Dopa also have been examined with respect to uptake and metabolism.

☆ *Abbreviations:* DDC, Dopa decarboxylase; Dopa, 3,4-(dihydroxyphenyl)alanine; DOPS, 3,4-(dihydroxyphenyl)serine; DA, dopamine; NE, norepinephrine; EPI, epinephrine; PLP, pyridoxal 5′-phosphate; PMP, pyridoxamine 5′-phosphate.

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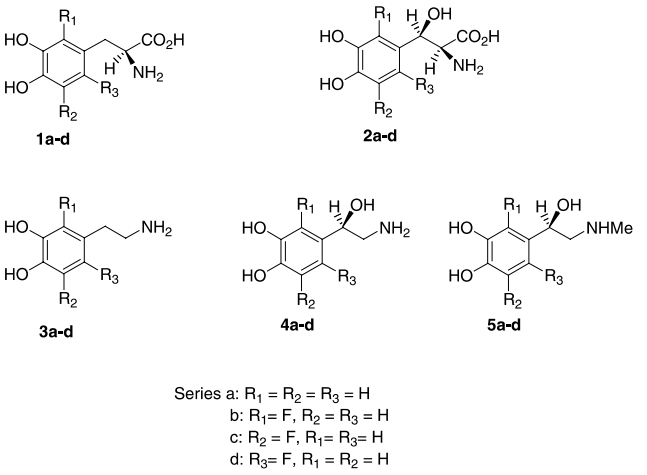
However, their potential use as PET-scanning agents is compromised by problematic pharmacodynamics [8–10]. Of course, critical to applications dependent on the biosynthesis of F-DA is the rate of enzyme-catalyzed decarboxylation relative to other metabolic fates. There is ample evidence for the facile enzymatic conversion of 6-F-L-Dopa (**1d**) to 6-F-DA (**3d**) [11,12]. In addition, there have been comparative studies in vivo and in vitro of the behavior of isomeric ring-fluorinated Dopas toward DDC. For example, a comparison of the kinetics of in vivo decarboxylation 2-[^{18}F]F-Dopa and 6-[^{18}F]F-Dopa in the hooded rat have been reported [10]. Weise et al. have compared the metabolic fates of 5-F and 6-F-L-Dopa in aggregating cell cultures reported in [13]. These and other reports have provided valuable information regarding the uptake and metabolism of these important fluorinated catechol amino acids. However, there appear to be no reports of the direct comparison of the kinetics of decarboxylation of Dopa and the three isomeric ring-fluorinated analogs. As part of our continuing research on the chemical and biological effects of fluorine substitution on catecholamines and amino acids, we have carried out efficient stereoselective syntheses of 2-, 5-, and 6-fluoro-L-Dopa [14]. We report here the examination of their behavior as substrates for DDC.

A close relative of Dopa also has received much attention. Over the past several years there has been substantial interest in L-*threo*-DOPS (**2**) as an alternative precursor of norepinephrine [15]. There is substantial evidence that administered L-*threo*-DOPS crosses the blood–brain barrier and is subsequently decarboxylated to produce norepinephrine in the central nervous system, bypassing dopamine as a biosynthetic intermediate. In fact, several clinical trials suggest that L-*threo*-DOPS may be beneficial in treating disorders of both the central and sympathetic nervous systems [16,17]. Despite evidence that administration of L-*threo*-DOPS increases CNS norepinephrine levels [18], other research indicates that in the brain, the major routes of metabolism are mediated by catechol *O*-methyl transferase and by DOPS aldolase [19,20].

The fluorinated analogs of L-*threo*-DOPS seemed attractive synthetic targets. After transport across the blood–brain barrier, decarboxylation of 2-F-L-*threo*-DOPS (**2b**) would deliver the β-adrenergic selective 2-FNE (**4b**) to the CNS, whereas similar processing of 6-F-L-*threo*-DOPS (**2d**) would produce the α-adrenergic selective 6-FNE (**4d**). It seemed possible that these analogs could thus be used to study the mechanisms of the physiological responses to administered L-*threo*-DOPS. After substantial effort, we achieved stereoselective syntheses of **2b**,**d** by two separate strategies [21,22]. A critical examination of their behavior as substrates for enzymatic decarboxylation seemed an appropriate initial study to evaluate their potential to deliver FNEs to the CNS. Accordingly, we included these analogs in our examination of fluorinated catechol amino acids as substrates for DDC.



Materials and methods

Compounds. L-Dopa, D,L-*threo*-DOPS, PLP, and PMP were purchased from Sigma. All other chemicals were of the highest purity available. Fluorinated analogs of L-Dopa (**1b–d**) and L-*threo*-DOPS (**2b**,**d**) were prepared as described in recent publications [14,21,22]. Fluorinated analogs of DA [23] (**3b–d**) and NE [2] (**4b**,**d**) were prepared using methods similar to the described procedures.

Enzyme preparation. Recombinant DDC was purified to homogeneity from *Escherichia coli* expressing pKKDDCΔ4Δ3′ as described [24] and used throughout. The enzyme concentration was determined using a molar extinction coefficient of $1.30 \times 10^5\,M^{-1}\,cm^{-1}$ [25].

Enzymic assays. DDC activity was determined by measuring amine production with a spectrophotometric assay outlined by Sherald et al. [26] and modified by Charteris and John [27]. The reaction mixture contained L-Dopa or fluorinated Dopa analogs in potassium phosphate 0.1 M, pH 6.8, and was incubated at 25 °C. The reaction was initiated by the addition of the enzyme. At various times aliquots were withdrawn and, after denaturation by boiling for 1 min at 100 °C, benzene and 2,4,6-trinitrobenzene-1-sulfonic acid were added. The reaction forming the trinitrophenylamine derivative was carried out at 42 °C for 1 h with continuous shaking. The concentrations of trinitrophenylamine derivative in the benzene layer were measured by using 12,400, 8700, 14,110, and 11,780 as the molar extinction coefficients for *N*-trinitrophenyldopamine, *N*-trinitrophenyl-2-F-dopamine, *N*-trinitrophenyl-5-F-dopamine, and *N*-trinitrophenyl-6-F-dopamine, respectively. The initial velocity of decarboxylation at varying concentrations of substrate (ranging from 0.1 to 5 mM) was calculated from the linear phase.

Alternatively, when the production of aromatic amine is very low, as with the decarboxylation of L-*threo*-DOPS and its fluorinated analogs, decarboxylase activity was measured by HPLC analysis. From the reaction mixture containing DDC and substrate in 0.1 M potassium phosphate buffer, pH 6.8, 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 mobile phase consisted of 20% acetonitrile containing the following: 0.1 M NaH_2PO_4, 0.34 mM EDTA, and 1.85 mM octanesulfonic acid adjusted to pH 3.6 with phosphoric acid. The flow rate was 0.6 ml/min, and the detection was set at 280 nm. The

concentration of non-fluorinated and fluorinated 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.

HPLC detection of PLP, PMP, and Pictet–Spengler adducts. Characterization of the reaction of PLP with fluorinated analogs of L-*threo*-DOPS, the chemical synthesis of PLP-fluorinated L-aromatic amino acids Pictet–Spengler adducts and also their isolation by HPLC were performed as described previously for L-aromatic amino acids [28]. The detection and quantification of PLP, PMP, and PLP-aromatic amino acid Pictet–Spengler adducts during the reaction of DDC with fluorinated analogs of L-Dopa or L-*threo*-DOPS were performed with the HPLC procedure described previously [28]. 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.

Results and discussion

The interactions of ring-fluorinated analogs of L-Dopa with DDC were examined. Fluorine is often considered to be an isosteric replacement for hydrogen in biological molecules. However, fluorinated compounds may have quite different physical, chemical, and biological properties compared to their natural counterparts [29]. The kinetic parameters for the decarboxylation of Dopa and 2-, 5-, and 6-F-Dopa are shown in Table 1. As can be seen, the predominant influence of fluorine substitution on decarboxylation is related to an increase in K_m for all fluorinated analogs (5-F > 2-F > 6-F > Dopa). This implies that the presence of fluorine substituents at ring position 2, 5, or 6 affects binding to the active site. In contrast, the effects of fluorine substitution on the k_{cat} for decarboxylation are minimal. Thus, the fluorinated analogs are decarboxylated less efficiently by DDC as a result of lower affinity for the enzyme. Of the analogs, 6-F-Dopa is the best substrate, with a k_{cat}/K_m some 10% that of L-Dopa. These data might appear to be in contrast with those of Weise et al. [13]. They found that, in aggregating cell cultures of fetal rat brain, while only 1% of injected 5-F-Dopa was converted to 5-F-DA, 6-F-Dopa was converted to decarboxylated metabolites to an extent comparable to that of L-Dopa. We have no clear explanation for this difference, although experimental conditions (whole cell vs purified enzyme, and/or concentration for L-Dopa, 6-F-Dopa, and 5-F-Dopa that are ~2-, 14-, and 65-fold lower than their respective K_m's) may be partially causative''. Interestingly, although 6-F-Dopa and 2-F-Dopa are decarboxylated in vitro with similar k_{cat}, as shown by our studies and those of Cumming et al. [10], numerous differences between the metabolism of these compounds have been observed. In fact, the peripheral formation of *O*-methylated derivative of L-2-^{18}F-Dopa was approximately twice as great as that for the 6-isomer, and L-2-^{18}F-Dopa passed less readily into brain than did L-6-^{18}F-Dopa. In addition, whereas significant amount of 6-^{18}F-DA and metabolites were formed in the striatum, no decarboxylated derivatives of L-2-^{18}F-Dopa were found [10].

It is noteworthy that a competing decarboxylation-dependent transamination reaction accompanies the decarboxylation of 2-F-Dopa with a partition ratio 1:350. In contrast, like L-Dopa [30], 5- and 6-F-Dopa do not undergo this abortive transamination. Considering that there is no direct electronic communication between the ring fluorine and the site of the chemical transformation, steric effects in the active site should be invoked. One may postulate that binding of 2-F-Dopa may produce minor distortions of enzyme structure and thereby alter slightly the spatial alignment of catalytic groups. In this case, according to Snell [31], abortive reactions become prominent. The observation that the isomerization reaction of tryptophan synthase toward D and L isomers of 5-fluorotryptophan is faster than with the natural substrate is consistent with this view [32].

It has already been reported that DDC catalyzes the oxidative deamination of dopamine [33]. Interestingly, while 5-F-DA and 6-F-DA undergo oxidative deamination with a rate (~1–2 mol product/min/mol enzyme) comparable to that of dopamine, 2-F-DA does not behave as a substrate in this reaction. This is further evidence that fluorine substitution in position 2 of the aromatic ring can perturb the alignment of catalytic groups in the enzyme.

We also examined the reactions of the fluorinated analogs of L-*threo*-DOPS with DDC. 6-F-*threo*-DOPS, at a concentration of 10 mM, is decarboxylated by DDC with an initial velocity of ~2 mol 6-FNE formed/min/mol enzyme (Fig. 1A), similar to that of the non-fluorinated compound under the same experimental conditions. In contrast, under these conditions there was no detectable decarboxylation of 2-F-*threo*-DOPS. As can be seen, the rate of decarboxylation of 6-F-*threo*-

Table 1
Kinetic parameters of DDC for L-Dopa and fluorinated Dopa analogs

Substrate	K_m (mM)	k_{cat} (s^{-1})	k_{cat}/K_m ($s^{-1}\,mM^{-1}$)
L-Dopa	0.091 ± 0.005	9.1 ± 0.2	100 ± 6
2-F-Dopa	0.95 ± 0.35	5.2 ± 0.5	5.47 ± 1.91
5-F-Dopa	3.35 ± 0.6	3.47 ± 0.3	1.04 ± 0.21
6-F-Dopa	0.7 ± 0.1	8.2 ± 0.6	11.7 ± 1.8

k_{cat} and K_m were obtained from non-linear regression fit to the Michaelis–Menten equation using Sigma Plot 2001 (SPSS). The errors reported are the standard error values derived by the curve fitting program.

DOPS gradually decreases despite the fact that substrate concentration is still high. The reason is that the coenzyme (PLP) is converted into a cyclic Pictet–Spengler adduct formed by condensation between PLP and 6-F-*threo*-DOPS. Fig. 1B also shows the rate of conversion of DDC-bound PLP into the cyclic adduct. As can be seen, there is correspondence between the decrease in decarboxylase activity and the conversion of PLP into the inactive adduct. Similarly, during the reaction of DDC with 2-F-*threo*-DOPS a cyclic adduct between coenzyme bound and the fluorinated compound takes place. It should be pointed out that no cyclic adduct formation is observed during the reaction of DDC with D,L-*threo*-DOPS. Mechanistic speculations aside, it is evident that the use of 2-fluorinated analog of L-*threo*-DOPS as in vivo precursor of 2-FNE may be compromised by this behavior with DDC.

Because of unexpected facility with which 2-F- and 6-F-*threo*-DOPS undergo Pictet–Spengler cyclization, we are exploring their reactivities with other aldehydes. For example, this may provide a tool for examination of possible in vivo formation of biogenic aldehyde-derived tetrahydroisoquinolines or in vivo formation of products resulting from a similar cyclization with D-glucose (Maillard reaction). Such reactions have been suggested as possible mechanisms for certain neurotoxic side effects of L-Dopa therapy [34].

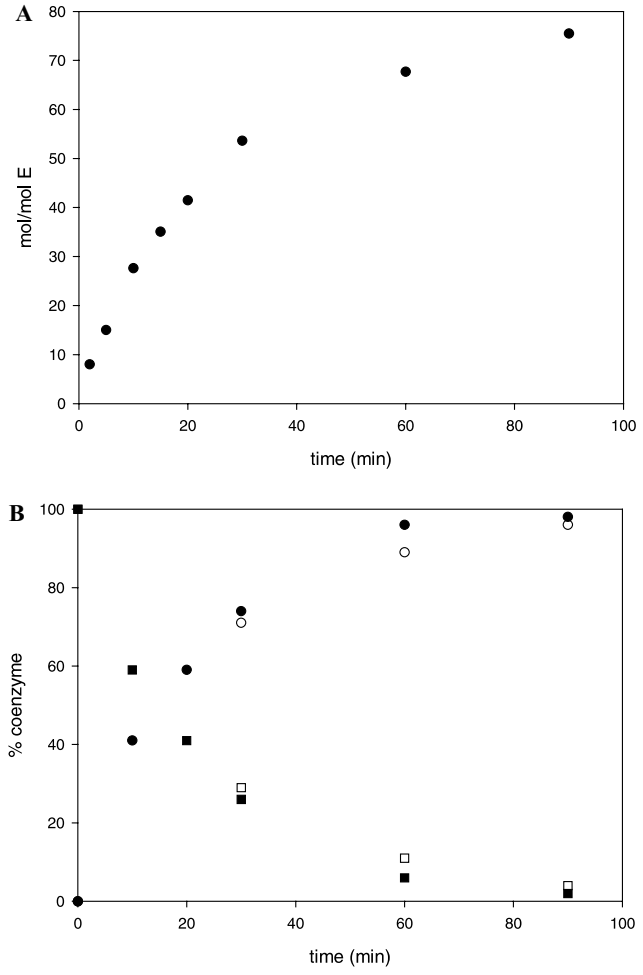


Fig. 1. Reaction of DDC with fluorinated *threo*-DOPS. (A) The enzyme (10 μM) was incubated with 10 mM 6-fluoro-DOPS in 100 mM potassium phosphate buffer, pH 6.8. Aliquots were removed at the indicated times and subjected to HPLC analysis for amine formation. (B) The enzyme (10 μM) was incubated with either 10 mM 2-F-*threo*-DOPS (open symbol) or 10 mM 6-F-*threo*-DOPS (closed symbol). Aliquots were removed at the indicated times and subjected to HPLC analysis for PLP (square) or cyclic adduct (circle).

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