SYNTHESIS AND ENZYMATIC DECARBOXYLATION OF 6-BROMO-L-DOPA, A POTENTIAL BRAIN TRACER FOR L-DOPA*

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SUMMARY

Direct bromination of L-dopa with stoichiometric amounts of molecular bromine was found to yield 6-bromo-L-dopa as the sole product. This determination was made using liquid chromatography with electrochemical detector, mass spectroscopy, and proton NMR. 6-Bromo-L-dopa was found to be a substrate of an isolated decarboxylase enzyme although the rate of decarboxylation was much slower than that for L-dopa. These results suggest that a radiobrominated L-dopa analog may be a suitable PET or SPECT tracer for L-dopa in the brain.

Keywords: 6-bromo-L-dopa, L-dopa tracer, emission tomography

INTRODUCTION

A radiolabeled analog of L-dopa, ^{18}F-fluoro-L-dopa, has been developed by Firnau et al., for imaging dopamine-containing structures in the human brain by positron emission tomography (PET)(1). This method has potential for studies on mechanisms which may underlie various neuropsychiatric disorders where abnormalities in dopamine transmission have been implicated (1,2). Although 6-^{18}F-fluoro-L-dopa has been useful, several methods to synthesize this radiotracer give low yields due to lack of regiospecificity of most syntheses. The aromatic radiofluorination of L-dopa using ^{18}F-F_2, for example, yields three isomers, 2-, 5-, and 6-^{18}F-fluoro-L-dopa (3). Chromatographic isolation of 6-^{18}F-fluoro-L-dopa

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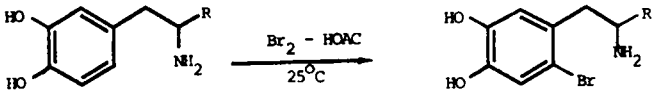
(^{18}F-6FD), the analog most suitable as cerebral L-dopa tracer, is then necessary for use in PET. Improved yields have recently been made in the synthesis of ^{18}F-6FD using ^{18}F-acetylhypofluorite, a more stereoselective radiofluorinating agent (4,5). The bromination of L-dopa, on the other hand, leads exclusively to one product, 6-bromo-L-dopa (6,7). Thus, it appears that a radiobrominated analog of L-dopa might serve as a more convenient compound for visualizing dopamine-containing brain structures using tomographic devices detecting either single photons or positron annihilation radiations.

Initial studies have indicated that radiobrominated L-dopa is decarboxylated to bromo-dopamine, in vivo, since rats given ^{77}Br-bromo-L-dopa were found to contain ^{77}Br-bromo-dopamine and ^{77}Br-bromo-DOPAC in their brains (8). This suggested that bromo-L-dopa may be a useful tracer in DA turnover studies when labelled with ^{75}Br using PET or with ^{77}Br using single photon tomography (SPECT). In this study, we confirm that the bromination of L-dopa with stoichiometric amounts of molecular bromine leads to only one product, 6-bromo-L-dopa. We also report a comparison of the rate of decarboxylation of 6-bromo-L-dopa to that of the native L-dopa, in vitro, using an isolated decarboxylase enzyme.

MATERIAL AND METHODS

Chemicals: L-Dopa, dopamine-HCL, and aromatic amino acid (L-tyrosine) decarboxylase (EC 4.1.1.25) were obtained from Sigma Chemical Co. (St. Louis, MO). Bromine and other chemicals were obtained from Fisher Scientific Co. Fairlawn, NJ.

HPLC Apparatus: High performance liquid chromatography (HPLC) was used to monitor both the bromination and decarboxylation reactions. The system consisted of an Alltech Adsorbosphere C18, 10 micron, 4.6 x 250 mm reversed-phase column, a Altex Model 110A pump, and Model LC-2A electrochemical detector with a glassy carbon electrode (Bioanalytical Systems, Lafayette, Indiana) was used to monitor both the bromination and decarboxylation reactions. The potential of the working electrode was maintained at +0.75 V vs. a Ag/AgCl reference electrode. The mobile phases used were: 90% .02M KOAc +10% MeOH, adjusted to pH 3.9 with acetic acid, for monitoring the bromination and decarboxylation of L-dopa; and 80% 0.02M KOAc + 20% MeOH, pH 3.9, for monitoring the decarboxylation of bromo-dopa.

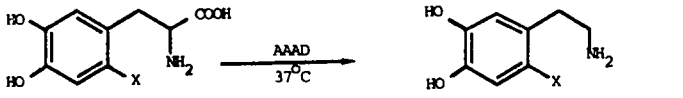


R = H, dopamine
= COOH, L-dopa

R = H, 6-bromo-dopamine
= Br, 6-bromo-L-dopa

Scheme 1

Bromination of L-dopa and dopamine (Scheme 1): 0.59 g of L-dopa was dissolved in 30 ml glacial acetic acid. Several drops of HCl were added to the mixture to aid in completely dissolving L-dopa. 3 ml 1M Br_2 in HOAc was then added dropwise to the stirred solution at room temperature. 2 µl sample of the reaction mixture was analyzed by HPLC at time intervals to follow the reaction. When the reaction was complete, the solvent was evaporated almost to dryness and distilled water and sodium bicarbonate were added to pH 5.0. The bromo-dopa product was crystallized at 4 °C, vacuum filtered, washed with distilled water, and, finally, dried. The identity and purity of the product was determined by HPLC, NMR spectroscopy, and mass spectrometry. The same procedure was followed to synthesize 6-bromo-dopamine from dopamine. This served as an HPLC marker for identifying the product of the decarboxylation of 6-bromo-L-dopa.



X = H, L-dopa
= Br, 6-bromo-L-dopa

X = H, dopamine
= Br, 6-bromo-dopamine

Scheme 2

Decarboxylation of L-Dopa and 6-Bromo-L-Dopa (Scheme 2): The reaction conditions used for the decarboxylation of both L-dopa and 6-bromo-L-dopa were procedures recommended by the supplier of the decarboxylase enzyme (Sigma Chem. Co.). The reaction medium contained 2 mmoles substrate, either L-dopa or 6-bromo-L-dopa, in a citrate-phosphate buffer, pH 5.5 at 37°C. Separate reactions were run using two enzyme concentrations, 0.7 mg and 1.4 mg L-tyrosine decarboxylase in 0.5 ml buffer. The reaction was started by adding the enzyme solution to the substrate solution and aliquots were analyzed by HPLC. A solution of the substrate was also monitored over the same time period in the absence of the enzyme to control for non-enzymatic degradation of the substrate.

Calculations: The rates of enzymatic decarboxylation of L-dopa and bromo-L-dopa were measured and compared by two parameters: (1) K_{enz}, rate constant at a given concentration of enzyme. This was calculated from:

$$K_{enz}[E] = K_{obs} - K_{buffer}$$

where [E] is the enzyme concentration, K_{obs} and K_{buffer} are rate constants for the disappearance of substrate (L-dopa or 6-bromo-L-dopa) in the reaction medium and buffer (enzyme blank), respectively, determined by the first order kinetic equation:

$$\ln A_t / A_o = - K_{obs}t$$

with (A_t / A_o) being the ratio of the reactant HPLC peak area at time t to that at zero time.

(2) $t_{1/2}$, the time it took a given amount of enzyme to convert half the substrate to product.

RESULTS AND DISCUSSION

Bromination of L-Dopa: Upon addition of the bromine to the L-dopa solution, instant decolorization of the dark red bromine solution was observed indicating that rapid bromination was occurring. Chromatograms of aliquots of the reaction mixture over time showed a disappearing L-dopa peak and a correspondingly growing single peak, presumably the bromo-L-dopa product peak. This reaction was found to go to completion. The product was precipitated by neutralizing the acetic acid to pH 5. The crystals were isolated and dried and found to decompose at 180°C. Chemical analysis of the recrystallized product by NMR showed aromatic protons, 6.65 ppm and 6.95 ppm, with para coupling. Negative ion FAB mass spectrometry showed $(M-H)^-$= 274,276(1:1) which is consistent with bromine isotopic ratio. These analyses indicate a mono-brominated product, 6-bromo-L-dopa.

The direct bromination of dopamine resulted in an oily product. NMR analysis of this oil showed aromatic protons with small (< 1Hz) para coupling while positive CI mass spectrometry showed $(M+H)^+$=232,234(1:1). These results are consistent with 6-bromo-dopamine as the product. No further effort was made to crystallized the product which was used as HPLC marker in this study.

Decarboxylation of L-Dopa and 6-Bromo-L-Dopa: The enzyme used in this study, L-tyrosine decarboxylase (AAAD), a bacterial enzyme from streptococcus faecalis, is specific for both L-tyrosine and L-dopa (9). Chromatograms monitoring the decarboxylation reactions of both L-dopa and 6-bromo-L-dopa over time showed a decreasing reactant peak and increasing product peak (Figure 1). The retention times of both the brominated and non-brominated reactants and products agree with those observed for the authentic compounds, (L-dopa - 1.2 min, DA - 2.1 min with 90% .02M KOAc +10%MeOH mobile phase; 6-Br-L-Dopa -1.6 min, Br-DA - 3.9 min with 80% .02M KOAc + 20% MeOH), thus, confirming their identity.

The plots of the ratio of HPLC peak area at time t, A_t, to that at time 0, A_0, A_t/A_0, versus time for L-dopa and 6-bromo-L-dopa both in the presence and absence of the enzyme are shown in Figure 2. Both the native and brominated L-dopa compounds were decarboxylated enzymatically but the reaction of 6-bromo-L-dopa was much slower than that for L-dopa. The calculated rates and half times for these reactions at two enzyme concentrations are listed in Table 1. These results show that decarboxylation of the brominated analog by the bacterial enzyme is about 260 times slower than that for the native compound.

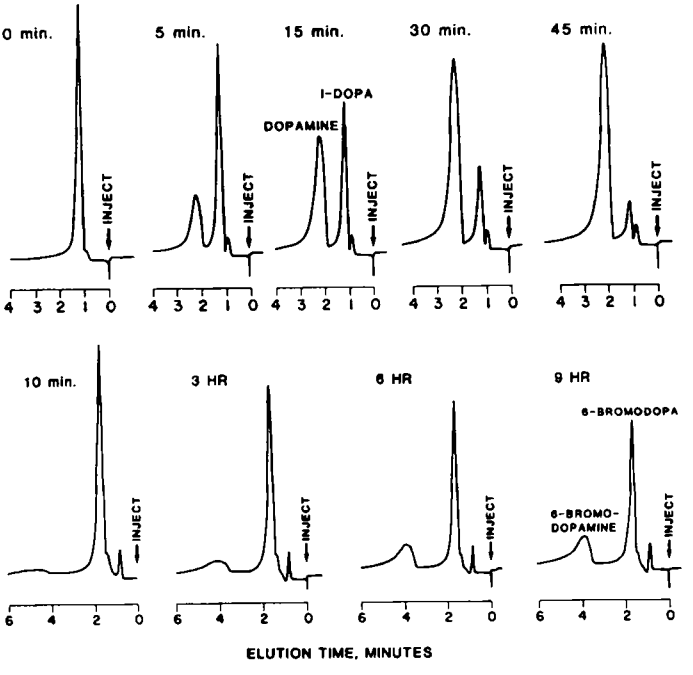


FIGURE 1. Enzymatic decarboxylation of L-dopa (upper chromatograms) and 6-bromo-L-dopa (lower chromatograms) as a function of reaction time monitored by HPLC-EC.

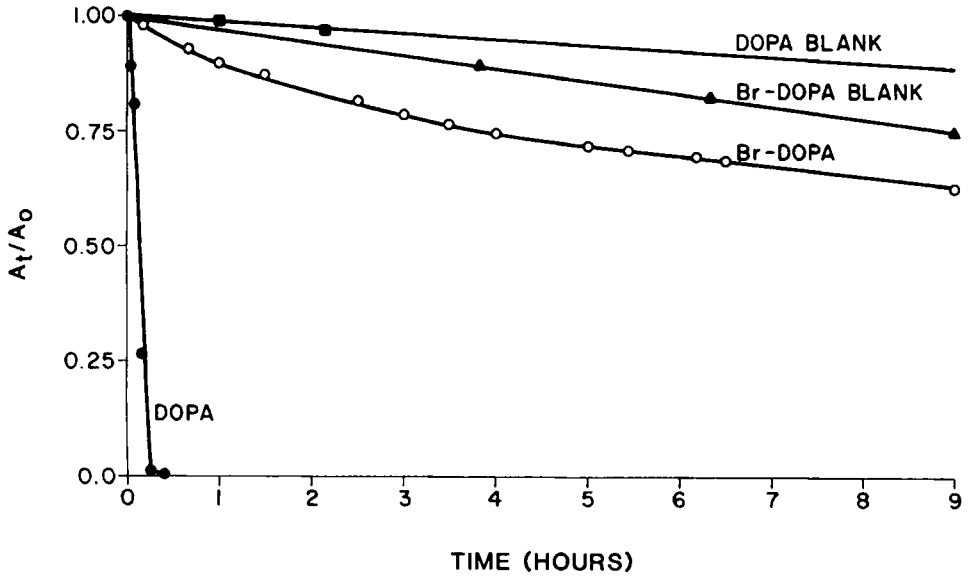


FIGURE 2. Kinetics of the enzymatic decarboxylation of L-dopa (●) and 6-bromo-L-dopa (o) and non-enzymatic decarboxylation of L-dopa (■) and 6-bromo-L-dopa (▲).

TABLE I

K_{enz} and $t_{1/2}$ for the Enzymatic Decarboxylation of L-Dopa and 6-Bromo-L-Dopa at Various Enzyme Concentrations

	L-dopa		6-bromo-L-dopa	
[E],mg/ml	K_{enz},min^{-1}	$t_{1/2}$,min	K_{enz},hr^{-1}	$t_{1/2}$,hr
0.0	0.001	46.6	0.032	21.7
0.7	0.173	4.01	0.040	17.3
1.4	0.311	2.23	0.073	9.5

CONCLUSION

The identification of 6-bromo-L-dopa as the sole product of a straightforward electrophilic bromination reaction indicates that this compound labelled with ^{75}Br or ^{77}Br can

be easily prepared. Since bromine isotopes are generally available as bromide, generation of electrophilic radiobromine species in situ using oxidizing agents such as N-chloro-succinimide in the presence of L-dopa is a viable scheme. We are currently investigating this radiobromination reaction using the diacetate of L-dopa as the starting material.

Our demonstration of enzymatic decarboxylation of 6-bromo-L-dopa suggests that this radiopharmaceutical may be suitable for PET or SPECT studies of the DA system. Although 6-bromo-L-dopa analog is a poor substrate for the bacterial enzyme, the specificity of the decarboxylase in mammalian brain may be broader as suggested by preliminary in vivo experiments where 6-bromo-DA and 6-bromo-DOPAC in addition to 6-bromo-L-dopa were detected in rat brain (8). Further studies on cerebral extraction and cerebral and peripheral metabolism are needed to explore the potential of this bromo analog of L-dopa. If 6-bromo-L-dopa is indeed decarboxylated very slowly by mammalian brain, but crosses the blood brain barrier easily, it might be useful for determination of local rates of L-dopa transport, analogously to the use of well-transported, poorly phosphorylated glucose analogs (10,11). Furthermore, because of slow decarboxylation, a greater fraction of 6-bromo-L-dopa may cross the blood brain barrier compared to L-dopa without the need for a peripheral decarboxylase inhibitor; this would lead to improved counting statistics. In conclusion, radiobrominated 6-bromo-L-dopa, easily and selectively prepared via electrophilic reactions, may be useful to study transport and disposition of L-dopa itself in the brain.

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