Synthesis of Radiobrominated m-Tyrosine

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Summary

The electrophilic radiobromination of m-tyrosine was investigated and found to be rapid and quantitative. Two positional isomeric products were found by radio-HPLC with a mass ratio of 4:1. Because of the inability of proton NMR to unequivocally identify the major product, ^{13}C-NMR analysis was performed. Results show that the major product was 6-bromo- D,L-m-tyrosine. Preliminary studies, in vivo, in mice suggest that radiobrominated m-tyrosine is a potential tracer for CNS dopamine.

Keywords: radiobromination, 6-bromo-m-tyrosine, PET scanning, dopamine turnover

Introduction

In our continuing efforts to develop L-dopa tracers for use in positron tomography (PET), we have previously reported the synthesis and enzymatic decarboxylation of 6-bromo-L-dopa (6-BD) (1) as an alternative radiotracer to 6-fluoro-L-dopa (6-FD). One advantage of 6-BD was its ease of preparation and regioselectivity of the electrophilic exchange yielding only the 6-BD isomer. However, these advantages of 6-BD over 6-FD have been diminished by the current availability of site-directed mercurated starting material for 6-FD (2,3) and also by our finding that 6-BD is decarboxylated about 250 times slower than L-dopa by the bacterial decarboxylase enzyme (1). This latter finding, however, may not be applicable to mammalian enzyme.

Positron tomography and ^{18}F-6-FD have been found to be useful in the study of Parkinson's disease (PD) (4), MPTP-induced PD (5), and dystonia (6). However, because of the rapid metabolism of 6-FD by the enzyme catechol O-methyl transferase (COMT), forming O-methyl- 6-FD which freely penetrates the blood brain barrier and adds "noise" to the PET image (7,8), full quantitative treatment of 6-FD PET scans has been difficult. Decarboxylase inhibitors (e.g. carbidopa) have been used in several laboratories as an adjunct to 6-FD PET scanning in order to

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improve counting statistics in the brain (5,9). These inhibitors, however, also increase the amount of ^{18}F which non-selectively enter all brain areas including those normally used as reference such as the cerebellum. This suggests that semi-quantitative measures using striatal and cerebellar uptake ratio may not be satisfactory. Recent work by Cumming et al. (10) found that the use of a COMT inhibitor, U-0521, resulted in the altered metabolism of ^{18}F-6-FD in the rat suggesting that if used in human PET studies, U-0521 could further complicate data analysis.

An alternative approach to the use of COMT inhibitors would be the use of L-dopa analogs which are not COMT substrates but nevertheless retain the metabolically important characteristics of L-dopa to be useful as dopamine tracer. Following this approach, we have begun studies evaluating the utility of a positron-emitting m-tyrosine analog as a tracer for CNS dopamine. Since m-tyrosine lacks the enediol moeity required of COMT substrates (11), formation of the unwanted O-methyl metabolite is then avoided. But unlike the more common p-tyrosine, m-tyrosine is a substrate of mammalian amino acid decarboxylase (AADC) enzyme retaining 73% of the activity of L-dopa as AADC substrate (12). Studies using experimental animals have found m-tyrosine to have effects similar to L-dopa including turning behaviour in rats with unilateral lesions of the nigrostriatal pathway and also preventing tremors in monkeys with unilateral lesions of the ventromedial tegmental area (13). Further, the major urinary metabolites found in humans given m-tyrosine were m-tyramine, m-hydroxyphenylacetic acid, m-hydroxyphenylpyruvic acid, and m-hydroxymandelic acid; metabolites thought to be products of the same enzyme reactions as those involved in dopamine (DA) synthesis and metabolism (14).

In this report, we have studied the radiobromination of m-tyrosine with the aim of preparing Br-75-labeled m-tyrosine for potential use as a PET tracer to assess central DA turnover.

Methods and Materials

DL-m-tyrosine was purchased from Sigma Chemical Co. (St. Louis, MO). ^{75}Br was produced by the (d,nα) reaction on enriched ^{78}Kr gas using 21.5 Mev deuterons produced by the ANL 60-inch cyclotron (15). The high performance liquid chromatograph used in this study consisted of an Altex 110A solvent metering pump, an Alltech C-18 Econosil 250 mm x 10 mm column, and dual detectors, Altex UV-Vis detector (254 nm) and a radiation flow detector with a NaI(Tl) crystal. Isocratic elution with 0.02M sodium acetate pH 3.75: methanol (95:5) at a flowrate of 2.7 ml/min was done. 50.32 MHz ^{13}C NMR spectra were taken on a Bruker WP-200 FT

spectrometer under proton-coupled and proton-decoupled conditions while 100 MHz ^{1}H NMR spectra were taken using a Varian XL-400 instrument. FAB mass spectrometer was used to analyze the product.

A. Bromination of m-Tyrosine

To a suspension of 0.5 g (2.76 mmol) DL-m-tyrosine (Sigma Chem. Co., St. Louis, MO) in 50 ml glacial acetic acid was added dropwise 5ml (3 mmol) bromine in acetic acid. The addition was complete in 30 minutes when most of the solid had gone into solution. The mixture was allowed to stir at room temperature for 2 hours. After removing acetic acid at reduced pressure, the crude solid was taken up in absolute ethanol (20 ml). A saturated solution of sodium bicarbonate (0.25 g) was added and the mixture was refrigerated. The first crop of crystals (0.45 g) had a melting point of 260-262 ^{o}C (decomposes). FAB-MS showed a pair of molecular ions $(M+1)^{+}$ at 260, 262 (relative intensity 100%) consistent with the molecular weight of natural ^{79}Br - and ^{81}Br -bromo-tyrosine. ^{1}H-NMR (D_2O, DCl) δ 4.19-4.33 (q, 1H, α-CH), δ 2.99- 3.29 (m, 2H, β-CH_2), δ 6.67 (d,1H, aromatic 2-CH), δ 6.57-6.59 (dd, 1H, aromatic 4-CH), δ 7.31 (d, 1H, aromatic 5-CH). ^{13}C-NMR (^{1}H decoupled, pH 1.1) δ 172.2 (-COOH), δ 53.8 (α-CH), δ 37.2 (β-CH_2), δ 135.6 (aromatic C-1), δ 119.5 (aromatic C-2), δ 156.4 (aromatic C-3), δ 117.9 (aromatic C-4), δ 135.0 (aromatic C-5), δ 114.9 (aromatic C-6).

B. Radiobromination of D,L-m-tyrosine

Several oxidizing agents, namely, N-chlorosuccinimide (NCS), $KBrO_3$, and H_2O_2-HOAc (1:1), were tested in studies to incorporate ^{75}Br-bromide into DL-m-tyrosine using the following reaction mixtures:

(a) 50 µl aqueous ^{75}Br-bromide (200 µCi) + 100 µl 0.1M NCS + 200 µl 1M H_2SO_4 + 100 µl 0.9 mg/ml m-tyrosine in dilute HOAc.

(b) 50 µl aqueous ^{75}Br-bromide (200 µCi) + 1 mg $KBrO_3$ in 200 µl 1M H_2SO_4 + 100 µl 0.9 mg/ml m-tyrosine in dilute HOAc.

(c) 50 µl aqueous ^{75}Br-bromide (200 µCi) + 100 µl H_2O_2-HOAc (1:1) + 100 µl 0.9 mg/ml m-tyrosine in dilute HOAc.

In order to monitor the extent of ^{75}Br substitution, an aliquot (10-15 µl) of each of the above mixtures was analyzed by radioHPLC at various reaction times at room temperature.

Results and Discussion

Direct bromination of DL-m-tyrosine in glacial HOAc was found to be rapid and quantitative. Results of proton NMR analysis of the recrystallized HPLC pure product proved inconclusive as to which position in the phenyl ring the bromine was in, either position 4 or 6. Thus, ^{13}C-NMR analysis was performed to make this identification using well known substituent effects on aromatic carbon shifts (20). The observed chemical shifts were compared with expected (calculated) values for the cases where either 6- or 4- bromination is assumed, i.e., with one mass spectrum, two sets of peak assignments of the ring carbons were made. A comparison of the difference in calculated and observed chemical shifts, in ppm, of the ring carbons of each assumed brominated m-tyrosine isomer is reported relative to the parent m-tyrosine compound. The results (Table I) show that the ^{13}C-NMR data are in excellent agreement with the product being 6-bromo-m-tyrosine (6-BMT).

Table I

Difference in calculated vs observed chemical shifts (ppm) between brominated-m-tyrosine and m-tyrosine

	6-bromo-m-tyrosine		4-bromo-m-tyrosine	
Carbon	calculated	observed	calculated	observed
1	3.4	-1.1	-1.6	-1.1
2	1.7	2.3	1.7	0.7
3	-1.6	-0.6	3.4	-0.5
4	1.7	2.1	-5.5	-0.9
5	3.4	3.5	3.4	3.6
6	-5.5	-7.4	1.7	-2.8

In order to introduce ^{75}Br into m-tyrosine, oxidizing agents to generate electrophilic ^{75}Br species *in situ* from ^{75}Br-bromide are required. The first oxidizing agent we tried for this purpose was N-chlorosuccinimide (NCS). Using HPLC to follow the reaction, after about one hour of reaction less than 10% of the radiobrominated product was formed (Figure 1A). In contrast, using $KBrO_3$ in 1M H_2SO_4 as oxidizing agent, complete incorporation of ^{75}Br within 4 minutes was observed (Figure 1B). However, the use of $KBrO_3$ introduces "cold" Br into the reaction thereby reducing the specific activity of the product (16).

For no carrier added radiobrominations, we have found H_2O_2-HOAc mixture to be an effective oxidizing agent (17,18). As seen in Figure 2, this method is likewise fast and effective in incorporating ^{75}Br into m-tyrosine wherein quantitative incorporation of ^{75}Br within 10 minutes was seen. The major product co-eluted by HPLC with authentic 6-BMT while a radiobrominated

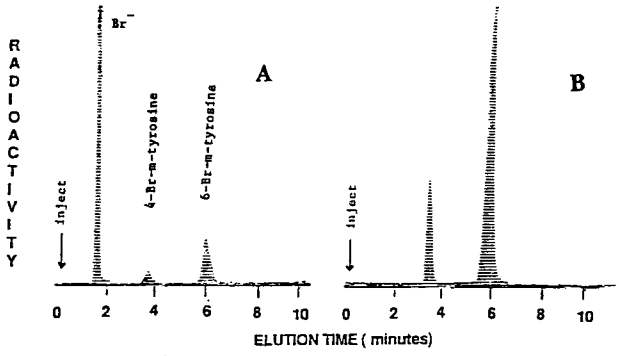

Fig. 1. Radiosynthesis of ^{75}Br-BMT using ^{75}Br-bromide and N-chlorosuccinimide (A) and $KBrO_3$ (B) as oxidizing agents as monitored by radio-HPLC.

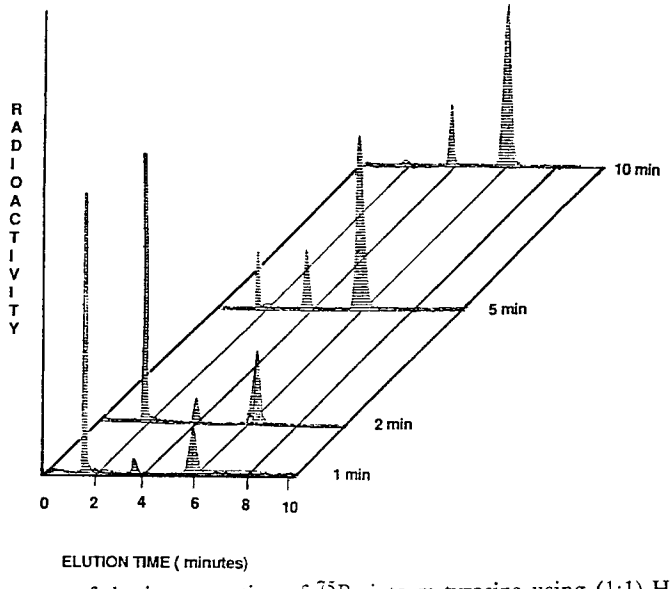

Fig.2. Time course of the incorporation of ^{75}Br into m-tyrosine using (1:1) H_2O_2-HOAc as oxidant as followed by radio-HPLC. Peaks are the same as in Fig.1.

minor product, which we have tentatively identified as 4-bromo-m-tyrosine amounting to about 20%, was also seen.

In preliminary studies to evaluate 6-BMT as a DA tracer, HPLC purified ^{75}Br-6-BMT was administered into several sets of mice and the time courses of the radiotracer in the blood, striatum, cerebellum, kidneys, and liver were determined (19). Results showed that (a) substantial amounts of ^{75}Br-6-BMT was found to be extracted by the brain, e.g., 3% injected dose/g was detected in the striatum, (b) clearance rates and regional cerebral uptake was found to be consistent with dopamine innervation, i.e., faster clearance from blood and cerebellum compared to striatum, and (c) striatum

to cerebellum (S/C) uptake ratio one hour after injection was 2.8. This S/C uptake ratio would be expected to improve with the use of the pure L-isomer.

In summary, we have prepared radiobrominated 6-bromo-D,L-m-tyrosine using ^{75}Br-bromide and 1:1 H_2O_2-HOAc resulting in 80% radiochemical yield at the no carrier added level. Preliminary *in vivo* murine studies suggest that our approach of using non-COMT substrates as tracers for dopamine turnover is viable but further studies in *vivo* and in *vitro* are necessary to validate the utility of this approach to PET scanning.

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