JOURNAL OF LABELLED COMPOUNDS AND RADIOPHARMACEUTICALS
J Label Compd Radiopharm 2006; **49**: 1061–1067.
Published online in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/jlcr.1123

Research Article

Synthesis of isotopomers of dopamine labeled with deuterium or tritium

M. Pająk and M. Kańska*
Department of Chemistry, University of Warsaw, Pasteur Str. 1, 02-093 Warsaw, Poland

Summary

The synthesis of four isotopomers of dopamine labeled with deuterium or tritium is reported. The ring labeled [2′,5′,6′-$^{2}H_3$]-, and [2′,5′,6′-$^{3}H_3$]-dopamine were obtained using acid catalyzed isotopic exchange between dopamine and heavy or tritiated water respectively. Two selectively labeled isotopomers, i.e. [1R-^{2}H]-, and [1R-^{3}H]-dopamine were synthesized by enzymatic decarboxylation of L-DOPA using the enzyme tyrosine decarboxylase (EC 4.1.1.25) from *Streptococcus faecalis*.

Received 27 June 2006; Revised 2 August 2006; Accepted 2 August 2006

Key Words: dopamine; deuterium; enzyme; labeling; tritium

Introduction

The biogenic dopamine, DA, plays an important role in many physiological functions as a neurotransmitter in the nervous system of mammals.[1–3] DA is formed in the brain by decarboxylation of L-DOPA catalyzed by enzyme *Aromatic* L-*Amino Acid Decarboxylase*[4–7] (EC 4.1.1.28), Figure 1. DA is also involved as a precursor in the synthetic enzymatic route of the other catecholamines as adrenaline and noradrenaline,[8–11] Figure 2.

In the recent years studies on the properties and pharmacological functions of DA have drawn special interest, particularly among biologists and medical personnel. DA is used as a drug in the treatment of many health problems,

*Correspondence to: M. Kańska, Department of Chemistry, University of Warsaw, Pasteur Str. 1, 02-093 Warsaw, Poland. E-mail: mkanska@alfa.chem.uw.edu.pl

Contract/grant sponsor: Committee for Scientific Research (Poland); contract/grant number: KBN 4 T09A 063 24

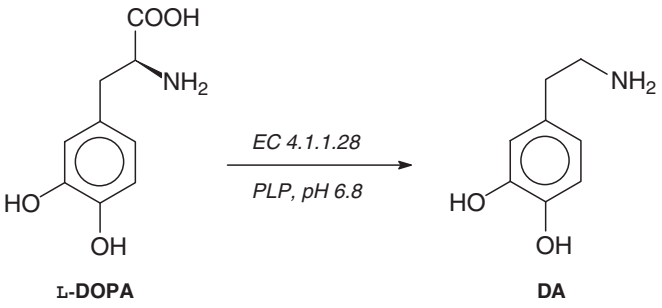

Figure 1. Enzymatic decarboxylation of L-DOPA

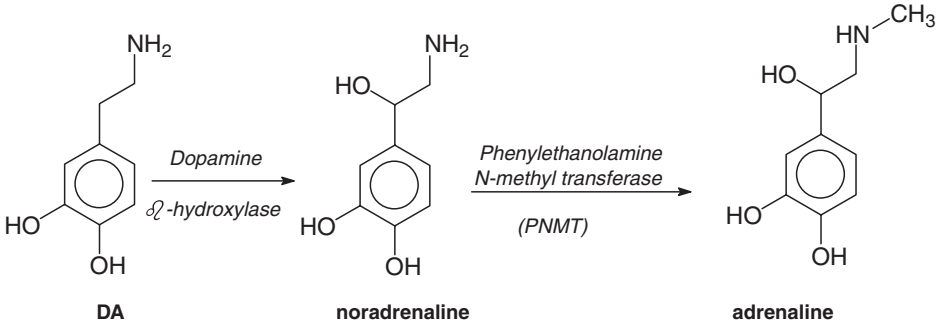

Figure 2. Dopamine as a precursor of neurotransmitters

such as Parkinson's disease.[12–15] As the mechanism of decarboxylation of L-DOPA (Figure 1), as well as the β-hydroxylation of DA (Figure 2) are not well understood it is of interest to study these aspects using the kinetic isotope effect method[16,17] (KIE).

As the first step for starting such a study we concentrated on developing methods for synthesizing deuterium and tritium ring labeled isotopomers of DA i.e. [2′, 5′, 6′-$^{2}H_3$]-, and [2′, 5′, 6′-$^{3}H_3$]-DA, which can then be used for KIE measurements. The acid-catalyzed exchange between aromatic compounds and isotopic water is a very useful method for introducing deuterium or tritium into non-labile positions of the desired molecules. This simple, convenient and one step procedure has been widely used for the synthesis of aromatic compounds labeled with isotopes of hydrogen.[18–20] The kinetics and degree of deuterium incorporation were easily monitored by ^{1}H NMR signal integration in the course of the exchange. The NMR approach is particularly useful as the deuterium distribution of deuterium can be easily determined over the course of the reaction. Investigation of H/D exchange also allows one to optimize the reaction conditions for tritiation.

DOI: 10.1002/jlcr

The next two isotopomers of DA specifically labeled with deuterium and tritium, i.e. [1R-^{2}H]-, and [1R-^{3}H]-DA, were obtained by enzymatic decarboxylation of L-DOPA catalyzed by the enzyme tyrosine decarboxylase (EC 4.1.1.25) from *Steptococcus faecalis* carried out in a deuteriated or tritiated medium respectively.

Results and discussion

The deuteriation of DA · HCl was carried out in heavy water acidified with deuterochloric acid at 50°C. The incorporation of deuterium was monitored by ^{1}H NMR spectroscopy over the course of reaction. The control blank experiment carried out at the same temperature for 5 days without acid added shows that under these conditions H/D exchange is not occurring.

The interesting part of the ^{1}H NMR spectrum of DA · HCl consists of signals from the two -CH_2- groups of the side chain and protons from the aromatic ring.

^{1}H-chemical shifts (δ in ppm relative to TMS in D_2O) and coupling constants (J in Hz) for these exchangeable protons in DA are given below:

- For the β-methylene group of the side chain; $\delta = 2.859$ (2 H, t, $J = 8$).
- For the α-methylene group of the side chain: $\delta = 3.209$ (2 H, t, $J = 7.5$).
- For the three protons in the aromatic ring: $\delta = 6.739$, 6.829 and 6.886.

No significant change of proton NMR signal integrations corresponding to methylene group (-CH_2-) have been noticed in the course of experiments. It has been found that incorporation of deuterium take place only into the aromatic ring of DA. Also the rates of H/D exchange are practically the same for the protons in the 2, 5, and 6 ring positions.

In Figure 3 a plot of the natural logarithm of the normalized ^{1}H NMR signal integration, I, for ring hydrogen corresponding to deuterium exchange in DA carried out in 6 M DCl/D_2O at 50°C is presented. It gives a good linear dependence (correlation coefficient = 0.98). The derived first-order rate constant k has a value of 0.31 h^{-1}.

The data from Figure 3 clearly indicate that the incorporation of deuterium into the ring of DA is practically complete after 15 h. For tritium labeling of DA we decided to carry out this reaction for 24 h.

Previous studies have shown that enzymatic decarboxylation of L-amino acids occurs with retention of configuration at the α-carbon,[21–23] Figure 4. This fact has been used to obtain two (*R*)-isotopomers of dopamine labeled with deuterium or tritium. For enzymatic decarboxylation of L-DOPA enzyme tyrosine decarboxylase[23] was used. The reaction carried out in a fully deuteriated medium produces [1R-^{2}H]-DA, and when carried out in tritiated water the tritium labeled isotopomer [1R-^{3}H]-DA is obtained.

DOI: 10.1002/jlcr

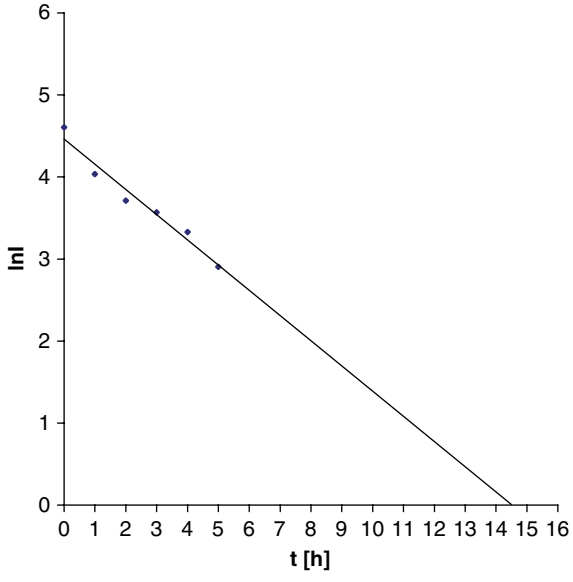

Figure 3. Semi-logarithmic plot of integrated signals Inl vs time for deuterium exchange between dopamine and 6 M DCI/D_2O

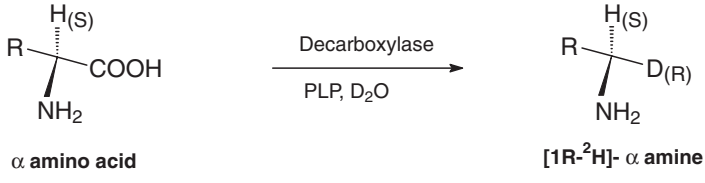

Figure 4. Retention of configuration in the course of enzymatic decarboxylation of α amino acids

Experimental

Materials

Tritiated water (specific act. 185 GBq/g) was purchased from ICN Pharmaceutical Inc, Irvine Ca, USA. Deuteriated water (99.9% deuterium), solutions of 37% DCl/D_2O, 83% D_3PO_4/D_2O, and 30% KOD/D_2O needed for preparation of fully deuteriated phosphate buffer were obtained from Polatom (Poland). Scintillation cocktail was purchased from Rotiszint (Germany). TLC plates *(DC Plastikfolien Aluminiumoxid 60 F_{256}, neutral, type E*) were from Merck. The enzyme tyrosine decarboxylase (EC 4.1.1.25) from *S. faecalis* and coenzyme pyridoxal 5-phosphate, PLP, were purchased from Sigma. DA · HCl, L-DOPA, and other chemicals, needed for the enzymatic synthesis and control experiments, were obtained from Sigma.

DOI: 10.1002/jlcr

Methods

The proton NMR spectra were recorded in D_2O using TMS as internal standard on a Varian 500 MHz Unity-Plus spectrometer. The radioactivity of all samples was determined using a liquid scintillation counter (LISA LSC PW470, Germany):

1. *Incorporation of deuterium into DA*. For the deuteriation kinetics experiments the 10 mg samples of DA · HCl were dissolved in 1 ml of 6 M solution of DCl in heavy water and placed in glass ampoules. The ampoules were connected to vacuum, frozen with liquid nitrogen, degassed, and after thawing, the cycle repeated. Next, the ampoules were sealed under vacuum and after thawing they were immersed and shaken for a short time in warm water (50°C) until the solution became homogeneous, and thermostated at 50°C. After a fixed time the ampoule was removed from the thermostat, cooled, opened, the contents lyophilized under vacuum. From the dry residue a 5 mg sample was taken for NMR assay.
2. *Synthesis of [2′,5′,6′-$^2H_3$]-DA · HCl,* **1**. A sample of 100 mg of dopamine hydrochloride dissolved in 10 ml 6 M solution DCl/D_2O was prepared and H/D exchange allowed to occur for 24 h at 50°C. The solvent was then evaporated to about 5 ml under reduced pressure, and the rest lyophilized to dryness leaving 93 mg of **1**. The purity of **1** was checked by TLC (*n*-butanol : acetic acid : water = 4:1:2 v/v).
3. *Synthesis of [2′,5′,6′-$^3H_3$]-DA · HCl,* **2**. To a glass ampoule 30 mg of DA · HCl, 0.5 ml of HTO (with total radioactivity 1.35×10^9 Bq), and 0.5 ml of conc. HCl were added. The ampoule was sealed under vacuum and thermostated at 50°C for 24 h. Next the reaction mixture was lyophilized to dryness under vacuum. The residue was dissolved in 1 ml of water, loaded on Amberlite IRC 50 H^+ column (100×10 mm), and washed with water to remove the rest of the HTO. Then, **2** was eluted with 0.5 M HCl and collected as 6 ml fractions. For each fraction a 100 µl sample was taken for radioactivity assay. The fractions containing **2** were combined, evaporated under reduced pressure to about 5 ml volume and extracted with *n*-butanol (4×4 ml). The organic layers were combined, dried over anhydrous $MgSO_4$, and evaporated under reduced pressure to about 5 ml volume. The *n*-butanol was removed by lyophilization leaving 26.6 mg (0.14 mmol) of tritiated **2** (about 88% chemical yield) with a total radioactivity 2.45×10^6 Bq (specific activity -1.75×10^7 Bq/mmol). The purity of **2** was checked TLC.
4. *Synthesis of [1R-^2H]-DA · HCl,* **3**. To a vial containing 20 ml of deuteriated phosphate buffer at pD 5.9 (0.1 M KD_2PO_4/D_2O) 30 mg of L-DOPA, 2.5 ml of 1 mM PLP/D_2O and 5 U of enzyme tyrosine

DOI: 10.1002/jlcr

decarboxylase were added. The mixture was incubated at room temperature for 30 h. The enzyme was removed by centrifugation and the reaction mixture loaded on to an Amberlite IRC-50 H^+ column (10×100 mm) previously equilibrated to pH 6.5 with 0.1 M KH_2PO_4. Unreacted L-DOPA was washed out with 0.1 M KH_2PO_4 (pH 6.5) and next **3** was eluted with 0.5 M HCl. The fractions containing **3** were combined and lyophilized. **3** was extracted with 15 ml of *n*-butanol, which in turn was lyophilized leaving 23.4 mg (0.123 mmol) of **3** (yield 81%). The near 100% incorporation of deuterium into the 1R position was ascertained from ^1H NMR spectrum.

5. *Synthesis of [1R-^3H]-DA · HCl,* **4**. To a vial containing 3 ml of 0.2 M phosphate buffer (pH 5.5),were added in turn: 5 mg of L-DOPA, 400 µl of 1mM PLP, 0.7 U of enzyme tyrosine decarboxylase, and 2 ml of HTO with a total radioactivity of approximately 18.5 GBq. The mixture was incubated at room temperature for 6 h. The enzyme was removed by centrifugation, and the mixture lyophilized to dryness. The residue was dissolved in 2 ml of water and loaded onto Amberlite IRC-50 column previously equilibrated to pH 6.5 with 0.1 M KH_2PO_4. Unreacted L-DOPA, the HTO, were washed out with 0.1 M KH_2PO_4 (pH 6.5). The desired product, **4**, was eluted with 0.5 M HCl and collected as 6 ml fractions. The fractions containing **4** were pooled and lyophilized to dryness. **4** was extracted with a 10 ml portion of n-butanol, which in turn was lyophilized leaving 3.9 mg (0.02 mmol) of **4** with total radioactivity of $6.1 \cdot 10^5$ Bq (specific activity of 3.05×10^7 Bq/mmol. Chemical yield was 82%.

Acknowledgements

This was sponsored by Committee for Scientific Research (Poland); grant no KBN 4 T09A 063 24.

References

1. Wick MM, Fitzgerald G. *Chem Biol Interactions* 1981; **38**: 99–107.
2. Premont RT, Gainetdinov RR, Caron MG. *Proc Natl Acad Sci* 2001; **98**: 9474–9475.
3. Nieoullon A, Coquerel A. *Curr Opin Neurol* 2003; **16**(Suppl. 2): 3–9.
4. Lovenberg W, Weissbach H, Udenfriend S. *J Biol Chem* 1962; **237**: 89–92.
5. Borri-Voltattorni C, Minelli A, Vecchini P, Fiori A, Turano C. *Eur J Biochem* 1979; **93**: 181–188.
6. Sourkes TL. *Methods in Enzymology*, vol. 147. Academic Press: New York, 1987; 170–187.
7. Waymire JC, Bjur R, Weiner N. *Anal Biochem* 1971; **43**: 588–600.

DOI: 10.1002/jlcr

8. Diliberto Jr EJ, Allen PL. *J Biol Chem* 1981; **256**: 3385–3393.
9. Friedman S, Kaufman S. *J Biol Chem* 1965; **240**: 4763–4773.
10. Nagatsu T, Levitt M, Udenfriend S. *J Biol Chem* 1964; **239**: 2910–2917.
11. Nielsen M, Eplov L, Scheel-Krüger J. *Naunyl-Schmiedeberg's Arch Pharmacol* 1974; **285**: 15–28.
12. Bjurling P, Watanabe Y, Oka S, Nagasawa T, Yamada H, Långström B. *Acta Chem Scand* 1990; **44**: 183–188.
13. Ceravolo R, Volterrani D, Gambaccini G, Bernardini S, Rossi C, Logi C, Tognoni G, Manca G, Mariani G, Bonuccelli U, Murri L. *Neural Transm* 2004; **111**: 1065–1073.
14. Li J, Zhu M, Manning-Bog AB, Di Monte DA, Fink AL. *FASEB J* 2004; **18**: 962–964.
15. Lindner KJ, Hartvig P, Tedroff J, Ljungström A, Bjurling P, Långström B. *J Pharmaceut Biomed Anal* 1995; **13**: 361–367.
16. Cook F. *Enzyme Mechanism From Isotope Effects*. CRS Press: Boca Raton, FL, USA, 1991.
17. Klinman JP, Humphries H, Voet JG. *J Biol Chem* 1980; **255**: 11648–11651.
18. Buncel E, Jones JR. *Isotopes in the Physical and Biomedical Sciences,* vol. 1*, Labelled compounds (part A*). Elsevier: Amsterdam, 1987; 122–155.
19. Evans EA. *Tritium and its Compounds*. Butterworth: London, 1974.
20. Tuck KL, Tan HW, Hayball PJ. *J Label Compd Radiopharm* 2000; **43**: 817–823.
21. Belleau B, Burba J. *JACS* 1960; **82**: 5751–5752.
22. Dunathan HC. *Adv Enzymolog* 1971; **35**: 79–134.
23. Vederas JC, Reingold ID, Sellers HW. *J Biol Chem* 1979; **254**: 5053–5057.

DOI: 10.1002/jlcr