Microwave enhanced synthesis of halogenated derivatives of L-tyrosine labeled with deuterium in aromatic ring

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Abstract

Three halogenated derivatives of L-tyrosine, selectively labeled with deuterium in aromatic ring, i.e., 3'-fluoro-[5'-²H]-, 3'-chloro-[5'-²H]-, and 3'-iodo-[2',5'-²H₂]-L-tyrosine, were synthesized using microwave assisted acid-catalyzed isotope exchange between 3'-fluoro-, 3'-chloro- and 3'-iodo-L-tyrosine and heavy water. The degree of deuterium incorporation was confirmed by ¹H NMR spectroscopy. The spectroscopic data indicate that isotope exchange depends on the method of heating and the power of microwaves. The deuterium enrichment of 3'-fluoro-[5'-²H]- and 3'-chloro-[5'-²H]-L-tyrosine amounted to 70% and 60%, respectively, while for 3'-iodo-[2',5'-²H₂]-L-tyrosine this value was about 50% and 95% for the 2'- and 5'-position. The isotopomers were obtained in good chemical yields of 50–70%.

Keywords Deuterium · Halogenated derivatives of L-tyrosine · Labeling · Microwave enhanced synthesis

Introduction

L-Tyrosine (L-Tyr) is an aromatic amino acid essential for many human metabolic processes and a precursor of other biologically active compounds. In the living cells L-Tyr is enzymatically oxidized to L-DOPA, another important amino acid [1], which is converted to dopamine, a neurotransmitter in the mammalian central nervous system [2], and melanins [3], the natural pigments of skin, hair and iris. Disturbed metabolism of L-Tyr contributes to the occurrence of Parkinson's disease, melanoma, vitiligo or albinism [4–6]. The reasons for these disorders are not entirely clear and cannot be explained without the knowledge of enzymatic reactions mechanisms involving L-Tyr or L-DOPA.

In the last decade, some halogenated derivatives of L-Tyr, labeled with short-lived radioisotopes of fluorine or iodine have been evaluated as radiopharmaceuticals in positron emission tomography (PET) or single photon emission computed tomography (SPECT) - to investigate the biodistribution of dopamine or as tumor imaging tracers [7–10]. Thus, before administration of a new kind of radiopharmaceuticals it is crucial to examine the metabolism of L-Tyr

halogenated derivatives. Our previous studies revealed that 3'-iodo-L-Tyr is an inhibitor of tyrosinase – the enzyme converting L-Tyr to L-DOPA [11]. One of the most powerful tools for such experiments is the kinetic (KIE) and solvent (SIE) isotope effects method. It requires the use of halogenated derivatives of L-Tyr - both unlabeled and selectively labeled with hydrogen isotopes. In the scientific literature, there are many methods for synthesis of labeled compounds, including deuteration in the presence of platinum catalyst [12], iridium-catalyzed H/D exchange [13], hydrodehalogenations, e.g. using Pd/C and gaseous tritium [14] or acid-catalyzed isotope exchange at high temperature [15]. Recently, also microwave assisted syntheses of labeled compounds are applied [16]. The aim of this study is the synthesis of 3'-fluoro-[5'-²H]-, 3'-chloro-[5'-²H]-, and 3'-iodo-[2',5'-²H₂]-L-tyrosine, as well as the comparison of the degree of deuterium incorporation into aromatic ring of L-Tyr halogenated derivatives using microwave enhanced and conventional heated acid-catalyzed isotope exchange.

Experimental

Materials

The solution of 37% ²HCl/²H₂O was purchased from POLATOM (Poland). ²H₂O (99.9% ²H) and Amberlite

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Fig. 1 Synthesis of halogenated derivatives of L-Tyr labeled with deuterium in aromatic ring

IR-120 (Na^+) resin were purchased from Aldrich. Plastic TLC plates, silica gel 60, coated with fluorescent indicator F_{254} were from Merck. 3'-Fluoro-L-tyrosine and 3'-chloro-L-tyrosine were purchased from Alfa Aesar and 3'-iodo-L-tyrosine was from Sigma.

Methods

^1H NMR spectra were registered in $^2\text{H}_2\text{O}$ with tetramethylsilane (TMS) as internal standard on Varian 500 MHz Unity-Plus spectrometer. Throughout the course of chromatographic column separation the presence of products was checked by TLC (developing solvent - acetonitrile:water, 4:1; v/v, visualization by UV lamp). The microwave enhanced syntheses were carried out in Whirlpool JT 379 microwave oven.

Synthesis

Synthesis of halogenated derivatives of L-Tyr labeled with deuterium in aromatic ring, i.e., 3'-fluoro-[5'- ^2H]-, **1**, 3'-chloro-[5'- ^2H]-, **2**, and 3'-iodo-[2',5'- $^2\text{H}_2$]-L-Tyr, **3**.

1. The use of conventional heating

For synthetic purpose a 20 mg (0.1 mmol) sample of 3'-iodo-L-Tyr was dissolved in 2 mL of 6 M $^2\text{HCl}/^2\text{H}_2\text{O}$ and placed in a glass vial. After freezing, the vial was connected to a vacuum apparatus, degassed and sealed. Then, it was placed in a thermostat and incubated at 120°C for 24 h. Next, the residue was lyophilized and purified as

described in [11]. The fractions containing the product (confirmed by TLC) were combined and lyophilized, leaving 8.8 mg (0.028 mmol) of **3** with chemical yield 44%. The compounds **1** and **2** were synthesized as described in [11]. The degrees of deuterium incorporation are presented in Table 1 and the differences in chemical shifts (^1H NMR data) for the authentic and deuterated derivatives are presented in Table 2.

2. The use of microwaves

For synthetic purpose a 10 mg sample of appropriate halogenated L-Tyr was dissolved in 1 mL of 6 M $^2\text{HCl}/^2\text{H}_2\text{O}$ in a polytetrafluoroethylene vessel, placed in a microwave oven, and heated for 2 min at 160 W. Then, the sample was cooled to RT and the cycle was repeated 8 times in total. The same procedure was used at 350 W for 1.5 min each. Next, the residues, containing **1**, **2**, or **3**, respectively, were lyophilized, loaded onto a chromatographic column (100 × 10 mm) containing the Amberlite IR-120 (H^+) resin. First, the column was washed with distilled water to remove the deuterium from labile positions. Then, the products were eluted with 1 M $\text{NH}_3(\text{aq.})$. The fractions containing **1**, **2** or **3** (confirmed by TLC) were combined and lyophilized, leaving 6.2 mg (0.031 mmol) of **1**, 7.3 mg (0.034 mmol) of **2** and 4.8 mg (0.016 mmol) of **3** with chemical yields 62%, 73% and 48%, respectively. Afterwards, the products **1**, **2** and **3** were taken for ^1H NMR analysis. The degrees of deuterium incorporation are given in Table 1 and the differences in chemical shifts (^1H NMR data) for the authentic and deuterated derivatives are presented in Table 2.

Table 1 Degree of deuterium incorporation into halogenated derivatives of L-Tyr

Method	Conditions	3'-fluoro-L-Tyr, 1			3'-chloro-L-Tyr, 2			3'-iodo-L-Tyr, 3		
		2'	5'	6'	2'	5'	6'	2'	5'	6'
		Degree of incorporation (%)								
Power [MW]	160 W	–	72	–	–	33	–	8	23	–
	350 W	–	66	–	–	62	–	48	95	–
Conventional heating	120 °C	–	100	–	–	100	–	70	96	–

Table 2 Chemical shifts (^1H NMR data) for authentic and deuterated compounds obtained. Chemical shifts are reported as follows: chemical shifts, multiplicity (s=singlet, d=doublet, dd=doublet of doublets, t=triplet, q=quartet, dq=doublet of quartets), type of hydrogen

Compound	Chemical shifts, δ (ppm)		
	Aromatic hydrogens	α -H	β -H
3'-fluoro-L-Tyr	7.06, dd 1H ₂ ; 6.99, s, 1H ₅ ; 6.96, t, 1H ₆	3.90, q, 1H	3.10, dq, 2H
3'-fluoro-[5'- ^2H]-L-Tyr, 1	7.09, d, 1H ₂ ; 6.97, s, 1H ₆	4.24, t, 1H	3.20, dq, 2H
3'-chloro-L-Tyr	7.30, d, 1H ₂ ; 7.09, dd, 1H ₆ ; 6.97, d, 1H ₅	3.91, q, 1H	3.09, dq, 2H
3'-chloro-[5'- ^2H]-L-Tyr, 2	7.25, s, 1H ₂ ; 7.06, s, 1H ₆	4.23, t, 1H	3.15, dq, 2H
3'-iodo-L-Tyr	7.71, d, 1H ₂ ; 7.18, dd, 1H ₆ ; 6.94, d, 1H ₅	3.92, q, 1H	3.10, dq, 2H
3'-iodo-[2',5'- $^2\text{H}_2$]-L-Tyr, 3	7.64, d, 1H ₂ ; 7.14, dd, 1H ₆ ; 6.86, d, 1H ₅	4.26, t, 1H	3.16, dq, 2H

Results and discussion

The synthesis of L-Tyr halogenated derivatives, labeled with deuterium in aromatic ring was carried out in 6 M $^2\text{HCl}/^2\text{H}_2\text{O}$ heated in conventional or microwave oven according to Fig. 1.

The degree of deuterium enrichment was checked by ^1H NMR spectroscopy (Table 2) and calculated on the basis of signals integration of the deuterated and authentic compounds. The spectroscopic data indicate that isotope exchange depends on the method of heating and power of microwaves (Table 1). The best results were obtained for halogenated derivatives of L-Tyr using the conventional heating (thermostat) and reached almost 100% at the 5'-position of compounds **1** and **2** aromatic rings (the disappearance of signals of protons at 5' position). The data obtained for compound **3** indicate that incorporation of deuterium takes place into the 5'- and 2'- positions of aromatic ring and reached 96% and 70%, respectively.

The microwave enhanced acid-catalyzed isotope exchange in aromatic ring of **1**, **2**, and **3** also occurs with sufficient deuterium enrichment and reached about 60–70% for compound **1** and **2**. The results received for compound **3** are very similar to those obtained with conventional heating (Table 1).

Although the KIE and SIE method requires the use of labeled compounds with almost 100% degree of incorporation, it is possible to use derivatives with lower level of deuterium enrichment [17]. The experimental data of KIEs and SIEs should be approximated to the values corresponding to 100% of deuterium incorporation using Eq. (1)

$$\alpha = p(\alpha_p - 1) + 1 \quad (1)$$

where p degree of enrichment with ^2H ; α corrected isotope effect to 100% of deuterium incorporation ($p=1$); α_p measured isotope effect for the compound with p degree of enrichment with ^2H .

Conclusions

The simple, convenient and time efficient method for synthesis of halogen derivatives of L-Tyr labeled with deuterium in aromatic ring was elaborated. The isotopomers were obtained with good chemical yields and high degree of deuterium incorporation. Microwave assisted isotope exchange gives products with sufficient deuterium enrichment in a very short time (8×1.5 min), which makes this method useful for synthesis of labeled compounds. The isotopomers obtained in this research may be used as substrates in further studies of enzymatic conversion of halogenated derivatives of L-Tyr using the kinetic isotope effects method.

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Compliance with ethical standards

Conflict of interest The author has no conflict of interest to declare that are relevant to the content of this article.

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