Applied Radiation and Isotopes 137 (2018) 118–122

Contents lists available at ScienceDirect

Applied Radiation and Isotopes

journal homepage: www.elsevier.com/locate/apradiso

Enzymatic synthesis of methyl derivatives of L-tryptophan selectively labeled with hydrogen isotopes

Elżbieta Winnicka[a,*], Marianna Kańska[b]

[a] Department of Chemistry, University of Warsaw, 1 Pasteur Str., 02-093 Warsaw, Poland
[b] Department of Biochemistry, 2nd Faculty of Medicine, Medical University of Warsaw, 1 Banacha Ave., 02-097 Warsaw, Poland

HIGHLIGHTS

- The method was elaborated for the synthesis of deuterium and tritium labeled in the α-position derivatives for the methyl derivatives of L-Trp.
- The ^{1}H NMR spectra were obtained for the methyl and deuteriated derivatives of L-Trp in the α-position.
- we determined the influence of deuterium substitution at the α-position of L-Trp on the chemical shift of protons.

ARTICLE INFO

Keywords:
Deuterium
Methylated L-tryptophan
Labeling
Tritium
Tryptophanase

ABSTRACT

We report the enzymatic synthesis of the derivatives of L-tryptophan methylated in indole moiety and labeled with deuterium and tritium in the 2-position of side chain. For kinetic studies twelve isotopomers, i.e., 1′-methyl-[2-^{2}H]-, 1′-methyl-[2–^{3}H]-, 1′-methyl-[2-^{2}H/^{3}H]-, 2′-methyl-[2-^{2}H]-, 2′-methyl-[2–^{3}H]-, 2′-methyl-[2-^{2}H/^{3}H]-, 5′-methyl-[2-^{2}H]-, 5′-methyl-[2–^{3}H]-, 5′-methyl-[2-^{2}H/^{3}H]-, 7′-methyl-[2-^{2}H]-, 7′-methyl-[2–^{3}H]-, and 7′-methyl-[2-^{2}H/^{3}H]-L-tryptophan are obtained by the enzymatic coupling of the appropriate methylated indole moiety with *S*-methyl-L-cysteine catalyzed by the enzyme tryptophanase.

1. Introduction

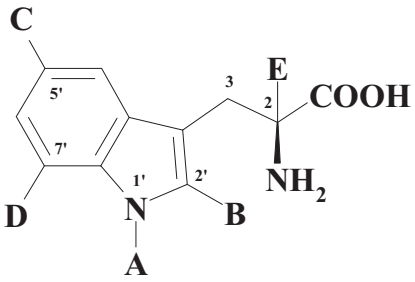
L-Tryptophan, L-Trp, an exogenous amino acid necessary for protein synthesis in living organisms, participates in three major biotransformations. As a result of enzymatic hydroxylation L-Try is converted to 5′-hydroxy-L-tryptophan (Slominski et al., 2002), an intermediate for production of serotonin and melatonin, the neurotransmitters responsible for occurrence of emotional states (aggression, mood, sleep, sexual behavior) (Beagles et al., 1998). The enzymatic decarboxylation of L-Trp leads to tryptamine, a backbone for group of compound known collectively as tryptamines. This group includes many biological active compounds, including niacin and neurotransmitters, regulating many processes affecting the nervous system (Kang et al., 2007). It is estimated that only 4% of the total L-Trp pool in the body is consumed for protein synthesis, about 1–2% is converted to serotonin, and the main part (c.a. 94%) is metabolized on the kinourеinine pathway (Stone, 1993). The adequate level of L-Try is necessary for the correct course of many physiological processes, which besides the nervous also include the digestive system (Van den Berg et al., 1990). This amino acid participates in secretion of growth hormone, increases the immunity, participates in the synthesis of vitamin B_3, and L-Trp is also a component of NADP and NAD^+ coenzymes, and many antibiotics (Amri-Heidari et al., 2007; Parry et al., 1992). Recently, the interest in the use of methyl derivatives of L-Trp in medicine has been increased significantly. 1-Methyl-L-tryptophan is an inhibitor of indolamine 2,3-dioxygenase (IDO), the rate-limiting enzyme of L-Trp catabolism on the kineurinine pathway, whose elevated activity is observed in human malignancies (Miyazaki et al., 2009; Wirthgen et al., 2016). Therefore, the enzyme IDO may be used in combination with cytostatic chemotherapy as a pharmacological inhibitor.

The purpose of our study is the synthesis of methylated derivatives of L-Trp labeled with hydrogen isotopes (Muller et al., 2005; Uyttenhove et al., 2003) to investigate the effect of methyl substituent on enzymatic reaction kinetics in which L-Trp is involved. The details of these reactions may be probed using kinetic (KIE) and solvent (SIE) isotopic effect methods (McFarland, 1991). The numerical values of KIE and SIE are useful for explaining some details of mechanism aforementioned enzymatic reaction such as bond breaking/forming and structure of active complex.

Selectively α-deuteriated and α -tritiated methyl derivatives of L-Trp, i.e., 1′-, 2′-, 5′-, and 7′-methyl-L-Trp (Fig. 1) were prepared according to slightly modified procedure consisting of the enzymatic coupling of *S*-methyl-L-cysteine with 1-, 2-, 5-, and 7-methylindole, catalyzed by enzyme tryptophanase in fully deuteriated or tritiated

* Corresponding author.

https://doi.org/10.1016/j.apradiso.2018.03.019
Received 21 July 2017; Received in revised form 26 January 2018; Accepted 24 March 2018
Available online 27 March 2018
0969-8043/



1'-methylindole (A = CH_3, B, C, D = H)	**5**, 1'-methyl-L-[2-^{2}H]-Trp (A = CH_3, B, C, D = H, E = ^{2}H) **9**, 1'-methyl-L-[2-^{3}H]-Trp (A = CH_3, B, C, D = H, E = ^{3}H) **13**, 1'-methyl-L-[2-^{2}H/^{3}H]-Trp (A = CH_3, B, C, D = H, E = ^{2}H/^{3}H)
2'-methylindole (B = CH_3, A, C, D = H)	**6**, 2'-methyl-L-[2-^{2}H]-Trp (B = CH_3, A, C, D = H, E = ^{2}H) **10**, 2'-methyl-L-[2-^{3}H]-Trp (B = CH_3, A, C, D = H, E = ^{3}H) **14**, 2'-methyl-L-[2-^{2}H/^{3}H]-Trp (B = CH_3, A, C, D = H, E = ^{2}H/^{3}H)
5'-methylindole (C = CH_3, A, B, D = H)	**7**, 5'-methyl-L-[2-^{2}H]-Trp (C = CH_3, A, B, D = H, E = ^{2}H) **11**, 5'-methyl-L-[2-^{3}H]-Trp (C = CH_3, A, B, D = H, E = ^{3}H) **15**, 5'-methyl-L-[2-^{2}H/^{3}H]-Trp (C = CH_3, A, B, D = H, E = ^{2}H/^{3}H)
7'-methylindole (D = CH_3, A, B, C = H)	**8**, 5'-methyl-L-[2-^{2}H]-Trp (D = CH_3, A, B, C = H, E = ^{2}H) **12**, 5'-methyl-L-[2-^{3}H]-Trp (D = CH_3, A, B, C = H, E = ^{3}H) **16**, 5'-methyl-L-[2-^{2}H/^{3}H]-Trp (D = CH_3, A, B, C = H, E = ^{2}H/^{3}H)

Fig. 1. Structure of methyl derivatives of L-Trp obtained.

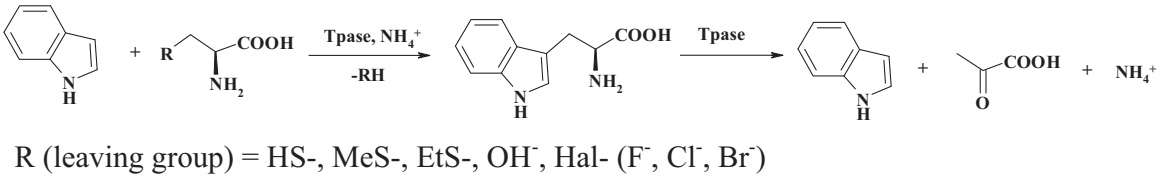


Fig. 2. Irreversible reaction of degradation/formation of L-Trp catalyzed by Tpase. R (leaving group) = HS-, MeS-, EtS-, OH^-, Hal- (F^-, Cl^-, Br^-).

media, respectively (Kiick and Phillips, 1988), (Fig. 2). The modified procedure involved use of tritiated water and a mixture of deuterated and tritiated water for a synthesis or methyl derivatives of L-Trp containing deuterium or tritium label. In addition, reaction time was extended, and product purification steps were changed.

2. Experimental

2.1. Materials

The enzymes tryptophanase (Tpase, EC 4.1.99.1) from *Escherichia coli* was purchased from Sigma. Tritiated water (5 Ci/mL) was purchased from ICN Pharmaceutical Inc, Irvine Ca, (USA). Deuteriated 30% KOD/D_2O and 85% D_3PO_4/D_2O were from POLATOM (Poland). Deuteriated water (99,9% D), *S*-methyl-L-cysteine and Amberlite IR 120 Na^+ were from Aldrich. Other chemicals like cofactor NADH, L-Trp, 1-, 2-, 5-, and 7-methylindoles, pyridoxal 5′-phosphate (PLP) and 2-mercaptoethanol were from Sigma. Scintillation cocktail was purchased from Rotiszint (Germany). Silica gel 60 for column chromatography and thin layer chromatography (TLC) plates (Keiselgel 60 F_{254}) were from Merck.

2.2. Methods

✓ The extent of deuterium incorporation in α-position of deuteriated isotopomers of appropriate methylated L-Trp was determined from the proton nuclear magnetic resonance (^{1}H NMR) spectra recorded on 500 MHz Varian Unity-plus spectrometer using ^2H_2O as solvent and tetramethylsilane (TMS) as an internal standard. The progress of the synthesis and identification of reagents during column chromatography separation were monitored using TLC on silica gel plates with acetonitrile: water 4:1, (v/v) as a developing solvent. UV-lamp and 0,1% solution of ninhydrin in ethanol were used as methods of chromatograms visualization. The concentration of methylated derivatives of L-Trp was determined spectrophotometrically according the procedure described earlier (Boroda et al., 2003; Winnicka et al., 2016). For separation and purification of products two kind of columns (10 × 100 mm) are used: either filled with silica gel or Amberlite IR 120 Na^+. The eluent mixture of acetonitrile: water, 5:1, (v/v) and 0.3 M NH_3(aq) were used. The spectrophotometric analyses were made with the use of the spectrometer UV–VIS (Schimadzu, UV-1800). The radioactivity of samples was measured using liquid scintillation analyzer (LSC) (Tri-Carb 2910 TR, Perkin Elmer).

2.3. Synthesis

2.3.1. The general incubation, separation, and purification procedure used for synthesis 1′-, 2′-, 5′-, and 7′-methyl-L-Trp (i. e. 1, 2, 3, and 4) and their deuteriated isotopomers, i.e, 1-', 2'-, 5'-, and 7'-methyl-[2-^{2}H]-L-Trp (i. e, 5, 6, 7, and 8)

To a capped vial the sample of appropriate methylindole (i.e., 1-, 2-, 5- and 7-methylindole) weighing c.a. 52 mg (0.4 mmol), 135 mg (1 mmol) of *S*-methyl-L-cysteine, 1.5 mg (6 μmol) of PLP, and 1 mg (306 U) of enzyme Tpase are added. The content of vial was dissolved in 20 mL of 0.1 M phosphate buffer (in the case of deuteriated **5**, **6**, **7**, and **8** the fully deuteriated phosphate buffer is used) and to this reaction mixture 7 μL of 2-mercaptoethanol was injected to prevent fungi growth. The incubation was carried out with constant stirring for 3 days at 37 °C. The progress of reaction was monitored by TLC. The reaction was quenched by acidification to pH 5 with glacial acetic acid. Next, the enzyme was centrifuged off, and unreacted methylindole was extracted from postreaction mixture with toluene (20 mL). The combined water layer containing product was loaded on the silica gel column (100 × 10 mm), and the product was eluted with acetonitrile:water,(5:1,v/v) and collected as 1,5 mL fractions. The presence of the product in each