Research Article

Journal of Labelled Compounds and Radiopharmaceuticals

Received 16 December 2008, Revised 5 March 2009, Accepted 7 April 2009 Published online 26 June 2009 in Wiley Interscience

(www.interscience.wiley.com) DOI: 10.1002/jlcr.1611

Enzymatic synthesis of N-methylhistamine labeled with deuterium and tritium

J. Šamonina and M. Kańska*

The isotopomers of N^{π}-methylhistamine (πMeHA) and N^{τ}-methylhistamine (τMeHA) labeled with deuterium and tritium at the α-carbon atom of the side chain were obtained using the enzyme histidine decarboxylase (HDC, EC 4.1.1.22) from *Lactobacillus 30a*. The deuterium labeled isotopomers [(αR)-^{2}H]-πMeHA and [(αR)-^{2}H]-τMeHA were synthesized by enzymatic decarboxylation of N^{π}-methyl-, and N^{τ}-methyl-L-histidines (respectively) in a fully deuteriated incubation medium. The same decarboxylation carried out in a tritiated medium resulted in tritiated [αR-^{3}H]-πMeHA and [αR-^{3}H]-τMeHA isotopomers of N-methylhistamine.

Keywords: deuterium; enzyme; N-methylhistamines; labeling; tritium

Introduction

The biogenic amine histamine (HA) found widespread in plant and mammalian cells plays an important role in various physiological functions.[1–3] In humans, it is one of key mediators of cell growth, gastric secretion, acute allergic inflammation, and neurotransmitter for blood pressure. The imbalance of HA metabolism was suspected in several diseases.[4–7] Therefore, the HA catabolism in humans and experimental animals[8,9] was carefully studied and two main metabolic pathways were found[10–12], Figure 1.

In humans, HA is mainly metabolized along two pathways. About three quarters of HA is methylated to τMeHA by enzyme N-methyltransferase (EC 2.1.1.8), and subsequently this intermediate is oxidized to N^{τ}-methylimidazole acetaldehyde by enzyme diamine oxidase (DAO, EC 1.4.3.6), while one quarter of HA is biotransferred indirectly into imidazole acetaldehyde by DAO. In rodent's brain, some of the histamine is also methylated to πMeHA and subsequently oxidized to N^{π}-methylimidazoleacetic acid.[13,14]

For a long time, the two numbering systems have been used by biochemists and organic chemists to designate nitrogen as well as carbon atoms in the ring and the side chain of histidine and its derivatives. Therefore, according to the recommendation of IUPAC[15], the nitrogen atoms of the imidazole ring are denoted by π and τ , and carbon atoms in the side chain as α, and β. The ring carbon atoms are numbered as 2, 4 and 5, respectively (Figure 2).

Despite many studies the mechanism of removal of HA excess from humans organism is not fully understood. Therefore, we decided to investigate some details of the methylation reaction presented in Figure 1 by applying the kinetic isotope effect (KIE) and solvent isotope effect (SIE) methods. Determination of numerical values for KIE and SIE could be useful to elucidate the intrinsic details of the reaction mechanism.[16,17] For this kind of study, the isotopomers of N-methylhistamine specifically labeled with deuterium and tritium are needed. In the literature there is description of the synthesis of τMeHA and πMeHA tritiated selectively in the methyl group.[18] Unfortunately, the product obtained was a mixture of two (τ and π) isomers with a ratio approximately 2:1. Chromatographic efforts to separate these two labeled isomers were unsuccessful. The preparation of tritium labeled (N^{τ}-C[^{3}H])-histamine · 2HCl from tritiated methyl iodide by chemical method is described[19] as well as the method for synthesis of τMeHA deuteriated in the methyl group (N^{τ}-C[$^{2}H_3$])-histamine · 2HCl.[20]

In this paper, a biosynthesis of N-methylhistamines labeled selectively at the α-carbon atom with hydrogen isotopes is reported.

Results and discussion

The enzymatic decarboxylation of N^{π}- and N^{τ}-methyl-L-histidines was chosen as the method of synthesis of the isomers of N-methylhistamine labeled selectively with deuterium and tritium at the α-carbon atom of the side chain (i.e. [(αR)-^{2}H], [(αR)-^{3}H]-πMeHA, and [(αR)-^{2}H]-, [(αR)-^{3}H]-τMeHA). Figure 3 presents the enzymatic biotransformation of N^{τ}-methyl-L-histidine into τMeHA. Similarly, the decarboxylation of N^{π}-methyl-L-histidine produces πMeHA.

The enzyme histidine decarboxylase (HDC, EC 4.1.1.22) from *Lactobacillus 30a* transforms L-histidine and some of its derivatives into corresponding amines in the presence of cofactor pyridoxal-5′-phosphate (PLP). However, the decarboxylation of N^{π}-, and N^{τ}-methyl-L-histidines occurs very slowly under the experimental conditions studied.[21] In our experimental conditions we observed that HDC converts N^{π}-methyl-L-histidine into πMeHA more effectively than in the case of N^{τ}-methyl-L-histidine. Over a period of one week the decarboxylation of

Department of Chemistry, University of Warsaw, Pasteur Str. 1. 02-093 Warsaw, Poland

**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

Journal of Labelled Compounds and Radiopharmaceuticals

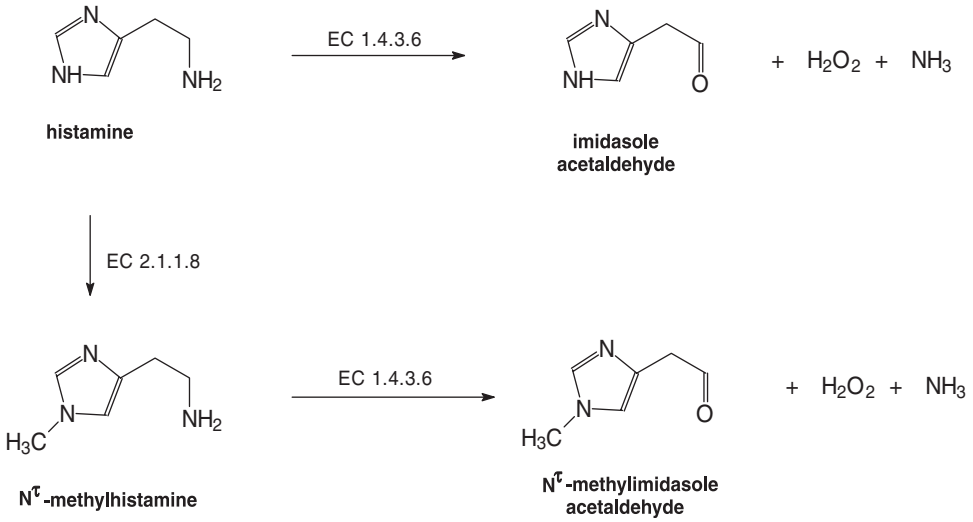


Figure 1. The fragment of histamine metabolism in man.

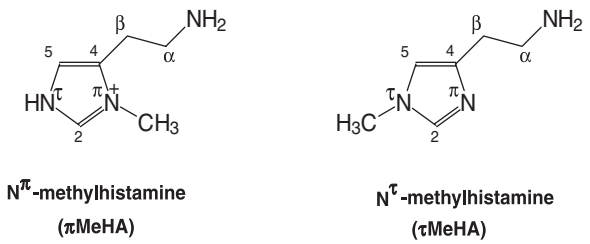


Figure 2. Denotation of nitrogen and carbon atoms in N-methylhistamine according to IUPAC.

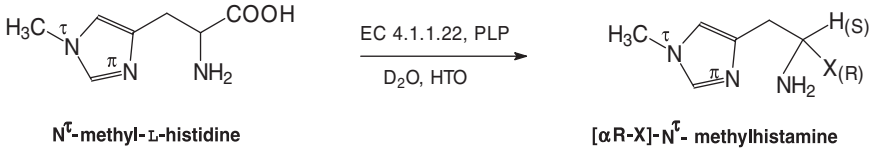
N^{π}-methyl-L-histidine the amine is formed with nearly 80% chemical yield. At the same time the N^{τ}-compound is transformed into τMeHA with about 60% yield. Such difference in chemical yields clearly shows that the rate of decarboxylation of N^{π}-methyl-L-histidine is significantly higher than that of its N^{π}-isomer. The prolongation of decarboxylation over one week is very risky because of the possible growth of fungi in incubation medium. Also, as a result of radioactive synthesis, the obtained products:[(αR)-^{3}H]-πMeHA and [(αR)-^{3}H]-τMeHA were significantly different in their specific activities (14.1 and 2.8 MBq/mmol, respectively). Probably, this is caused by the higher kinetic H/T isotope effect for tritium incorporation step into [(αR)-^{3}H]-τMeHA leading to enrichment of the product with protium and thus lowering its specific activity. If H/T KIEs were similar for these two reactions then the specific activities should be close to each other since the specific activities of incubation media were the same. Previous research showed that decarboxylation of L-histidine catalyzed by HDC proceeds with the introduction of one hydrogen atom from the solvent to the newly generated amine and preservation of the configuration at the carbon atom in position α of the side chain.[22,23] Therefore, the decarboxylation of N^{π}-, and N^{τ}-methyl-L-histidines carried out in a fully deuteriated or tritiated incubation medium can be used to obtain the (αR)-isotopologues of N-methylhistamine labeled with deuterium or tritium. When the decarboxylation is carried out in a fully deuteriated incubation medium, nearly 100% incorporation of deuterium in (αR)-position of both isomers of methylhistamine is achieved. It is explicable as in this case other sources of protium do not exist.

In the course of preliminary studies the optimal experimental conditions were elaborated for decarboxylation of L-histidines. The concentration of N-methylhistamines in the course of the reaction was determined indirectly by spectrophotometric measurements of decreasing (395 nm) and increasing (326 nm) absorbencies of two different forms of PLP.[24] We studied the dependence of the reaction yield on time, pH, concentration of buffer, enzyme and substrates. All trial studies were carried out in phosphate buffer at 37°C. However, most of the results we get by monitoring the progress of decarboxylation of L-methylhistidine using TLC method. In preset time, in the course of reaction, the samples were taken from the incubation medium and developed on TLC plates. The resolved spots (L-methylhistidine and methylhistamine) were visualized by ninhydrin. The disappearance of the spot corresponding to histidine was compared with the appearance of the spot associated with methylhistamine. Finally, the control decarboxylation experiments were performed after the optimal reaction conditions were determined. The resulted methylhistamine was purified from the reaction medium by the column chromatography. The reaction yield was determined by weighing the material after the removal of the solvent. The maximal yield of methylated histamines (*ca.* 60–80%) can be reached after 8 days of incubation in 0.1 M phosphate buffer (pH 4.7) and at the concentration of substrate and enzyme *ca.* 5×10^{-2} M and 1.8 U/mL, respectively.

The enzymatic decarboxylation of N-methylated L-histidines was monitored by ^{1}H NMR, in which identification of the magnitude and the deuteriation site of the labeled compound were achieved. In this case, the decarboxylation of L-histidines was carried out in a fully deuteriated phosphate incubation medium adjusted to pD 4.7 using KOD/D_2O and D_3PO_4/D_2O.

During trial deuteriation assays we observed that deuterium atom is also incorporated to a large extent (*ca.* 30%) into 2 position of imidazole ring of histamines. However, that substitution of protium by deuterium is labile. We saw complete removal of deuterium after five days long equilibration of the isolated products with the excess of water at room temperature, thus leaving amines labeled in the α-position only.

^{1}H NMR-chemical shifts (δ in ppm in D_2O relative to TMS, 500 MHz and coupling constants (J in Hz) are listed in Table 1.



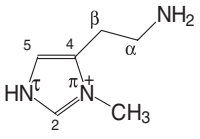
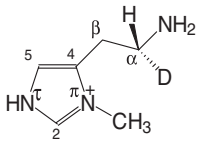
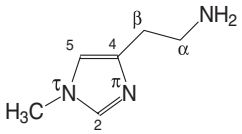
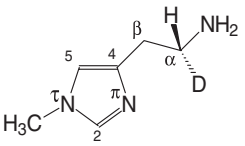
N^{τ}-methyl-L-histidine

[αR-X]-N^{τ}- methylhistamine

where X = ^{2}H or ^{3}H

Figure 3. Synthesis of R-isotopologue of N^{τ}-methylhistamine labeled with deuterium or tritium.

Table 1. ^{1}H NMR chemical shifts of authentic and deuteriated N^{π}- and N^{τ}-methylhistamines (δ in ppm in D_2O and coupling constants J in Hz)

Compound Protons	 πMeHA	 [αR-^{2}H]-πMeHA	 τMeHA	 [αR-^{2}H]-τMeHA
α	3.272 (2H, t; $J = 14$)	3.317 (1H, tt; $J_1 = 14$, $J_2 = 4$)	2.891 (2H, t; $J = 14$)	3.125 (1H, tt; $J_1 = 13$, $J_2 = 4$)
β	3.041 (2H, t; $J = 14$)	3.080 (2H, d, $J = 14$)	2.704 (2H, t; $J = 14$)	3.002 (2H, d; $J = 14$)
N^{π}-CH_3 -group	3.690 (3H, s)	3.706 (3H, s)	—	—
N^{τ}-CH_3 -group	—	—	3.737 (3H, s)	3.665 (3H, s)
2 ring position	7.653 (1H, s)	7.759 (1H, s)	7.605 (1H, s)	7.653 (1H, s)
5 ring position	6.940 (1H, s)	6.976 (1H, s)	6.993 (1H, s)	6.899 (1H, s)

Decarboxylation of N-methyl-L-histidines and subsequent substitution of the carboxylic group by deuterium atom is followed by a change of signals of ^{1}H NMR spectra of deuteriated πMeHA and τMeHA in the region corresponding to their respective side chains. The triplet at C_β changes into a doublet and the triplet at C_α – into triple triplets. Also, the signal integration at C_β is twice that of at C_α. There were also some changes in the chemical shifts of the protons of the side chain as well as of those within the imidazole ring. These ^{1}H NMR data strongly suggests that to C_α are bonded one protium and one deuterium atom.

Experimental

Materials

Tritiated water was purchased from INC Pharmaceutical Inc, Irvine, USA. Deuteriated water (99.9% D), 30% KOD/D_2O, 83% D_3PO_4/D_2O were obtained from Polatom (Poland). Scintillation cocktail was purchased from Rotiszint (Germany). TLC silica gel 60 (0.063–0.200 mm) plates were from Merck. Ion exchange resin Amberlit IR-120 for column chromatography was from Aldrich. Enzyme histidine decarboxylase (HDC), (EC 4.1.1.22) type III-S from *Lactobacillus* 30a, pyridoxal-5′-phosphate (PLP), N^{π}-, and N^{τ}-methyl-L-histidines, and N^{π}-, and N^{τ}-methylhistamines were from Sigma.

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 samples was recorded by an automatic liquid scintillation counter LISA LSC PW470 (Germany). The concentration of N-methylhistamines in the course of decarboxylation was determined on a Shimadzu 1020 UV–VIS spectrometer.

The presence of N-methylhistamines and histidines was checked qualitatively by TLC using silica gel and developing solvent – methanol:water:25% ammonia−7:2.5:0.5 v/v; visualization by ninhydrin.

Synthesis

[(αR)-^{2}H]-πMeHA, ***1***

In a capped vial, to N^{π}-methyl-L-histidine (50 mg, 0.296 mmol) dissolved in 6 mL of fully deuteriated 0.1 M phosphate buffer adjusted to pD 4.7 were added PLP (3.8 mg) and the enzyme HDC (10.8 units), and the reaction mixture was incubated at 37°C for 8 days with continuous shaking. The enzyme was removed by centrifugation, then the solvent was evaporated to dryness under reduced pressure at 45°C. The residue was dissolved in 10 mL of water and left for 5 days at room temperature in order to remove the deuterium from the labile position of imidazole ring. Next, the volume of this mixture was reduced to *ca.* 1 mL by evaporation under reduced pressure and the residue was loaded onto Amberlit IR-120 (H^+) column (100 × 10 mm). In the first step, the buffer salts were washed out with 150 mL of water, next the unreacted N^{π}-methyl-L-histidine

was eluted with 0.3 M NH_3(aq), and finally the product **1** was washed out with 2 M NH_3(aq) and collected as 5 mL fractions. The presence of **1** in eluted fractions was checked by TLC (see *Methods*). The fractions containing **1** were combined and evaporated under reduced pressure at 45°C and lyophilized to dryness under vacuum (1×10^{-3} mm Hg). As a result 24.2 mg (0.195 mmol) of **1** was obtained with 66% chemical yield. The incorporation of deuterium atom into α position of **1** was verified by changes in the ^{1}H NMR spectrum. At C_α the triplet (for πMeHA) changed into a doublet (for [αR-^{2}H]-πMeHA) and signal integration showed for one proton at C_α; at C_β – the triplet (for πMeHA) changed into triple triplets for [αR-^{2}H]-πMeHA.

[(αR)-^{3}H]-πMeHA, ***2***

In a capped vial, to N^π-methyl-L-histidine (8 mg, 0.05 mmol) dissolved in 1 mL of 0.1 M phosphate buffer adjusted to pH 4.7 were added PLP (1.6 mg), of enzyme HDC (10.8 units) and 0.2 mL of tritiated water with total radioactivity of about 6.7 GBq. The reaction mixture was incubated at 37°C for 8 days with continuous shaking. Next, the tritiated water from the post-reaction mixture was removed by lyophilization and dry residue was dissolved in 10 mL of water in order to remove the labile tritium from imidazole ring, and was left for 5 days at room temperature. Next, the volume of the mixture was reduced (to about 1 mL) and loaded onto Amberlit IR-120 (H^+) column (100 × 10 mm). The chromatographic procedure of separation of product **2** was the same as described in section '*1.[(αR)-^{2}H]-πMeHA,* **1**'. From each eluted fraction, 100 μL sample was taken for radioactivity assay. The fractions containing **2** were combined and evaporated to dryness as in section '*1.[(αR)-^{2}H]-πMeHA,* **1**'. As a result 4.8 mg (39 μmol) of **2** was obtained with total radioactivity of 0.55 MBq (sp. activity 14.1 MBq/mmol) and 81% chemical yield.

[(αR)-^{2}H]-τMeHA, ***3***

The decarboxylation of N^τ-methyl-L-histidine was carried out in the same experimental conditions as described in section '*1.[(αR)-^{2}H]-πMeHA,* **1**'. For the reaction, a sample of N^τ-methyl-L-histidine (50 mg, 0.296 mmol) was taken and as a result 19.5 mg (0.157 mmol) of **3** was obtained with 58% chemical yield. Similar changes in the ^{1}H NMR spectrum of **3** (as in the case of **1**) were found (a triplet changed into a doublet at C_α and a triplet changed into triple triplets at C_β).

[αR-^{3}H]-τMeHA, ***4***

For the reaction, a sample of N^τ-methyl-L-histidine (8 mg, 0.05 mmol) was used. The amounts of the other reactants and radioactivity of tritiated water, also incubation, separation, and purification protocol were the same as described in '*2.[(αR)-^{3}H]-πMeHA,* **2'**. As a result 2.1 mg (17 μmol) of **4** was obtained with total radioactivity of 4.8×10^4 Bq (sp. activity 2.8 MBq/mmol) and 35% chemical yield.

Acknowledgement

This work was supported by grant 501/64-BST-132623 from Ministry of Science and Higher Education (Poland).

References

[1] *Histamine and Anti-Histaminics, Part I. Handbook of Experimental Pharmacology*, vol. XVIII/1, (Ed.: M. Rocha e Silva), Springer, Berlin, **1966**.
[2] *Histamine and Anti-Histaminics, Part I. Handbook of Experimental Pharmacology*, vol. XVIII/2, (Ed.: M. Rocha e Silva), Springer, Berlin, **1978**.
[3] D. D. Metcalfe, D. Baram, Y. A. Mekori, *Physiol Rev.* **1997**, *77*, 1033–1079.
[4] C. Maslinski, *Agents Actions, Part I.* **1975**, *5*, 89–107.
[5] C. Maslinski, *Agents Actions, Part I.* **1975**, *5*, 183–225.
[6] C. J. Schwartz, M. J. Arrang, Chapter 14: histamine, in *Neuropsychopharmacology*: *The Fifth Generation of Progress*, Lippincott Williams & Wilkins, **2002**, 179–190.
[7] P. Górski, *Alergia.* **2007**, *4*, 33–35.
[8] L. B. Hough, J. K. Khandelwal, J. P. Green, *J Neurochem.* **1982**, *38*, 1593–1599.
[9] Y. Itoh, R. Oishi, M. Nishibori, K. Saeki, *J Neurochem.* **1989**, *53*, 844–848.
[10] D. D. Brown, J. Axelrod, R. Tomchick, *Nature* (*London*). **1959**, *183*, 680–680.
[11] G. N. Beall, P. P. Jr Vanarsled, *J Clin Invest.* **1960**, *39*, 676–683.
[12] P. C. Waldmeier, Feldtrauer, L. Maître, *J Neurochem.* **1977**, *29*, 785–790.
[13] R. E. Jr West, R-L. Wu, M. M. Billah, R. W. Egan, J. C. Anthes, *Eur J Pharmacol.* **1999**, *377*, 233–239.
[14] K. S. Herman, R. R. Bowsher, D. P. Henry, *J Biol Chem.* **1985**, *260*, 12336–12340.
[15] Nomenclature and Symbolism for Amino Acids and Peptides, IUPAC recommendations **1983**. Available from: http://www.chem.qmul.uk/iupac/AminoAcid/.
[16] D. B. Northrop, *Methods*: *Companion Methods Enzymol.* **2001**, *24*, 117–124.
[17] W. W. Cleland, *Arch Biochem Biophys.* **2005**, *433*, 2–12.
[18] T. Iwashina, P. G. Scott, E. E. Tredget, *Appl Radiat Isot.* **1997**, *48*, 1187–1191.
[19] R. I. Knight, D. J. Morecombe, D. Saunders, I. R. Smith, *J Label Compd Radiopharm.* **1979**, *16*, 761–769.
[20] E. E. Tredget, T. Iwashina, P. G. Scott, A. Ghahary, *J Chrom B.* **1997**, *694*, 1–9.
[21] J. Rosenhalter, B. M. Guirard, G. W. Chang, E. E. Snell, *Proc Natl Acad Sci USA.* **1965**, *54*, 152–158.
[22] A. R. Battersby, M. Nicoletti, J. Stauton, R. Vlegaar. *J Chem Soc Perkin I.* **1980**, 43–51.
[23] E. Santaniello, A. Manzocchi, *J Chem Soc Perkin I.* **1981**, 307–309.
[24] K. D. Schnackerz, C-H. Tai, R. K. W. Pötsch, P. F. Cook, *J Biol Chem.* **1999**, *274*, 36935–36943.