NUKLEONIKA 2014;59(3):91–95
doi: 10.2478/nuka-2014-0015

ORIGINAL PAPER

DE GRUYTER
OPEN

Enzymatic oxidation of substituted tryptamines catalysed by monoamine oxidase

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Abstract. The enzymatic deamination of 5-fluorotryptamine and 5-hydroxytryptamine, 5-HT, catalysed by enzyme monoamine oxidase A (MAO-A, EC 1.4.3.4) was investigated using the kinetic (KIE) and solvent (SIE) isotope effects methods. The numerical values of deuterium isotope effects in the (*1R*) positions of 5-F-tryptamine were determined using non-competitive spectrophotomeric method. Isotopologue 5-F-[(*1R*)-$^{-2}$H]-tryptamine, needed for kinetic studies was obtained by enzymatic decarboxylation of 5′-fluoro-L-tryptophan, 5′-F-L-Trp, in fully deuteriated medium.

Key words: monoamine oxidase (MAO) • tryptamine • serotonin • isotope effects

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Received: 1 March 2013
Accepted: 26 June 2014

Introduction

Monoamine oxidase (MAO, amine:oxygen oxidoreductase, EC 1.4.3.4) plays a central role in the oxidation of the essential bioamines involved in the metabolic processes in living organisms [1]. MAO exists in two isoforms: MAO-A and MAO-B differing in specificities for substrates and inhibitors and tissue distributions.

MAO, a flavin-dependent enzyme catalyses the oxidation of primary and secondary amines to the corresponding aldehydes [2]. The mechanisms of oxidation of amines catalysed by MAO were divided into three pathways [3, 4]: a single electron transfer (SET), a hydrogen atom transfer (HAT) and nucleophilic or polar mechanism.

Also, the amine oxidases were classified into three groups with respect to stereospecificity of amines oxidation. First group catalyses the removing of *pro-R* hydrogen bonded to the amine α-carbon atom (MAO-A, MAO-B isolated from all tissue), the second group eliminates *pro-S* hydrogen from α-carbon atom [benzylamine oxidase or diamine oxidase (DAO) from hog kidney and pea seedling], when third group of oxidases catalyses deamination without the requirement of absolute specificity (oxidase from bovine plasma) [5–7].

Oxidation of serotonin, 5-HT and norepinephrine is preferentially catalysed by MAO-A, but benzylamide and phenylethylamide are oxidised in

the presence of MAO-B. Dopamine, tyramine and tryptamine are substrates for both forms of MAO. The acetylenic inhibitor clorgyline is a selective inhibitor of MAO-A while MAO-B is irreversibly inactivated by pargyline and deprenyl. MAO-A is found in high levels in the liver, placenta, intestine and some brain regions, but is absent in blood platelets, lymphocytes, and chromaffin cells. MAO-B is found mainly in high levels in the liver, brain and platelets, while it is weakly expressed in the placenta only [4, 8]. MAO controls the neurotransmitter levels by catabolising endogenous monoamines, e.g. serotonin (5-hydroxytryptamine, 5-HT). This amine is involved in a wide range of physiological and biochemical processes in mammalian, as the blood pressure control, mood, appetite and sleep, and acts as a neurotransmitter in central and peripheral nervous system. In mammalian including human, the source of 5-HT is enterochromaffin cells occurring in the digestive and respiratory tracts, serotonergic neurons of the brain, pineal gland and platelets where synthesised from L-tryptophan in two step multienzymatic biotransformations involving: tryptophan hydroxylase (TPH, EC 1.14.16.4) and aromatic L-amino acid decarboxylase (AADC, EC 4.1.1.28). In plants, 5-HT, found in fruits, vegetables and nuts [9, 10], is synthesised by hydroxylation of tryptamine catalysed by tryptamine 5-hydroxylase (T5H) [11], Fig. 1. Also tryptamine, a derivative of 5-HT found in trace amounts in the brains of mammals is believed to play a role as a neuromodulator or neurotransmitter [12]. Tryptamine is also the backbone for a group of compounds collectively known as tryptamines showing bioactivity including neurotransmitter and psychotropic properties [13]. Tryptamine and 5-HT are metabolised to: 5-hydroxyindoleacetaldehyde (5-HIAL) [9] and 3-indoleacetaldehyde (3-IAL) [14], and subsequently can be oxidised to corresponding acids (5-hydroxyindoleacetic acid, 5-HIAA and 3-indoleacetic acid, 3-IAA) with the participation of aldehyde dehydrogenase (ALDH, EC. 1.2.1.3, Fig. 1).

From a biological point of view, the halogen derivatives of tryptamine are not important for metabolism, as they are not found in the tissue and have no biological functions. Nevertheless, in many molecules of biological importance, biogenic hydrogen can be easily replaced by another element mainly a halogen, without much changing the activity of these species. The introduction of ^{18}F and ^{131}I in the place of hydrogen atom is widely used for production of radiopharmaceutics for positron emission tomography (PET) [15], a non-invasive, nuclear imaging technology enabling to investigate the internal organ function, also allowing early diagnosis of cancer, monitoring the response to treatment and improvement or worsening of the disease. From nuclear medicine in PET diagnosis it is essential to investigate the metabolism of halogen-substituted derivatives of biologically active compounds. Such potential radiopharmaceutics like ^{18}F-serotonin, ^{18}F-tryptamine and ^{18}F-dopamine are the promising agents for monitoring of abnormal brain states occurring in Parkinson and Alzheimer diseases and schizophrenia [16].

Before using ^{18}F-labelled compounds for PET studies should be carry out a several studies on the inactive compounds to determine their involving in metabolic reactions. The object of our study was to investigate whether MAO catalyses the oxidation of substituted derivatives of inactive tryptamine, i.e. 5-fluorotryptamine (5-F-tryptamine) and 5-hydroxytryptamine (5-HT). To study some intrinsic details of mechanism of deamination of abovementioned amines the kinetic (KIE) and solvent (SIE) isotope effect methods were used [17, 18].

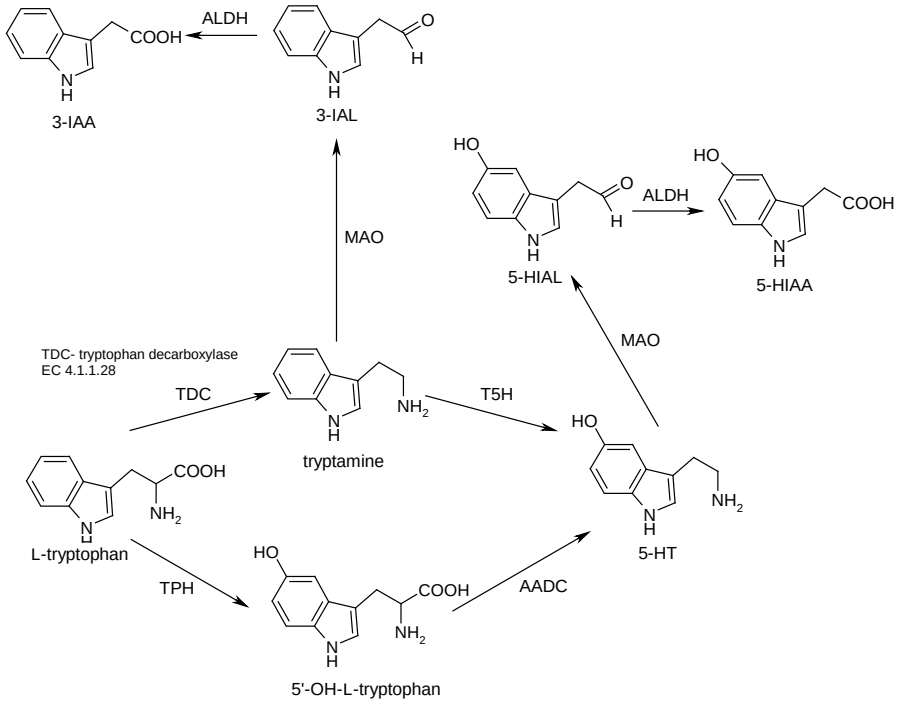

Fig. 1. Biosynthesis pathway of L-tryptophan.

Experimental

Materials

Deuteriated water (99.9% D), 83% D_3PO_4/D_2O and 30% KOD/D_2O were from Polatom (Poland). The enzymes: L-Phenylalanine decarboxylase (EC 4.1.1.53) from *Streptococcus faecalis*, MAO-A (EC 1.4.3.4) *human*, Horseradish peroxidase type VI-A′ HRP (EC 1.11.1.7) from horseradish and coenzyme PLP (pyridoxal 5′-phosphate) were from Sigma-Aldrich. The chemicals, needed for trial experiments, enzymatic synthesis, and kinetic assays such as 5′-F-L-Trp, 5-hydroxytryptamine, 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), sodium azide (NaN_3) and all other chemicals were from Sigma-Aldrich. TLC plates (DC-Alufolien Kieselgel 60) and silica gel for column chromatography (Kieselgel 60, 0.063–0.200 mm) were from Merck.

Methods

The ^1H nuclear magnetic resonance, NMR, spectra were recorded in D_2O using tetramethylsilane, TMS, as internal standard on a Varian 500 MHz Unity-plus spectrometer. The kinetic assays were performed using a Shimadzu-UV-1800 spectrophotometer. The progress of oxidations was monitored indirectly by measuring the absorbency of oxidised form of ABTS formed and the coupled reaction catalysed by enzyme HRP. The progress of decarboxylations was monitored by TLC using as developing solvent; acetonitrile:water; 4:1, v/v.

Synthesis

Synthesis of 5-F-[(1R)-^{2}H]-tryptamine

The isotopologue 5-F-[(*1R*)-^{2}H]-tryptamine was obtained by one step reaction, Fig. 2, by enzymatic decarboxylation of 5′-F-L-Trp in fully deuteriated Tris·DCl buffer (pD 5.5). The reaction was carried out and product was separated and purified as described earlier [19]. To an encapped vial with 50 mg (0.24 mmol) sample of 5′-F-L-Trp dissolved in 15 mL of deuteriated 50 mM Tris·DCl buffer (pD 5.5) to which 4 mg (16 μmol) of PLP (dissolved in 3 mL of Tris·DCl buffer) and 50 mg of enzyme decarboxylase phenylalanine (1 U) were added. As a result 40 mg (0.25 mmol) sample of 5-F-[(*1R*)-^{2}H]-tryptamine was obtained (80% chem. yield). The extent of deuterium incorporation (near 100%) at the (*1R*) position of tryptamine was determined by ^1H NMR spectrum (chem. shifts: δ 3.36 ppm for $1H_\alpha$, t, J = 10 Hz; 3.18 ppm for $2H_\beta$, d, J = 20 Hz).

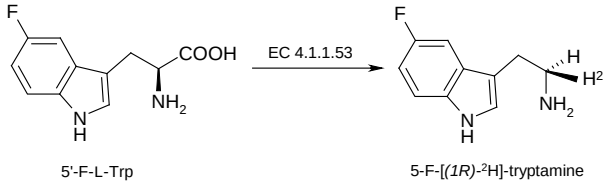

Fig. 2. Synthesis of 5-F-[(*1R*)-^{2}H]-tryptamine by enzymatic decarboxylation of 5′-F-L-Trp in deuteriated medium.

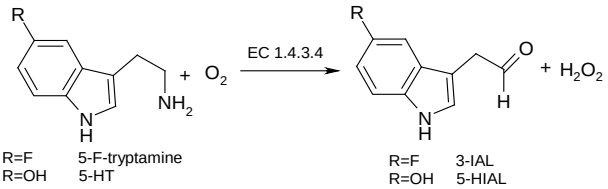

Fig. 3. Enzymatic oxidation of 5-HT and isotopologues of tryptamine catalysed by enzyme MAO-A.

Synthesis of 5-fluorotryptamine

This isotopomer was obtained similarly as described in synthesis of 5-F-[(*1R*)-^{2}H]-tryptamine. The sample of 5′-F-L-Trp (25 mg, 110 μmol) was dissolved in 7 mL of 50 mM Tris·HCl buffer (pH 5.5) to which 1 mg (4 μmol) of PLP and 26.5 mg (1 U) decarboxylase phenylalanine were added. Finally, obtained 22.6 mg (10.2 mmol) of 5-F-tryptamine (90% chem. yield). The structure of compounds was confirm by ^1H NMR spectrum (chem. shifts: δ 7.67 ppm for H^4, dd; 7.30 ppm for H^6, dd; 7.03 ppm for H^7, td; 7.34 for H^2, s; 3.38 ppm for $2H_\alpha$, t, J = 20 Hz; 3.2 ppm for $2H_\beta$, t, J = 20 Hz).

Kinetic assays

For studying the kinetic of enzymatic oxidative deamination of 5-HT and isotopologues of 5-F-tryptamine, [20], Fig. 3, the following buffered solution was used:

a) 0.1 M Tris·HCl buffer, pH 7.6.
b) 0.1 M Tris·DCl buffer deuteriated, pD 8.
c) 10 mM of ABTS (as solution in 0.1 M Tris·HCl buffer).
d) 0.1 M NaN_3 (as solution in 0.1 mM Tris·HCl buffer).
e) Solution of enzyme HRP VI-A′ (EC 1.11.1.7; 1000 U/ml) prepared by dissolving of 1 mg of commercial product in 1 mL of Tris·HCl buffer.
f) Solution of enzyme MAO-A (EC 1.4.3.4; 100 U/ml) in 0.1 M phosphate buffer, prepared from commercial product.

Separately, for experiments carried out in deuteriated media the reagents listed in points ‘c’ to ‘e’ were dissolved in deuteriated 0.1 M Tris·DCl, pD 8, and enzyme MAO-A was dissolved in deuteriated 0.1 M phosphate buffer, pD 7.8. The exact pD values of the buffered solutions were adjusted using D_3PO_3/D_2O, DCl/D_2O, and KOD/D_2O solutions.

Each kinetic run consisted of six measurements carried out in 1 mL quartz spectroscopic cuvettes for different concentrations of 5-HT and isotopologues of 5-F-tryptamine (from 0.03 to 0.1 mM). The quantities of enzymes MAO-A and HRP VI-A′ in each cuvette were 1 and 5 U/mL, respectively. The progress of reaction was registered spectrophotometrically by measuring the increase of absorbance of the oxidised form of ABTS at λ = 410 nm at 1 min intervals for 20 min.

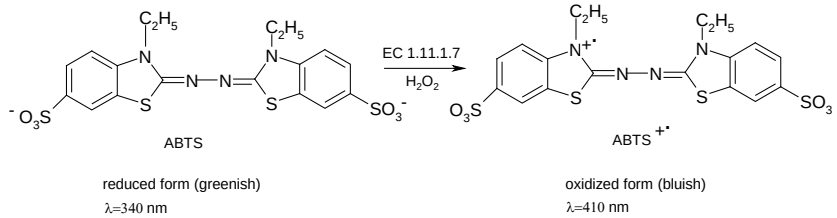

Fig. 4. Enzymatic oxidation of ABTS by H_2O_2 catalysed by the enzyme Horseradish peroxidase, HRP.

Results and discussion

Synthesis

The sample of 5-F-tryptamine, needed for kinetics studies was obtained by enzymatic decarboxylation of 5′-F-L-Trp catalysed by enzyme L-phenylalanine decarboxylase (EC 4.1.1.53). The isotopologue of 5-F-tryptamine, i.e., 5-F-[(*1R*)-^{2}H]-tryptamine was synthesised by enzymatic decarboxylation of 5′-F--L-Trp, respectively, using the modified procedures described earlier [21]. The decarboxylation of 5′-F--L-Trp was carried out using fully deuteriated and buffered medium, corrected to pD 5.5 due to higher pK (D_2O) [22]. All substrate for this reaction were dissolved in almost entirely deuteriated Tris·DCl 0.1 M buffer (394 mg, 2.5 mmol of Tris·HCl were dissolved in 50 ml of D_2O; and calculated fraction of H^+/D^+ ions in this way prepared incubation medium was equal to 0.0005).

Unfortunately, we could not calculate isotope effects for the attempts to obtain [(*1R*)-^{2}H]-5-HT, i.e. [(*1R*)-^{2}H]-serotonin, because the attempts to obtain this compound by decarboxylation of 5′-OH-L-Trp with the use of L-phenylalanine decarboxylase, as well as, tyrosine decarboxylase (EC 4.1.1.25) was failed.

Kinetic assays

The kinetic of oxidative deamination of 5-HT and isotopologues of 5-F-tryptamine (Fig. 3) at room temperature was investigated using non-competitive spectrophotometric method [23].

The numerical values of deuterium KIE and SIE for conversion of 5-HT and isotopologues of 5-F--tryptamine to the corresponding aldehydes were determined indirectly by measuring the absorbency of oxidised form of 2,2′ azino-di-(3-ethylbenzothiazoline-6-sulphonate), ABTS, formed by the coupled reaction of H_2O_2, (released in course of oxidation) catalysed by the enzyme peroxidase (Horseradish peroxidase, type VI-A′, HRP, EC 1.11.1.7) with reduced form of ABTS (strong absorbing at λ = 410 nm), [24; Fig. 4].

One of the pot enzymatic reactions was carried out in the same measuring spectrometric cuvette. The progress of the oxidative deamination of 5-F--tryptamine and 5-HT to aldehydes was measured by recording the increase in the absorbency of oxidised form of ABTS at λ = 410 nm. The kinetic reaction of oxidation labelled 5-F-tryptamine was measured in the spectrophotometer. The progress of reaction was indirectly monitored by the growth of absorbency in the UV-VIS region using a coupling reaction. Hydrogen peroxide resulting from deamination of tryptamine derivatives, converts reduced ABTS into the oxidised form (Fig. 4). This reaction was catalysed by enzyme peroxidase (Horseradish peroxidase, type VI-A′, HRP, EC 1.11.1.7). The incubation medium containing derivatives of tryptamine, NaN_3, ABTS, the enzymes peroxidase and MAO-A was introduced into one spectrophotometer cuvette. The increase of absorbance of oxidised form ABTS was measured at λ = 410 nm.

The KIE's and SIE's in the enzymatic deamination of labelled tryptamine and its derivatives were determined using a non-competitive method. The kinetic parameters i.e. K_m and V_{max} in the Michaelis–Menten Eq. (1) were generated using Enzifitter™ 1.05 software from the experimentally determined reaction rates at given concentration of substrate (S). The initial rates, ν, for corresponding isotopologues of tryptamine were calculated from Eq. (2) [25, 26].

$$K_m = [S]\left(\frac{V_{max}}{\nu} - 1\right) \quad (1)$$

$$\nu = \frac{[S]}{\frac{1}{V_{max}/K_m} + \frac{[S]}{V_{max}}} \quad (2)$$

where K_m is the Michaelis constant, [S] is the concentration of substrate (S is the concentration of enzyme), ν is the reaction rate at concentration S, and V_{max} the maximum reaction rate.

The SIE was calculated by dividing the parameters for the reaction with 5-F-tryptamine, 5-F-[(*1R*)-^{2}H]-tryptamine or 5-HT by parameters obtained in Tris·HCl buffer and deuteriated Tris·DCl buffer, Table 1. Similarly, KIE was calculated by dividing the parameters for the reaction for unlabelled 5-F--tryptamine by parameters for the reaction for labelled 5-F-tryptamine, Table 2.

Table 1. The solvent isotope effect (SIE) for the oxidation of 5-F-tryptamine and 5-HT by MAO-A

	SIE	
	on V_{max}	on V_{max}/K_m
5-F-[(*1R*)-^{2}H]-tryptamine	1.3 ± 0.26	1.9 ± 1.1
5-OH-tryptamine	1.7 ± 0.18	1.1 ± 0.34
5-F-tryptamine	2.6 ± 0.33	3.9 ± 1.9

Table 2. The kinetic isotope effect (KIE) for the oxidation of 5-F-tryptamine by MAO-A

	KIE		
	on V_{max}	on V_{max}/K_m	Solvent
5-F-[(*1R*)-^{2}H]--tryptamine	3.6 ± 0.49	3.7 ± 2.1	H_2O
	1.9 ± 0.36	1.8 ± 0.35	^2H_2O

The values SIE's and KIE's are higher than unity in each investigated of compounds suggesting that MAO-A catalysed deamination substituted tryptamines in indole ring.

The determined value of the SIE's on V_{max} shows that the solvent has effects on the transformation of the enzyme–substrate complex into enzyme–product complex. Whereas, slightly smaller but significant values of SIE's on V_{max}/K_m suggests that the solvent plays a role during the formation of enzyme–substrate complex [18].

That is worth noticing that the value of the solvent isotope effect for the 5-OH-tryptamine are higher than in the case of fluorine-substituted, unlabelled tryptamine.

The high effect of KIE on V_{max} = 3.6 of 5-F-[(*1R*)-^{2}H]-tryptamine shows the enzyme MAO-A catalyses the disconnection of proton at the (*R*) position at the α-carbon atom of amines, similarly as described early [5]. The value of the determined KIE's on V_{max}/K_m of 5-F-[(*1R*)-^{2}H]-tryptamine suggests that deuterium substitution at the (*R*) position significantly affect the conversion of the enzyme–substrate complex into the enzyme–product complex [27].

Acknowledgment. This work was supported by grant BST 501/86-DSM-100100.

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