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Analysis of monoamine oxidase enzymatic activity by reversed-phase high performance liquid chromatography and inhibition by β-carboline alkaloids occurring in foods and plants

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Abstract

Monoamine oxidase (MAO) is a flavin adenine dinucleotide (FAD)-containing enzyme located at the outer membranes of mitochondria that catalyzes the oxidative deamination of biogenic and xenobiotic amines. We have used a chromatographic method to measure MAO-enzymatic activity by using kynuramine as a non-selective substrate with its MAO-oxidation product subsequently analyzed by RP-HPLC–DAD and HPLC–mass spectrometry (MS). This method was applied to study the kinetic parameters, inhibition and reaction products of MAO recombinant enzymes in presence of tetrahydro-β-carboline and β-carboline alkaloids occurring in foods, plants and mammals. Analysis by HPLC showed that tetrahydro-β-carbolines or β-carbolines were not modified by MAO. Several β-carbolines such as tryptoline (1,2,3,4-tetrahydro-β-carboline) and 1-methyltryptoline (1-methyl-1,2,3,4-tetrahydro-β-carboline) were inhibitors of MAO-A; instead their corresponding 6-hydroxy-derivatives (6-hydroxytryptoline and 6-hydroxy-1-methyltryptoline) lacked this activity. Tetrahydro-β-carboline-3-carboxylic acids were unable to inhibit MAO enzymes. In contrast, their oxidation products, i.e. the fully aromatic β-carbolines (norharman and harman), acted as good inhibitors of MAO. Two tetrahydro-β-carbolines (i.e. tryptoline and 1-methyltryptoline) occurring in foods were isolated by solid-phase extraction (SPE) and RP-HPLC from selected samples of sausages and the corresponding extracts exhibited good inhibition properties over MAO-A. These results suggest that β-carbolines from foods, plants, and mammals may exert inhibitory actions on MAO enzymes.

Keywords: Monoamine oxidase; MAO; Enzyme assay; MAO inhibitors; β-Carbolines; Tetrahydro-β-carbolines; Alkaloids; Foods; Plants; Sausages; RP-HPLC; MS

1. Introduction

Monoamine oxidase (MAO) (EC 1.4.3.4) is a flavin adenine dinucleotide (FAD)-containing enzyme located at the outer membranes of mitochondria in the brain, liver, intestinal mucosa, and other organs. It catalyzes the oxidative deamination of biogenic amines (neuroamines, vasoactive and exogenous amines), including dopamine, serotonin, norephinephrine, tyramine, tryptamine and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) neurotoxin. The end products of MAO are aldehydes, H_2O_2 and ammonia that are involved in oxidative cellular and cytotoxic processes. MAO appears as two isozymes, MAO-A and MAO-B, distinguished by their differences in substrate and inhibitor selectivities [1,2]. MAO-A preferentially catalyzes the oxidation of serotonin and norephinephrine, and is inhibited by clorgyline, whereas MAO-B selectively catalyzes the oxidation of phenylethylamine and benzylamine and is inhibited by (*R*)-deprenyl. Tyramine, dopamine, and tryptamine appear to be substrates for both isozymes. MAO is involved in the regulation and turnover of neuroamines in the central nervous system and peripheral organs. Abnormal MAO-B is implicated in neurological disorders such as Parkinson's and Alzheimer's diseases, whereas MAO-A is involved in behavior and psychiatric conditions such as depression [3–6]. Identification of MAO inhibitors is of great interest in drug discovery [2,7,8]. MAO-A inhibitors are useful as antidepressants [3,9], and MAO-B inhibitors in the treatment of Parkinson's disease [7,10].

A number of analytical methods have been used for the determination of MAO enzymatic activity [7]. Those are generally based on the detection of oxygen consumption, H_2O_2 or ammonium generated, and the measurement of oxidized monoamine products by direct absorbance or fluorescence detection [11,12].

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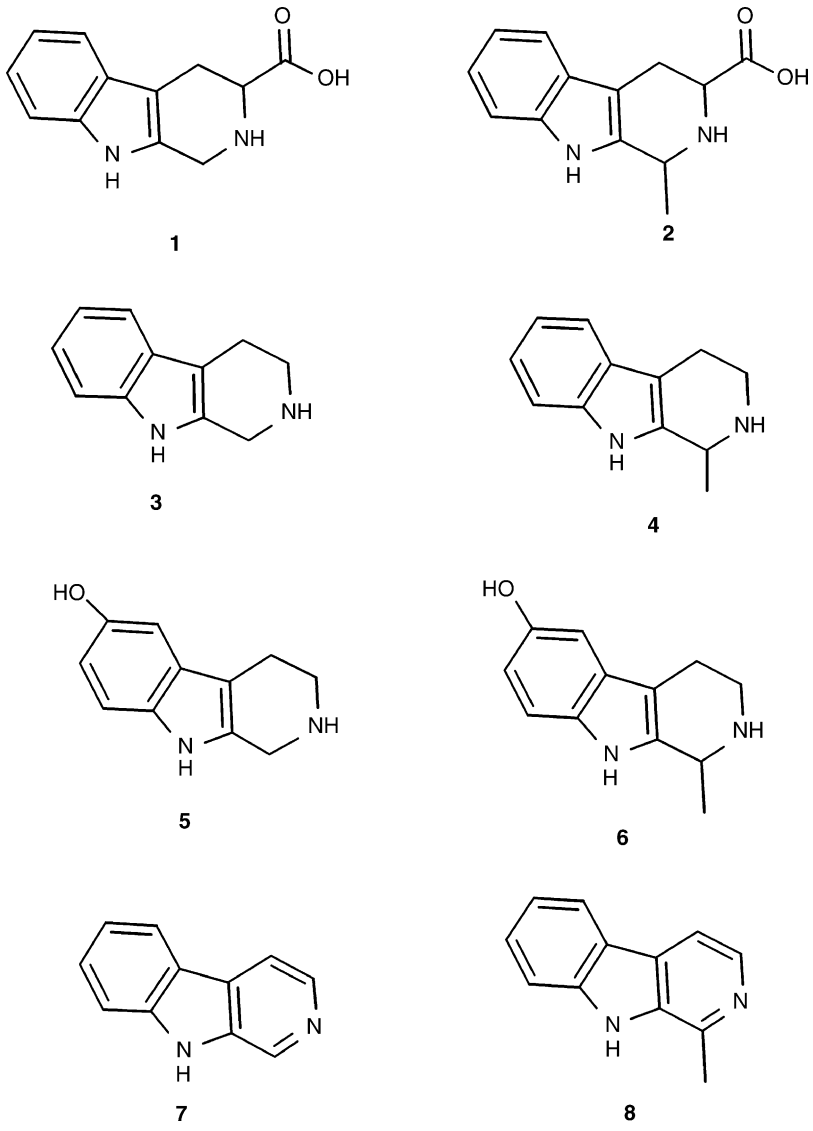

Fig. 1. β-Carboline alkaloids found in foods, plants, and mammalian systems. The chemical names are as follows: 1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid (**1**); 1-methyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid (**2**); 1,2,3,4-tetrahydro-β-carboline (**3**); 1-methyl-1,2,3,4-tetrahydro-β-carboline (**4**); 6-hydroxy-1,2,3,4-tetrahydro-β-carboline (**5**); 6-hydroxy-1-methyl-1,2,3,4-tetrahydro-β-carboline (**6**); norharman (**7**); and harman (**8**).

The results obtained with them, however, are often compromised by low selectivity or sensitivity and also by the presence of interfering compounds in samples and standards. Assays based on the determination of MAO deamination products by chromatographic methods such as HPLC and HPLC–MS/MS are time-consuming [8,13], but instead they afford the needed selectivity and specificity required in some studies. Furthermore, analytical chromatographic separation also allows for the screening of possible MAO metabolites produced from those compounds or samples of interest. HPLC based assays can also be adapted for high-throughput screening in pharmaceutical research [8]. Following, we report an assay to determine MAO enzymatic activity based on HPLC–DAD and mass spectrometry determination of monoamine deamination products. This assay is further applied to investigate whether a family of β-carboline alkaloids affect MAO enzymes as well as their substrate and inhibitor characteristics. β-Carbolines are heterocyclic pyridoindole alkaloids produced through a Pictet–Spengler condensation from indolethylamines and carbonylic compounds. Two types of structural compounds, e.g. tetrahydro-β-carbolines and β-carbolines were studied for such purpose (Fig. 1). Various of these alkaloids occur in foods, plants and environmental samples [14–20]. Moreover, they may appear endogenously in biological fluids and tissues, including the brain [21,22]. β-Carbolines exhibit a wide spectrum of pharmacological and neuroactive actions; e.g. they bind to several receptors in the brain such as benzodiazepine, imidazoline, serotonin, and alter the levels of neurotransmitters [23–25]. This class of compounds may act as radical scavengers [26,27] and may also interact with MAO enzymes [28]. On the other hand, MAO enzymes might hypothetically participate in the metabolic oxidation of tetrahydro-β-carbolines as it occurs with the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine [29]. Following we report that various β-carboline alkaloids ocurring in food, plant, environmental