Chemico-Biological Interactions 208 (2014) 37–46

Contents lists available at ScienceDirect

Chemico-Biological Interactions

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

Effect of Cd, Zn and Hg complexes of barbituric acid and thiouracil on rat brain monoamine oxidase-B (*in vitro*)

Nadia Z. Shaban [a,*], Mamdouh S. Masoud [b], Doaa Awad [a], Mai A. Mawlawi [a], Omayma M. Sadek [a]

[a] Biochemistry Department, Faculty of Science, Alexandria University, Alexandria, Egypt
[b] Chemistry Department, Faculty of Science, Alexandria University, Alexandria, Egypt

ARTICLE INFO

Article history:
Received 21 June 2013
Received in revised form 14 November 2013
Accepted 25 November 2013
Available online 1 December 2013

Keywords:
Monoamine oxidase-B
Inhibition
Rat brain
Barbiturates complexes
Thiouracils complexes

ABSTRACT

Metal pyrimidine complexes (MPCs) including cadmium–barbiturate (Cd–BA), zinc–barbiturate (Zn–BA), cadmium–thiouracil (Cd–TU) and mercury–thiouracil (Hg–TU) were prepared and their analysis was carried out. These MPCs were evaluated as monoamine oxidase-B (MAO-B) inhibitors. Rat brain MAO-B was inhibited (*in vitro*) by Cd–BA, Zn–BA, Cd–TU and Hg–TU complexes. The inhibition of MAO-B by these complexes was time and concentration dependent. The values of IC_{50} of Zn–BA, Cd–BA, Hg–TU and Cd–TU were 10.2, 15.8, 16.2 and 20.4 nM, respectively. The effect of different substrate concentrations in the absence and in the presence of MPCs was determined. Lineweaver–Burk plots were plotted and the values of apparent Michaelis constant (K_m), maximum velocity (V_{max}), the dissociation constant of enzyme inhibitor complex (K_i) and the percent of inhibition ($i\%$) were calculated. The data showed that the inhibition of MAO-B by all studied MPCs was the non-competitive type. The sequence of inhibition zone was: Zn–BA > Cd–BA and Hg–TU > Cd–TU affected by the chemistry of both the metal and the ligand. Otherwise, the results of the present study showed that the inhibition of MAO-B by all MPCs was fully reversible. The data showed that the presence of Cd–BA, Zn–BA, Cd–TU and Hg–TU complexes changed the optimum temperature and pH of MAO-B.

1. Introduction

Monoamine oxidases (MAOs, EC 1.4.3.4) are flavoenzymes which play an important role in the oxidative catabolism of amine neurotransmitters such as dopamine, serotonin and norepinephrine [1,2]. Monoamines (R-NH_2) are transformed into the corresponding aldehydes and the byproducts (hydrogen peroxide, H_2O_2, and ammonia). The aldehydes are oxidized into acids by aldehyde dehydrogenase (ALDH). The byproducts, in particular H_2O_2, are potentially neurotoxic can trigger the production of reactive oxygen species (ROS) and induce mitochondrial damage and neuronal apoptosis [3]. All mammals contain two distinctive MAO enzymes, MAO-A and MAO-B, which are bound to the outer membrane of the mitochondria [2,3]. These enzymes are encoded by separate genes [4]. They differ in their selectivity for substrates, inhibitors and cellular localization. MAO-A preferentially oxidizes serotonin and norepinephrine [5] and is irreversibly inhibited by low concentration of clorgyline, while MAO-B preferentially oxidizes histamine and β-phenylethylamine (β-PEA) and is reversibly inhibited by low concentration of deprenyl. Both isoforms oxidise dopamine [6,7]. Both isozymes are highly conserved, with the amino acid sequences of the two isoforms showing 70% sequence identity [8–10]. Functional MAO-A and MAO-B proteins are thought to consist of two identical subunits each, with molecular weights of 59 and 58 kilo daltons, respectively, consisting of 527 and 520 amino acids, respectively [10,11]. Binding of FAD is essential for enzyme function, and each subunit binds one FAD molecule through the cysteine residue by a sulfhydryl bond [10,12,13]. Cerebral MAO-B activity increases with aging and in neurodegenerative disorders such as Alzheimer, Parkinson and Huntington's diseases [14,15]. Thus, the inhibition of MAO-B in these patients not only increases the levels of monoamines which activate the membrane receptors but also decreases H_2O_2 production and the potential for hydroxyl radical formation and consequently the oxidative stress is decreased [16].

Pyrimidine is the parent heterocycle of a very important group of compounds that have been extensively studied due to their occurrence in living systems [17], which exist in nucleic acids, several vitamins, coenzymes and antibiotics. Pyrimidine derivatives are reported to have a broad spectrum of biological activities such as anticancer [18], antiviral [19], antibacterial [20], antioxidant [21], anti-inflammatory [22], analgesic activities [23], anxiolytic [24] and antidepressant activities [25]

The transition and inner transition metal ions are known to have small radii and variable coordination number ranging from 3 to 12, which make them excellent spacers in assembling fascinating metal organic frameworks. Pyrimidines and their derivatives

* Corresponding author. Tel.: +20 1227425785; fax: +20 33911794.

0009-2797/$ - see front matter © 2013 Elsevier Ireland Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.cbi.2013.11.012

Table 1
Elemental analysis and physical properties of the metal pyrimidine complexes.

Metal complexes MPCs	Color	m.p. °C	Calculated/(found)%				
			C	H	N	S	M
Zinc barbituric acid $Zn(HL)_2$	Buff	>360	30.07 (30.10)	1.89 (1.30)	17.53 (17.90)	– (–)	20.46 (20.30)
Cadmium barbituric acid $Cd(HL)_2{\cdot}H_2O$	Buff	>360	23.87 (23.90)	2.50 (2.30)	13.92 (13.90)	– (–)	27.92 (28.10)
Mercury thiouracil $HgL{\cdot}H_2O$	Yellow	314	22.93 (23.10)	1.92 (2.10)	13.37 (13.40)	15.30 (15.30)	31.21 (31.30)
Cadmium thiouracil $CdL{\cdot}H_2O$	White	314	18.73 (18.90)	1.57 (1.80)	10.92 (11.30)	12.50 (12.80)	43.81 (43.80)

Table 2
Fundamental infrared bands (cm^{-1}) of barbituric acid and its complexes.

Barbituric acid (BA)		$Zn(BA)_2$		$Cd(BA)_2.2H_2O$		Assignments
3552 (s)		–		3521 (sh)		υ_{OH}
3478 (s)		3497 (sh)		–		
3182 (m)	(sp)	3171 (m)		3187 (m)		υ_{NH}
3096 (w)		–		–		
–		–		–		
2876 (m)		–		–		υ_{CH}
2830 (w)		2813 (w)		2810 (vw)		
1744 (w)	(sp)	–		–		$\upsilon_{C=O}$
1718 (w)						
1617 (m)		1647 (m)	(sp)	1645 (m)	(sp)	$\upsilon_{C=N}$
		1607 (m)		1607 (m)		
1526 (m)		1536 (vw)		1536 (vw)		$\upsilon_{C=C}$
1410 (m)		–		1415 (vw)		δ_{NH}
1366 (vm)		1392 (s)		1391 (m)		υ_{C-O}, δ_{CH}
1349 (w)		1349 (s)		1348 (s)		
1285 (m)		1300 (s)		1300 (s)		υ_{C-O}, δ_{OH}
1232 (s)		–		–		υ_{C-N}
1193 (m)		1214 (m)		1214 (m)		
–		1115 (vs)		1086 (w)		υ_{C-O}, υ_{C-N}
1028 (s)		1008 (m)		1008 (m)		υ_{C-C}
936 (s)		–		–		
–		–		–		ρ_{CH}, ρ_{OH}
–		920 (s)		821 (s)		
–		–		–		
733 (s)		776 (vs)		776 (vs)		
739 (m)		–		–		
656 (m)		683 (m)		683 (m)		
–		–		–		
632 (s)		–		–		
–		524 (vs)		524 (vs)		υ_{M-O}
–		316 (m)		315 (m)		υ_{M-N}

m = medium, s = strong, sh = shoulder, sp = splitted, v = very and w = weak.

provide potential binding sites for metal ions. The interaction of metal ions with nucleobases is of great interest because of their relevance to the essential, medical or toxic bioactivity of metal, where nucleobase molecule can coordinate as exogenous ligands in metalloproteins [18]. Various metal complexes with bi and tridentate Schiff bases containing nitrogen and oxygen donor atoms play an important role in biological systems [26]. Schiff base complexes incorporating phenolic group as chelating moieties in the ligand are considered as models for executing important biological reactions and mimic the catalytic activities of metalloenzymes [26]. The metal complexes of purines, pyrimidines and their nucleotides play a dominant role in many biochemical systems [27]. Moreover, they have been found to exhibit anticancer and fungicidal properties [28]. Furthermore, it has been suggested that the presence of metal ions in biological fluids, could have a significant effect on the therapeutic action of drugs [29]. In recent years it has been shown that, in many cases, certain metal complexes of a drug are proved to be more potent than the pure drug. The increase in potency is because binding of a drug with metal ions conferred it with some special physicochemical properties helpful in its biological activities; such as low dissociation constant, special redox potential, electron distribution and lipid solubility [20,21].

Barbiturates and their derivatives are widely used as sedative hypnotic drugs and are also employed for anaesthesia [30]. Compounds containing nitrogen and sulphur as donor atoms like thiouracil, have an important role to be used as anti-cancer and anti-viral activities [27]. Synthesis and characterization of barbituric and thiobarbituric acid complexes derived from cobalt(II), nickel(II) and copper(II) salts were reported by Masoud et al. [31]. Barbiturate and thiouracil Cd(II), Hg(II), and Zn(II) complexes have been prepared and characterized by elemental analyses, infrared (IR), electronic spectra, magnetic susceptibility and electron spin resonance spectra [32]. The present study was undertaken to study the effects of Cd (II), Zn (II) and Hg (II) complexes of barbiturate and thiouracil on MAO-B activity *in vitro* to discover novel inhibitors for