PHYSICAL CHEMISTRY 2018

14th International Conference
on Fundamental and Applied Aspects of
Physical Chemistry

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Volume I

September 24-28, 2018
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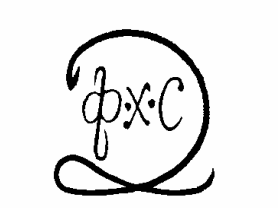

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INFLUENCE OF 12-TUNGSTOSILICIC ACID AND 12-TUNGSTOPHOSPHORIC ACID ON THE ACTIVITY AND SECONDARY STRUCTURE OF ACETYLCHOLINESTERASE

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ABSTRACT

Inhibition of acetylcholinesterase (AChE) is presented as a promising strategy in the treatment of Alzheimer disease providing inspiration for new discoveries and investigations less toxic and more effective potential anti Alzheimer drugs. In this paper, it demonstrated that the activity of acetylcholinesterase can be effectively inhibited by polyoxometalates (POMs), 12-tungstosilicic acid (WSiA) and 12-tungstophosphoric acid (WPA) without significant changes on the secondary structure of this enzyme. The obtained values of partition coefficient implicated on smooth pass of these POMs trough cell membrane and satisfied necessary criteria for the drugs used in the treatment of the central nervous system disease. Based on these obtained results it is possible to conclude that POM could represent new generation of potential anti Alzheimer drugs.

INTRODUCTION

Acetylcholinesterase (AChE) is a key enzyme of cholinergic brain synapses and neuromuscular junctions which the major biological role is the termination of impulse transmission by rapid hydrolysis of the cationic neurotransmitter acetylcholine. Besides that, AChE affects cell proliferation, differentiation and responses to various insults, including stress **[1]**. In medicine, AChE inhibitors are currently used in the therapy of Alzheimer's disease. Polyoxometalates (POMs) are inorganic cluster compounds that possess a number of pharmacological properties such as antibacterial, antiviral and anticancer activities. However, their molecular mechanism of

action is still unknown. The numerous literature data relate their biological activity with the inhibition of enzymes. For example, their antiviral properties are closely associated with inactivation of reverse transcriptases **[2]** while the activity of the POM decavanadate against leishmania may be due to the inhibition of phosphoglycerate mutase and various phosphatases **[3]**. In order to confirm possible role of POMs in the treatment of Alzheimer disease we were investigating their influence on the activity and secondary structure of acetylcholinesterase, target enzyme in the treatment of this common neurological disease.

EXPERIMENTAL

Chemicals. Acetylcholinesterase, acetylthiocholine iodide (AChI), 5,5'-dithio-bis(2-nitrobenzoic acid) (DTNB), sodium dodecyl sulfate (SDS), WSiA and WPA were purchased from Sigma-Aldrich (Germany). The other medium assay chemicals, were obtained from Merck (Germany).

AChE assay. AChE activity was determined in 0.1 M phosphate buffer pH 8, containing 20µl 2U/ml commercial enzyme and 70µl desired inhibitor concentrations in the final volume of 650µl, using Ellman's procedure **[4]** and expressed as the mean percentage of enzyme activity relative to the corresponding control value (REA).

Measurement of the partition coefficient

The partition coefficient of the selected POMs was measured using "shake-flask" method, in two phase system composed of n-octanol and deionised water **[5]**. The absorbance of POMs in the aqueous phase was measured spectrophotometrically at 263 nm for WSiA and at 257 nm for WPA, and used for the determination of the concentration of POMs left in the aqueous phase.

Fluorescence measurements were performed using a Fluorolog-3 model FL3-221 spectrofluorimeter (HORIBA Jobin- Yvon) in front face mode. Excitation and emission monochromators were of double grating design, with a dispersion of 2.1 nm mm 1 (1200 grooves per mm), blazed at 295 nm for excitation and 315 – 420 nm for emission. A xenon lamp provided excitation and a Horiba TBX-04 PMT detector was used for the emission measurements in a right angle configuration using a 1 cm path cuvette.

Circular Dichroism spectra were recorded using a Jasco 1500 spectrophotopolarimeter equipped with a Peltier thermostatic system under constant nitrogen flux at 25 ºC, with a 0.1 cm quartz cuvette in the range of 190 – 260 nm. The CD spectrum of each sample was recorded three times at a scan rate of 10 nm min-1. For the base line, a solution which contain 0.1M phosphate buffer, pH = 8 was used.

RESULTS AND DISCUSSION

In order to gather information about the influence of selected POMs on AChE activity, the commercially available enzyme was exposed to increasing concentrations of POMs, in the range from 1×10^{-9} to 1×10^{-4} M. Sigmoid-shaped inhibition curves were obtained in both cases (**Fig. 1**). Inhibitory parameters, half-maximum inhibitory concentration values (IC_{50}) and Hill coefficient (n) were obtained using Hill analysis of inhibition curves (**Inset, Fig. 1**) and presented in the **Table 1**.

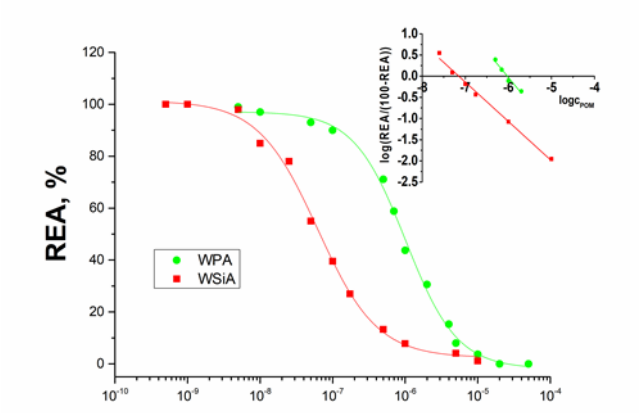


Figure 1. Inhibition of AChE induced by selected POMs. Inset: Hill analysis of inhibition curves.

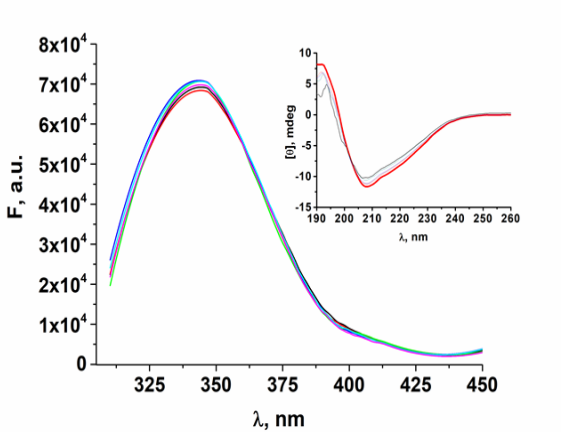
The obtained IC_{50} values for the selected POMs complexes suggest a powerful inhibition of AChE activity, which is in the submicromolar range. The values of Hill coefficient n ~1 indicate no cooperative binding of inhibitor to the enzyme and one binding site of POMs. The obtained values of partition coefficient (**Table 1**) implicated on smooth pass of these POMs trough cell membrane and satisfied necessary criteria for the drugs used in the treatment of the central nervous system disease.

Table 1. Parameters of AChE inhibition induced by selected POMs and their log P values.

POM	log P	*Hill analysis*	
		IC_{50}, M	n
WSiA	-0.47	$(7{,}23 \pm 0{,}02) \times 10^{-8}$	$0{,}93 \pm 0{,}09$
WPA	-0.29	$(1{,}23 \pm 0{,}01) \times 10^{-6}$	$1{,}23 \pm 0{,}17$

Fluorescence and CD spectroscopy studies performed on these systems pointed out that the inhibition of AChE activity with selected POMs does not related with the changes in the secondary structure of this enzyme (**Fig. 2a and 2b**). In our experiments commercial AChE in phosphate buffer (pH 8) has its characteristic emission peak at 443 nm under excitation at 295 nm and characteristic negative maxmum at 208 nm in the CD spectrum. After incubation with POMs at the range of concentrations $1 \times 10^{-7} - 1 \times 10^{-6}$ for

15 min, the peak of AChE in fluorescence and CD spectra showed no shift, which indicated that AChE retained its natural secondary structure.



CONCLUSION

Investigated POMs present potent inhibitors of AChE activity with IC_{50} values in the submicromolar range of concentrations, and their inhibitory potency is not related to changes in the secondary structure of this enzyme. The log P values satisfied necessary criteria for smooth pass through cell membrane and criteria for the drugs used in the treatment of the central nervous system disease. Based on these obtained results, the conclusion can be made that POMs could represent new generation of potential anti Alzheimer drugs.

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