PHYSICAL CHEMISTRY 2018

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

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September 24-28, 2018
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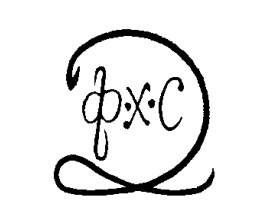

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OXIDATIVE STRESS RESPONSES OF 12-TUNGSTOSILICIC AND 12-TUNGSTOPHOSPHORIC ACID

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ABSTRACT

In vitro oxidative stress responses of two Keggin-type polyoxotungstates, 12-tungstosilicic (WSiA) and 12-tungstophosphoric acid (WPA), were investigated using rat synaptosomes as a model system. WSiA induced concentration-dependent increase in catalase activity, up to about 6 times compared to the control activity in untreated synaptosomes, and glutathione peroxidase was not significantly affected after WSiA treatment. On the contrary, WPA treatment resulted in the increase of glutathione peroxidase activity, while synaptosomal catalase was even reduced related to the control, at all investigated WPA concentrations. Both investigated polyoxotungstates did not significantly change malondialdehyde content in synaptosomal preparations. It could accordingly be concluded that WSiA and WPA probably induce reactive oxygen species generation, resulting in the activation of the antioxidant defense enzymes. However, these polyoxotungstates are not strong prooxidants being able to cause oxidative stress, and consequently synaptosomal membrane lipid damage.

INTRODUCTION

12-tungstosilicic acid, $H_4SiW_{12}O_{40}$ (WSiA) and 12-tungstophosphoric acid, $H_3PW_{12}O_{40}$ (WPA) are Keggin structure polyoxotungstates belonging to a family of polyoxometalates (POMs). These compounds are polyanionic oligomeric aggregates with a high density of negative charge, which contain transition metal ions held together by oxygen bridges [1]. They were synthesized in order to be applied in catalysis, separations, analysis, and as electrondense imaging agents [2]. Additionally, *in vitro* and *in vivo* studies indicate that some of these complexes exhibit biological activity [3].

The results of our previous *in vitro* study [4] demonstrated that WSiA and WPA affect the activities of Na^+/K^+-ATPase and E-NTPDase in micromolar concentrations, key enzymes in cancer cell migration and purinergic signaling

[5]. However, the main limitation for polyoxometalate application in biomedicine is their approved toxic action [6]. Taking into account this fact, the purpose of this study is to test oxidative stress responses of various doses of WPA and WSiA by determining antioxidant enzyme activities, catalase (CAT) and glutathione peroxidase (GPx), and lipid peroxidation in rat brain synaptosomes.

EXPERIMENTAL

WSiA and WPA are commercially available (Sigma-Aldrich, Germany). Synaptosomes were isolated from the whole brains of *Wistar albino* rats [7]. Aliquots of synaptosomal preparations were incubated at 37 °C for 2 h in water bath in the absence (control) and presence of desired concentrations of WSiA and WPA, and then used for biochemical analysis.

Catalase activity was measured by the H_2O_2 degradation assay [8]. GPx activity was measured in a coupled enzyme method by measuring the decrease of NADPH at 340 nm [9]. The level of lipid peroxidation was estimated as the concentration of thiobarbituric acid reactive product MDA by using the method of Aruoma et al. [10].

RESULTS AND DISCUSSION

The influence of exposure toward increasing concentrations (within the range 10^{-6}–10^{-3} mol/L) of WSiA and WPA on the specific activity of synaptosomal antioxidant enzymes, CAT and GPx is presented in Figure 1(a and b). The obtained results show that WSiA significantly increased synaptosomal CAT in a dose-dependent manner. The lowest investigated WSiA concentration (1×10^{-6} mol/L) induced about 4 times higher specific CAT activity compared to the control value (specific CAT activity in the untreated synaptosomes), while the highest WSiA concentration resulted in more than 6 times increase. On the contrary, WPA caused a noticeable decrease in CAT activity at all investigated concentrations (Fig. 1a), indicating the enzyme inhibition induced by WPA. Unlike CAT, synaptosomal GPx activity was not significantly affected by WSiA compared to the control (Fig. 1b). However, WPA induced significantly increase in GPx activity. It can be observed (Fig. 1b) that the lowest investigated WPA concentration (1×10^{-6} mol/L) caused the maximal increase (about 2 times in comparison with the control value). On the other hand, the GPx activity obtained in the presence of 1×10^{-3} mol/L WPA was almost equal to the control value, suggesting the potential inhibition of GPx at high WPA concentrations. The obtained increase in CAT and GPx activities (Fig. 1) could be assigned to the production of reactive oxygen species (ROS) in the presence of WSiA and WPA, respectively.

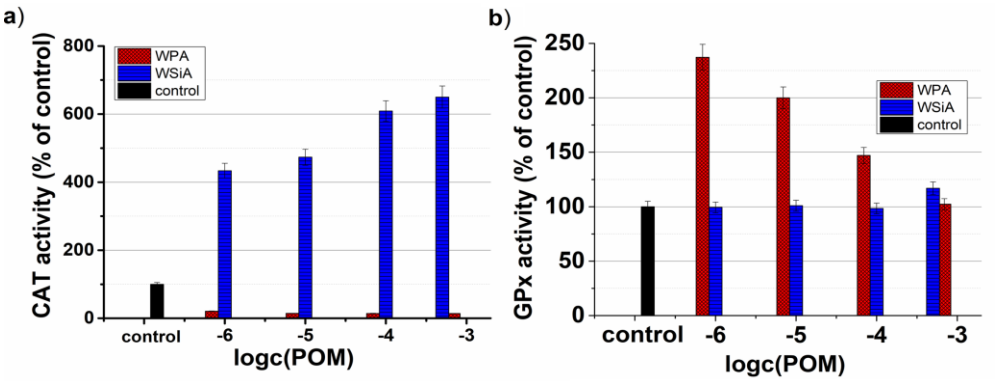

Figure 1. CAT (a) and GPx (b) activities in rat brain synaptosomes obtained in the absence (control) and presence of different WPA and WSiA concentrations. The specific enzyme activities are expressed as a percentage of the control value (100%). Values are mean ± SD.

The effect of synaptosomal exposure toward WPA and WSiA (within the range 10^{-6}–10^{-3} mol/L) on the level of MDA, as a marker of synaptosomal lipid peroxidation, is shown in Fig. 2. It can be seen that all investigated concentrations of both WPA and WSiA do not induce significant changes in MDA level related to the control value (untreated synaptosomes). The similar values of synaptosomal MDA obtained in both absence and presence of the investigated compounds indicate that these polyoxotungstates do not induce lipid peroxidation. Actually, the induced WSiA/WPA increase in CAT and GPx activities (Fig. 1) suggests that the activation of these antioxidant enzymes prevents oxidative stress, and consequently synaptosomal membrane damage.

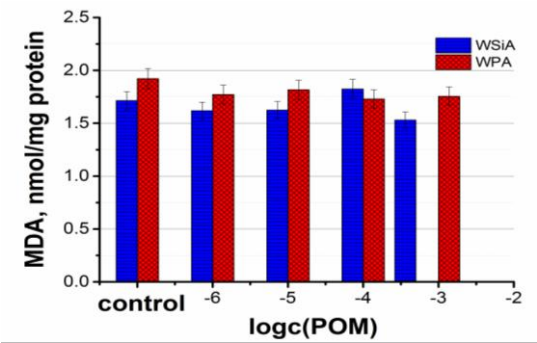

Figure 2. MDA contents in rat brain synaptosomes obtained in the absence (control) and presence of different WPA and WSiA concentrations. Values are mean ± SD.

CONCLUSION

The studied polyoxotungstates in this study, WSiA and WPA, induce the activation of the antioxidant enzymes, CAT and GPx respectively, but do not significantly affect MDA level in rat synaptosomes, the marker of oxidative stress and membrane lipid peroxidation. Accordingly, it could be concluded that both WSiA and WPA cannot be considered as strong prooxidants capable to induce oxidative stress and membrane damage.

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