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INFLUENCE OF HETEROPOLY ACIDS ON RAT SYNAPTIC PLASMA MEMBRANE ATP -ASE ACTIVITY

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Abstract

The *in vitro* influence of 12-tungstosilicic acid (WSiA) and 12-tungstophosporic acid (WPA) on Na^+/K^+–ATPase activity, using rat synaptic plasma membrane (SPM) as a model system was investigated. The half-maximum inhibition (IC_{50}) of the enzyme activity was achieved with $5.80 \cdot 10^{-5}$mol/L of WPA and $1.17 \cdot 10^{-4}$ mol/L of WSiA. The both examined compounds showed a dose-dependent inhibitory effect on the enzyme activity in the concentration higher than 1 μmol/L.

Introduction

Polyoxometalates (POMs) are polyanionic, condensed oligomeric aggregates of transition-metal ions, usually in their d^o electronic configurations, and oxide ions, held together only by metal-oxygen bonds. POMs made up of a great number of structures and compositions, constitute a large category of compounds interesting for theoretical investigations and practical applications. Heteropoly oxometalates which possess the Keggin-type anion, such as WPA and WSiA, attract the greatest interest. In medicinal chemistry, polyoxometalates exhibit biological activity, such as highly selective inhibition of enzymes, *in vitro* and *in vivo* antitumor, antiviral, and antiretroviral activities [1,2]. Considering the key role of Na^+/K^+- ATPase in normal functioning of most animal cells as well as pivotal roles in cancer cell migration, the aim of this work was to examine the influence of heteropoly acids $H_3PW_{12}O_{40}$ (WPA) and $H_4SiW_{12}O_{40}$ (WSiA) on Na^+/K^+- ATPase activity.

Material and methods

WPA was prepared by a previously described method [3] and confirmed by infrared spectroscopy, while WSiA was commercially available (Fluka). Both acids were recrystallized prior to use. The enzymatic activity of commercial porcine cerebral Na^+/K^+- ATPase was followed in the absence and presence of increasing concentration of WPA and SiWA (within the range of 10^{-8} - 10^{-3} mol/L). The SPM were isolated from the whole rat (albino, vistar) brain according to the standard method. The standard assay medium for investigation of SPM Na^+/K^+-ATPase activity contained: 50 mM Tris–HCl buffer, pH 7.4; 100 mM NaCl; 20 mM KCl; 5 mM $MgCl_2$; 2 mM ATP; and 25 μg SPM protein. Assay mixtures were preincubated for 10 min at 37°C in the presence of investigated compounds or distilled water (control). Reaction was

initiated by addition of ATP and stopped after 10 min by addition of 22 μL ice cold $HClO_4$ (3 M) and immediate cooling on ice. The released Pi (inorganic orthophosphate) released from the enzymatic hydrolysis of ATP was determined by spectrophotometric method. The spectrofotometric measurements were performed on a Perkin Elmer Lambda 35UV VIS spectrophotometer.

Results and discussion

The influence of WPA and WSiA on SPM ATPases activity was investigated by *in vitro* exposure to enzymes in the concentration range from $1 \cdot 10^{-8}$ to $1 \cdot 10^{-3}$ mol/L. The results show that increasing concentrations of WPA and WSiA induced inhibition of enzymatic activity in a concentration-dependent manner in both cases (Fig.1). The half-maximum inhibitory concentrations (IC_{50}) of the investigated compounds for Na^+/K^+-ATPases were determined by sigmoid fitting of the experimental results as well as by Hill analysis and are summarized in Table 1. The half-maximum inhibition (IC_{50}) of the enzyme activity was achieved at $5.80 \cdot 10^{-5}$ mol/L for WPA acid and $1.17 \cdot 10^{-4}$ mol/L for WSiA. Complete inhibition of the enzyme was achieved at the concentration of $5 \cdot 10^{-4}$ mol/L WPA, while the same effect was achieved at a two times higher concentration of SiWA.

The obtained dose-dependent inhibition of sodium pump by heteropoly acids is in agreement with previously reported findings that decameric vanadate species block the active side of P-type ATPases and consequently prevent formation of the phosphoenzyme intermediary [4,5].

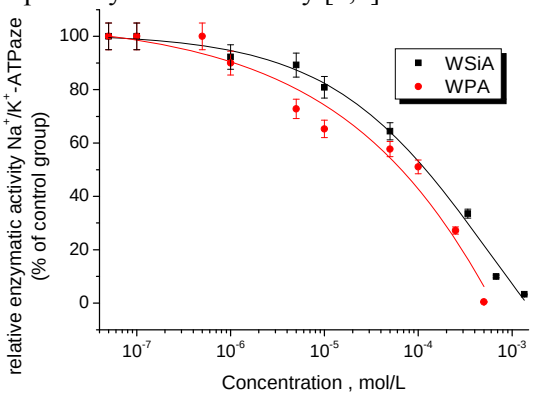

Fig. 1. Na^+/K^+-ATPase specific activity in dependence of the concentration of the WPA and SiWA.

Table 1. IC_{50} values of WPA and WSiA for ATPase obtained by fit of sigmoidal inhibition curves and by Hill analysis.

Compound	IC_{50}, mol/L	
	Sigmoidal fit	Hill analysis
$H_4SiW_{12}O_{40} \cdot 6H_2O$	$(1.15 \pm 0.05) \times 10^{-4}$	$1.17 \cdot 10^{-4}$
$H_3PW_{12}O_{40} \cdot 6H_2O$	$(6.57 \pm 0.80) \times 10^{-5}$	$5.80 \cdot 10^{-5}$

Conclusion

It could be concluded that WPA and SiWA induce inhibition of SPM ATPases activity in a concentration-dependent manner, probably directly affecting phosphorylation step in the enzyme cycle of P-type ATPases.

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