PHYSICAL CHEMISTRY 2008

Proceedings

of the 9th International Conference on Fundamental and Applied Aspects of Physical Chemistry

Volume I

The Conference is dedicated to the 200th Anniversary of the University in Belgrade

UNIVERSITY OF BELGRADE
1808 • 2008

September 24-26, 2008,
Belgrade, Serbia

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ISBN	978-86-82475-16-3
Title:	Physical Chemistry 2008. (Proceedings)
Editor:	Prof. dr A. Antić-Jovanović
Published by:	The Society of Physical Chemists of Serbia, Studentski trg 12-16, P.O.Box 47, 11158 Belgrade, 218, Serbia
Publisher:	Society of Physical Chemists of Serbia
For publisher:	Prof. dr S. Anić, president of the Society of Physical Chemists of Serbia
Printed by:	"Jovan" Printing and Published Comp; 250 Copies; Number of Pages: x + 468; Format B5; Printing finished in September 2008.
Text and Layout:	Aleksandar Nikolić

250 – copy printing

The Conference is organized by
the Society of Physical Chemists of Serbia

in cooperation with
Institute of Catalysis, Bulgarian Academy of Sciences

Boreskov Institute of Catalysis,
Siberian Branch of the Russian Academy of Sciences

Faculty of Physical Chemistry, University of Belgrade

Institute of Chemistry, Technology and Metallurgy, Belgrade

Institute of General and Physical Chemistry, Belgrade

INFLUENCE OF DECAVANADATE ON RAT SYNAPTIC PLASMA MEMBRANE ATPASES ACTIVITY

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Abstract

The *in vitro* influence of decameric vanadate species on Na^+/K^+–ATPase, plasma membrane Ca^{2+}-ATPase (PMCA)-calcium pump and ecto-ATPase activity, using rat synaptic plasma membrane (SPM) as a model system was investigated. The concentration-dependent responses to decavanadate of these enzymes were obtained. The half-maximum inhibition (IC_{50}) of the enzyme activity was achieved at $(4.74 \pm 1.15) \times 10^{-7}$mol/l for Na^+/K^+–ATPase and $(3.13 \pm 1.70) \times 10^{-8}$ mol/l for Ca^{2+}-ATPase, while ecto-ATPase is significantly less sensitive toward decavanadate (IC_{50}- $(1.05 \pm 0.10) \times 10^{-4}$ mol/l) than investigated P-type ATPases.

Introduction

Interest in the interaction of vanadate oxoanions with biological systems has inceased since it has been demonstrated to have a variety of physiological effects acting either as a phosphate analogue in the monomeric form ($H_2VO_4^-$) [1] or through oligomeric vanadate species which interact with biomolecules with various and versatile activity (enzymes inhibitor or activator). Several studies report that decavanadate has a stronger effect on various enzymes, when compared to other vanadate oligomers and include the possibility of its use as a tool in the in the understanding of molecular mechanism of muscle contraction as well as inhibition of several ATPases such as P-type ATPases [2,3].

The aim of this work was the investigation of the *in vitro* effect of ammonium decavanadate, $(NH_4)_6V_{10}O_{28}.5H_2O$ on Na^+/K^+–ATPase (sodium pump), plasma membrane Ca^{2+}-ATPase (PMCA)-calcium pump and ecto-ATPase (Mg^{2+}-ATPase) activity, using rat synaptic plasma membrane (SPM) as a model system.

Material and Methods

Ammonium decavanadate was prepared by dissolving ammonium trioxovanadate(V) NH_4VO_3 (0.4 g, 3.42 mmol) in distilled water (20 ml) as described in [1] to obtain decavanadate anions. The solution was stirred and heated until complete dissolution of NH_4VO_3 (about 2h). The pH was adjusted to 5.90 by

dropwise addition of NH_4OH. Orange crystals were formed within four days from the solution and kept at room temperature.

The SPM were isolated from the whole rat (albino, Vistar) brain according to the standard method [4]. The standard assay medium for investigation of SPM Na^+/K^+-ATPase activity contained (in mM) 50 Tris–HCl, pH 7.4; 100 NaCl; 20 KCl; 5 $MgCl_2$; 2 ATP; 25 μg SPM proteins, while the rat synaptic PMCA activity was assayed in standard medium containing (in mM): Tris-HCl (pH 7.4), 0.5 EGTA, 0.5 $CaCl_2$, 5 $MgCl_2$, 2 ATP and 25 μg SPM proteins in a final volume of 200 μl. Incubation mixtures were preincubated for 10 min at 37°C in the presence of investigated compound or distilled water (control). The inorganic orthophosphate (P_i) liberated from the hidrolysis of ATP, was measured using spectrophotometry at 690 nm. The activity obtained in the presence of Mg^{2+} alone was attributed to ecto-ATPase (Mg^{2+}-ATPase) activity. SPM Na^+/K^+ -ATPase as well as SPM Ca^{2+}-ATPase activities were calculated by subtracting the Mg^{2+}-ATPase activity from the total ATPase activity in the presence of Na^+, K^+ and Mg^{2+} ions (i.e. in the presence of Ca^{2+} and Mg^{2+} ions).

Results and Discussion

The influence of decavanadate on SPM ATPases activity was investigated by *in vitro* exposure to enzymes in the concentration range from $1x10^{-10}$ to $1x10^{-1}$ mol/L. The results show, that increasing concentrations of decavanadate induced inhibition of enzymatic activity in a concentration-dependent manner in all cases (Fig. 1a). The dependence of enzyme activity, expressed as a percentage of the control value (obtained without inhibitor) on inhibitor concentration fit a sigmoidal function for all enzymes. The half-maximum inhibitory concentrations (IC_{50}) of the investigated compound for all ATPases were determined by sigmoidal fitting of the experimental results as well as by Hill analysis (Fig.1b) and are summarized in Table 1. It is obvious that plasma membrane calcium and sodium pumps are more sensitive toward decavanadate anion than ecto – ATPase. At the concentration of $1x10^{-5}$ mol/l decavanadate inhibited Na^+/K^+–ATPase as well as Ca^{2+}-ATPase up to 80%, while the effect of the same concentration of decavanadate on the ecto-ATPase activity was negligible. The half-maximum inhibition (IC_{50}) of the enzyme activity was achieved at $(4.74 \pm 1.15) \times 10^{-7}$ mol/l for Na^+/K^+–ATPase and $(3.13 \pm 1.70) \times 10^{-8}$ mol/l for Ca^{2+}-ATPase, while the same effect for ecto-ATPase was observed at a few (several) orders of magnitude higher concentration of decavanadate - $(1.05 \pm 0.10) \times 10^{-4}$ mol/l. The obtained dose-dependent inhibition of PMCA and sodium pump by decavanadate 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 [5]. However, this mechanism could not be responsible for obtained ecto-ATPase inhibition (a member of E-NTPDases) and probably occurs via different mechanism resulting in lower sensitivity toward decavanadate, when compared to P-type ATPases (sodium and calcium pump).

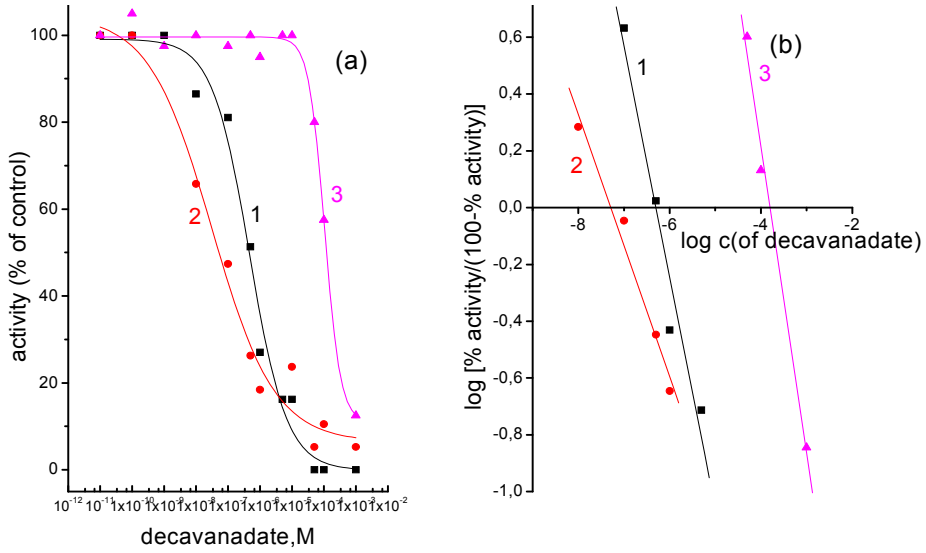

Fig. 1. The concentration dependent (a) and Hill analysis (b) inhibition of Na^+/K^+–ATPase (1), Ca^{2+}-ATPase (2) and Mg^{2+}-ATPase (3) by ammonium decavanadate

Table 1. IC_{50} values of ammonium decavanadate for all ATPases obtained by fit of sigmoidal inhibition curves and by Hill analysis

Enzyme	IC_{50}, M Hill	IC_{50}, M
Na^+/K^+-ATPase	4.79×10^{-7}	$(4.74 \pm 1.15) \times 10^{-7}$
Ca^{2+}-ATPase	4.68×10^{-8}	$(3.13 \pm 1.70) \times 10^{-8}$
Mg^{2+}-ATPase	1.58×10^{-4}	$(1.05 \pm 0.10) \times 10^{-4}$

Conclusion

It could be concluded that the decameric vanadate species induce inhibition of SPM ATPases activity in concentration-dependent manner, probably directly affecting phosphorylation step in the enzyme cycle of P-type ATPases (sodium and calcium pump).

References

[1] N.D. Chasteen, Struct. Bonding, 1983, **53**, 105-138.
[2] M. Aureliano and R.M.C. Gândara, J. Inorg. Biochem., 2005, **99 (5)**, 979-985.
[3] M. Aureliano, J. Inorg. Biochem., 2000, 80, 145-147.
[4] R.S. Kohen, F. Blomberg, K. Berzins, P. Siekevits, J. Cell Biol., 1977, **74**, 181-203.
[5] S. Varga, P. Csermely, A. Martonosi, Eur. J. Biochem., 1985, **148**, 119-126.