PHYSICAL CHEMISTRY 2004

Proceedings

of the 7th International Conference on Fundamental and Applied Aspects of Physical Chemistry

Volume I and II

September 21-23, 2004
Belgrade, Serbia and Montenegro

PHYSICAL CHEMISTRY 2004

Proceedings

of the 7th International Conference on Fundamental and Applied Aspects of Physical Chemistry

Volume I and II

Editors
A. Antić-Jovanović and S. Anić

ISBN	86-82457-12-x
Title:	Physical Chemistry 2004. (Proceedings)
Editors	A. Antić-Jovanović and S. Anić
Published by:	The Society of Physical Chemists of Serbia, Studentski trg 12-16, P.O.Box 137, 11001 Belgrade, Serbia and Montenegro
Publisher:	Society of Physical Chemists of Serbia
Printed by:	"Jovan" Printing and Published Comp; 300 Copies; Number of Pages: x + 906; Format B5; Printing finished in September 2004.
Text and Layout:	Aleksandar Nikolić

300 – copy printing

EFFECTS OF METAL IONS ON ECTO-ATPase ACTIVITY IN PLASMA MEMBRANE ISOLATED FROM THE RAT OVARY

M.Milošević, S.Petrović and A.Horvat

Laboratory of Molecular Biology and Endocrinology, "Vinča" Institute of Nuclear Sciences, P.O. Box 522, 11001 Belgrade, Serbia and Montenegro

Abstract

Effects of increasing concentrations of metal salts $SrCl_2$, CsCl, $CrCl_3$, $CdCl_2$, and $HgCl_2$ on rat ovarian plasma membrane ecto-ATPase activity were investigated. $CrCl_3$, $CdCl_2$, and $HgCl_2$ exert total inhibition of enzyme activity in the presence of 0.01 M and 0.1 M respectively. $SrCl_2$ and CsCl exhibit up to 25% of inhibition. According to the IC50, ecto-ATPase possesses greater sensibility to Cd^{2+} (IC_{50} is 0.887 mM) > Cr^{3+} (IC50 is 1.936 mM) > Hg^{2+} (IC50 is 4.39 mM). All investigated ions exert negative cooperativity (n<1). Physico-chemical properties of the metal are of importance in metal toxicity. Cr^{3+} and Cd^{2+} ions, with lower radius may inhibit ecto-ATPase activity by binding to hydrolytic site or by replacing Mg^{2+} in Mg-ATP, a substrate of the enzyme. Hg^{2+}, as a larger ion probably inhibits the enzyme activity through conformational changing the enzyme by binding to S-S or -SH groups on the site distinct to hydrolytic one. By inhibiting the enzyme activity these metals may affect maturation and release of oocytes as well as synthesis and release of gonadal hormones and decrease the fertility of mammals.

Introduction

Metals are widely dispersed throughout the environment. Environmental pollution by metals increase the exposure of organisms, which results in their accumulation in various tissues, including the ovary [1]. Metals have a number of toxic mechanisms including interference with enzyme function either by binding competitively with binding sites or by modifying metal-binding proteins. A plenitude of evidence indicates that heavy metals such as lead, mercury, cadmium, arsenic, chromium, nickel and several others, are developmental and reproductive toxicants acting on DNA transcription and production of reactive oxygen species [1]. The ecto-adenosine triphosphatase (ecto-ATPase, EC 3.6.1.3) is a membrane-bound enzyme, which in the presence of divalent cations (Ca^{2+} or Mg^{2+}) plays a role in the extra cellular metabolism of ATP. By controlling the concentration of the extra cellular ATP, it influences a large variety of P2 receptor-mediated processes [2]. ATP may influence maturation of ovarian cells and synthesis of gonadal hormones. The specific inhibitor(s) of ecto-ATPase has not been found up to now. In this work, we investigated possible metal toxicity on the reproductive system of mammals. With this aim we examined the effects of $CrCl_3$, $SrCl_2$, CsCl, $CdCl_2$, and $HgCl_2$, on plasma membrane ecto-ATPase activity from the rat ovary, as a model system.

Experimental Procedure

Experiments were performed on 3-month-old female Wistar albino rats obtained from a local colony. Ovarian plasma membranes (OPM) were isolated as described previously [3]. The activity of ecto-ATPase was determined by the spectrophotometric method by measuring the inorganic phosphate liberated from hydrolysis of ATP. OPM (70μg) were preincubated at 37^0C without or in the presence of increasing concentrations of $CrCl_3$, $SrCl_2$, CsCl, $CdCl_2$, and $HgCl_2$, for 20 min in an enzyme assay medium containing (in mM) 50 Tris-HCl, pH 7.4; 1 EDTA; 5 $MgCl_2$. After incubation, the enzyme reaction was started by the addition of 2 mM ATP, allowed to proceed for 15 min and stopped by the addition of 3 mol/l perchloric acid. All measurements were performed in triplicate. The results are expressed as the mean percentage of enzyme activity compared to the corresponding control.

Results and Discussion

Chloride salts of the investigated metals were added to the reaction mixture in the concentration range from $1x10^{-7}$ to 0.1 M. The effects of increasing concentrations of metal salts $CrCl_3$, $CdCl_2$, and $HgCl_2$ on OPM ecto-ATPase activity shows total inhibition relative to the control samples while, $SrCl_2$ and CsCl exhibits up to 25% of inhibition. Concentrations of metals for 50% of enzyme activity inhibition (IC50) were calculated from the Hill analysis of the experimental results.

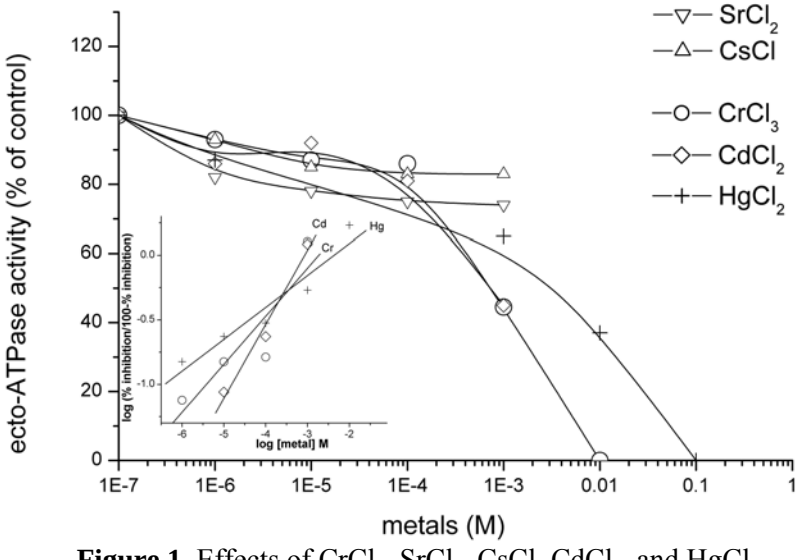

Figure 1. Effects of $CrCl_3$, $SrCl_2$, CsCl, $CdCl_2$, and $HgCl_2$ on ecto-ATPase activity. Hill graph presented as inset.

Cr^{3+}, Cd^{2+} and Hg^{2+} ions exert total inhibition of control enzyme activity (0.130 μmol Pi/mg/min) in the presence of 0.01 M and 0.1 M respectively (Fig. 1.). According to the IC50, ecto-ATPase possesses greater sensibility to Cd^{2+} (IC50 is 0.887 mM) followed by Cr^{3+} (IC50 is 1.936 mM), and Hg^{2+} (IC50 is 4.39 mM). According to the Hill coefficient, n, all investigated ions exert negative cooperativity (n<1). When dealing with defining the metal toxicity, particular attention should be focused on metal chemical specificity in the physico-chemical properties of the metal coordination sphere. The absence of ecto-ATPase inhibition by Cs and Sr may be explained by large ionic radius, compared to Mg^{2+}, so they probably cannot substitute Mg^{2+} in the Mg-ATP complex. Also they lack the ability to form complexes with –SH, -NH_2, -OH or other groups in protein. Transition metals may modify the secondary structure of the protein, compete with free Mg^{2+} or bind to functional catalytic groups, such as SH-groups and exert the inhibition of enzyme activity. Due to similarity in ionic radius, Cd^{2+} and Cr^{3+} can probably substitute Mg^{2+} ions in Mg-ATP, which is the substrate for ecto-ATPase, or binding to the hydrolytic site. Hg^{2+}, possessing high radius may form complexes with –SH or –S-S- residues, out of active center of enzyme, affecting protein conformation and inhibition of hydrolytic activity.

Conclusion

When dealing with metal ion toxicology, particular attention should be focused on metal chemical speciation in that the physico-chemical properties of the metal coordination sphere are of importance in defining the metal toxicity. Metals, Cd^{2+} and Cr^{3+}, with lower a radius may inhibit ecto-ATPase activity by binding to hydrolytic site or replacing Mg^{2+} in Mg-ATP, a substrate of the enzyme. Hg^{2+}, as the larger ion probably inhibits enzyme activity by conformational changing the enzyme protein by binding to S-S or -SH groups on the site distinct to the hydrolytic one. By inhibiting the enzyme activity, these metals may affect maturation and release of oocytes as well as synthesis and release of gonadal hormones and consequently decrease the fertility of mammals. Further investigations will be undertaken with the aim to define inhibition mechanisms of the studied metals.

Acknowledgements

This study was supported by the Ministry of Science and Environmental Protection of the Republic of Serbia, Grant No. 1956

References

[1] P. Kovacic, J.D. Jacintho, Curr. Med. Chem., 2001, 8, 863.
[2] H. Zimmermann, N. Braun, B. Kegel, P. Heine, Neurochem. Intern., 1998, 32, 421.
[3] A. Horvat, G. Nikezić, J. Martinović, Experientia 1995, 51, 11.