PHYSICAL CHEMISTRY 2006

Proceedings

of the 8th International Conference on Fundamental and Applied Aspects of Physical Chemistry

September 26-29,
Belgrade, Serbia

ISBN	86-82139-26-X
Title:	Physical Chemistry 2006. (Proceedings)
Editors	Prof. dr A. Antić-Jovanović
Published by:	The Society of Physical Chemists of Serbia, Studentski trg 12-16, P.O.Box 137, 11001 Belgrade, 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 + 442; Format B5; Printing finished in September 2006.
Text and Layout:	Aleksandar Nikolić

250 – copy printing

EFFECT OF EDTA ON THE INHIBITION OF RAT MYOMETRIAL ECTO-ATPASE ACTIVITY IN THE PRESENCE OF HEAVY METAL IONS I. MERCURY

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Abstract

The effects of increasing concentrations of $HgCl_2$ on rat uterine plasma membrane ecto-ATPase activity, in presence and absence of ethylenediamine tetra acetic acid (EDTA) were studied. The aim was to examine the ability of EDTA to prevent mercury induced inhibition of ecto-ATPase activity. Our results show that addition of 1mmol/l EDTA to the reaction mixture potentiates Hg^{2+} induced inhibition of enzyme activity. We may concluded that formation of the HgEDTA complex increased capacity of Hg^{2+} to inhibit enzyme activity.

Introduction

Ecto-adenosine triphosphatase (ecto-ATPase) is an integral plasma membrane glycoprotein, with transmembrane domain at both the N- and C-terminus, and one large extracellular domain containing the active site. As a member of E-NTPDases (EC 3.6.1.5); a family of ectoenzymes, it hydrolyzes terminal phosphoanhydride bonds of extracellular nucleoside tri- and diphosphates in the presence of divalent cations (Ca^{2+} or Mg^{2+}). ATP and its products of hydrolysis are involved in signaling processes through interaction with extracelullar receptors. These purinergic receptors control many physiological processes, including neurotransmission, blood clotting, pain perception and smooth muscle contraction. Also, the existence of purinoceptors in the rat female reproductive tract was confirmed [1]. High ecto-ATPase activity in myometrium tissue indicates its role in cell mechanisms of controlling extracellular concentrations of nucleotides.

Plenty of evidence indicates that Hg^{2+} is reproductive tissue toxicant [2]. The aim of this work was to investigate *in vitro* the ability of EDTA to prevent Hg^{2+} induced inhibition of ecto-ATPase activity.

Experimental

Experiments were performed on 3-months-old female Wistar albino rats obtained from the local colony. Myometrial plasma membranes (MPM) were isolated as described previously [3]. Incubation medium (200μl) contained 50mmol/l Tris-HCl (pH 7.4), 1mmol/l $MgCl_2$, 7μg MPM protein and increasing concentration of $HgCl_2$ in absence or presence of 1mmol/l EDTA. Incubation with $HgCl_2$ lasted 30 min at 37^0 C and the enzyme reaction was started by the addition of 1mol/l ATP and allowed to proceed for

additional 10 min. The enzyme reaction was stopped by the addition 22µl of ice cold 3mol/l perchloric acid. The inorganic phosphate (Pi) liberated from the hydrolysis of ATP was determined by the spectrophotometric method.

Results and Discussion

Mercury salt ($HgCl_2$) was added to the reaction mixture in concentration ranges from $1x10^{-7}$ to 0.1 mol/l. The effects of increasing concentrations of Hg^{2+} were measured in the absence and in the presence of 1mM EDTA. In both cases, ecto-ATPase activity was not affected in the presence of $1x10^{-4}$ mol/l of added $HgCl_2$ and lower. Almost total inhibition was achieved in the presence of 0.1 mol/l of $HgCl_2$ and the presence of EDTA potentiates the inhibitory effect of Hg^{2+} ions (Fig 1.). The half-maximum inhibitory activities (IC_{50}) determined by Hill analysis of experimental curves were $1.06x10^{-3}$ in the presence and $3.38x10^{-3}$ mol/l in the absence of EDTA.

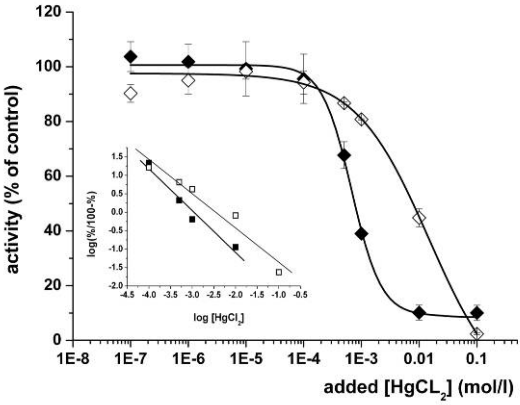

Fig. 1. Inhibition of ecto-ATPase activity by $HgCl_2$ in the absence (open symbol) and in the presence (solid symbols) of 1×10^{-3} mol/l EDTA. Inset: Hill plot of experimental data. The values given are the mean of at least three experiments ±S.E.M.

The concentrations of ionic species were calculated, taking into account equilibrium reactions involving Mg^{2+}, ATP, EDTA and Hg^{2+}. The stability constants were found in the literature [4, 5] and calculated according to Sorer and Cornish-Bowden [6]. In absence of EDTA, since Hg^{2+} formed no complex with ATP, the experimental (added) and free concentrations of Hg^{2+} were the same. Metals have a number of toxic mechanisms including interaction with -SH, $-NH_2$, -COOH, -OH groups in protein, the conformation changing or competition with free Mg^{2+} in substrate $MgATP^{2-}$ complex. Hg^{2+} binds well to -SH groups and this beeing most expected mechanism of its toxicity. E-NTPDases contain 10 Cys residues in their extracellular domain but have no free sulpfhidryl groups. These data may explain ecto-ATPase resistance to inhibition by Hg^{2+} ions and a relatively high IC_{50} value in the absence of EDTA.

Since EDTA binds Hg^{2+} with high affinity, almost all Hg^{2+} are trapped in the chelate. These results indicate that the HgEDTA complex (Fig.2.), not free Hg^{2+} ions (10^{-24}-10^{-15} mol/l; data not shown), is responsible for inhibition. The toxicity of HgEDTA and HgEGTA was described previously [7]. Duhr at al. reported that complex HgEDTA, but not Hg^{2+}, induced inhibition of microtubule polymerization by inhibi-

tion of the brain β-tubulin activity by disrupting the interaction of the substrate, GTP (nucleotide triphosphate) with the enzyme-binding site. We propose similar mechanism in our experimental model. Binding of HgEDTA to or near to ATP (nucleotide triphosphate) hydrolysing site on the ecto-ATPase disable binding of substrate, ATP, and thereby inhibits the ecto-ATPase activity.

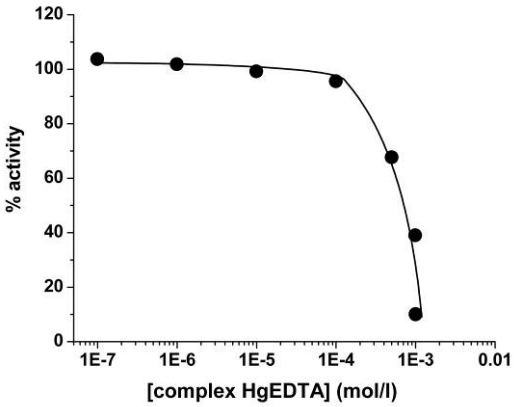

Figure 2. Inhibition of ecto-ATPase activity by HgEDTA complex in reaction mixture (calculated values). The values given are the mean of at least three experiments.

Conclusion

In the reaction medium containing Hg^{2+}, addition of the EDTA formed the HgEDTA complex which increases the capacity of Hg^{2+} to inhibit ecto-ATPase activity. According to our results and literature data, we may propose the possible mechanism of HgEDTA induced inhibition of ecto-ATPase activity: (i) HgEDTA prevents interaction of substrate $MgATP^{2-}$ with active site of the enzyme and/or (ii) displaces bound $MgATP^{2-}$ from the enzyme substrate binding site.

Acknowledgements

This study was supported by the Serbian Ministry of Sciences and Environmental Protection, Project No. 143044.

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