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Editors
A. Antić-Jovanović and S. Anić

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TIME DEPENDED INHIBITION OF SPMs Na^+/K^+-ATPase INDUCED BY CADMIUM AND LEAD

D. Kojić and D. Stojić

Department of Physical Chemisty Institute of Nuclear Sciences "Vinča", 11001 Belgrade, Serbia and Montenegro

Abstract

In this work, time depended interaction of SPMs Na^+/K^+-ATPase with Cd^{2+} and Pb^{2+} by single exposure and in the mixture, was investigated. The possibility to selectively detect these ions on the basis on the rate of their interaction with the protein was studied. The decreasing protein concentration increased the sensibility of Na^+/K^+-ATPase towards both metals. The selectivity was obtained by variation of metal-enzyme contact time.

Introduction

The enzyme Na^+/K^+-ATPase is integral part of plasma membranes of all higher organisms, especially in synaptic plasma membranes (SPM), and transfer chemical energy of hydrolysis of ATP to potential energy of electrochemical ion gradients [1]. Because heavy metals and many organic compounds inhibit the activity of this enzyme in concentration dependent manner [2], development of respective biosensing system, using this enzymatic system, for selective detection of toxic agents becomes interesting. The measure of ATPase activity is temporal change of the concentration of inorganic ortho-phosphate (P_i) and is usually determined by spectrophotometric methods [3]. Development of test method for inhibitors detection based on the colour reaction for orthophosphate determination seems reasonable. The aim of this work was to investigate the possibility for selective recognition of inhibitors of Na^+/K^+-ATPase activity by Cd^{2+} and Pb^{2+} based on influence of time on the inhibition of the enzymatic activity.

Experimental

The SPM were isolated from the whole brain of 3-month-old male Wistar albino rats as described previously [4]. Total ATPase activity was determined in a standard incubation medium [2] containing 125μg/ml protein concentration, and final volume of 2 ml containing 12.5μg/ml protein. Incubation mixtures were incubated before enzymatic reaction for different incubation time and metal-enzyme contact time, at 37°C. The enzymatic reaction started with addition of ATP. The concentration of inorganic orthophosphate was determined using the modified spectrophpotometric procedure based on the modified stannous chloride method [3]. Na^+/K^+-ATPase activity was calculated as a difference between the total ATPase and Mg^{2+} - ATPase activities, obtained without NaCl and KCl.

Results and Discussion

The activity of Na^+/K^+-ATPase was investigated by varying the concentrations of $Pb(NO_3)_2$ and $Cd(NO_3)_2$ from $1x10^{-9}$ to $1x10^{-3}$ M in the reaction mixture containing 125 μg/ml or 12.5 μg/ml protein. The tubes were incubated for 10 minutes at 37°C before starting enzymatic reaction. In all cases concentration dependent sigmoidal inhibition curves were obtain. The IC_{50} values (metal ion concentration which produced 50% inhibition of the enzymatic activity) were calculated from experimental results. As the results indicate, in the presence of 125 μg/ml protein concentration, IC_{50} value was $(2.0\pm0.1)x10^{-5}$M for Cd^{2+} and $(1.0x\pm0.1)x10^{-4}$M for Pb^{2+}. By lowering protein concentration to 12.5μg/ml IC_{50} value was also ten fold lower, for Cd^{2+} was $(2.0\pm0.2)x10^{-6}$M and $(7.0x\pm0.3)x10^{-6}$M for Pb^{2+}. However, in the presence of 12.5 μg/ml protein the metal concentration which produced 50% of enzyme activity inhibition was lower than the maximal allowed concentration of this metal in water [3].

Inhibition of Na^+/K^+-ATPase activity in the presence of low protein concentration (12.5 μg/ml) was investigated in the presence of $1x10^{-4}$ M Pb^{2+}, $2x10^{-6}$ M Cd^{2+} and combination of these metals as a function of the metal-enzyme contact time. Results are present in Fig.1. The results show that the variation of the contact time (time before starting of enzyme reaction) between the enzyme and metal ions produced the various effects on the enzyme activity. As can be seen from Fig.1 (curve 1), Cd^{2+} induced about 35% inhibition during the first 5 minutes, by single exposure. The prolonged contact time did not influence the change of the enzyme inhibition. On contrary, in the first five minutes the change of the enzyme activity by single exposure to Pb^{2+} remained in the range of the experimental error (Fig.1. curve 2). Prolonged contact time caused that inhibition of activity, that continually decreased to 100% after 40 min. Various effects of contact time on the inhibition of Na^+/K^+-ATPase activity by Cd^{2+} and Pb^{2+} could be explained by the difference in the reaction rate of solvent exchange (water) in coordination sphere of the metal ion during the ligand-protein interaction. Namely, the rate of binding of Cd^{2+} ions to inhibitory sites of enzyme was faster compared to Pb^{2+} ions, and the results show that the equilibration was achieved in the first five minutes. On contrary, the reaction of Pb^{2+} ions with enzyme inhibitory sites was much slower, but this metal exerted more toxic effect, since it inhibited the enzyme activity completely after 40 min exposure. Simultaneous exposure of Na^+/K^+-ATPase to Pb^{2+}/Cd^{2+} showed that both salts induced an additive effect (Fig.1., curve 3). Experimental data (Fig.1. open triangl) fit with calculated data (fig.1. dash line). The results show that the both metals induced the inhibition of the enzyme activity that depended on the metal-enzyme contact time.

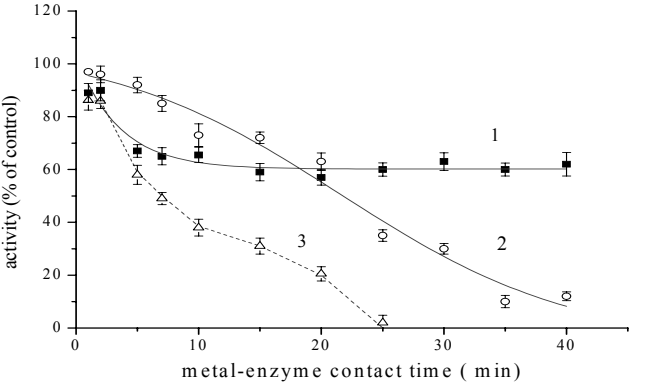

Fig.1 Effect of metal-enzyme contact time on Na^+/K^+-ATPase activity in presence of $2x10^{-6}$M Cd^{2+}(curve 1), $1x10^{-6}$M Pb^{2+} (curve 2) and simultaneous exposure $2x10^{-6}$M $Cd^{2+}/1x10^{-6}$M Pb^{2+} (curve 3-experimental data open triangle and calculated data dash line)

Conclusion

By decreasing protein concentration from 125 μg/ml to 12.5 μg/ml in standard medium mixture, IC_{50} values of Cd^{2+} and Pb^{2+} induced inhibition of Na^+/K^+-ATPase decreased ten fold. The variation of metal-enzyme contact time by simultaneous exposure enabled the selective recognition of inhibition induced by these ions. Keeping the short contact time between the mixture of these ions and protein, the inhibition is mainly due to Cd^{2+} ions. The increasing of the inhibition by prolonged contact time under the same experimental conditions can probably be induced by the presence of Pb^{2+} ions.

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