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Interaction of some Pd(II) complexes with Na^+/K^+-ATPase: Inhibition, kinetics, prevention and recovery

KATARINA S. KRINULOVIĆ & VESNA M. VASIĆ

Vinca Institute of Nuclear Sciences, Department of Physical Chemistry, P.O.Box 522, Belgrade 11001 Serbia & Montenegro

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Abstract

The aim of this work was to investigate the influence of $[PdCl_4]^{2-}$, $[PdCl(dien)]^+$ and $[PdCl(Me_4dien)]^+$ complexes on Na^+/K^+-ATPase activity. The dose-dependent inhibition curves were obtained in all cases. IC_{50} values determined by Hill analysis were 2.25×10^{-5} M, 1.21×10^{-4} M and 2.36×10^{-4} M, respectively. Na^+/K^+-ATPase exhibited typical Michelis-Menten kinetics in the presence of Pd(II) complexes. Kinetic parameters (V_{max}, K_m) derived using Eadie–Hofstee transformation indicated a noncompetitive type of Na^+/K^+-ATPase inhibition. The inhibitor constants (K_i) were determined from Dixon plots. The order of complex affinity for binding with Na^+/K^+-ATPase, deducted from K_i values, was $[PdCl_4]^{2-} > [PdCl(dien)]^+ > [PdCl(Me_4dien)]^+$. The results indicated that the potency of Pd(II) complexes to inhibit Na^+/K^+-ATPase activity depended strongly on ligands of the related compound. Furthermore, the ability of SH-donor ligands, L-cysteine and glutathione, to prevent and recover the Pd(II) complexes-induced inhibition of Na^+/K^+-ATPase was examined. The addition of 1 mM L-cysteine or glutathione to the reaction mixture before exposure to Pd(II) complexes prevented the inhibition by increasing the IC_{50} values by one order of magnitude. Moreover, the inhibited enzymatic activity was recovered by addition of SH-donor ligands in a concentration-dependent manner.

Keywords: *Na^+/K^+-ATPase, Pd(II) complexes, inhibition, kinetics, prevention and recovery*

Introduction

Na^+/K^+-ATPase (EC. 3.6.3.9) is an integral membrane protein found in the cells of all higher eukaryotes [1]. It maintains the active transport of monovalent cations (Na^+, K^+) across membrane utilizing energy liberated in the hydrolysis of ATP as driving force [2]. The activity of this enzyme is very sensitive to the action of various bioregulators, such as cardiac steroids, transition and heavy metals, as well as metal complexes [3–10]. Literature references indicate that inhibition of Na^+/K^+-ATPase by metal ions is realized through their binding to enzyme sulfhydril groups [6,7]. There is a good correlation between metal toxicity, expressed as an IC_{50} value, and stability constants of metal-sulfhydril ligand complexes [6].

The complexes of platinum group elements have received much attention over the last two decades, because of their potential antitumor activity and increasing application in chemotherapy [11,12]. Their antitumor activity is governed by chemical reactions with DNA, but the interactions of Pt(II) complexes with the –SH groups of peptides and proteins have often been ascribed to the resistance in antitumor therapy by Pt(II) based drugs [12–14]. Literature data indicate that nephrotoxicity of cisplatin and its derivatives is related to the inhibition of Na^+/K^+-ATPase, but the mechanism of enzyme-inhibitor interaction is still under the investigations [15,16]. For the study of the reaction mechanism of platinum(II) complexes their palladium analogs are often used as model compounds, since they exhibit about 10^4–10^5 fold higher reactivity, while their structural and equilibrium behavior is very similar [17]. Recently published results concentrated on the reactions of platinum ($PtCl_{4-n}L_n$) and palladium ($PdCl_{4-n}L_n$) complexes with sulphur—bonding ligands, such as L-cysteine and glutathione (GSH),

Correspondence: V. M. Vasić, Vinca Institute of Nuclear Sciences, Department of Physical Chemistry, P.O.Box 522, Belgrade 11001, Serbia & Montenegro. Tel: 381 11 2453 967. Fax: 381 11 2447 207. E-mail: evasic@vin.bg.ac.yu

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which could be of fundamental importance for understanding the toxicity of related platinum complexes [18–22]. It was shown that these complexes have a great affinity for substitution reactions of the Cl^- ligand by SH-donor ligand. Moreover, it was reported that GSH and L-cysteine are potent enzyme activity reactivators, since they recover the metal ions-induced inhibition of some enzymes [23–25]. The reactivation is usually due to the complex formation between thiols and the metal ion bonded to the –SH groups of the enzyme [26]. Furthermore, modification of cysteine residues in proteins due to its ability to strongly coordinate transition metal ions is one of the arguments of critical importance for the design of novel types of pharmacological agents [27,28].

Our study was undertaken with the aim to examine the effects of Pd(II) complexes on the enzymatic activity and kinetic properties of Na^+/K^+-ATPase. The extensive kinetic analyses were done in order to determine the kinetic parameters and type of Na^+/K^+-ATPase inhibition by Pd(II) complexes as model compounds for platinum anticancer drugs. The affinity of this enzyme for binding to Pd(II) complexes with structurally different ligands has been discussed. Furthermore, the ability of sulphur-donor ligands (L-cysteine and glutathione) to prevent the Pd(II) complexes-induced inhibition of Na^+/K^+-ATPase and to recover enzymatic activity was investigated.

Materials and methods

Chemicals and equipment

All chemicals were of analytical grade. Na^+/K^+-ATPase (specific activity 2.71 μmol P_i/h/mg protein) from porcine cerebral cortex and ATP were purchased from Sigma Co, as well as some chemicals for the assay medium (magnesium chloride and TRIS–HCl). Other chemicals for the assay medium (sodium chloride, potassium chloride) and P_i determination (stannous chloride and ammonium molybdate) were from Merck (Darmstadt, Germany). The acidity of the incubation medium was controlled by the addition of 1 M TRIS–HCl buffer (pH 7.4). Triply distilled water was used throughout. L-cysteine (Fluka, 99.5%) and GSH (Fluka, 99%) were commercial products of the highest purity available. Pd(II) complexes, $[PdCl(dien)]^+$ and $[PdCl(Me_4dien)]^+$, were prepared according to the standard procedure [29]. The spectrophotometric measurements were done on a PerkinElmer Lambda 35 UV/VIS spectrophotometer. The pH values of the solutions were measured by a Metrohm pH—meter, Model 713.

ATPase assay

Na^+/K^+-ATPase activity was determined in a standard incubation medium (200 μl), containing 50 mM Tris–HCl (pH 7.4), 100 mM NaCl, 20 mM KCl, 5 mM $MgCl_2$, 2 mM ATP and protein (2 μg/μl) in the presence or absence (control) of the desired concentration of Pd(II) complexes. Incubation mixtures were preincubated for 15 min at 37°C. The reaction was started by the addition of ATP, allowed to proceed for 15 min, and interrupted by the addition of ice cold $HClO_4$ and immediate cooling on ice. The inorganic orthophosphate (P_i) liberated from the hydrolysis of ATP was measured using a modified spectrophotometric procedure [4].

The ability of SH-containing ligands to prevent the Pd(II) complexes-induced Na^+/K^+-ATPase inhibition was investigated under the same conditions as described above, with L-cysteine or GSH added to the medium before the enzyme exposure to inhibitor. The recovery of the inhibited enzyme activity by SH-containing ligands was examined by adding L-cysteine or GSH to the medium assay after 15 min. preincubation in the presence of Pd(II) complexes. The controls contained the corresponding concentration of thiols without inhibitor.

Kinetic analysis

Kinetic analysis undertaken to determine the nature of the enzyme inhibition induced by Pd(II) complexes was carried out according to a slightly modified method of Philips et al. [30]. The initial reaction rate was measured in the same incubation medium, as a function of increasing substrate ($MgATP^{2-}$) concentration (0.15–4.00 mM). The measurements were performed in the absence or presence of selected concentrations of Pd(II) complexes. The experimental data were fitted to the Michaelis-Menten equation by non-linear regression analysis using Origin 6.1. V_{max} and K_m values with standard errors were derived from a Eadie–Hofstee plot. A series of kinetic assays were carried out at a variety of inhibitor concentrations but at fixed concentrations of substrate in order to obtain Dixon plots. Graphs of $1/V_{max}$ values against inhibitor concentration were plotted for each Pd(II) complex and the inhibitor constants, K_i, were determined from these plots.

Results

Effect of Pd(II) complexes on Na^+/K^+-ATPase activity

The influence of $[PdCl_4]^{2-}$, $[PdCl(dien)]^+$ and $[PdCl(Me_4dien)]^+$ on Na^+/K^+- ATPase activity was investigated after the addition of the complexes to the reaction mixture in the concentration range 1×10^{-8}–1×10^{-2} M. Sigmoid-shaped inhibition curves were obtained in all cases (Figure 1). The half-maximum inhibitory activities (IC_{50}) were determined by Hill analysis (Figure 1. inset) of the

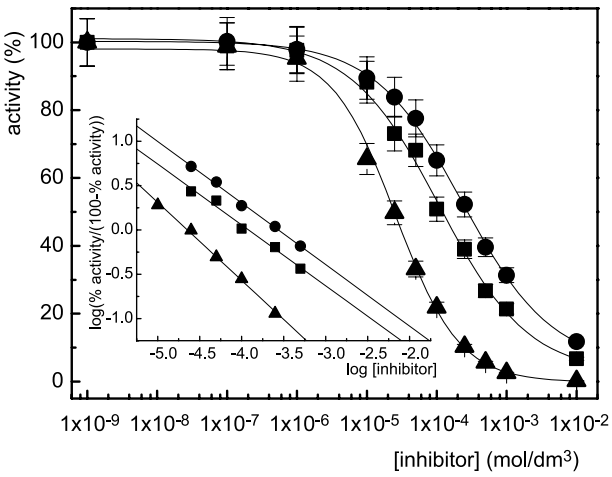

Figure 1. Inhibition of Na^+/K^+-ATPase activity by $[PdCl_4]^{2-}$ (up triangles), $[PdCl(dien)]^+$ (squares) and $[PdCl(Me_4dien)]^+$ (circles). The values given are the mean of at least three experiments ± S.E.M. The Hill plots constructed from the data obtained by inhibition experiments is shown in the inset. Regression lines were calculated by means of the least square method. The R values were in the range 0.9796–0.9995.

experimental curves according to the equation:

$$log \frac{REA}{100 - REA} = n \cdot log [I] - n \cdot log IC_{50} \qquad (1)$$

where REA is relative enzymatic activity (the specific activity of Na^+/K^+-ATPase at a particular concentration of Pd(II) complex divided by the specific activity of control sample without inhibitors) expresed as %, n is the Hill coefficient, [I] is the inhibitor concentration and IC_{50} is half-maximum inhibitory activity (i.e. inhibitor concentration that induced 50% Na^+/K^+-ATPase inhibition). The results are presented in Table I together with the Hill coefficients.

The IC_{50} values for Pd(II) complexes with various ligands indicated that their inhibition power depended on the structure of ligands. Complexes with massive tridentate ligands and high steric hindrance, such as $[PdCl(dien)]^+$ and $[PdCl(Me_4dien)]^+$, showed lower potency to inhibit Na^+/K^+-ATPase activity. Moreover, IC_{50} values for the Pd(II) complexes-induced inhibition of Na^+/K^+-ATPase were at least ten fold higher than values previously reported for transition and heavy metal ions that are in the range 10^{-5}–10^{-7} M [5–7]. This incongruity is evidently based on the variances of ligands in the coordination sphere of metal ions. However, aqua complexes of transition and heavy metals have higher affinity for interaction with Na^+/K^+-ATPase, since the rate of water exchange is usually high compared to the ligands that form strong coordinative bonds.

Hill coefficients for the investigated Pd(II) complexes were below unity ($n < 1$) and indicated negative cooperation for binding of these inhibitors to Na^+/K^+-ATPase. Previously reported Hill coefficient values for Hg^{2+}- and Cd^{2+}-induced inhibition of Na^+/K^+-ATPase activity fulfilled the relation $1 < n < 2$ and indicated that there is cooperative interaction between a minimum of two binding sites on the enzyme for binding of metal ions [31]. In the case of Pd(II) complexes, steric hindrance seems to be responsible for a decrease in n value below 1 and the loss of the cooperativity.

Kinetic analysis

The nature of Na^+/K^+-ATPase inhibition by $[PdCl_4]^{2-}$, $[PdCl(dien)]^+$ and $[PdCl(Me_4dien)]^+$ and the kinetic parameters, K_m and V_{max}, were determined by varying the concentration of $MgATP^{2-}$ in the range of 0.15–4.00 mM, while maintaining the concentrations of other substances in the enzymatic mixture constant. The kinetic properties of the enzyme were determined in the presence of inhibitor concentrations (5×10^{-5} M for $[PdCl_4]^{2-}$, 1×10^{-4} M for $[PdCl(dien)]^+$ and 1×10^{-4} M for $[PdCl(Me_4dien)]^+$), that were chosen from the inhibition curves, as the concentrations that inhibited 40–60% of the enzyme activity. The dependence of the initial reaction rate (v) *vs.* $MgATP^{2-}$ concentration in the absence and the presence of Pd(II) complexes exhibited typical Michelis-Menten kinetics (Figure 2). Kinetic parameters (V_{max} and K_m) were

Table I. IC_{50} values and Hill coefficients (n) for Pd(II) complexes-induced inhibition of Na^+/K^+-ATPase in the absence and the presence of 1 mM L-cysteine and GSH.

Complex	thiol	IC_{50} (M)	n
$[PdCl_4]^{2-}$	/	*2.25 ± 0.21 × 10^{-5}	*0.88 ± 0.03
	L-cys	2.5 ± 0.6 × 10^{-4}	
	GSH	3,1 ± 0.3 × 10^{-4}	
$[PdCl(dien)]^+$	/	*1.21 ± 0.13 × 10^{-4}	*0.70 ± 0.05
	L-cys	7.4 ± 0.8 × 10^{-4}	
	GSH	8.0 ± 0.5 × 10^{-4}	
$[PdCl(Me_4dien)]^+$	/	*2.36 ± 0.30 × 10^{-4}	*0.69 ± 0.02
	L-cys	8.2 ± 0.9 × 10^{-4}	
	GSH	1,4 ± 0.2 × 10^{-3}	

* data obtained by Hill analysis.

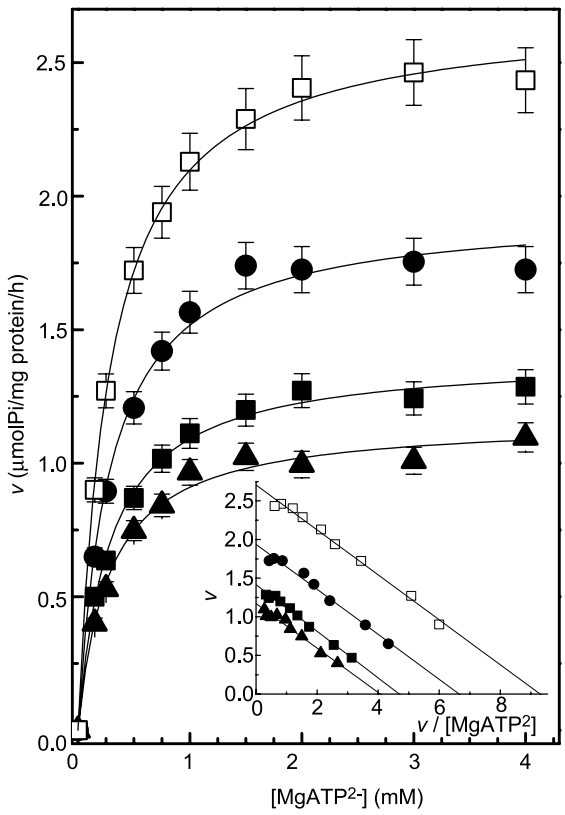

Figure 2. Initial reaction rate (v) vs. $MgATP^{2-}$ concentration in the absence (open squares) and presence of 5×10^{-5} M $[PdCl_4]^{2-}$(solid up triangles), 1×10^{-4} M $[PdCl(dien)]^{+}$ (solid squares) and 1×10^{-4} M $[PdCl(Me_4dien)]^{+}$ (solid circles). The values given are the mean of at least three experiments ± S.E.M. The Eadie–Hofstee transformation of the data is shown in the inset.

derived using Eadie–Hofstee transformation of the experimental data (Figure 2 inset). It is obvious from the results presented in Table II that V_{max} values in the presence of investigated Pd(II) complexes decreased, while K_m values remained the same as in the control sample (without Pd(II) complexes). As can be seen, the inhibitors significantly decreased maximal reaction rate without change in the enzyme's affinity for substrate i.e. substrate and inhibitor were bonded to different sites on enzyme, and the binding of the inhibitor did not affect binding of the substrate. This kind of enzyme behavior in the presence of Pd(II) complexes indicated a noncompetitive type of inhibition [32].

In order to determine Na^+/K^+-ATPase affinities for binding with Pd(II) complexes Dixon plots [33] were created (Figure 3.). The initial reaction rate was measured at four fixed concentrations of substrate ($MgATP^{2-}$) in the presence of various inhibitor concentrations ($0–5 \times 10^{-4}$ M). Graphs of the reciprocal reaction rate against inhibitor concentration were plotted and the inhibitor constants (K_i) for the Pd(II) complexes were read off directly from intersections of lines with the x-axis (Table II). The following sequence of Pd(II) complex affinity for binding to Na^+/K^+-ATPase: $[PdCl_4]^{2-} > [PdCl(dien)]^{+} > [PdCl(Me_4dien)]^{+}$, was deducted from K_i values. Most likely, the main reason for low enzyme-inhibitor affinity of $[PdCl(dien)]^{+}$ and $[PdCl(Me_4dien)]^{+}$ was steric bulkiness that hindered contact of these complexes with enzyme. The values of stability constants of enzyme-inhibitor complex, generated as reciprocical inhibitor constants ($K_s = 1/K_i \cong 10^4$), are close to the value of overall binding constants that have been reported for the interaction of Na^+/K^+-ATPase with Pt(II) complexes. As an example, cisplatin showed the low affinity for the interaction with Na^+/K^+-ATPase, with overall binding constant $K = 1.93 \times 10^4 M^{-1}$ [34]. Furthermore, the inhibitory constants for Pd(II) complexes indicated low inhibitor–enzyme affinity compared to the high affinity for reaction of these complexes with L-cysteine or GSH [21]. This comparison indicated a high potency of L-cysteine and GSH to extrude and substitute enzyme from the inhibitor–enzyme complex.

Effect of L-cysteine and glutathione on the prevention and recovery of Na^+/K^+-ATPase activity exposed to Pd(II) complexes

Previously published results demonstrated that Pd(II) complexes have a great affinity for substitution of the Cl^- ligand by SH–donor ligands, GSH and L-cysteine[18–20], according to the relation (2):

$$[PdCl_{4-n}L_n]^{(2-(4-n))} + \text{thiol} \rightleftarrows [PdCl_{3-n}L_n(\text{thiol})]^{(2-(3-n))} + Cl^- \quad (2)$$

$$\text{thiol} = \text{L} - \text{cysteine or GSH, L} = \text{ligand}$$

Table II. Kinetic analysis of Na^+/K^+-ATPase in the absence (control) and presence of Pd(II) complexes.

Inhibitor	Conc. (M)	K_m (mM)	V_{max} (μM P_i/h/mg)	K_i (M)
Control	0	0.29 ± 0.01	2.71 ± 0.03	–
$[PdCl_4]^{2-}$	5×10^{-5}	0.29 ± 0.02	1.17 ± 0.03	3.97×10^{-5}
$[PdCl(dien)]^{+}$	1×10^{-4}	0.29 ± 0.01	1.39 ± 0.02	1.04×10^{-4}
$[PdCl(Me_4dien)]^{+}$	1×10^{-4}	0.29 ± 0.01	1.94 ± 0.03	4.20×10^{-4}

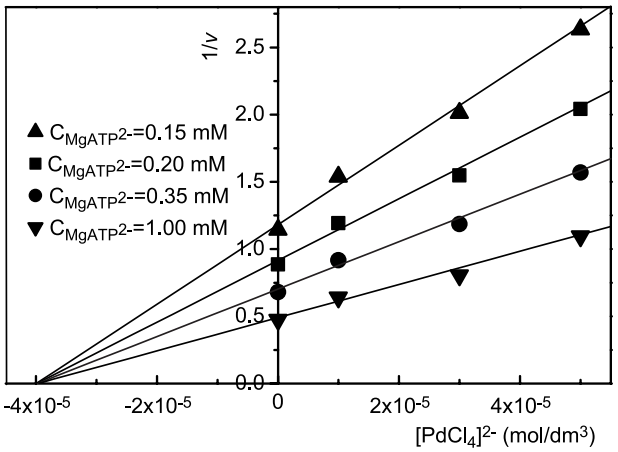

Figure 3. The Dixon transformation of the kinetic analysis data: reciprocal value of initial reaction rate ($1/v$) vs. $[PdCl_4]^{2-}$ concentration in the presence of several $MgATP^{2-}$ concentrations. The symbols representing $MgATP^{2-}$ concentrations are shown in the inset.

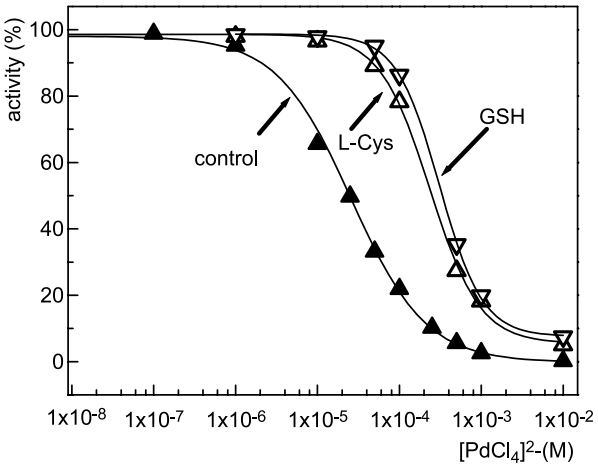

Figure 4. Preventive effect of 1 mM L-cysteine (open up triangles) and 1 mM GSH (open down triangles) on $[PdCl_4]^{2-}$-induced inhibition of Na^+/K^+-ATPase activity. The activity of control sample without SH-donor ligands is given as solid up triangles.

The stoichiometry of the complexes was determined by a molar ratio method (Pd(II):thiol = 1:1).

Furthermore, Na^+/K^+-ATPase can be observed as a SH-donor ligand since the enzyme has 36 SH groups which are held responsible for interactions of this enzyme with various metal ions [7]. It is generally considered that the non-specific bonding of metal ions to enzyme sulfhydril groups is accompanied by the inhibition of enzymatic activity [6,7]. Moreover, the inhibitor constants for Pd(II) complexes, obtained by kinetic analysis, indicated low stability of inhibitor–enzyme complex compared to the stability of $[PdCl_{3-n}L_n(GSH)]^{(2-(3-n))}$ or $[PdCl_{3-n}L_n(L\text{-cys})]^{(2-(3-n))}$ [21]. Based on these facts we assumed that presence of L-cysteine or GSH in the enzymatic mixture will result in substitution of the enzyme from the inhibitor–enzyme complex and a decrease in the concentration of $[PdCl_{4-n}L_n]^{(2-(4-n))}$ complexes that can interact with Na^+/K^+-ATPase and induce inhibition of enzymatic activity.

The effect of $[PdCl_4]^{2-}$, $[PdCl(dien)]^+$ and $[PdCl(Me_4dien)]^+$, within the range 10^{-8}–10^{-2} M, on ATP hydrolysis catalyzed by Na^+/K^+-ATPase was investigated in the absence and presence of 1 mM L-cysteine or GSH. The presence of these thiols in the reaction mixture prevented enzyme inhibition at Pd(II) complexes concentrations below 0.1 mM and reduced inhibition at higher concentrations. Figure 4 illustrates the preventive effects of L-cysteine and GSH on $[PdCl_4]^{2-}$ -induced inhibition of Na^+/K^+-ATPase. Both thiols exerted a similar ability to prevent inhibition, since the inhibition curves overlapped. Analogous inhibition curves in the presence of thiols were observed for the other two Pd(II) complexes. The IC_{50} values determined from inhibition curves in the absence and presence of 1 mM L-cysteine and GSH for all investigated Pd(II) complexes are presented in Table I. The results show that the sensitivity of Na^+/K^+-ATPase to Pd(II) complexes decreased in the presence of SH-containing ligands. More likely, the reason for prevention of inhibition under these experimental conditions was the formation of the stable, inactive $[PdCl_{3-n}L_n(thiol)]^{(2-(3-n))}$ complex [21] replacing of the inhibitory potent $[PdCl_{4-n}L_n]^{(2-(4-n))}$.

The recovery of the enzyme activity inhibited by Pd(II) complexes was investigated by varying L-cysteine or GSH concentrations in the range of 1×10^{-6}–1×10^{-2} M. The results, presented in Figure 5, show that L-cysteine and GSH had a dose-dependent recovery effect on Na^+/K^+-ATPase activity exposed to 5×10^{-5} M $[PdCl_4]^{2-}$, 1×10^{-4} M $[PdCl(dien)]^+$ or 1×10^{-4} M $[PdCl(Me_4dien)]^+$. Full recovery was achieved when the concentration of SH-containing ligands was equal or higher than the Pd(II) complex concentration. The recovery of the inhibition can be explained by the high potency of thiols to extrude Na^+/K^+-ATPase from the enzyme-inhibitor complex and formation of stable $[PdCl_{3-n}L_n(thiol)]^{(2-(3-n))}$ complexes [18–21]. Since the intracellular concentration of GSH is up to 8 mM, and is usually much greater than those of cysteine [35], the ability of these ligands to detoxify after chemotherapy seems to be an interesting point for further investigations.

Discussion

Results presented in this work confirmed that Pd(II) complexes, as previously reported for Pt(II) complexes [9,15,16], induced the inhibition of Na^+/K^+-ATPase

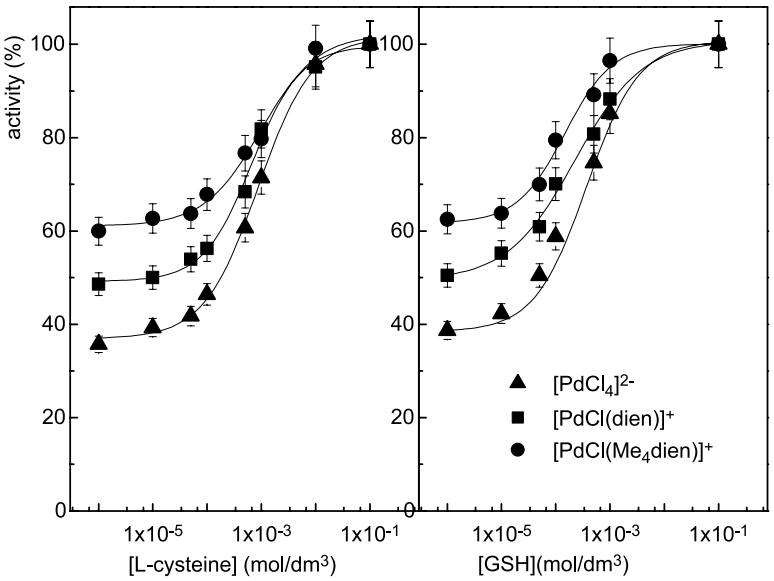

Figure 5. Recovery effect of L-cysteine and GSH on the Na^+/K^+ -ATPase activity inhibited in the presence of 5×10^{-5} M $[PdCl_4]^{2-}$ (up triangles), 1×10^{-4} M $[Pd(dien)Cl]^+$(squares) and 1×10^{-4} M $[Pd(Me_4dien)Cl]^+$(circles).

activity. The IC_{50} values for the investigated series of Pd(II) complexes are the same order of magnitude compared to the values reported for Pt(II) complexes [9,16]. Moreover, IC_{50} values obtained for Pd(II) complexes and the values reported for aqua complexes of transition and heavy metals showed that the aqua complexes have 10–1000 fold higher potency to inhibit Na^+/K^+-ATPase [5–7]. Since most of the heavy and transition metals, as well as Pt(II) and Pd(II) compounds have a strong affinity to bind to –SH groups of proteins, their potency to inhibit Na^+/K^+-ATPase depended on the rate of ligand exchange in the coordinative sphere of the metal ion. It is reasonable to assume that the inhibitor potency depended strongly on the nature of the metal ion, as well as the nature of the ligand. However, the stability constants of enzyme-Pd(II) complexes determined in the present work ($K_s = 1/K_i \cong 10^4\,M^{-1}$) are close to the value of the overall binding constant that was reported for the interaction of Na/K-ATPase with cisplatin ($K = 1.93 \times 10^4\,M^{-1}$) [34], but are also two orders of magnitude lower compared to the aqua complexes of heavy and transition metals [36]. It is obvious in the Pd(II) series the steric bulkiness of ligands may be the main reason for decrease in the Pd(II) complex's ability to inhibit Na^+/K^+-ATPase. Besides, the low inhibitory power compared to aqua complexes of transition and heavy metals [5–7], could be attributed to the effect of high concentration of Cl^- ions that was present in the incubation medium, as well as in the physiological conditions.

Kinetic analysis indicated a noncompetitive type of inhibition and suggested that Pd(II) complexes did not affect the binding of the substrate. Considering the fact that Pd(II) complexes are model compounds for their Pt(II) analogs [17], which showed similar inhibitory effects on Na^+/K^+-ATPase [9,15,16], the conclusion can be drawn that the same kind of enzyme-inhibitor interaction can be expected for the widely-used platinum anticancer drugs. More likely, the mechanism of interaction of enzyme, L-cysteine and GSH with investigated Pd(II) complexes is similar, since it involves substitution of the Cl^- ligand by SH-donor ligands [18–21]. However, the affinity of L-cysteine or GSH for this reaction is much higher compared to the Na^+/K^+-ATPase affinity. This is in good agreement with the previously reported mechanism of inhibition of Na^+/K^+-ATPase by various metal ions which is realized through their binding to enzyme sulfhydril groups [6,7].

The inhibitory effect of Pd(II) complexes can be prevented and recovered by the addition of L-cysteine or GSH, which show high potency to extrude and substitute the enzyme from the Pd(II) complex. Is seems that prevention and recovery of Pd(II) complexes-induced Na^+/K^+-ATPase inhibition are realized due to the competition between the SH functional groups of protein and thiols (L-cysteine or GSH) for substitution of the Cl^- ligand in the coordination sphere of Pd^{2+}. These results are consistent with the kinetic analysis, suggesting that these complexes are reversible noncompetitive inhibitors of this enzyme. Since platinum anticancer drugs react in the same manner as their palladium analogs, it was suggested that L-cysteine or GSH might have the ability to detoxify after chemotherapy.

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References

[1] Lingrel JB, Kuntzweiler T. Na^+,K^+-ATPase. J Biol Chem 1994;269:19659–19662.

[2] Mobasheri A, Avila J, Cozar-Castellano I, Brownleader MD, Trevan M, Francis MJO, Lamb JF, Martin-Vasallo P. Na^+,K+$^+$-ATPase isozyme diversity; comparative biochemistry and physiological implications of novel functional interactions. Biosci Rep 2000;20:51–91.

[3] Krstić D, Krinulović K, Joksić G, Momić T, Vasić V. Effects of digoxin and gitoxin on the enzymatic activity and kinetic parameters of Na^+/K^+-ATPase. J Enz Inhib Med Chem 2004;19:409–415.

[4] Vasić V, Jovanović D, Krstić D, Nikezić G, Horvat A, Vujisić LJ, Nedeljković N. Prevention and recovery of $CuSO_4$ induced inhibition of Na,K-ATPase and Mg-ATPase in rat brain synaptosomes by EDTA. Toxicol Lett 1999;110:95–104.

[5] Vasić V, Krinulović K, Krstić D, Momić T, Horvat A. ATPases as multi-response sensing system for various organic and inorganic analytes. Monatsch Chem 2004;135:605–614.

[6] Vujisić LJ, Krstić D, Krinulović K, Vasić V. The influence of transition and heavy metal ions on ATP-ases activity in rat synaptic plasma membranes. J Serb Chem Soc 2004;69:541–547.

[7] Carfagna MA, Ponsler GD, Muhoberac BB. Inhibition of ATPase activity in rat synaptic plasma membranes by simultaneous exposure to metals. Chem Biol Interact 1996;100:53–65.

[8] Boldyrev A, Bulygina E, Kramarenko G, Vanin A. Effect of nitroso compounds on Na/K-ATPase. Biochim Biophys Acta 1997;1321:243–251.

[9] Nechay BR, Neldon SL. Characteristics of inhibition of human renal adenosine triphosphatases by cisplatin and chloroplatinic acid. Cancer Treat Rep 1984;68:1135–1141.

[10] Louie AY, Meade TJ. Metal complexes as enzyme inhibitors. Chem Rev 1999;99:2711–2734.

[11] Jakupec MA, Galanski M, Keppler BK. Tumour-inhibiting platinum complexes–state of the art and future perspectives. Rev Physiol Biochem Pharmacol 2003;146:1–54.

[12] Lippert B. Cisplatin, chemistry and biochemistry of a leading anticancer drug. Zürich: Wiley-VCH; 1999.

[13] Reedijk J. Why does cisplatin reach Guanine-N7 with competing S-donor ligands available in the cell? Chem Rev 1999;99:2499–2510.

[14] Djuran MI, Dimitrijević DP, Milinković SU, Bugarčić ŽD. Reactions of platinum(II)complexes with sulfur-and histidine-containing peptides: A model for selective platination of peptides and proteins. Trans Metal Chem 2002;27:155–158.

[15] Sakakibara N, Suzuki K, Kaneta H, Yoshimura Y, Deyama Y, Matsumoto A, Fukuda H. Inhibition of Na^+,K^+-ATPase by cisplatin and its recovery by 2-mercaptoethanol in human squamous cell carcinoma cells. Anticancer Drugs 1999;10:203–211.

[16] Uozumi J, Litterst CL. The effect of cisplatin on renal ATPase activity *in vivo* and *in vitro*. Cancer Chemother Pharmacol 1985;15:93–96.

[17] Rau T, van Eldik R. Interactions of metal ions with nucleotides, nucleic acids, and their constituents. In: Sigel A, Sigel H, editors. Metal ions in biological systems. Vol. 32, New York: Marcel Dekker; 1996. p 339–378.

[18] Bugarčić ŽD, Liehr G, van Eldik R. Kinetics and mechanism of the reaction of chelated Pd(II) complexes with thiols in acidic aqueous solution. Synthesis and crystal structure of [Pd(bpma)Cl]Cl·H_2O (bpma = bis(2-pyridylmethyl)amine). J Chem Soc Dalton Trans 2002;:951–956.

[19] Vasić V, Čakar M, Savić J, Petrović B, Nedeljković J, Bugarčić Ž. Influence of sodium dodecyl sulfate on the kinetics of complex formation between $[PdCl(dien)]^+$ and sulfur containing ligands L-cysteine and glutathione. Polyhedron 2003;22:279–285.

[20] Vasić VM, Živanović MA, Čakar MM, Savić JZ, Nedeljković JM, Bugarčić ŽD. Influence of acidity on the reaction between $[PdCl(dien)]^+$ and L-cysteine or glutathione in the presence of sodium dodecyl sulfate micelles. J Phys Org Chem 2005;18:441–447.

[21] Bugarcic ZD, Jancic DM, Shoukry AA, Shoukry MM. Rate and equilibrium data for substitution reactions of [Pd(dien)Cl]+with L-Cysteine and glutathione in aqueous solution. Monatsch Chem 2004;135:151–160.

[22] Bugarčić ŽD, Heinemann FW, van Eldik R. Substitution reactions of $[Pt(terpy)X]^{2+}$ with some biologically relevant ligands. Synthesis and crystal structure of [Pt(terpy)(cyst-S)]$(ClO_4)2{\cdot}0.5H_2O$ and [Pt(terpy)(guo-N7)]$(ClO_4)2{\cdot}0.5$guo $\cdot 1.5H_2O$. J Chem Soc Dalton Trans 2004;:279–286.

[23] Clausen AE, Kast C, Bernkop-Schnürch E. The role of glutathione in the permeation enhancing effect of thiolated polymers. Pharmac Res 2002;19:602–608.

[24] Rabenstein DL, Guevremont R, Evans CA. Glutathione and its metal complexes. In: Sigel H, editor. Metal ions in biological systems. Vol. 9, New York: Marcel Dekker; 1979. p 103–107.

[25] Hussain S, Anner RM, Anner BM. Cysteine protects Na,K-ATPase and isolated human lymphocytes from silver toxicity. Biochem Biophys Res Commun 1992;189: 1444–1449.

[26] Henderson GB, Fairlamb AH, Ulrich P, Cerami A. Substrate specificity of the flavoprotein trypanothione disulfide reductase from Crithidia fasciculata. Biochemistry 1987;26:3023–3027.

[27] Scozzafava A, Mastrolorenzo A, Supuran CT. Agents that target cysteine residues of biomolecules and their therapeutic potential. Expert Opin Ther Pat 2001;11:765–787.

[28] Scozzafava A, Casini A, Supuran CT. Targeting cysteine residues of biomolecules: New approaches for the design of antiviral and anticancer drugs. Curr Med Chem 2002;9:1167–1185.

[29] Breet ELJ, van Eldik R. The isolation and identification of an unusual palladium(II) substituted dien complex [1]. Inorg Chim Acta 1983;76:L301–L303.

[30] Philips TD, Hayes AW, Ho IK, Desiah D. Effects of rubrotoxinB on the kinetics of cationic and substrate activation of Na,K-ATPase and p-nitrophenylphosphetase. J Biol Chem 1978;253:3487–3493.

[31] Vasić V, Jovanović D, Horvat A, Momić T, Nikezić G. Effect of $Cd^{2+}$and Hg^{2+} on the activity of Na^+/K^+-ATPase and Mg^{2+}-ATPase adsorbed on polystyrene microtiter plates. Anal Biochem 2002;300:113–120.

[32] Garrett RH, Grisham CM. Biochemistry. 2nd edition Orlando; Florida: Harcourt Brace; 1999. p 443–447.

[33] Al-Jafari AA, Kamal MA, Greig NH, Alhomida AS, Perry ER. Kinetics of human erythrocyte acetylcholinesterase inhibition by a novel derivative of physostigmine: Phenserine. Biochem Biophys Res Comm 1998;248:180–185.

[34] Neault JF, Benkirane A, Malonga H, Tajmir-Riahi HA. Interaction of cisplatin drug with Na,K-ATPase: Drug binding mode and protein secondary structure. Drug J Inorg Biochem 2001;86:603–609.

[35] Friedman M. The chemistry and biochemistry of the sulfhydryl group Amino acids, peptides and proteins. New York: Pergamon Press; 1973. p 52–87.

[36] Krstić D, Krinulović K, Vasić V. Inhibition of Na^+/K^+-ATPase and Mg^{2+}-ATPase by metal ions and prevention and recovery of inhibited activities by chelators. J Enz Inhib Med Chem 2005;20:469–476.