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Reduction of some Pt(IV) complexes with biologically important sulfur-donor ligands

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Abstract

The reduction of the Pt(IV) complexes [$PtCl_4$(bipy)], [$PtCl_4$(dach)] and [$PtCl_4$(en)] by glutathione (GSH), L-cysteine (L-Cys) and L-methionine (L-Met) was investigated by stopped-flow spectrophotometry at pH 2.0 (in 0.01 M perchloric acid) and at pH 7.2 (in 25 mM Hepes buffer). Kinetic measurements were performed under *pseudo*-first order conditions with an excess of the reducing agent. The order of the reactivity of the studied complexes was [$PtCl_4$(bipy)] > [$PtCl_4$(dach)] > [$PtCl_4$(en)], and reactivity of investigated reducing agents followed the order GSH > L-Cys > L-Met. All the reactions between the selected Pt(IV) complexes and the sulfur donor biomolecules proceeded by a reductive elimination process that included nucleophilic attack by the reducing agent on one of the mutually *trans*-coordinated chloride ligands, which led to a two-electron transfer process. The final products of the redox reactions were the corresponding reduced Pt(II) complexes and the oxidized form of the reducing agents.

Keywords: Pt(IV) complexes; reduction; glutathione; L-cysteine; L-methionine

Introduction

It is well known that the clinical efficiency of cisplatin, *cis*-[$PtCl_2(NH_3)_2$], is limited by many toxic side effects, such as dose-limiting nephrotoxicity, drug resistance of the tumor cells and a narrow range of activity.[1] Further studies in this field revealed a number of different Pt(II) complexes, structurally similar to cisplatin, with reduced toxicity, but still not suitable for longer application.[2-4] To date, about 3000 different platinum complexes have been synthesized and investigated in an attempt to improve the antitumor activity, to lower the toxicity and to design a drug that is able to overcome cell resistance. However, only about 30 platinum complexes have hitherto entered clinical trials.[5-7] Significant among them are Pt(II) complexes with a *trans*-geometry, dinuclear Pt(II) complexes and Pt(IV) complexes.[1,2,4]

Platinum(IV) complexes have shown considerable promise as antitumor agents. Their octahedral geometry introduces two additional ligand sites, and the high kinetic inertness of the complexes lowers their reactivity as well as the prospect of side reactions. Thus, the potential advantages of Pt(IV) complexes are based on the fact that they remain in the higher oxidation state in the bloodstream due to their lower reactivity. This can diminish the loss of the active drug and lower the number of side reactions that lead to toxic side effects.[8-10]

Compared to the Pt(II) complexes, some complexes of Pt(IV) can be orally administrated since the amount of the complex that is lost or deactivated on the way to the target cells is relatively small. For example, satraplatin is the first orally applicable Pt drug in a clinical trial.[11] On the other hand, the lipophilicity of the Pt(IV) complexes is an important factor for their oral administration.[8,12]

Pt(IV) complexes are inert in ligand substitution reactions relative to their Pt(II) analogues.[13-15] Upon entering into the cell, there are two metabolic pathways for Pt(IV) complexes, *viz*. reduction by agents present in the cell, such as glutathione, L-cysteine, L-methionine and ascorbic acid, or direct interaction with DNA molecules in the cell nucleus. The first pathway leads to the well-known reactions of Pt(II) complexes,[16] whereas the second pathway involves the formation of an adduct between Pt(IV) and DNA.[17,18]

Elding and co-workers[19-23] studied in detail the reduction of various Pt(IV) complexes, such as *trans,trans,trans*-$[PtCl_2(OH)_2(NH_3)(cha)]$, *cis,trans,cis*-$[PtCl_2(OH)_2(NH_3)(cha)]$, *trans*-$[PtCl_4(NH_3)(thiazole)]$, *trans*-$[PtCl_4(NH_3)(cha)]$, *cis*-$[PtCl_4(NH_3)(cha)]$, *cis*-$[PtCl_4(NH_3)_2]$, and *trans,cis,cis*-$[Pt(OCOCH_3)_2Cl_2(NH_3)(cha)]$, where cha = cyclohexylamine, with different thiols and ascorbic acid as reducing agents. The most of these reactions followed an inner-sphere reductive-elimination mechanism that involved attack of the reducing agent on the halide coordinated *trans* to the good leaving group, leading to a two-electron transfer process. The final products of these reactions were the corresponding reduced Pt(II) complexes and the oxidized form of the reducing agents.

Although most evidence indicates that Pt(IV) compounds are reduced to Pt(II) by potential cellular reducing agents, the underlying reaction mechanism is still poorly understood. The relation between the structures of the Pt(IV) complexes and the rates and mechanisms of their reduction should be strongly connected. Published results for the reduction of different Pt(IV) complexes showed that their reactivity depended on their reduction potential.[8,17,18,24,25] For diammine complexes of platinum, the structure

variation of the diammine ligand had less effect on the reduction potential, whereas the nature of the axial ligands exerted a stronger influence.[21,22,26] When the axial ligands were chloride, the reduction was very fast when compared to those of carboxylate or hydroxide complexes.[27]

In this study, the reactions of [$PtCl_4$(bipy)], [$PtCl_4$(dach)] and [$PtCl_4$(en)] with glutathione (GSH), L-cysteine (L-Cys) and L-methionine (L-Met) were investigated by stopped-flow spectrophotometry. The influence of acidity on the rate of reduction of the Pt(IV) complexes could reveal more information on the relationship between their structure and chemical behavior due the interaction with the selected biomolecules. The structures of the studied complexes and reducing agents are presented schematically in Fig. 1.

Fig. 1

Results and discussion

The redox reactions of [$PtCl_4$(bipy)], [$PtCl_4$(dach)] and [$PtCl_4$(en)] with GSH, L-Cys and L-Met were studied at pH 2.0 (0.01 M $HClO_4$) in the presence of 0.2 M NaCl, by following the changes in absorbance at suitable wavelengths as a function of time. Single-exponential traces were obtained under all experimental conditions. The calculated values for the *pseudo*-first order rate constants, k_{obsd}, are given in Tables S1-S3 (ESI). The suggested pathways for the reduction of the selected Pt(IV) complexes by the thiols and thioether at pH 2.0 are presented in Scheme 1. In the first step, the reductive elimination by RSH results in the production of RSCl, which hydrolyzes rapidly in the

subsequent step to form RSOH, which in turn reacts with another RSH molecule to form the final product RSSR.[21] The first step of this reaction sequence is the rate-determining process.

$$[PtCl_4(N\text{-}N)] + RSH \xrightarrow{k_2} [PtCl_2(N\text{-}N)] + RSCl + H^+ + Cl^- \quad \text{slow}$$

$$\left.\begin{array}{l} RSCl + H_2O \longrightarrow RSOH + H^+ + Cl^- \\ RSOH + RSH \longrightarrow RSSR + H_2O \end{array}\right\} \text{fast}$$

$$[PtCl_4(N\text{-}N)] + MeSR + H_2O \xrightarrow{k_2} [PtCl_2(N\text{-}N)] + MeS(O)R + 2H^+ + 2Cl^-$$

N-N = bipy, dach, en; RSH = GSH, L-Cys; MeSR = L-Met

Scheme 1

The dependence of the observed rate constants, k_{obsd}, on the concentration of the reducing agents can be express by Eqn. (1), where k_2 represents the second-order rate constants for the rate-determining step of all reaction systems:

$$k_{obsd} = k_2[\text{Reducing agent}] \quad (1)$$

All plots of k_{obsd} *vs.* reducing agent concentration were linear and passed through the origin. This indicates that possible parallel or back reactions are insignificant or absent

(see Fig. 2, Figs. S1 and S2 (ESI)). The second-order rate constants (k_2) obtained from the slope of the observed plots are summarized in Table 1.

Fig. 2

Table 1

The obtained results clearly show that the reactivity of the studied complexes followed the order: $[PtCl_4(bipy)] > [PtCl_4(dach)] > [PtCl_4(en)]$. The order of reactivity of the selected reducing agents was: GSH > L-Cys > L-Met.

To determine the influence of added chloride on the reduction rate at pH 2.0, the same reactions were investigated in the absence of NaCl. The calculated rate constants are also given in Table 1, whereas the linear plots of k_{obsd} *vs.* reducing agent concentration are shown in Figs. S3-S5 (ESI). The values of k_{obsd} are given in Tables S4-S6 (ESI). It was found that the rate constant for reduction, k_2, was independent of the chloride concentrations, which is in agreement with previously published results.[23]

Since Pt(IV) compounds are in general inert toward substitution, reduction of Pt(IV) complexes by thiols and thioether occurs by the attack of a sulfur atom on one of the *trans* coordinated ligands (inner-sphere mechanism). The reduction of Pt(IV) halide compounds by reducing agents follows a reductive elimination process. It was proposed that this mechanism involves the attack of the reductant on one of the mutually *trans* coordinated chlorides in the Pt(IV) complex, which leads to a two-electron transfer *via* a chloride-bridged complex. Furthermore, the reduction is followed by loss of two *trans* chloride ligands and the formation of the corresponding Pt(II) complex.[8,20,21,28,29] The rate

of the reduction of Pt(IV) complexes strongly depends on the type of ligands in the axial and equatorial positions. The axial bridging ligands are usually halide ions, which correlates with their electronegativity. More electronegative ligands promote destabilization of the Pt(IV) complexes, which resulted in a faster reduction process.[26] In the case of complexes that do not have axial chloride ligands but some others, such as acetate ligands, the reduction was slower and followed an outer-sphere mechanism.[22,28] On the other hand, bulky inert equatorial ligands destabilized six-coordinated Pt(IV) complexes and allowed a faster reduction to the four-coordinated Pt(II) complexes.[26,30] This was confirmed by the results obtained in the present study since ethylenediamine is less bulky than the other two chelates, bipy and dach, and the reduction of the $[PtCl_4(en)]$ complex was the slowest, whereas $[PtCl_4(bipy)]$ showed the fastest reduction rate.

The rate of reduction of Pt(IV) complexes depended on their cathodic reduction potential.[17,18,24-26] Namely, the cathodic potential depends on the electron-withdrawing power of the axial ligands as well as on the bulkiness of the inert equatorial ligands. The published value for the cathodic potential for $[PtCl_4(en)]$ was -160 mV and was -90 mV for $[PtCl_4(dach)]$.[26] This trend is in good agreement with the obtained order of reactivity in the studied case, since the higher value for the cathodic redox potential should correlate with a faster reduction process.[8,26]

The lowest reactivity observed for the thioether (L-Met) of all studied reducing agents can be accounted for since thioethers are in general less powerful reducing agents than thiols. Furthermore, another possible reason is the steric hindrance of the methyl group, which is directly linked to the sulfur atom.[8]

The reduction of $[PtCl_4(bipy)]$, $[PtCl_4(dach)]$, $[PtCl_4(en)]$ with the selected biomolecules was also investigated at pH 7.2 (25 mM Hepes buffer) in the presence of 0.2 M NaCl by stopped-flow spectrophotometry at 310 K. Single-exponential traces were obtained for all the reactions. The calculated values for the *pseudo*-first order rate constants, k_{obsd}, are reported in Tables S7-S9 (ESI). A linear dependence of k_{obsd} on the reducing agent concentration was observed for all studied reactions (Fig. 3, Figs. S6 and S7, ESI), which can be described by Eqn. (2).

Fig. 3

$$k_{obsd} = k_1 + k_2[\text{Reducing agent}] \quad (2)$$

From the slopes of the observed lines, the second-order rate constants, k_2, for the redox reactions were calculated, see Table 1. The obtained results showed the same order of reactivity for the studied complexes and reducing agents at pH 7.2 as was observed for the reactions at pH 2.0. However, there was a significant difference in that all the observed dependencies at pH 7.2 did not pass through the origin and showed significant intercepts (k_1). In order to investigate this observation further, the reactions between the studied complexes and glutathione were studied at different chloride concentrations. The values for the *pseudo*-first order rate constants obtained under such conditions are reported in Tables S10–S12 (ESI), and the plots of k_{obsd} as a function of glutathione concentration for the $[PtCl_4(bipy)]$ complex in the presence of different concentrations of chloride are reported in Fig. S8 (ESI). The values of the intercept (k_1) and slope (k_2) for

the studied reactions as a function of chloride concentration are summarized in the Table 2. Both these rate constants increased strongly with increasing chloride concentration. The dependences of the rate constants k_1 and k_2 on the chloride concentration are shown in Figs. S9 and S10 (ESI), respectively, from which it follows that both rate constants depended linearly on the chloride concentration. This suggests that the higher chloride concentration mediates the reductive elimination process *via* both reaction paths represented by k_1 and k_2. Thus, Eqn, (2) can be extended to Eqn. (3) for the reaction between $[PtCl_4(bipy)]$ and glutathione, where k_3 and k_4 were determined from the slopes of the plots in Figs. S9 and S10 and had the values $(2.4 \pm 0.1) \times 10^2$ M^{-1} s^{-1} and $(5.7 \pm 0.5) \times 10^3$ M^{-2} s^{-1}, respectively.

Table 2

$$k_{obsd} = k_3[Cl^-] + k_4[Cl^-][\text{glutathione}] \quad (3)$$

The proposed pathway for the reaction at pH 7.2 is shown in Scheme 2.

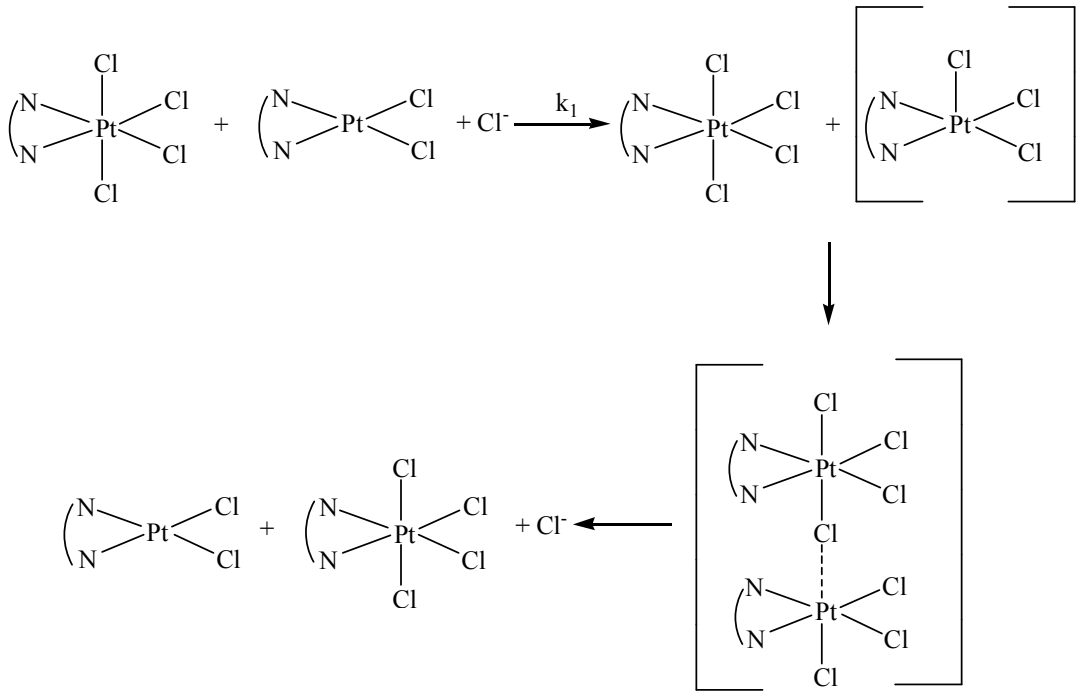
$$[PtCl_3(H_2O)(N\text{-}N)]^+ + RS^- + Cl^- \xrightarrow{k_2} [PtCl_2(N\text{-}N)] + RSCl + Cl^- + H_2O \text{ slow}$$

$$\left.\begin{array}{l} RSCl + H_2O \longrightarrow RSOH + H^+ + Cl^- \\ RSOH + RS^- \longrightarrow RSSR + OH^- \end{array}\right\} \text{fast}$$

$$[PtCl_3(H_2O)(N\text{-}N)]^+ + MeSR + Cl^- \xrightarrow{k_2} [PtCl_2(N\text{-}N)] + MeS(O)R + 2Cl^- + 2H^+$$

where:

$$[PtCl_3(H_2O)(N\text{-}N)]^+ + Cl^- \rightleftharpoons [PtCl_4(N\text{-}N)] + H_2O$$



N-N = bipy, dach, en; RS^- = deprotonated GSH, L- Cys; MeSR = L-Met

Scheme 2

In the first step, the rate-determining step, the aqua complex, $[PtCl_3(H_2O)(N\text{-}N)]^+$ is present in the system that reacts with chloride ions to give the $[PtCl_4(N\text{-}N)]$ complex. This complex reacts further with the reducing agents leading to the formation of the corresponding Pt(II) complexes. The higher chloride concentration in the solution, the greater is the stability of the $[PtCl_4(N\text{-}N)]$ complex.

In order to confirm the hydrolysis of the complexes under the investigated experimental conditions, the change of absorbance as a function of wavelength was monitored for a series of solutions of the $[PtCl_4(en)]$ complex (1×10^{-3} M) in the absence of chloride and in the presence of different concentrations of chloride. Three NaCl concentrations were investigated: 0.05, 0.1 and 0.2 M (Fig. S11, ESI). The spectra of each solution of the complex was recorded after 1 h, 3 h, 4 h and 1 day, and then compared. It can be seen that the absorbance significantly decreased when chloride were absent from the solution and hydrolysis of the $[PtCl_4(en)]$ complex was rapid, while higher concentrations of chloride suppresed the hydrolysis process.

In a separate experiment, an aliquot (3 ml) of the solution of complex $[PtCl_3(H_2O)(en)]^+$, was transferred into a cuvvete and UV-Vis spectrum was recorded. An appropriate amount of solid NaCl was added in the cuvvete to the concentration 0.2 M NaCl, and UV-Vis spectrum was recorded, after 1 day (Fig. S12, ESI). Comparing the spectra it can be seen that the absorbance increases with addition of chloride in a solution of complex $[PtCl_3(H_2O)(en)]^+$, and this complex is converted to the $[PtCl_4(en)]$.

The appearance of the intercept could be explained by a dinuclear transition state between Pt(II) and Pt(IV) complexes through chloride as a bridging ligand. The above-mentioned transition state, as a result of a two-electron transfer between the divalent and the tetravalent metal ions, the formerly Pt(IV) species loses the two axial chloride ligands, leading to the reduced Pt(II) species. However, the Pt(II) species will be oxidized to Pt(IV) species that have two chloride ligands in the axial positions[30,31] (Scheme 2). As the formation of a bridge between complexes of Pt(II) and Pt(IV) occurs through a chloride ligand, higher chloride concentrations in the solution could facilitate the

formation of the bridge. The reduction to the Pt(II) complexes is faster at pH 7.2 than at pH 2.0, which leads to the formation of larger amounts of complex Pt(II). In addition, due to the faster reduction, the chlorides were released faster at pH 7.2 than at pH 2.0, which led to higher chloride concentrations in the solution.

The obtained results also showed that the rate of reduction strongly depended on the acidity of the solution. At pH 7.2, the reductions were about 5-30 times faster than at pH 2.0. This observation is connected with the structures of the ligands and their deprotonation constants. The acid dissociation constants for GSH are $pK_{a1} = 2.05$, $pK_{a2} = 3.40$, $pK_{a3} = 8.79$ and $pK_{a4} = 9.49$, for L-Cys, they are $pK_{a1} = 1.9$ $pK_{a2} = 8.1$ and $pK_{a3} = 10.9$, and for L-Met, they are $pK_{a1} = 2.65$ and $pK_{a2} = 9.08$.[32]

The employed amino acids and peptide represent different protolytic forms depending on the pH (Figs. S13-S15, ESI). At pH 2.0, the formation of a bridged activated complex is very difficult, but at pH 7.2, deprotonation of the thiol group increased the reaction rate by a factor of between 5 and 30. Elding *et al.* defined the reduction rates of Pt(IV) complexes that include all protolytic constants of the thiols.[21] Their results for GSH and L-Cys are in very good agreement with the present data in Table 1, *viz*., the reactivity of the thiolates (RS^-) was directly related to the proton basicity, whereas the steric factor had little influence on the reaction rate. The oxidation of the thioether, L-Met, is also favored at higher pH. Transformation of thioether to sulfoxide occurs through attack of a hydroxyl group on the sulfur atom and further deprotonation of the obtained product. At pH 2.0, the occurrence of this process is more difficult, which could be the explanation for the higher reactivity of L-Met at neutral pH.

To confirm that the reduction of the Pt(IV) complexes leads to the formation of Pt(II) analogues through the reductive elimination pathway, the reaction between [$PtCl_4$(en)] and GSH was studied by ^{1}H NMR in D_2O at 295 K and at pD 6.4 (pD = pH + 0.4). The spectra of (a) free glutathione, (b) oxidized glutathione and (c) the product of the reduction of [$PtCl_4$(en)] by GSH are summarized in Fig. 4. The formation of GSSG during the reaction could be verified by the disappearance of the multiple peaks at 3 ppm, assigned to the Cys-βCH_2 group of GSH, and the appearance of doublet of doublets at 3.06 ppm and 3.36 ppm, typical for free GSSG. In this region, the signal of the corresponding Pt(II) complex appeared at 3.35 ppm, while the signal at 3.37 ppm was ascribed to the remaining Pt(IV) complex in solution.

Fig. 4

Experimental

Materials

Potassium tetrachloridoplatinate(II) (K_2PtCl_4) was purchased from Strem Chemicals. The ligands ethylenediamine (en) (Merck), (*1R,2R*)-1,2-diaminocyclohexane (dach) (Acros Organics) and 2,2′-bipyridyl (Aldrich), and the nucleophiles glutathione (Acros Organics), L-cysteine and L-methionine (Aldrich) were used without further purification. Hepes buffer (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) was obtained from Aldrich. All other chemicals, such as NaCl (Lachema), $AgClO_4$ (Aldrich), $HClO_4$ (Reanal), EDTA (Acros Organics) and D_2O (Aldrich) were of the highest commercially available purity. The complexes [$PtCl_4$(bipy)], [$PtCl_4$(dach)] and [$PtCl_4$(en)] were

prepared according to published procedures.[33,34] All solutions were prepared in doubly distilled water.

The aqua complex, $[PtCl_3(H_2O)(en)]^+$, was prepared by the addition of 1 equivalent $AgClO_4$ into the solution of the complex $[PtCl_4(en)]$ in perchloric acid (1×10^{-3} M) at pH 3.0, under stirring in the dark at room temperature, for 2 h. The precipitated AgCl was filtered off using Milipore filters. The obtained aqua complex was used as proof for the hydrolysis of the $[PtCl_4(en)]$ complex.

Instrumentation

The UV-Vis spectra were recorded on Shimadzu UV 250 and Hewlett-Packard 8452A diode-array spectrophotometers with thermostated 1.00 cm quartz Suprasil cells. The kinetic measurements were performed on an Applied Photophysics SX.18MV stopped-flow instrument coupled to an on-line data acquisition system. Nuclear magnetic resonance (^{1}H NMR) spectra were acquired on a Varian Gemini-200 spectrometer at 295 K. All chemical shifts are referenced to trimethylsilylpropionic acid (TSP).

Kinetic measurements

The redox reactions were investigated by stopped-flow spectrophotometry at pH 2.0 (0.01 M $HClO_4$ solution) and at pH 7.2 (25 mM Hepes buffer) in the presence of 0.2 M NaCl. To determine the effect of chloride on the reduction rate, the same reactions were investigated at pH 2.0 in the absence of chloride. All kinetic measurements were performed by following the change in absorbance at suitable wavelengths as a function of time at 310 K. The selected wavelength for each system was determined by recording the

spectra of the reaction mixture over the wavelength range between 220 and 500 nm and is given in Tables S1–S9 (ESI). Reactions were initiated by mixing equal volumes of both solutions of the complex and reducing agent directly in the stopped-flow instrument and were followed for at least five half-lives of the reaction. Kinetic measurements were performed under *pseudo*-first order conditions (10-30 fold excess of the reducing agent). Higher concentrations of the reducing agent could not be used due to differences observed in the recorded spectra that pointed to a more complex reaction sequence (see Fig. S16, ESI). The *pseudo*-first order rate constants, k_{obsd}, were calculated as the average value from five to seven independent kinetic runs. Experimental data are reported in Tables S1–S9 (ESI).

It is known that molecules that contain thiol groups are sensitive to autoxidation catalyzed by metal ions such as Cu(II) and Fe(III).[35] The rate of autoxidation increases with increasing pH.[23] In order to check whether autoxidation had an effect on the present kinetic measurements, control experiments were conducted for the reactions of the $[PtCl_4(bipy)]$ complex with glutathione at pH 7.2. In these experiments, both the complex and glutathione solutions contained 0.5 mM EDTA in order to eliminate the possible catalytic effect of any traces of metal ions. Before the reactions were initiated in the stopped-flow instrument, solutions of the $[PtCl_4(bipy)]$ complex and glutathione were flushed with nitrogen for at least 30 min in order to remove dissolved oxygen. No significant discrepancy between the values of k_{obsd} obtained in these experiments and those performed in air-saturated solutions was observed (see data in Table S7, ESI). This finding is in agreement with the results reported by Elding *et al*.[23] For the calculation of the kinetic data, Microsoft Excel and Origin 6.1 programs were used.

Conclusion

The present results show that the reactivity of the studied complexes follows the order $[PtCl_4(bipy)] > [PtCl_4(dach)] > [PtCl_4(en)]$. The reactivity depends of the structure of the spectator ligands, because bulky inert equatorial ligands destabilize six-coordinated Pt(IV) complexes and enable faster reduction to the corresponding Pt(II) complex. The obtained order of reactivity of the investigated ligands is GSH > L-Cys > L-Met. L-Met is a less powerful reductant than the thiols. The steric hindrance of the methyl group could be a further reason for the lower reactivity.

The rate of reduction strongly depends on the acidity of the solution. At higher pH values, the process is much faster due to the easier formation of the bridged intermediate with the deprotonated form of the thiols. In addition, the thioether converts to the sulfoxide faster in neutral than in acidic medium. At pH 7.2, hydrolysis of the starting complex occurs and the formed aqua complex, $[PtCl_3(H_2O)(N\text{-}N)]^+$, reacts with chloride ions forming $[PtCl_4(N\text{-}N)]$. Furthermore, an increase in chloride concentration leads to increasing of intercepts, due to the facilitated formation of a bridge between Pt(IV) and Pt(II) complexes. All these results confirm the fact that the reduction of complexes Pt(IV) involves a two-electron transfer process and the loss of axial ligands to form the corresponding square-planar Pt(II) complex.

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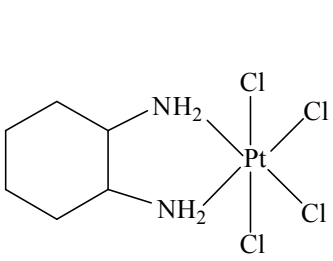

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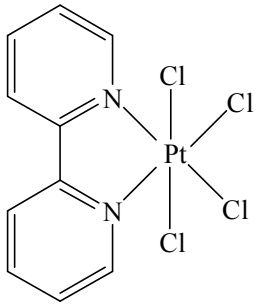

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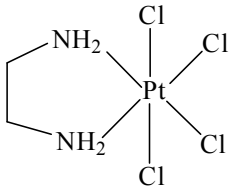

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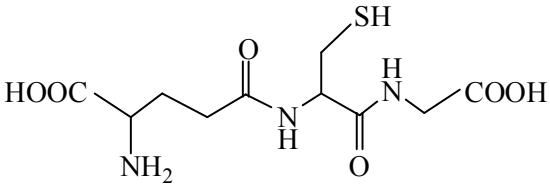

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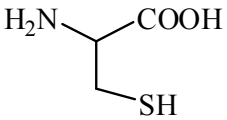

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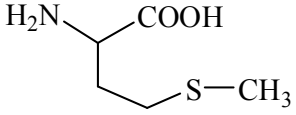

L-Methionine (L-Met)

Fig. 1 Schematic structures of the investigated Pt(IV) complexes and reducing agents.

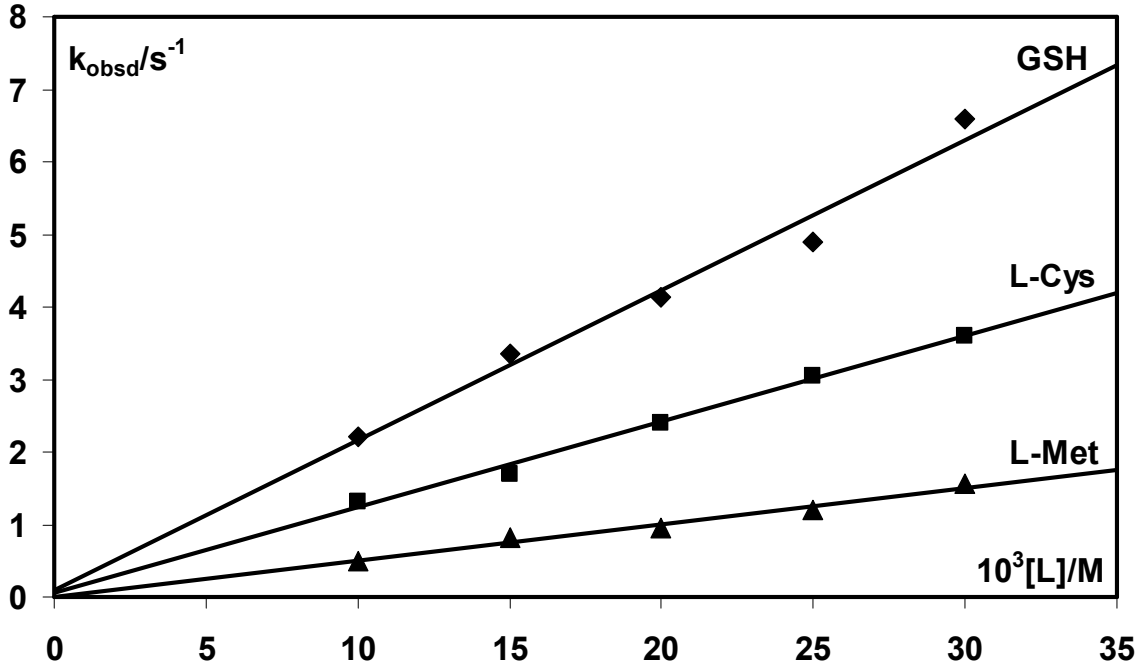

Fig. 2 *Pseudo*-first order rate constants plotted as a function of reducing agent (L) concentration for the reactions of the $[PtCl_4(bipy)]$ complex at pH 2.0 (0.01 M $HClO_4$) in the presence of 0.2 M NaCl at 310 K.

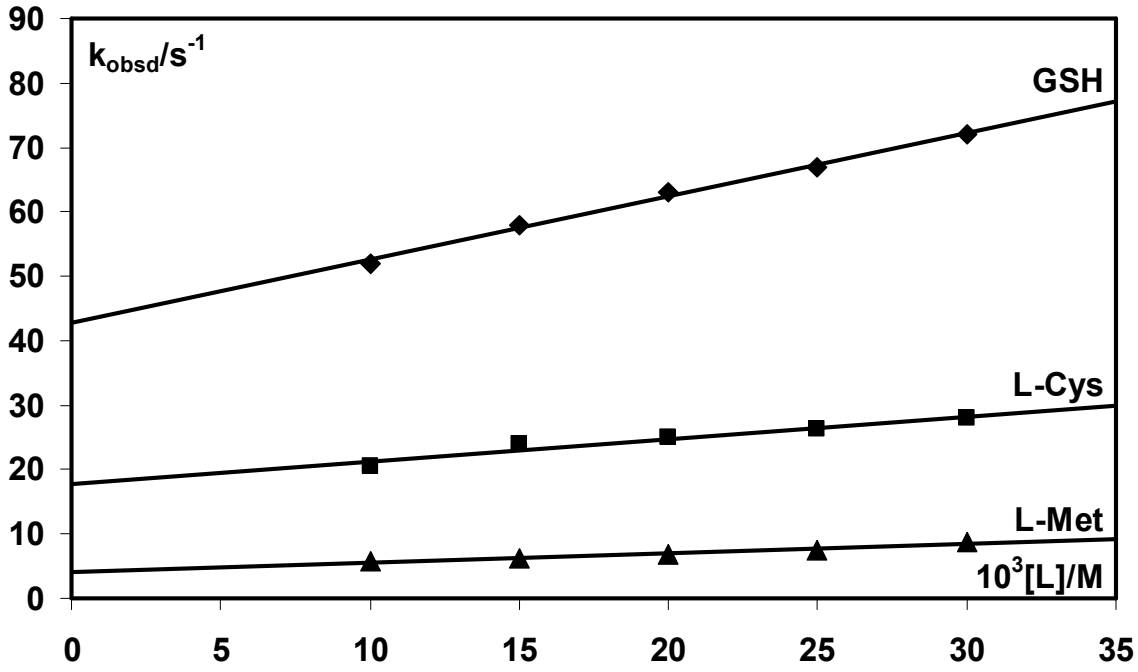

Fig. 3 *Pseudo*-first order rate constants plotted as a function of reducing agent (L) concentration for the reactions of the $[PtCl_4(bipy)]$ complex at pH 7.2 (25 mM Hepes buffer) in the presence of 0.2 M NaCl at 310 K.

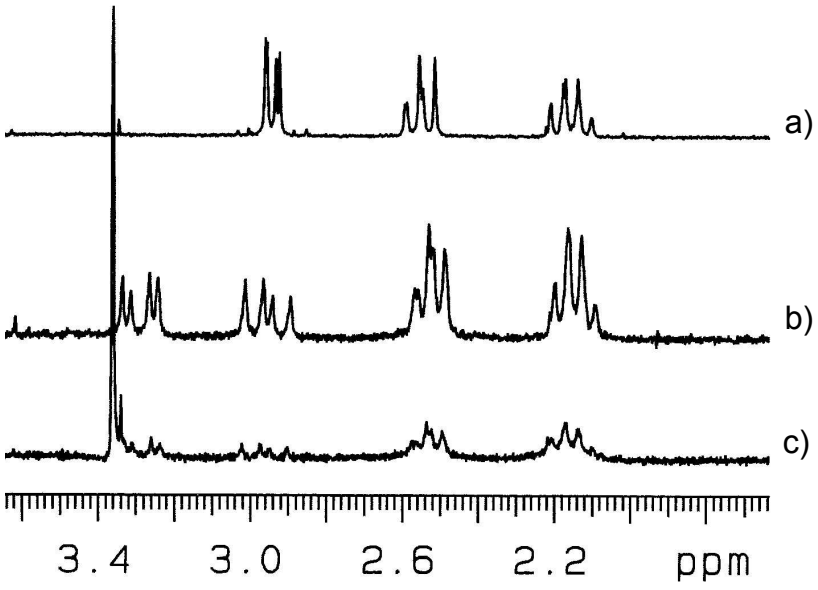

Fig. 4 ^{1}H NMR spectra recorded in D_2O at 295 K and pD 6.4 (pD = pH + 0.4) of a) 4 mM GSH, b) 2 mM oxidized glutathione (GSSG), c) the final spectrum of the reaction between 2 mM [$PtCl_4$(en)] and 4 mM GSH.

Table 1 Second-order rate constants for the reactions of the selected Pt(IV) complexes with GSH, L-Cys and L-Met at different pH at 310 K.

Complex	pH	GSH	L-Cys	L-Met
		$k_2/M^{-1}s^{-1}$		
$[PtCl_4(bipy)]$	2.0[a]	210 ± 20	118 ± 6	50 ± 4
	2.0[b]	190 ± 20	107 ± 5	48 ± 5
	7.2[c]	980 ± 40	350 ± 50	150 ± 20
	7.2[d]	10 ± 1	-	-
$[PtCl_4(dach)]$	2.0[a]	62 ± 3	57 ± 3	39 ± 3
	2.0[b]	60 ± 3	54 ± 1	35 ± 2
	7.2[c]	920 ± 70	340 ± 40	134 ± 5
	7.2[d]	1.5 ± 0.5	-	-
$[PtCl_4(en)]$	2.0[a]	31 ± 2	20 ± 1	14 ± 1
	2.0[b]	30 ± 2	19 ± 2	13 ± 1
	7.2[c]	870 ± 90	330 ± 40	130 ± 20
	7.2[d]	1.1 ± 0.1	-	-

[a] 0.01 M $HClO_4$ in the presence 0.2 M NaCl; [b] 0.01 M $HClO_4$ in the absence of NaCl; [c] 25 mM Hepes buffer in the presence 0.2 M NaCl; [d] 25 mM Hepes buffer in the absence of NaCl

Table 2 The values of the rate constants k_1 and k_2 for the reactions between the Pt(IV) complexes and glutathione (Scheme 2) in the presence of different concentrations of chloride at pH 7.2 and 310 K.

	$[Cl^-]$/M	k_1/s^{-1}	$k_2/M^{-1} s^{-1}$
$[PtCl_4(bipy)]$	In the absence of NaCl	0.51 ± 0.02	10 ± 1
	With 0.02	4.9 ± 0.2	114 ± 9
	With 0.05	10.8 ± 0.5	230 ± 40
	With 0.10	17.7 ± 0.5	450 ± 20
	With 0.15	25 ± 2	760 ± 80
	With 0.20	42.8 ± 0.5	980 ± 40
$[PtCl_4(dach)]$	In the absence of NaCl	0.038 ± 0.005	1.5 ± 0.5
	With 0.02	3.7 ± 0.2	91 ± 7
	With 0.20	35.2 ± 0.5	920 ± 70
$[PtCl_4(en)]$	In the absence of NaCl	0.0034 ± 0.0005	1.1 ± 0.1
	With 0.02	2.8 ± 0.1	87 ± 8
	With 0.20	27 ± 2	870 ± 90

Graphical Contents Entry

Reduction of some Pt(IV) complexes with biologically important sulfur-donor ligands

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The reduction of the Pt(IV) complexes [$PtCl_4$(bipy)], [$PtCl_4$(dach)] and [$PtCl_4$(en)] by glutathione (GSH), L-cysteine (L-Cys) and L-methionine (L-Met) was investigated by stopped-flow spectrophotometry at pH 2.0 (in 0.01 M perchloric acid) and at pH 7.2 (in 25 mM Hepes buffer).