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ORIGINAL PAPER

Equilibrium studies on complex formation reactions of dichlorido[(*R*,*R*)-*trans*-1,2-diaminocyclohexane]platinum(II) complex with ligands of biological significance

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Abstract The hydrolysis and complex formation equilibria of $[Pt(dach)(H_2O)_2]^{2+}$, where dach is (*R*,*R*)-*trans*-1,2-diaminocyclohexane, with some sulfur- and nitrogen-bonding ligands, such as L-methionine, glutathione, inosine, inosine-5′-monophosphate, and guanosine-5′-monophosphate, were studied in aqueous 0.10 M $NaClO_4$ solution at 298 K by potentiometric titrations. The experimentally determined pK_a values for the studied diaqua complex were 6.00 and 10.03, respectively. The acid dissociation constants of the ligands were also determined. The stoichiometry and stability constants of the formed complexes are reported, as well as the concentration distribution of the various complex species evaluated as a function of pH. In all studied systems, species with one coordinated molecule of ligand were detected. However, only in systems with L-methionine and inosine, complexes with two molecules of ligand directly coordinated to the Pt(II) ion were found. The results also show that glutathione formed the most stable complexes. These results could contribute to better understanding of the interactions between Pt(II) complexes and biologically significant molecules.

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Introduction

Successive generations of platinum-based anticancer drugs (cisplatin, carboplatin, and oxaliplatin) have demonstrated that metal coordination complexes can play an important role in anticancer treatment [1–8]. The antitumor activity of cisplatin or carboplatin is ascribed to interactions between the complex and DNA [1–11]. However, full understanding of the mode of action of platinum-based antitumor drugs requires detailed study of their interaction with all possible biological targets including amino acids, hormones, peptides, and proteins.

Today it is not clear how the platinum(II) species reaches DNA, because the Pt(II) ion has high affinity for sulfur donors. Cysteine–platinum products are quite inert [10–12], whereas L-methionine bonded to platinum can be replaced by thiols or nucleobases [13, 14], forming thermodynamically more stable products [15–19]. This assumption is supported by different experiments carried out with models of cisplatin [10, 14–17, 20–23].

Oxaliplatin produces a similar type of DNA adduct as cisplatin and carboplatin, but oxaliplatin–DNA products contain rather 1,2-$[Pt(dach)]^{2+}$-d(GpG). In addition, oxaliplatin forms a hydrogen bond between an NH_2 group of the dach ligand and the O6 atom of the guanine base. This bond interaction can be formed only with the biologically active (*R*,*R*)-isomer of oxaliplatin. So, these conformational differences between oxaliplatin and cisplatin adducts of DNA may be responsible for their different behavior in protein recognition and cellular processing of platinum antitumor compounds [6, 9, 24].

The mechanism of cellular oxaliplatin accumulation is still unclear. During metabolism, the oxalato leaving group is left unchanged. Also, incorporation of different groups at positions close to the metal center or even at the coordinated nitrogen atoms of amine groups would significantly influence the structure of DNA adducts [25].

Platinum complexes undergo hydrolysis under physiological conditions. Thus, study of the interactions between $[Pt(dach)(H_2O)_2]^{2+}$ complex with nitrogen- and sulfur-bonding ligands could provide better understanding of the specific and selective role of metal ions in biological systems.

In this work we studied the complex formation between $[Pt(dach)(H_2O)_2]^{2+}$, where dach is (*R*,*R*)-*trans*-1,2-diaminocyclohexane, and some sulfur- and nitrogen-bonding ligands, such as L-methionine (L-Met), glutathione (GSH), inosine (Ino), inosine-5′-monophosphate (5′-IMP), and guanosine-5′-monophosphate (5′-GMP) in aqueous solution by potentiometric measurements. This set of nucleophiles was selected because of their differences in nucleophilicity, steric hindrance, binding properties, and biological relevance. The structures of the investigated complex and nucleophiles are given in Fig. 1.

Results and discussion

The speciation in the three-component system [Pt(dach)-$(H_2O)_2]^{2+}$–H^+ (or OH^-)–ligand (L-Met, GSH, Ino, 5′-IMP, and 5′-GMP), generally denoted $M_pH_qL_r$, for convenience is denoted by three stoichiometric coefficients (*p*,*q*,*r*) given in the order M, H, L. At the beginning of our work it was necessary first to characterize the binary equilibria, i.e., hydrolysis of $[Pt(dach)(H_2O)_2]^{2+}$ and protonation of ligands under exactly the same experimental conditions as for the complexation study.

Hydrolysis of $[Pt(dach)(H_2O)_2]^{2+}$

The acid–base equilibrium for the system $[Pt(dach)(H_2O)_2]^{2+}$ was characterized by fitting the potentiometric titration data to

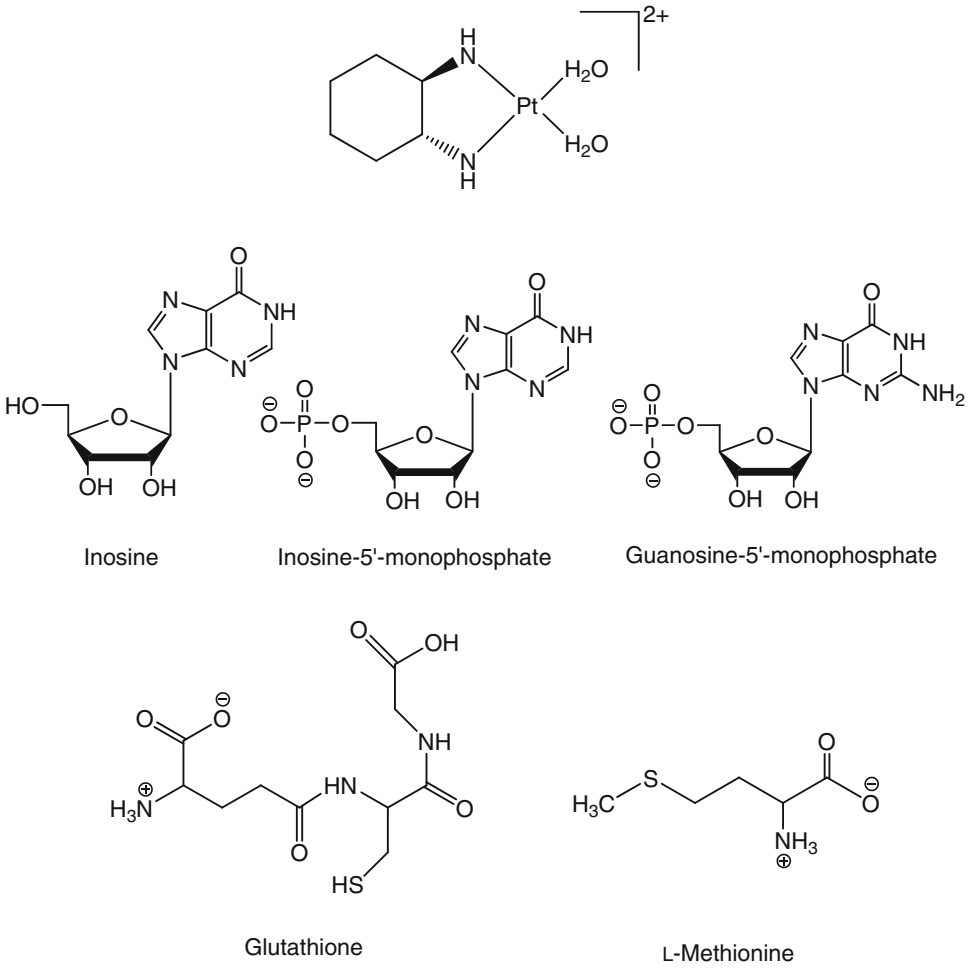

Fig. 1 Structures of the investigated complex and nucleophiles

various acid–base models. The studied complex undergoes stepwise deprotonation of two coordinated water molecules, as given in Eqs. (1) and (2).

$$\underset{(1,0,0)}{\left[\mathrm{Pt(dach)(H_2O)_2}\right]^{2+}} \rightleftharpoons \underset{(1,-1,0)}{\left[\mathrm{Pt(dach)(H_2O)(OH)}\right]^{+}} + \mathrm{H^+} \quad (1)$$

$$\underset{(1,-1,0)}{\left[\mathrm{Pt(dach)(H_2O)(OH)}\right]^{+}} \rightleftharpoons \underset{(1,-2,0)}{\left[\mathrm{Pt(dach)(OH)_2}\right]} + \mathrm{H^+} \quad (2)$$

The experimentally determined pK_a values of coordinated water molecules were 6.00 and 10.03, respectively. The distribution diagram of species is shown in Fig. 2.

The diaqua complex (1,0,0) is a dominant species in the pH range between 2 and 5. Further increase of pH moves equilibrium (1) to the right-hand side. At pH around 6, the concentrations of the (1,0,0) and (1,−1,0) species are almost the same, while at physiological pH value the $[Pt(dach)(H_2O)_2]^{2+}$ complex exists mostly as monohydroxo complex (1,−1,0). The dihydroxo complex (1,−2,0) starts to form at pH around 8. The calculated values for the stability constants, given in Table 1, confirm the greater stability of monohydroxo complex than dihydroxo. However, a dinuclear μ-hydroxido complex (2,−1,0) has been detected at pH 6. The formation of the dinuclear complex is described by Eq. (3).

$$\underset{(1,0,0)}{\left[\mathrm{Pt(dach)(H_2O)_2}\right]^{2+}} + \underset{(1,-1,0)}{\left[\mathrm{Pt(dach)(H_2O)(OH)}\right]^{+}} \rightleftharpoons \underset{(2,-1,0)}{\left[\mathrm{Pt_2(dach)_2(H_2O)_2(OH)}\right]^{3+}} + \mathrm{H_2O} \quad (3)$$

The equilibrium constant for the dimerization reaction is calculated by Eq. (4) and amounts to 2.24.

$$\log K_{\mathrm{dimer}} = \log \beta_{2-10} - \log \beta_{1-10} \quad (4)$$

The pK_a values of coordinated water molecules could be a very good indicator for the electron density around the metal center. Comparing pK_a values for the diaqua form of cisplatin $[Pt(NH_3)_2(H_2O)_2]^{2+}$ (pK_{a1} = 5.37, pK_{a2} = 7.21) [26] and $[Pt(en)(H_2O)_2]^{2+}$ (en = 1,2-diaminoethane) (pK_{a1} = 5.97, pK_{a2} = 7.47) [26] with those for $[Pt(dach)(H_2O)_2]^{2+}$ shows that decreasing of σ-donor ability of the inert ligand decreases the pK_a value of the complex. Also, the incorporation of the inert ligand with pyridine rings, as in the case of $[Pt(bipy)(H_2O)_2]^{2+}$ (bipy = 2,2′-bipyridine) (pK_{a1} = 4.80, pK_{a2} = 6.32) [26], decreases the electron density around the metal center and strongly increases its acidity. However, the pK_a values of $[Pt(dien)(H_2O)]^{+}$ (dien = 3-azapentane-1, 5-diamine) (pK_a = 6.94) [27], $[Pt(terpy)(H_2O)]^{+}$ [terpy = 2,6-bis(2-pyridyl)pyridine] (pK_a = 4.42) [28], and $[Pt(SMC)(H_2O)_2]^{+}$ (SMC = *S*-methyl-L-cysteine) (pK_{a1} = 3.49) [29] show that the presence of ligands with a strong *trans*-effect leads to an increase in acidity. Generally, the structure of the inert ligand has a strong influence on the acid–base properties of Pt(II) complexes.

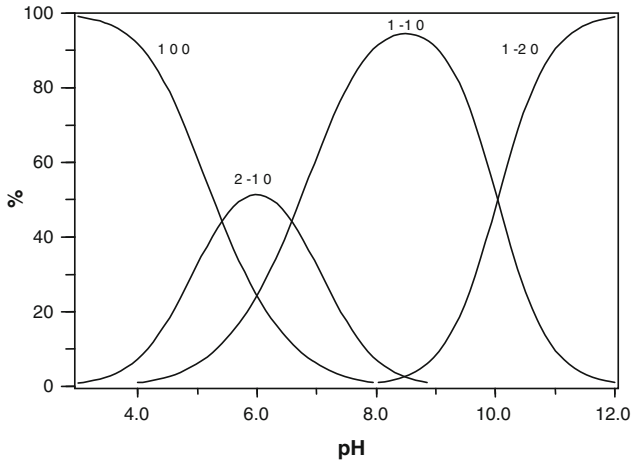

Fig. 2 Distribution of $[Pt(dach)(H_2O)_2]^{2+}$ hydrolytic species in 0.1 mol dm^{-3} $NaClO_4$ ionic medium at 298 K; $c^{2+}_{[Pt(dach)(H2O)2]}$ = 2.50 mmol dm^{-3}

Protonation of ligands (L)

Protonation constants, β_n, of the ligands (L = L-Met, GSH, Ino, 5′-IMP, and 5′-GMP) are defined according to the equilibrium

$$n\mathrm{H} + \mathrm{L} \rightarrow \mathrm{H}_n\mathrm{L};\ \beta_n (n = 1, 2, 3, 4). \quad (5)$$

Glass electrode potentiometric titrations were carried out with 1.0, 2.5, and 5.0 mM total ligand concentrations in the pH range between 2.0 and 10.5. The calculated values of protonation constants are given in Table 1.

Complex formation equilibrium of $[Pt(dach)(H_2O)_2]^{2+}$ with L-Met

A representative graph for the change of pH under titration of the system with different molar ratios of $[Pt(dach)(H_2O)_2]^{2+}$ to L-Met (1:1 and 1:2) is shown in Fig. 3.

Data were evaluated with the aid of the program Hyperquad2006. The finally accepted set of complexes is given in Table 1 together with the statistical parameters which determine the quality of fit.

The distribution diagram of species in the $[Pt(dach)(H_2O)_2]^{2+}$–L-Met system is shown in Fig. 4. The dominant complex at lower pH is the (1,1,1) complex. Upon increasing the pH, complex (1,1,1) releases a proton, giving the complex (1,0,1) with a maximum concentration at pH 6.5. At higher pH values in the system, complex (1,0,2) is present, with a maximum in concentration at pH around 11.5. The hydrolytic complex (1,−1,0) forms in a very small amount at pH 10. The complex (1,−2,0) begins to form at pH 9, and its concentration increases with further increase of pH.

The pK_a values of protonated L-Met coordinated to Pt(II) is 3.12 ($\log \beta_{111} - \log \beta_{101}$). This value corresponds to

Table 1 Stability constants of $[Pt(dach)(H_2O)_2]^{2+}$–L complexes formed in 0.1 mol/dm^3 $NaClO_4$ ionic medium at 298 K

log $\beta_{p,q,r} \pm \sigma$						
Species (p,q,r)[a]	$[Pt(dach)(H_2O)_2]^{2+}$					
		L-Met	GSH	Ino	5′-IMP	5′-GMP
(1,−1,0)	−6.00(2)					
(1,−2,0)	−16.03(4)					
(2,−1,0)	−3.76(6)					
(0,1,1)		8.89(1)	9.73(5)	8.68(6)	9.14(5)	9.57(7)
(0,2,1)		10.81(5)	18.06(7)	–	15.25(3)	15.73(5)
(0,3,1)		–	21.58(9)	–	16.33(8)	18.06(6)
(0,4,1)		–	23.68(9)	–	–	–
(1,0,1)		9.75(2)	12.25(2)	6.94(6)	7.70(6)	7.66(2)
(1,1,1)		12.87(9)	22.11(9)	14.36(9)	16.95(5)	16.67(3)
(1,2,1)		–	25.55(14)	–	23.64(7)	22.96(4)
(1,0,2)		13.94(7)	–	14.58(12)	–	–
(1,1,2)		–	–	22.04(12)	–	–
Statistics	$\chi^2 = 12.01$	$\chi^2 = 13.03$	$\chi^2 = 13.05$	$\chi^2 = 11.06$	$\chi^2 = 11.25$	$\chi^2 = 12.78$
	$s = 1.10$	$s = 1.68$	$s = 1.15$	$s = 1.80$	$s = 1.35$	$s = 1.76$

[a] p, q, and r are the stoichiometric coefficients corresponding to $[Pt(dach)(H_2O)_2]^{2+}$, H^+, and ligand, respectively

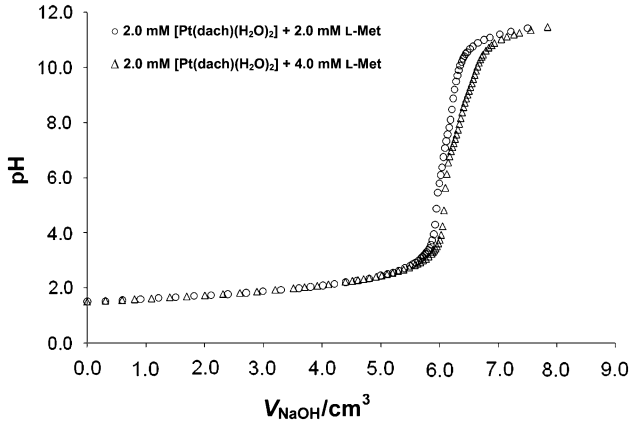

Fig. 3 Potentiometric titration of $[Pt(dach)(H_2O)_2]^{2+}$–L-Met solutions with standard NaOH in 0.1 mol dm^{-3} $NaClO_4$ ionic medium at 298 K. The concentration in mmol dm^{-3} is denoted as mM

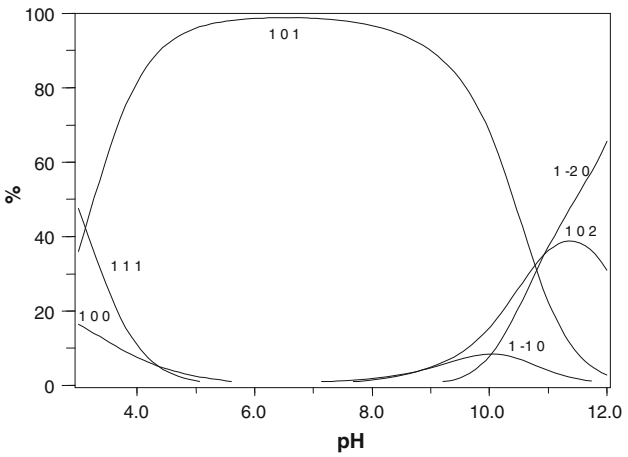

Fig. 4 Distribution diagram of $[Pt(dach)(H_2O)_2]^{2+}$–L-Met species at ligand-to-metal concentration ratio of 1:1 and total $[Pt(dach)(H_2O)_2]^{2+}$ concentration of 2.0 mmol dm^{-3}

deprotonation of the –COOH group of the amino acid. In addition, the pK_a values of L-Met increase significantly upon coordination to Pt(II) ion [26]. The acid dissociation constants determined for free L-Met are pK_{a1} = 1.92 and pK_{a2} = 8.89.

However, the coordination to Pt(II) occurs via the sulfur atom of the thioether group of the amino acid. In the studied system, two major types of complexes can be mentioned: one, at lower pH, where there is just one molecule of amino acid in the coordination sphere of the complex, and the other at higher pH, with two molecules of L-Met. Previously published results confirm that L-Met could form a six-membered *S*,*N*-chelate with the studied complex [30]. So, the coordination via the nitrogen atom from an amine group is more efficient at lower proton concentration in the solution, although pK_{a2} has the greater value [26].

Complex formation equilibria of $[Pt(dach)(H_2O)_2]^{2+}$ with GSH

The system consisting of $[Pt(dach)(H_2O)_2]^{2+}$ complex and GSH in 0.1 M $NaClO_4$ medium was titrated with standard NaOH solution at 25 °C. To find the model that gives the best fit to the experimental data, various complexes and combinations were included in Hyperquad2006 calculations. The final accepted sets of complexes are given in Table 1.

The distribution diagram of species in the $[Pt(dach)(H_2O)_2]^{2+}$–GSH system is shown in Fig. 5. In acidic medium the dominant species is the (1,2,1) complex with the maximum in concentration at pH lower than 3. The complex (1,1,1) is present in the pH region between 2.0 and

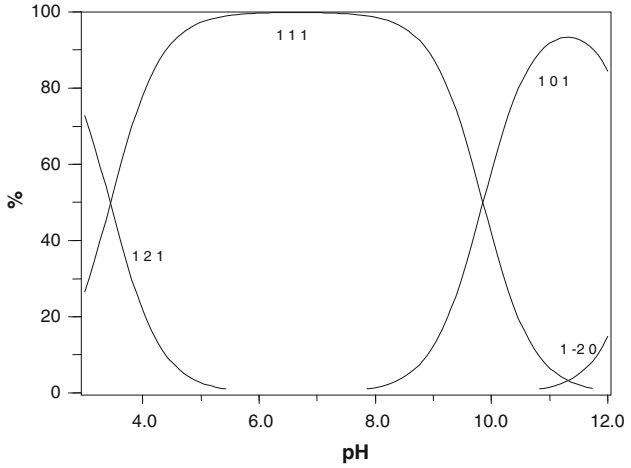

Fig. 5 Distribution diagram of $[Pt(dach)(H_2O)_2]^{2+}$–GSH species at ligand-to-metal concentration ratio of 1:1 and total $[Pt(dach)(H_2O)_2]^{2+}$ concentration of 2.0 mmol dm^{-3}

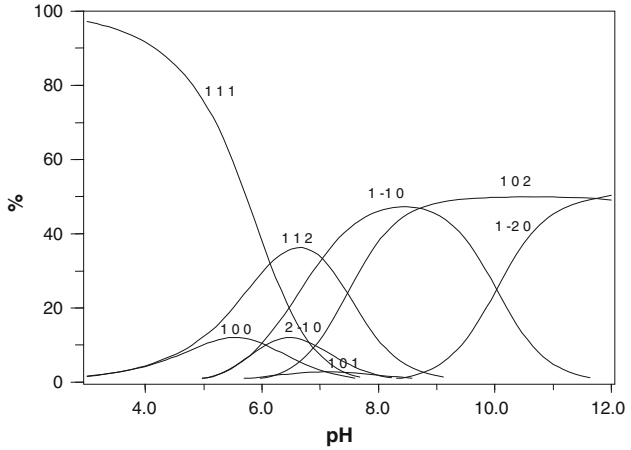

Fig. 6 Distribution diagram of $[Pt(dach)(H_2O)_2]^{2+}$–Ino species at ligand-to-metal concentration ratio of 1:1 and total $[Pt(dach)(H_2O)_2]^{2+}$ concentration of 2.5 mmol dm^{-3}

12.0, with a maximum in concentration at pH 7. The species (1,0,1) begins to form at pH 8 and has a maximum in concentration at pH about 11. However, in very alkaline medium the hydrolytic complex (1,−2,0) has been detected in very small concentration.

It is well known that Pt(II) has high affinity toward sulfur donor ligands, forming very stable products. In addition, comparing the values of the stability constants of (1,1,1) species for all studied systems, given in Table 1, it can be seen that log β for the system $[Pt(dach)(H_2O)_2]^{2+}$–GSH has the highest value. The higher stability of the GSH adduct can be attributed to the electronic characteristics of the metal ion and ligand (soft acid–soft base) as well as the presence of intramolecular hydrogen bonds which further stabilize a product of the substitution.

Pt(II) has high affinity for binding to sulfur donors, especially thiols, compared with nitrogen donor ligands such as DNA bases [19, 31]. The result of the reaction between Pt(II) complexes and GSH is formation of thermodynamically very stable products which could inactivate some of the Pt(II) complexes. The huge stability of the $[Pt(dach)(H_2O)_2]^{2+}$–GSH product contributes to an explanation for competitive reaction of substitution of thioethers (L-Met) from the Pt(II)-S(thioether) complex by GSH [17, 32].

Complex formation equilibria of $[Pt(dach)(H_2O)_2]^{2+}$ with Ino

The calculated values for stability constants of the formed species in the $[Pt(dach)(H_2O)_2]^{2+}$–Ino system are given in Table 1, whereas the distribution diagram is shown in Fig. 6. In acidic medium the dominant species is the (1,1,1) complex. In the pH range between 4 and 8, a few species such as (1,1,2), (1,0,0), (2,−1,0), and a very small amount of (1,0,1) could be seen. The complex (1,1,2) is dominant at pH 6.5 (about 40%). At pH 8 there are two species present: (1,−1,0) and (1,0,2). The complex (1,−2,0) begins to form at pH 8, and its concentration increases with further increase of pH.

It is well known that purine constituents of DNA have two metal ion binding sites, N1 and N7 atoms [1–7]. The pH dependence for binding of DNA subunits to Pt(II) complexes has been reported before [33]. Our results clearly show the composition of the complexes for different pH values. At physiological pH the dominant species in the system are (1,1,2) and (1,−1,0). In the complex (1,1,2), two molecules of Ino are coordinated to the Pt(II) center. The other is the monohydroxo complex $[Pt(dach)(H_2O)(OH)]^{+}$.

Generally, all complex species found in this system are unstable, considering and comparing the stability constant values in Table 1.

Complex formation equilibria of $[Pt(dach)(H_2O)_2]^{2+}$ with 5′-IMP

The accepted results of calculation for the $[Pt(dach)(H_2O)_2]^{2+}$–5′-IMP system are also given in Table 1, while the distribution diagram of species is shown in Fig. 7.

In acidic medium the predominant species is the (1,2,1) complex. This complex, upon increasing the pH, gives the (1,1,1) complex with a maximum in concentration at pH around 8 (about 90%). Also, at higher pH, three different species are present in the system: (1,0,1) dominant in the pH range between 9 and 11, (1,−1,0) in a very small concentration at pH around 10, and (1,−2,0) dominant at pH higher than 11.

5′-IMP could coordinate to the Pt(II) complex via N1 and N7 atoms [1–7]. The binding via N7 position is verified

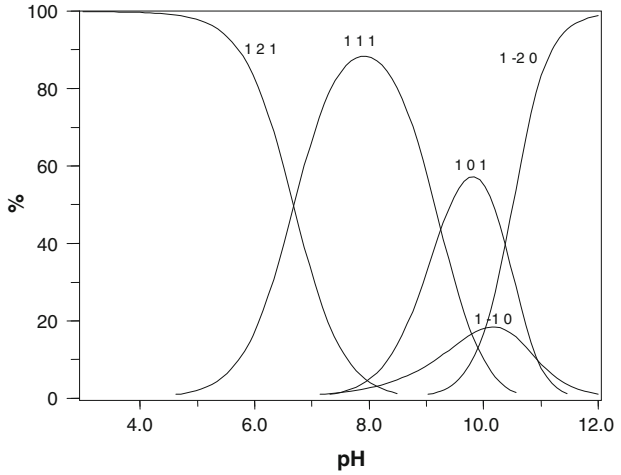

Fig. 7 Distribution diagram of $[Pt(dach)(H_2O)_2]^{2+}$–5′-IMP species at ligand-to-metal concentration ratio of 1:1 and total $[Pt(dach)(H_2O)_2]^{2+}$ concentration of 2.0 mmol dm^{-3}

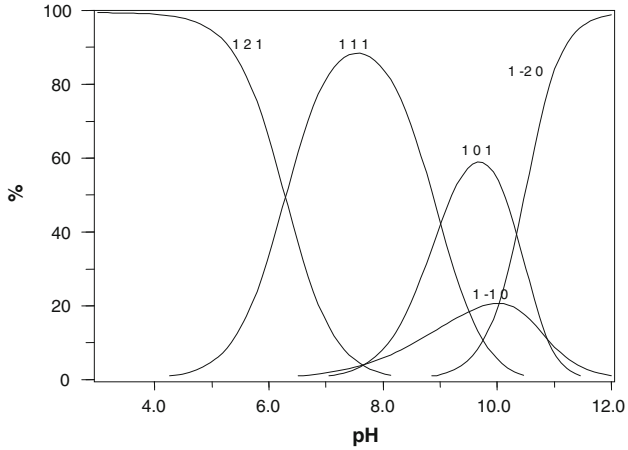

Fig. 8 Distribution diagram of $[Pt(dach)(H_2O)_2]^{2+}$–5′-GMP species at ligand-to-metal concentration ratio of 1:1 and total $[Pt(dach)(H_2O)_2]^{2+}$ concentration of 2.0 mmol dm^{-3}

in neutral and acidic medium, because at low pH values N1 is still protonated [34, 35]. At physiological pH the (1,1,1) complex was found, what is in agreement with previously reported results [30]. Also, the formed complexes are more stable than complexes with Ino. This could be explained based on the presence of well-known noncovalent interactions which stabilize the product. On the other hand, in this system the complex with two molecules of base is not detected, as was the case in the system with Ino.

Complex formation equilibria of $[Pt(dach)(H_2O)_2]^{2+}$ with 5′-GMP

The results of calculation for the $[Pt(dach)(H_2O)_2]^{2+}$–5′-GMP system are given in Table 1. The distribution diagram of species in this system is shown in Fig. 8.

This diagram is very similar to the diagram observed for the $[Pt(dach)(H_2O)_2]^{+}$–5′-IMP system. In acidic medium the dominant species is (1,2,1). In the pH range between 4 and 9, (1,1,1) is found, having maximum concentration at pH around 7.5 (90%). Also, at pH values between 9 and 10, the (1,0,1) complex and a small amount of (1,−1,0) complex are formed. At pH higher than 10 there is just (1,−2,0) species.

The obtained diagram is almost the same as we found for the $[Pt(dach)(H_2O)_2]^{+}$–5′-IMP system. This similarity is confirmed by the fact that 5′-GMP and 5′-IMP have a slight difference in structure as well as forming the same type of product with Pt(II) complexes with almost the same stability constants. Here, (1,0,2) or (1,1,2) types of complexes are not detected. Recently published results show that some *trans*-diammine Pt(II) complexes can form the bis-adduct with 5′-GMP by photoactivation [36].

Conclusions

We present results for the composition and stability constants of the products formed in the reaction between the $[Pt(dach)(H_2O)_2]^{+}$ complex and selected biologically relevant nucleophiles at different pH values. The bifunctional Pt(II) complex in the reaction with sulfur donor ligands (L-Met and GSH) forms very stable products, especially with the tripeptide GSH. The complexes found in the reactions between $[Pt(dach)(H_2O)_2]^{+}$ and purine constituents of DNA show great similarities in composition, and the difference in their stability is negligible. Also, under the selected conditions in all studied systems, substitution occurs. So, these results could be a good supplement to previously published results of the kinetics of the substitution reactions between oxaliplatin and the same biologically relevant nucleophiles [26]. Finally, knowledge of the composition and stability of the species, especially at physiological pH, could contribute to better understanding of some interactions present in biological systems.

Experimental

Materials

The complex $[PtCl_2(dach)]$ was prepared according to the literature method [37]. Chemical analysis, ultraviolet–visible (UV–Vis), and ^1H nuclear magnetic resonance (NMR) spectra were in good agreement with previously obtained data. The chlorido complex was converted into the aqua analog $[Pt(dach)(H_2O)_2]^{2+}$ in solution by addition of two equivalents of $AgClO_4$, heating to 50–60 °C for 1 h, and removing the precipitated AgCl by filtration through a 0.10-μm-pore membrane filter. Great care was taken to

ensure that the resulting solution was free of Ag^+ ions and that the chlorido complex had been converted completely into the aqua species. Since it is known that perchlorate ions do not coordinate to Pt(II) and Pd(II) in aqueous solution [38], the equilibria and complex-formation reactions were studied in perchlorate medium.

Ligand stock solutions were prepared shortly before use by dissolving the chemicals L-methionine (Fluka, assay >99%), guanosine-5′-monophosphate sodium salt hydrate (Sigma-Aldrich), inosine (Sigma-Aldrich), inosine-5′-monophosphate (Sigma-Aldrich), and glutathione (Accros Organics) in purified, deionized water. The other reagents were of analytical reagent grade.

The ionic strength of the solutions was adjusted to 0.10 M using $NaClO_4$ (Merck, p.a.). The salt was dissolved in double-distilled water. A known volume of this solution was evaporated to dryness for calculation of the real concentration of the solution.

The pH of the solutions was adjusted using $HClO_4$ and NaOH. The sodium hydroxide solution was prepared from concentrated volumetric solutions (Merck, p.a.) by diluting with freshly boiled double-distilled water, cooled under constant flow of purified nitrogen. The alkali concentration was checked by titration against potassium hydrogenphthalate. For the preparation of perchloric acid solution, $HClO_4$ (Merck, "Suprapure", p.a.) was used. The concentration of the resulting solution was determined by potentiometric titration against tris(hydroxymethyl)aminomethane. The concentration of $HClO_4$ solution was 0.1028 M, and the concentration of NaOH solution was 0.1000 M.

Nitrogen gas, used for stirring solutions and providing an inert atmosphere during the titrations, was purified by passing it through 10% NaOH, then 10% H_2SO_4, and finally distilled water.

Apparatus

Chemical analyses were performed on a Carlo Erba elemental analyzer 1106. UV–Vis spectra were recorded using a PerkinElmer Lamda 35 spectrophotometer, while NMR spectra were recorded on a Varian Gemini 200 MHz. Potentiometric measurements were made on a Hanna Instruments HI9017 microprocessor pH meter equipped with a Radiometer combined electrode. A Metrohm Dosimat model 665 automatic burette with antidiffusion tip was used for delivery of the titrant.

Potentiometric measurements

Potentiometric titrations were carried out on the solutions in a double-walled glass vessel at 25.0 ± 0.1 °C. A magnetic stirrer was used, and purified nitrogen was bubbled through the solution during titrations. The test solution was titrated with standard CO_2-free NaOH. The ionic strength of all test solutions was adjusted to 0.1 M using $NaClO_4$.

To reduce the concentration of the hydrogen ion, the alkali was added stepwise from an auto burette in small aliquots (0.005–0.1 cm^3). The potential was monitored after each addition of titrant. The concentration of free hydrogen ion, h, at each point of the titration is related to the measured electromotive force, E^0, of the cell by the Nernst equation

$$E = E^0 + Q\log h + E_j, \tag{6}$$

where E^0 is a constant which includes the standard potential of the glass electrode, Q is the slope of the glass electrode response, and E_j is the liquid junction potential. The parameters E_0, Q, and E_j were determined by strong acid–strong base titration to check the system suitability. The value of E^0 for the electrode was determined from a Gran's plot derived from a separate titration of perchloric acid with a standard NaOH solution under the same temperature and medium conditions. The water autoprotolysis constant was taken as $pK_w = 13.78$.

The solutions titrated can be presented according to the following scheme: (a) $HClO_4$ (10 mM) + (L = L-Met, GSH, Ino, 5′-IMP, and 5′-GMP) (2.5 and 5.0 mM); (b) $HClO_4$ (10 mM) + $[Pt(dach)(H_2O)_2]^{2+}$ (1.0, 1.5, 2.5, and 5.0 mM); (c) $HClO_4$ (10 mM) + $[Pt(dach)(H_2O)_2]^{2+}$ (2.0 and 2.5 mM) + (L = L-Met, GSH, Ino, 5′-IMP, and 5′-GMP) (2.0, 4.0, 2.5, and 5.0 mM). A constant ionic strength was adjusted to 0.10 M by $NaClO_4$, and the total volume was kept constant at 20 cm^3.

Data treatment

The species formed in the studied systems could be characterized by the general equilibrium

$$p\left[Pt(dach)(H_2O)_2\right] + qH + rL \rightleftharpoons \left[\{Pt(dach)\}_pH_qL_r\right] + 2H_2O;$$
$$\beta_{p,q,r}(L = L-Met,\ GSH,\ Ino,\ 5'-IMP,\ \text{and}\ 5'-GMP), \tag{7}$$

and the corresponding constants are given by Eq. (8):

$$\beta_{p,q,r} = \frac{\left[\{Pt(dach)\}_pH_qL_r\right]}{\left[\{Pt(dach)(H_2O)_2\}\right]^p[H]^q[L]^r}, \tag{8}$$

where L is the deprotonated molecule of ligand.

In this study, the convention has been adopted whereby a complex containing a $[Pt(dach)(H_2O)_2]^{2+}$ ion (M), proton (H), and ligand (L) takes the general formula $M_pH_qL_r$, where p, q, and r are the stoichiometric indices of the components in the complex. Negative values of q refer to proton removal or hydroxide ion addition during formation of the complex. Thermodynamically, these two processes are equivalent and cannot be distinguished

potentiometrically. The equilibrium constant for the formation of this complex from its components is then designated by the symbol $\beta_{p,q,r}$. For simplicity, the charges of these species are omitted.

Three kinds of equilibria should be considered in the present study: (a) protonation of ligands, (b) hydrolysis of $[Pt(dach)(H_2O)_2]^{2+}$ ion, and (c) general three-component equilibria, which include the case where $q = 0$, i.e., the formation of pure binary complexes of $[Pt(dach)(H_2O)_2]^{2+}$. Negative values of q denote hydroxo complexes. The overall protonation constants of ligands and stability constants of hydrolytic complexes of $[Pt(dach)(H_2O)_2]^{2+}$ ion were determined in separate experiments. Thus, in evaluation of three-component equilibria (c), the binary models (a) and (b) were considered as known. The concentration stability constants of complexes $\beta_{p,q,r}$ were calculated with the aid of the Hyperquad2006 suite of computer programs [39]. In Hyperquad calculations the identity and stability of complexes which gave the best fit to the experimental data were determined by minimizing the objective function U defined by

$$U = \Sigma_i W_i (E_{\text{obs}} - E_{\text{calc}})^2, \tag{9}$$

where E_{obs} and E_{calc} refer to the measured potential calculated from Eq. 6. The weighting factor W_i is defined as the reciprocal of the estimated variance of the measurement.

$$W_i = 1/\sigma^2 = 1/\left[\sigma_E^2 + (\delta E/\delta V)^2 \sigma_V^2\right], \tag{10}$$

where σ_E and σ_V are the estimated variances of the potential and volume readings, respectively. The quality of the fit was judged by the values of the sample standard deviation S and the goodness of fit χ^2 (Pearson's test). At $\sigma_E = 0.1$ mV (0.001 pH error) and $\sigma_V = 0.005$ cm^3, the values of S in different sets of titrations were between 1.0 and 1.8 and χ^2 was between 11.0 and 13.0. The scatter of residuals ($E_{\text{obs}} - E_{\text{calc}}$) versus pH was reasonably random, without any significant systematic trends, indicating a good fit of the experimental data.

The results are summarized in Table 1. Distribution of species in solution were calculated using the program Hyss2006 [40].

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