Competition of Various *cis*-Pt(II) Diamines for the N1 and N7 Sites of Adenosine and 9-(β-D-Ribofuranosyl)purine

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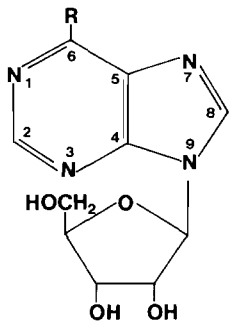
Abstract

Two different methods are employed to determine the N1 *versus* N7 binding ratio of several aquated *cis*-Pt(II) diamine compounds (diamine = $(NH_3)_2$, $(CH_3NH_2)_2$, $[(CH_3)_2NH]_2$ and tetramethylethylenediamine) with adenosine and 9-(β-D-ribofuranosyl)-purine at pH 4. The first method involves separation of the Pt–nucleoside 1:1 complexes bearing the ^3H label at C8 of the nucleobase by LC and subsequent determination of the complexes by a scintillation detector. The second procedure employs a controlled reaction of the isolated 1:1 complexes with thioacetamide, which gives a correlation factor between the signal height of the complex observed by LC and the concentration of the complex. The quantified stock solutions of the complexes are then used to calibrate the reaction products of the nucleosides with Pt(II) separated by LC. With both nucleosides all Pt(II) compounds studied seem to slightly favor the N7 site over the N1 site. The $C6NH_2$ group of adenosine appears to prevent N1 binding more efficiently than coordination to N7 even with sterically less hindered Pt(II) diamines.

Introduction

Purine nucleosides offer several potential binding sites for metal ions. Hard Lewis acids generally coordinate to the glycon moiety, while soft acids prefer the heterocyclic ring nitrogens (Scheme 1) [1]. The latter interactions have received particular interest, since certain Pt(II) antineoplastic agents are supposed to complex with purine residues in the DNA of malignant cells [2]. Although guanine N7 seems to be the main target for Pt(II), coordination also to adenine residues has been observed [3]. In the latter case, however, both N1 and N7 binding is expected under neutral and slightly acidic conditions. For example, monofunctionally binding dienPt(II) distributes almost equally between the N1 and N7 sites in neutral adenosine [4], whereas it slightly favors N7 in 5′-AMP at pH 5 [5]. At pH ≥ 4 monofunctional $[Pt(NH_3)_3Cl]Cl$ exhibits almost equal tendencies to bind to the N1 and N7 sites, while *cis*-$Pt(NH_3)_2Cl_2$ has a larger affinity for the N7 site [6]. The aim of the present study was to elucidate to what extent the preference of the coordination site is affected by the size of the amine ligand tightly bound to Pt(II). For this purpose the N1 *versus* N7 binding mode of the aqua derivatives of various bifunctional *cis*-Pt(II) diamine compounds (diamine = $(NH_3)_2$, $(CH_3NH_2)_2$, $[(CH_3)_2NH]_2$ and tetramethylethylenediamine) with adenosine (**I**) has been studied at pH 4. To study the role of the $C6NH_2$ group in **I** to the binding behaviour of Pt(II), 9-(β-D-ribofuranosyl)purine (**II**) was used as a reference compound. The N1 *versus* N7 binding was investigated employing two different methods. The first method involves the use of HPLC equipped with a liquid scintillation detector and ^3H labeled nucleosides. In the second procedure the solutions of isolated 1:1 complexes of **I** and **II** were quantified by their controlled reaction with thioacetamide, after which they were used to calibrate the reaction products of the nucleosides with Pt(II) separated by HPLC. In both cases the assignment of the coordination site was based on pH-dependent product distribution.



I = Adenosine; R = NH_2

II = 9-(β-D-ribofuranosyl)-purine; R = H

Scheme 1.

Experimental

Materials

K_2PtCl_4 was purchased from Degussa, *N,N,N′,N′*-tetramethylethylenediamine (tmen) was delivered by Aldrich and the nucleosides were commercial products

0020-1693/89/$3.50

TABLE 1. UV Spectroscopic Data for the Isolated Pt(II)–Nucleoside 1:1 Complexes

Amine	$(A)_2Pt^{II}$(nucleoside-N1)		$(A)_2Pt^{II}$(nucleoside-N7)	
	λ_{max} (nm)	λ_{min} (nm)	λ_{max} (nm)	λ_{min} (nm)
9-(β-D-ribofuranosyl)purine				
NH_3	278.5	251.2	263.2	241.1
CH_3NH_2	278.5	250.9	262.4	239.3
$(CH_3)_2NH$	278.4	249.2	263.0	239.2
tmen	277.7	249.8	263.3	240.4
Adenosine				
NH_3	260.0	240.6	269.4	238.0
CH_3NH_2	260[a]	240.5	268[b]	240.0
$(CH_3)_2NH$	260.0	241.2	266.9	239.2
tmen	259.7	240.7	267.8	241.4

[a] A broad band 259–261 nm. [b] A broad band 266.5–269 nm.

of Sigma. They all were used as received. **I** and **II** bearing the ^3H label at C8 were prepared by stirring the unlabeled nucleoside in ^3H_2O (NEN) for 4 h at 363.2 K. The progress of the reactions was followed by monitoring the ^1H NMR spectrum of the nucleosides in D_2O. Repeated water treatments and evaporations were used to protiate the rapidly exchangeable hydrogen atoms in the tritiated products.

cis-$Pt(A)_2Cl_2$, where A = NH_3, CH_3NH_2 or $(CH_3)_2$-NH, were prepared and their geometry and purity checked as described previously [7]. Pt(tmen)Cl_2 was obtained by a slight modification of the procedure reported by Mann and Watson [8]. 0.3 g (2.6 mmol) of free amine was added to the solution of 1.0 g (2.4 mmol) of K_2PtCl_4 in 20 cm^3 water. The addition of 2 cm^3 of 10% HCl gave almost immediately orange crystals which had elemental composition corresponding to that of Pt(tmenH_2)Cl_4. After cooling the mixture in a refrigerator the crystals were filtered off and washed with a small amount of cold water. The product was dissolved in 40 cm^3 water, 1.2 g (10 mmol) of free amine added and the solution stirred in room temperature for 24 h. After cooling in a refrigerator, yellow crystals of Pt(tmen)Cl_2 were collected by filtration. Recrystallization from hot water gave 0.35 g (38%) of air dried product. *Anal.* Calc. for $C_6H_{16}N_2Cl_2Pt$: C, 18.86, H, 4.22; N, 7.33; Cl, 18.55. Found: C, 18.82; H, 4.22; N, 7.36; Cl, 18.66%. IR spectra: 545(sh) and 527 cm^{-1} (ν Pt–N), 328 cm^{-1} (ν Pt–Cl)*.

Treatment of the Pt(II) dichloro compounds with 2 equivalents of $AgNO_3$ in the dark gave the corresponding aqua derivatives. To avoid the dimerization and decomposition of Pt(II) aqua ions, HNO_3 was added to the solutions (pH about 2.5) and they were stored in the dark. The following procedure was employed to prepare and isolate the Pt(II)–nucleoside 1:1 complexes. About 1.5 mg (6 mmol) nucleoside was added to the solution of aquated Pt(II) diamine (200 mm^3, 10 mmol) and pH of the mixture was adjusted to 4 with 0.1 mol dm^{-3} NaOH. After suitable reaction time (15 to 120 min, depending on the amine**) the mixture was injected into HPLC (Spectra-Physics 3500 equipped with Kratos Spectraflow 757 UV-detector and a 150 mm^3 loop) and the 1:1 complexes were separated using the eluents and analytical columns described below. About 500 mm^3 of the eluent showing maximum absorption for the complex was collected in a test tube, which was stored in ice. The purity of the isolated compounds was checked chromatographically under analytical conditions showing the formation of 1:1 complexes as the main products. In addition, the complexes were characterized UV-spectroscopically with a Hitachi U 2000 Spectrophotometer (Table 1). Due to the limited amount of the isolated material the measurement of NMR spectra was not possible.

Determination of the Binding Ratio

Method 1. The ratio of N1 and N7 binding in the reactions of aquated Pt(II) diamines with tritriated nucleosides was determined at pH 3.90–4.20 at ambient temperature. A typical reaction solution contained 15–20 mmol Pt(II) and 3 mmol ligand. The reaction products were separated at suitable time intervals with an RP-18 column (Serva or HPLC-Technology) using 5×10^{-4} mol dm^{-3} HNO_3 and 5×10^{-2} mol dm^{-3} $NaClO_4$ or 5×10^{-2} mol dm^{-3} $NaClO_4$ and acetic acid buffer (1×10^{-2} mol dm^{-3},

*The appearance of only a single ν Pt–Cl band is consistent with other *cis*-$Pt(A)_2Cl_2$ compounds studied (see ref. 7a).

**The reactivity order is $CH_3NH_2 > NH_3 \gg (CH_3)_2NH >$ tmen.

pH 4.3) in water/methanol mixtures (100/0–95/5) as eluents with a flow rate of 0.8 ml min^{-1} given by a Waters 501 HPLC pump. Compounds were detected by a UV monitor (LKB 2158 Uvicord equipped with LKB 2210 recorder) after which the eluent was mixed with scintillation liquid (Luma flow III, 2.4 ml min^{-1}) and passed through a radioactivity monitor (LKB 1208 Betacord equipped with Waters 740 Data Module) in order to detect Pt complexes bearing the ^3H label. Peak areas were used to calculate the ratio of the complexes. The corresponding analysis from the mixtures made in acidic conditions (pH 2.0 for adenosine, pH 0.3 for 9-(β-D-ribofuranosyl)purine) was used to ascertain the binding sites.

Method 2. Different amounts of the isolated 1:1 complex were added into a series of test tubes containing a fixed amount of thioacetamide (ta, 0.1 mmol) giving thioacetamide/complex ratios from 1.5 to 6. The stoppered tubes were kept at room temperature until the signal of the complex disappeared as deduced by LC analysis (from 2 to 6 h, depending on the amine**), after which water was added to the mixture to give a constant volume and the tubes were immersed in an ice bath. The amount of unreacted ta in each tube was measured chromatographically using peak height as a measure of the concentration and known samples for calibration, which gave a plot of ta reacted *versus* the complex added. Assuming 1:1 reaction between ta and the complex, the slope of the plot is equal to the concentration of the stock solution of the isolated Pt–nucleoside complex. In all cases the series of N1 and N7 bound complexes were analyzed at the same time. The quantified stock solutions of the 1:1 complexes were employed to measure the correlation factor between the signal height and the concentration. Identical LC conditions were then used to measure the peak height of the complexes formed under analytical conditions in Pt(II) excess and the actual product distribution was observed by the correlation factor.

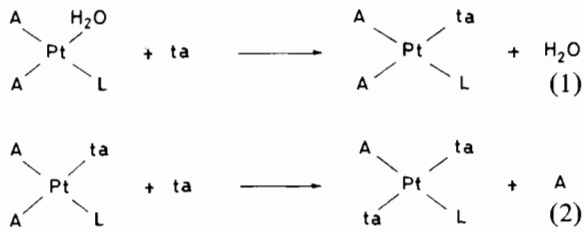
Kinetic Measurements

LC was employed to study the kinetics of the reaction between ta and the isolated 1:1 complexes in aqueous solution at 298.2 K. In all cases pH of the reaction mixture was 3.5–4.0, adjusted with HNO_3 if necessary. The reactions were carried out in stoppered tubes immersed in a water bath, the temperature of which was kept constant within 0.05 K. Aliquots of 0.2 cm^3 withdrawn from the reaction mixture at suitable time intervals were immediately analyzed by LC using the columns and eluents described above and signal height as a measure of the concentration. The disappearance of the signal of the complex under 10–15 fold molar excess of ta (up to 5×10^{-4} mol dm^{-3}) gave pseudo first-order rate constant, k_1, for the replacement of the water molecule bound to Pt(II) with ta. A considerably higher ta excess (up to 2×10^{-2} mol dm^{-3}) was employed to follow the disappearance of the product formed in the first reaction. In the case of the second reaction, the first sample was injected after the completion of the first reaction (about 10 half-lives). Division of the pseudo first-order rate constants obtained with the total concentration of the incoming ta ligand gave the second-order rate constant for both reactions.

Results and discussion

Kinetic Studies

The well-known *trans* directing effect of thioacetamide bound to platinum via sulfur atom strongly labilizes the ligand *trans* to it. Instead, the remaining groups positioned *trans* to each other are not labilized. In the reaction between *trans*-$Pt(NH_3)_2Cl_2$ and thiourea, for example, the Cl^- ions are easily replaced with thiourea binding via the sulfur atom, whereas only prolonged treatment at elevated temperatures in high thiourea excess results in a removal of NH_3 ligands [7b]. Accordingly, eqns. (1) and (2) can



(1)

(2)

safely be supposed to describe the reactions between Pt–nucleoside 1:1 complexes and thioacetamide. Here L denotes a nucleoside, which is bound to platinum via a nitrogen atom. The charges are omitted for clarity, because all the Pt complexes indicated are expected to be dications at about pH 4. The second order rate constants, k_1 and k_2 for eqns. (1) and (2) in the case of **II** are listed in Table 2. The

TABLE 2. Rate Constants, $k_i(10^{-2}$ M^{-1} $s^{-1})$, for the Reactions of Thioacetamide with Isolated 1:1 Pt Complexes of 9-(β-D-Ribofuranosyl)purine According to eqns. (1) and (2)

Amine	$(A)_2Pt^{II}$(L-N1)		$(A)_2Pt^{II}$(L-N7)	
	k_1	k_2	k_1	k_2
NH_3	620	6.3	520	5.4
CH_3NH_2	930	6.8	680	5.9
$(CH_3)_2NH$	190	1.4	190	2.9
tmen	140	[a]	130	[a]

[a] No reaction observed during 3 h in the presence of 0.02 mol dm^{-3} thioacetamide.

data reveal, that reaction (1) proceeds about 100 times faster than reaction (2), which is also expected considering the stability of nitrogen bound ligands compared to that of the water molecule. Consequently, the analytical procedure described above predominantly represents a 1:1 reaction, because only a moderate excess of thiourea is employed. Reactions (1) and (2) both gave only a single product, the retention time of which was longer in the latter case. In addition, the products of isomeric Pt–nucleoside complexes were well resolved. LC analysis revealed furthermore no detachment of the nucleosides, which gives support to the validity of reactions (1) and (2). When the amine was tmen, however, only reaction (1) was observed. In this case the addition of the second ta molecule involves the rupture of a chelate structure, which is thermodynamically very stable and hence the reaction rate is considerably retarded.*

Analysis with Thioacetamide

Figure 1 shows as examples the plots of ta uptake *versus* the complex added when the amine is NH_3. Usually good linear plots going through origo were observed, but too small ta excess gave minor deviations from linearity. Table 3 lists the correlation factors given by the analysis and the apparent ratio of the complexes observed by LC for each system, the multiplication of which gives the actual binding ratio.

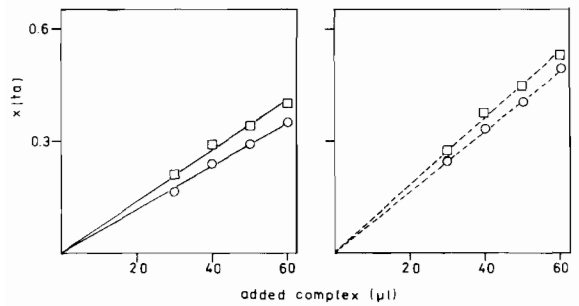


Fig. 1. The plots of thioacetamide reacted *vs.* the complex added. Solid line for I, dashed line for II. ○ represents the N1 bound complexes, □ N7 bound species.

Chromatographic Studies with UV and Scintillation Detectors

Figure 2 shows typical chromatograms recorded with UV and radioactivity detectors from the reaction mixture of aquated Pt(II) diamines and radiolabeled nucleosides. The signal and the shoulder that appear on the UV chromatograms at about 4 min result from Pt(II) diamine and a diplatinated complex, respectively. Their absence on the radioactivity chromatogram is consistent with this assignment. In addition, and authentic Pt(II) sample gave a retention time identical to that observed for the main signal. The shoulder is assigned as a diplatinated form because (i) increased charge and polarity results in shorter retention times with RP columns and (ii) it begins to form only after the appearance of the main products denoted as 1 and 2 in all cases. It should be noted that the sensitivity of the UV monitor exceeds that of the scintillation detector employed. A sharp but weak signal given by the UV monitor can thus disappear in the latter mode especially when the eluent bearing the compound is mixed with an excess of scintillation liquid before radioactive detection. With both nucleosides product 1 is assigned to N1 bound Pt complex, whereas 2 denotes complex where Pt(II) is coordinated to N7. The considerable diminution of the first product in acidic medium strongly supports this assignment, because both ligands most probably protonate at N1 according to ^{15}NMR studies made with 9-substituted purines [9]. As a consequence, Pt(II) binding to this site is hindered under these conditions. These assignments are valid also for the thioacetamide method, because identical LC conditions were employed in both cases.

Ratio of N1 and N7 Bound Complexes

The ratio of N1 and N7 bound 1:1 complexes observed with both methods for the reactions of various Pt(II) compounds with adenosine and 9-(β-D-ribofuranosyl)purine are listed in Table 4. The values observed by the two different procedures employed are in a good agreement with each other, which lends support to the validity of these methods. With both nucleosides N7 seems to be more susceptible for bifunctional Pt(II) ions than N1 as observed earlier for *cis*-$Pt(NH_3)_2Cl_2$ [6], although this behaviour is in contrast to the basicity of these nitrogens. In the case of I, however, protonation of N1 may partially hinder platination at this site. The pK_a value of 3.86 reported for I in 1.0 mol dm^{-3} $NaClO_4$ [10] indicates

TABLE 3. Apparent Ratio of the N1 and N7 Bound Pt–Nucleoside 1:1 Complexes Observed by LC and the Analytically Determined Correlation Factor Between the Concentration of the Isolated Complexes and their Peak Height Given by LC

Amine	Adenosine		9-(β-D-ribofuranosyl)purine	
	a	b	a	b
NH_3	0.83	0.78	0.69	1.4
CH_3NH_2	0.90	0.77	0.48	1.5
$(CH_3)_2NH$	0.94	0.67	0.45	1.4
tmen	0.69	0.78	0.38	1.7

[a]Apparent ratio given by peak heights at 260 nm as a mean value of three different LC separations. [b]Correlation factor.

*According to our findings the reaction of aquated Pt^{II}(tmen) with a 20-fold molar excess of thiourea (0.1 mol dm^{-3}) requires about 120 h at 40 °C for completion.

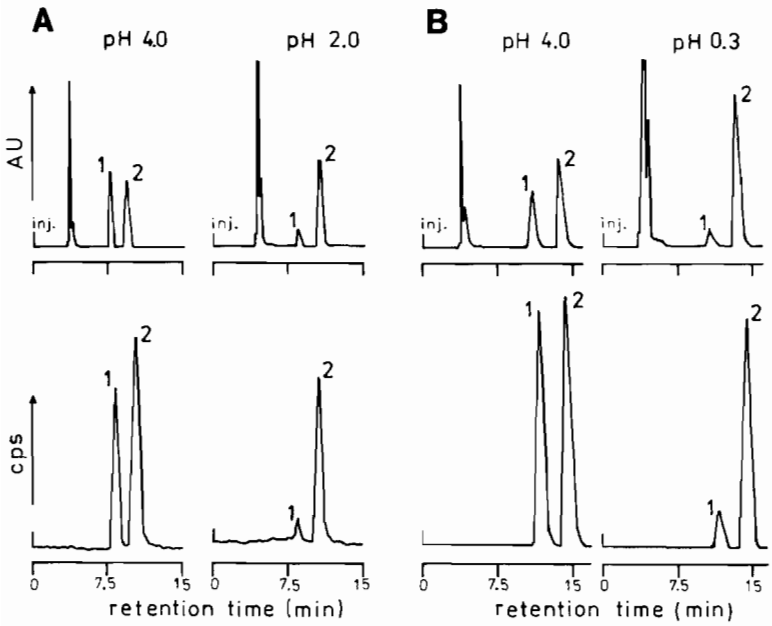


Fig. 2. Reaction mixtures of [8-^{3}H]adenosine with *cis*-$Pt^{II}(NH_3)_2$ aqua ion (A) and [8-^{3}H]9-(β-D-ribofuranosyl)purine with *cis*-$Pt^{II}(CH_3NH_2)_2$ aqua ion (B) at two different pHs separated after suitable reaction time with an RP-18 column. The upper chromatograms are from UV detector, the lower from radioactivity monitor. Both scales are arbitrary.

TABLE 4. The Ratio of N1 and N7 Bound 1:1 Complexes of Adenosine and 9-(β-D-Ribofuranosyl)purine with Various *cis*-Pt(II) Diamine Compounds in Aqueous Solution at pH 3.90–4.20

Amine	$r_{N1/N7}$					
	Adenosine			9-(β-D-ribofuranosyl)purine		
	Method		Mean	Method		Mean
	1	2		1	2	
NH_3	0.67	0.65	0.66	0.93	0.97	0.95
CH_3NH_2	0.65	0.69	0.67	0.77	0.72	0.75
$(CH_3)_2NH$	0.68	0.63	0.65	0.66	0.63	0.65
tmen	0.57	0.54	0.55	0.62	0.65	0.63

that far less than half of the free ligand is protonated under the conditions employed (pH 3.90–4.20, $I \approx 0.06$ mol dm^{-3}). It is therefore assumed that the protonation equilibrium has no major effect on the product distribution observed. It thus appears that besides the basicity of a given coordination site other factors such as the size and nature of the Lewis acids need to be considered in the complexation. However, the results observed show no clear trend between the size of the amino group tightly bound in Pt(II) and the coordination site. Instead, the $C6NH_2$ group of adenosine seems to retard more efficiently binding to N1 than to N7 even with sterically less hindered Pt(II) compounds. The same behaviour has previously been observed for the corresponding 3d transition metal complexes [10].

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