Articles

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Kinetics of Complexation of Aquated Pt^{II}(dien) with Inosine and 1-Methylinosine as a Function of pH

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Kinetics of the complexation of aquated Pt^{II}(dien) (dien = diethylenetriamine) with inosine and 1-methylinosine has been studied by HPLC in aqueous solution at 298.2 K (pH = 4.2–8.4). With both nucleosides the complex formation occurs via the [Pt(dien)$(H_2O)]^{2+}$ ion, which is the reactive species of aquated Pt^{II}(dien). 1-Methylinosine forms only a N7-bound 1:1 complex with Pt(II) throughout the pH range studied. 1:1 complexes of inosine also favor this binding mode below pH 6, the affinity of Pt(II) toward the N7 site being equal with neutral inosine and 1-methylinosine. At higher pH N1H of inosine is deprotonated and, hence, offers an additional coordination site. Nevertheless, Pt(II) also seems to favor N7 over N1 in anionic inosine. Attachment of Pt(II) to N7 of inosine acidifies the N1H proton by 1.6 log units, while the displacement of the N1H proton by Pt(II) makes the N7 site about 1.1 log units more basic. The intrinsic formation reactions of N1,N7-bound diplatinum complex via N1- and N7-bound 1:1 complexes are kinetically equal. No sign of N3 coordination was observed under these conditions.

Introduction

Binding of Pt(II) to purine nucleosides and related compounds has received considerable interest in the past two decades.[1] Much attention has been paid especially to the coordination properties of the base moieties because certain anticarcinogenic Pt(II) compounds are believed to interact directly with the purine bases in DNA of malignant cells.[1,2] In 6-oxo-substituted nucleosides the predominant coordination site in acidic medium is the ring nitrogen N7 because the prevailing keto tautomer requires a proton at N1 and, hence, metal ion binding to this site is prevented.[1-4] By contrast, the N1 binding mode may become significant in neutral and slightly basic solution upon deprotonation of N1H.[3,4] Under these conditions excess Pt(II) leads to monomeric N1,N7-diplatinated[5] and N1,N3,N7-triplatinated[5c] complexes, while equimolar mixtures of ligand and Pt(II) yield polymeric species involving Pt(II) N1,N7 bridging.[5a,6] In more alkaline solutions mixtures of N1-platinated, N7-platinated, and N1,N7-diplatinated complexes are formed.[7] Alkylation of N7 results in exclusive N1 coordination in 6-oxo-substituted purine derivatives.[8] The thermodynamically controlled distribution of Pt(II) between these sites is difficult to estimate because of the general inertness of Pt(II) compounds toward substitution reactions and the high stability of the Pt–N bond.[9] However, the different binding modes can be conveniently studied by using a kinetic approach.

In a few cases quantitative kinetic data have been reported for the complexation of Pt(II) with nucleosides[10] and nucleotides[11] in slightly acidic medium. By contrast, no such data seem to exist under neutral conditions. Here we wish to report a systematic HPLC study on the complexation of aquated Pt^{II}(dien) with inosine (InoH) and 1-methylinosine (MeIno) in the pH range 4.2–8.4 in aqueous solution at 298.2 K. The main purposes can be summarized as follows: (i) the effect of pH on the reactivity of aquated Pt^{II}(dien), (ii) the pH-dependent distribution of Pt(II) between the N1 and N7 sites in inosine, (iii) the influence of coordinated Pt(II) on the acidity of the ring nitrogens N1 and N7 of inosine, and (iv) the formation of diplatinated inosine complexes.

Experimental Section

Materials. Inosine and its methyl derivatives were purchased from Sigma, 2-ethylpyridine was purchased from Koch-Light Laboratories, 2,6-dimethylpyridine was obtained from EGA-Chemie, 2,4,6-trimethylpyridine was purchased from Fluka AG, and triethanolamine was obtained from Merck; they all were used as received. A known amount of [Pt(dien)I]I[12] was converted to the corresponding aqua derivative by treating the aqueous suspension of the salt with 1.98 equiv of $AgNO_3$ overnight in the dark. To prevent the possible dimerization of aquated Pt^{II}(dien) through OH bridging,[13] the pH of the stock solution was adjusted below 3 with HNO_3 and the solution was stored in the dark. Solutions of N1- and N7-bound Pt^{II}(dien)–inosine 1:1 complexes were obtained by the following procedure. To a suspension of about 15 mg (40 mmol) of inosine in 50 μL of 1 M NaOH was added 350 μL (17.5 mmol) of aquated $[Pt(dien)]^{2+}$, and the mixture (pH 8.9) was gently warmed to dissolve the inosine. After 6 h the pH was adjusted to about 3 with 1 M HNO_3 and the mixture was fractionated by LC as previously described[14] using aqueous $NaClO_4$ (0.05 M) as an eluent. The N7-bound Pt complex of 1-methylinosine was obtained analogously after 30 min reaction at pH 3. For NMR measurements the fractions (pH about 5)

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Table I. UV and ^1H NMR Spectroscopic Data for the Isolated Pt(II)–Nucleoside Complexes and Inosine Derivatives

compd[a]	UV:[b] $\lambda_{max}/\lambda_{min}$ pH 1.0	pH 7.0	pH 12.0	^1H NMR[c] H2	H8	Remarks
inosine	249.0/221.6	248.8/222.2	251.2/223.6	8.18	8.30	
1-methylinosine	250.4/223.8	250.0/224.6	249.6/224.8	8.33	8.29	*d*
		271 sh	270 sh			
7-methylinosine	252.8/223.6	262.2/228.4	266.4/231.0[e]	8.12	8.38	*f*
Pt(dien)(MeIno-*N7*)	254.8/231.4	254.8/231.0	254.8/231.2	8.46	8.72	*g*
Pt(dien)(Ino-*N1*)	263.0/245.4	254.4/245.0	254.8/239.2	8.31	8.16	*h*
		272 sh	273 sh	8.52	9.26	*i*
Pt(dien)(Ino-*N7*)	254.0/231.0	258.4/238.0	261.6/237.6	8.29	8.74	
$\{Pt(dien)\}_2(\mu$-Ino-*N1*,*N7*)	261.2/245.8	261.2/245.2	261.4/246.0			

[a] Charges are omitted for clarity. [b] Data (nm) were obtained with a Hitachi U 2000 spectrophotometer. [c] Data (ppm) were obtained by employing *t*-BuOH as internal standard (1.24 ppm downfield from DSS), but the chemical shifts are quoted with respect to DSS. The assignment of H2 and H8 was based on deuteration of H8. [d] N1–CH_3: 3.63 ppm. [e] Decomposes. [f] N7–CH_3: 4.23 ppm. [g] N1–CH_3: 3.68 ppm. [h] At pD 7. [i] At pD 1.

were evaporated to dryness and the ^1H NMR spectra were recorded in D_2O on a JEOL GX-400 spectrometer. Table I gives the UV and ^1H NMR spectroscopic data for the complexes isolated.

Kinetic Measurements. HPLC was employed to follow the complexation of aquated Pt^{II}(dien) with both nucleosides in buffered aqueous solution (pH = 4.2–8.4) at 298.2 K. The following nitrogen bases and HNO_3 mixtures were used as buffers (pH range employed in parentheses; $[B]_T$:$[L]_T$ > 40:1): 2-ethylpyridine (4.5–6.0), 2,6-dimethylpyridine (5.8–6.8), 2,4,6-trimethylpyridine (6.6–8.0), and triethanolamine (6.9–8.4). Conventional acetate or phosphate buffers could not be used to maintain the pH value of the reaction mixture because their anions are known to coordinate to Pt(II).[15] Instead, 2-substituted pyridines have been shown to react with Pt(II) only very slowly.[16] The 20:1 ($[Pt]_T$:$[L]_T$) excess of Pt^{II}(dien) provided pseudo-first-order conditions for the complex formation. The reactions were carried out in stoppered tubes immersed in a water bath, the temperature of which was kept constant within 0.05 K. Either samples from the reaction mixture were directly chromatographed at suitable time intervals, as described earlier,[10c] or the samples were made alkaline to stop the complex formation (pH > 11, ice bath) and then chromatographed. With both nucleosides the separations were carried out on an RP-18 column using aqueous $HCOONH_4$ (0.12 M), containing 4% MeOH and 0.2% HCOOH, or aqueous $NaClO_4$ (0.05 M) in HOAc/NaOAc buffer (0.02 M, pH = 4), containing 10% MeOH, as eluents. Signal height was used as a measure of concentration in all cases.

Pseudo-first-order rate constants, k_i', for the disappearance of the free ligand, or 1:1 complex, were calculated from the integrated first-order rate equation (1), where $[X]_0$ denotes the initial concentration of the

$$\ln [X]_t = -k_i' t + \ln [X]_0 \quad (1)$$

ligand, or the complex, and $[X]_t$ is the concentration at moment t. Usually 8–12 samples withdrawn from the reaction mixture during about 3 half-lives were analyzed by LC, which gave correlation coefficients of at least 0.998 for plots of ln [X] vs t. Above pH 6.5 the time-dependent concentration of the N7-bound 1:1 complex was employed to calculate the rate constants $(k_1 + k_2)_{obs}$ and $k_{4,obs}$ for the formation and disappearance of this species. The rate constants were obtained by eq 2 with

$$[ML]_t = \frac{(k_1 + k_2)_{obs}}{k_{4,obs} - k_{d,obs}}[InoH]_T(e^{-k_{d,obs}t} - e^{-k_{4,obs}t}) \quad (2)$$

least-squares fitting.[17] Here $[InoH]_T$ is the initial ligand concentration and $[ML]_t$ is the concentration of the complex at the moment t. The term $k_{d,obs}$ denotes the sum constant for the formation of all 1:1 complexes. A calibration sample, prepared in acidic medium (pH < 2.5) from a known amount of the ligand in Pt(II) excess, was employed to transform the signal heights into concentrations.

Results and Discussion

1-Methylinosine. Chromatographic analysis revealed the formation of a single reaction product for the system Pt^{II}(dien)–MeIno throughout the pH range studied. The ^1H NMR spectrum of the isolated product strongly suggests Pt(II) coordination to N7, as evidenced by the 0.5 and 0.2 ppm shifts for H8 and H2, respectively. The observed second-order rate constant for the formation of the $[Pt(dien)(MeIno\text{-}N7)]^{2+}$ ion decreases with increasing pH, as can be seen in Table II. Since 1-methylinosine acts as a neutral ligand in the pH range studied, this observation can be attributed to the deprotonation of the $[Pt(dien)(H_2O)]^{2+}$ ion, which yields substitution-inert hydroxo species,[18a] analogous to the corresponding $[Pd(dien)(H_2O)]^{2+}$ ion.[18b] Accordingly, the complexation of aquated Pt^{II}(dien) with 1-methylinosine under these conditions may be depicted by Scheme I. The pH-dependent rate constant, $k_{1,obs}$, can thus be expressed by eq 3, where k_1

$$k_{1,obs} = k_1 \frac{[H^+]}{[H^+] + K_a} \quad (3)$$

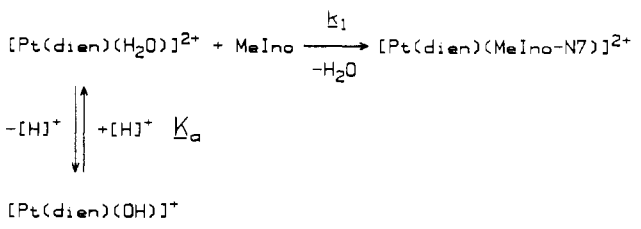
represents the second-order rate constant for the formation of the $[Pt(dien)(MeIno\text{-}N7)]^{2+}$ ion and K_a is the acidity constant of the $[Pt(dien)(H_2O)]^{2+}$ ion. The values obtained by least-squares fitting were $k_1 = 0.54\ M^{-1}\ s^{-1}$ and $K_a = 10^{-6.24}$ M. The latter

Table II. Observed Rate Constants, $k_{i,obs}/10^{-2}\ M^{-1}\ s^{-1}$, for the Formation of 1:1 Complexes between Aquated Pt^{II}(dien) and Inosine or 1-Methylinosine in Buffered Aqueous Solution (pH = 4.2–8.4) at 298.2 K[a]

1-methylinosine pH	$k_{1,obs}$[b]	inosine pH	$k_{d,obs}$[b]	$(k_1 + k_2)_{obs}$[c]
4.27[d]	54.0	4.20[d]	55.0	
4.40	53.4	4.82	53.4	
4.78	51.0			
5.32	46.8	5.16	52.3	
5.40	46.9			
5.84	40.9	5.76	40.8	
5.85	38.0	5.98	37.3	
6.25	25.7	6.25	26.7	
6.44	22.2	6.56	18.1	18.0
6.49	18.2	6.62	17.0	
6.86	9.6			
6.96	9.6	7.10	7.7	7.3
7.13	4.8	7.12	8.0	7.8
7.40	3.4	7.36	4.7	4.4
7.43	3.5	7.48	3.5	3.0
7.82	1.3	7.50	3.7	3.0
7.88	1.1	7.98	2.0	1.5
8.38	0.4	8.42	1.5	1.1

[a] In 0.1 M $NaClO_4$. [b] Obtained by eq 1. [c] Obtained by eq 2. [d] Unbuffered solution.

Scheme I



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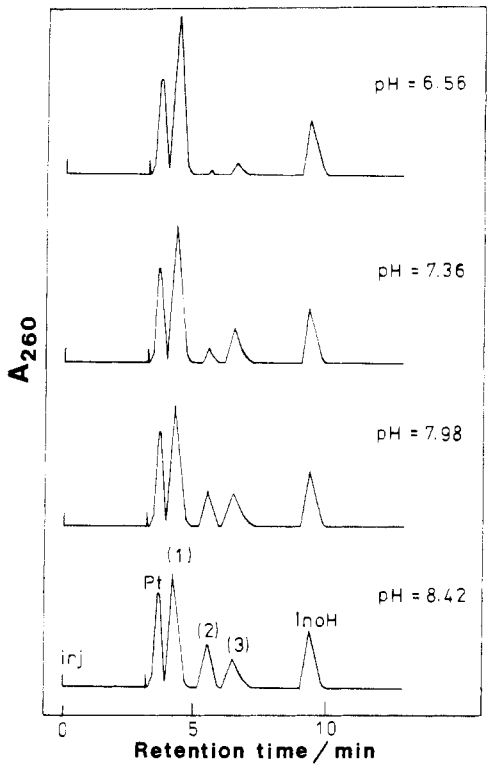
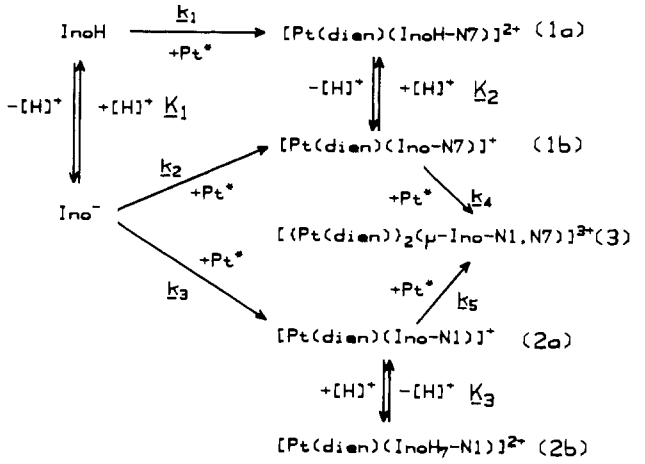


Figure 1. HPLC analyses of the system Pt^{II}(dien)–inosine after 1 half-life at different pH values using aqueous $HCOONH_4$ (0.12 M) containing 4% MeOH and 0.2% HCOOH as an eluent. Notation: (**1**) N7-bound 1:1 complex; (**2**) N1-bound 1:1 complex; (**3**) N1,N7-diplatinated complex.

agrees well with value $10^{-6.13}$ reported in the literature.[19] In neutral solution aquated Pt^{II}(dien) has been found to dimerize slowly (K_d = 108 M^{-1} and $k_d = 3.3 \times 10^{-3}$ M^{-1} s^{-1} at 308.2 K).[13] However, this dimerization process seems not to affect significantly the complexation of the $[Pt(dien)(H_2O)]^{2+}$ ion with inosine derivatives. The preceding data reveal that at pH < 7.25 the complexation rate is at least 20-fold greater than the dimerization rate. Above this pH the rates are of the same order of magnitude but the amount of the dimer strongly diminishes. For example, at pH 8.25 the equilibrium concentration of the dimer is less than 1% from the amount of Pt(II) employed, viz. 6×10^{-3} M.

Pt(II)–Inosine 1:1 Complexes. Figure 1 shows typical chromatograms of the system Pt(dien)–InoH after 1 half-life at selected pH values. Below pH 6 only one product (**1**) is formed in detectable amounts. Above this pH a second product (**2**) with a slightly longer retention time than **1** begins to form and the relative amount of **2** increases on going to pH 8.4. Comparison with 1-methylinosine suggests that product **1** is the N7-bound 1:1 complex, whereas in **2** Pt(II) coordinates to N1 of the base moiety. Since the formation of the latter requires deprotonation of the N1H of inosine, this binding mode is effectively blocked in more acidic medium. Both UV and ^1H NMR spectra of the isolated products strongly support these assignments (Table I). UV spectroscopic properties of **1** are quite similar to those of 7-methylinosine, whereas compound **2** behaves analogously to 1-methylinosine. In compound **1** Pt(II) induces a stronger change in the H8 than in the H2 shift, as compared to the spectrum of the free nucleoside, which is in line with N7 coordination. The ^1H NMR spectrum of compound **2** is consistent with N1 coordination. At pD 7 the H2 resonance is shifted more downfield than that of N7, when compared to the spectrum of the free nucleoside. By contrast, the H8 resonance is shifted 0.9 ppm downfield upon acidification to pD 1, while the observed shift for the H2 resonance is only 0.2 ppm. This behavior clearly reveals

Scheme II



protonation at N7, as expected for the N1-bound complex. In addition to **1** and **2**, a third product (**3**) with the longest retention time among the products is found. The time-dependent appearance of **3** (not shown) suggests that it is a diplatinated complex, the formation of which is discussed below.

Rate constants for the disappearance of free inosine as a function of pH are listed in Table II. Comparison of these data with those of 1-methylinosine shows that at pH 4.2 the rate constants are practically equal, whereas at pH 8.4 the complexation of inosine is about 3 times faster than that of the methyl derivative. This behavior can be attributed to the deprotonation of N1H of inosine, which enhances complex formation. The complexation pathway assumed for inosine in Pt(II) excess is depicted in Scheme II. Here Pt* denotes the $[Pt(dien)(H_2O)]^{2+}$ ion, the reactive form of aquated Pt^{II}(dien). The reactions in Scheme II may be divided into groups, which can be studied independently. (i) First-generation rate constants, k_1 and $k_2' = k_2 + k_3$, for the formation of 1:1 complexes **1a** and **1b + 2**, respectively, can be obtained from the disappearance of the free ligand by employing the rate data in Table II. The rate law for the disappearance of the uncomplexed ligand (L) may be expressed by eq 4, and the observed second-order rate constant, by eq 5.

$$-d[L]/dt = k_1[InoH][Pt^*] + k_2'[Ino^-][Pt^*] \quad (4)$$

$$k_{d,obs} = \frac{k_1[H^+] + k_2'K_1}{K_1 + [H^+]} \frac{[H^+]}{K_a + [H^+]} \quad (5)$$

Here K_a and K_1 are the known acidity constants of the $[Pt(dien)(H_2O)]^{2+}$ ion and inosine, respectively. (ii) pH-dependent formation of the N7-bound complex gives k_2 by eq 5, when $k_{d,obs}$ and k_2' are replaced by $(k_1 + k_2)_{obs}$ and k_2, respectively. Steps i and ii directly give $k_3 = k_2' - k_2$.

Least-squares fitting of the kinetic data yields the value of 0.55 M^{-1} s^{-1} for k_1, 2.7 M^{-1} s^{-1} for k_2, and 1.4 M^{-1} s^{-1} for k_3. Accordingly, monofunctional Pt^{II}(dien) exhibits equal affinities for the N7 site of neutral inosine and 1-methylinosine. This agrees with the behavior of aquated cis Pt(II) diamines in slightly acidic medium, though the reactivity of the $[Pt(dien)(H_2O)]^{2+}$ ion is slightly higher than that of the diaqua cations of cis Pt(II) diamines.[10c] Although deprotonation of N1H of inosine offers an additional binding site for Pt(II), it also appears to increase the susceptibility of N7 to platinum, as evidenced by the rate constants k_2 and k_3 of anionic inosine. The N1:N7 binding ratio obtained, viz. 0.5, is in agreement with that found for the analogous complexes of 5′-AMP,[20] as well as with the binding pattern of aquated cis Pt(II) diamines with adenosine and 9-(β-D-ribofuranosyl)-purine.[14] No migration of Pt(II) between the N1 and N7 sites was observed under the experimental conditions. This process appears not to affect the complexation kinetics at 298.2 K, although the N1 and N7 complexes were detected to undergo in-

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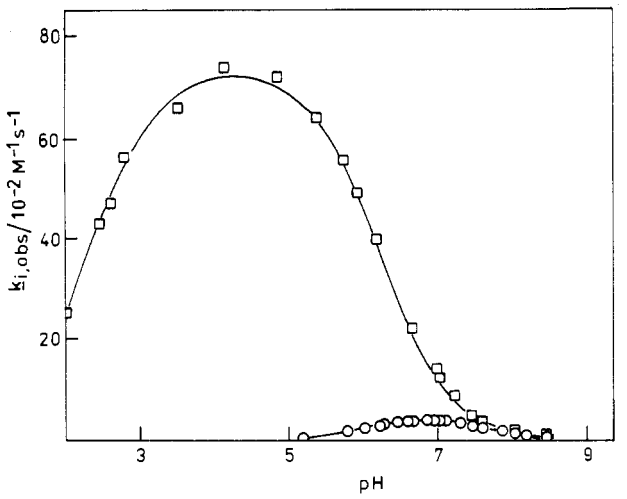

Figure 2. Observed rate constants for the disappearance of different inosine 1:1 complexes in Pt(II) excess as a function of pH. Notation: (□) N1-bound complex; (○) N7-bound complex. Calculated values obtained via eq 6 by least-squares fitting.

terconversion at elevated temperatures, as suggested previously.[7b,21]

Pt(II)–Inosine 2:1 Complexes. The preceding discussion reveals that upon deprotonation of N1H, inosine offers two potential binding sites for platinum. The formation of diplatinated species in Pt(II) excess is thus expected under these conditions. As shown in Figure 1, the chromatographic analysis revealed the formation of compound **3**, which was assigned to a diplatinated complex. The fact that both isolated 1:1 complexes gave this same product strongly supports this assignment. In addition, the $\lambda_{max}/\lambda_{min}$ values (Table I) remain practically constant in the pH range 1–12, which points to platination of both N1 and N7. Unfortunately the ^1H NMR spectrum of this compound could not be recorded because of the limited amount of the material. Figure 2 shows the observed rate constants for the disappearance of **1** and **2** as a function of pH. Interestingly, the rate constant of both complexes has a maximum value but at different pHs. This can be best explained by the competition of proton and Pt(II) for the same site, i.e. the N1 site in **1** and the N7 site in **2**. The relative rate constant shows that the former must be far more basic than the latter.

Table III records the observed second-generation rate constants, $k_{4,obs}$ and $k_{5,obs}$, for the disappearance of **1** and **2**. The least-squares fitting to the former data gives the value of 0.74 M^{-1} s^{-1} for k_4 and $10^{-7.24}$ M for K_2 by employing eq 6. Comparison of the latter

$$k_{4,obs} = \frac{k_4K_2}{K_2 + [H^+]} \frac{[H^+]}{K_a + [H^+]} \qquad (6)$$

to the acidity constant of the ligand, viz. 8.8,[4] reveals that Pt(II) binding to N7 lowers the basicity of N1H about 1.6 log units. This agrees well with the findings reported earlier for Pt(II) complexes of ligands of the same type.[5-7,22] A similar treatment of the pH-dependent rate data for **2** yields the values 0.75 M^{-1} s^{-1} for k_5 and 2.30 for pK_3 by employing an analogous equation, in which $k_{5,obs}$ stands for $k_{4,obs}$, k_5 for k_4, and K_3 for K_2. The rate constants

Table III. Observed Rate Constants, $k_{i,obs}/10^{-2}$ M^{-1} s^{-1}, for the Binding of the Second Pt(II) Ion to the N1- and N7-Bound 1:1 Complexes of Pt^{II}(dien) with Inosine in Aqueous Solution (pH = 2.0–8.4) at 298.2 K[a]

Pt(dien)(Ino-*N7*)[b]		Pt(dien)(Ino-*N1*)[b]	
pH	$k_{4,obs}$[c]	pH	$k_{5,obs}$[c]
5.16	0.3	2.00	25[d]
5.76	1.0	2.45	43[d]
5.98	1.6	2.60	47[d]
6.21	2.7	2.78	57[d]
6.25	2.9	3.52	66[d]
6.42	3.3	4.13	74[d]
6.56	3.4[e]	4.84	72
6.63	3.6	5.37	64
6.82	3.7	5.72	55
6.94	3.6	5.98	49
7.00	3.8	6.17	40
7.10	3.6[e]	6.63	22
7.12	3.7[e]	6.95	14
7.12	3.7[e]	7.00	12
7.28	3.1	7.20	8.9
7.36	2.7[e]	7.44	4.5
7.44	2.5	7.57	3.7
7.48	2.2[e]	7.83	2.1
7.50	2.3[e]	8.17	0.9
7.57	2.0		
7.83	1.5		
7.98	1.0[e]		
8.17	0.7		
8.42	0.5[e]		

[a] In 0.1 M $NaClO_4$. [b] Charges are omitted for clarity. [c] Data obtained by eq 1. [d] Unbuffered solution. [e] Obtained by eq 2.

obtained show that the intrinsic abilities of both 1:1 complexes to bind a second Pt(II) are practically equal, which parallels the behavior of anionic inosine, though the latter slightly favors coordination to N7. Replacement of the proton at N1 with Pt(II) makes the N7 site of inosine about 1.1 log units more basic. A similar increase in the basicity of N7 has been previously observed for N1-bound Pt(II) complexes of 9-substituted purines.[7,23]

Concluding Remarks

The complexation of aquated Pt^{II}(dien) with nucleosides proceeds via the aqua cation, while the deprotonated $[Pt(dien)(OH)]^+$ ion can be considered to be inert toward substitution reactions. In acidic medium aquated Pt^{II}(dien) binds exclusively to N7 to both nucleosides. With increasing pH the N1 site of inosine becomes an additional coordination site because of the deprotonation of N1H. Nevertheless, Pt(II) also seems to kinetically favor N7 over N1 in anionic inosine. Introduction of Pt(II) to N7 of inosine acidifies the N1H proton by 1.6 log units as compared to the free nucleoside. In contrast, the replacement of the N1 proton with Pt(II) makes the N7 site about 1.1 log units more basic. The intrinsic formation reactions of the N1,N7-bound diplatinum complex via N1- and N7-bound 1:1 complexes are kinetically equal. Under these conditions N3 coordination was observed in neither case. However, some preliminary experiments have shown that at very high Pt(II) concentration and at elevated temperatures, the diplatinated inosine complex slowly yields a product that is susceptible to decomposition.

Registry No. $Pt(dien)(H_2O)^{2+}$, 48102-16-5; InoH, 58-63-9; 1-methylinosine, 2140-73-0.

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