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Interaction of (dien)Pd(II) Complexes with the Amino Group of Cytidine: a Kinetic and NMR Study

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Abstract

Interaction between cytidine and (dien)Pd(II) has been studied at high pH by spectrophotometry and by ^{1}H, ^{13}C and ^{15}N NMR. With increasing pH interaction of (dien)Pd(II) with the exocyclic NH_2 group is observed, and this (dien)Pd–N4 complex is more stable at high pH than the (dien)Pd–N3 complex, which exists at neutral pH. Furthermore, at the higher concentrations used in the NMR spectroscopy (0.1–0.2 M) a binuclear complex implying both the N3 and N4 (exocyclic amino) sites is present. pH-jump experiments revealed two kinetically distinct reactions. The faster 'Reaction I' is catalysed by OH^- and is attributed to the formation of the (dien)Pd–N4 complex. This reaction proceeds by a mechanism in which fixation of Pd(II) at the N3 site is required for the deprotonation of the NH_2 group by OH^-. This process is followed by an intramolecular transfer of (dien)Pd(II) from N3 to the deprotonated N4. The slower 'Reaction II' is attributed to dissociation of both Pd–N3 and Pd–N4 complexes at high pH and leads to the thermodynamically stable (dien)Pd-$(OH)^+$. Kinetic data for this process are in good agreement with those observed for the formation of (dien)Pd–N3.

Introduction

Palladium complexes interact with various nucleic acid constituents. The simplest systems involve derivatives of cytosine, uracile and thymine, because their reaction with (dien)Pd(II) complexes involve usually only one site. Studies in solution, principally based on NMR, led to elucidate binding sites of Pd(II) and of analogous Pt(II) complexes with cytidine [1–9], CMP [10] and cytosine [11] and have shown that the N3 site is involved in neutral or near neutral solutions. Several crystal structure studies have confirmed this binding site [12–16].

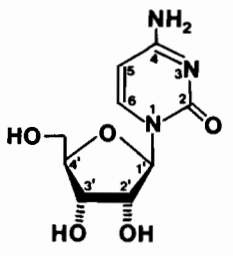
It was believed until recently that the exocyclic NH_2 group does not interact with metal ions under mild conditions. However, in a few particular cases, coordination to this site was evidenced [17, 18]. The complex formed implies formation of a chelate, with a metal-complex having two labile sites in *cis* configuration, and two cytidine or 1-methyl cytosine ligands.

In this paper we wish to report on the existence of such a complex, with (dien)Pd(II) species, having only one labile site and, therefore, unable to form such chelates. The (dien)Pd(II)–cytidine system has been studied from thermodynamic and kinetic points of view in a previous work in near physiological conditions [19]. In the present work the system is investigated at high pH, far from physiological conditions. Although equilibrium conditions are quite different in the two cases, kinetic data are in good agreement. Structures and distribution of the different species are confirmed by ^{1}H, ^{13}C and ^{15}N NMR studies.

Experimental

Materials

Cytidine (free base) **1** was obtained from Sigma Chemical Co and it was used without further purification. Both [(dien)PdCl]Cl and $[(dien)Pd(H_2O)](ClO_4)_2$ were prepared as described previously [19]. The pH of the solutions was adjusted using $HClO_4$ or NaOH and ionic strength was maintained at 0.2 M by means of $NaClO_4$.



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Kinetic Methods

Reaction kinetics at high pH were studied using a pH-jump technique. A solution, containing cytidine

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and (dien)Pd(II) at equilibrium at pH = 6, was mixed with an appropriate NaOH solution in order to achieve the desired pH value between pH = 11 to 13. Changes in absorbances *versus* time were monitored and correspond to the transformation of the neutral pH species into those existing at high pH. In the case of fast reactions a Dionex Model D-130 stopped-flow spectrophotometer with data acquisition was used. This system with corresponding data-treatment was described previously [19]. The slower reactions were followed by means of a Perkin-Elmer 552 spectrophotometer with constant temperature cell compartment.

NMR Spectra

NMR spectra were recorded on a Bruker WH-400 High resolution spectrometer at Université de Montréal's Regional NMR Facilities. Concentrations of (dien)Pd(II) and cytidine were of 0.033 M and 0.030 M respectively in the ^{1}H NMR experiments. Total Cl^- concentration was 0.066 M and pH was adjusted by means of NaOD in D_2O.

For H_2O solutions, a symmetric Redfield pulse sequence was used to suppress the solvent signal. For the saturation transfer experiments, difference spectra were obtained to best visualize the results; transients were collected after preirradiation (5 s) at selective frequencies and recycled to average the drift effect. pH values for solvent isotope effects were corrected as usual. The integration on partially overlapping peaks was carried out using a deconvolution program. ^{13}C experiments were carried out at 100.6 MHz using dioxane as external reference (67.40 ppm), 0.1 M cytidine and 0.11 M (dien)Pd(II), while ^{15}N data were obtained at 40.62 MHz in 85:15 (*v/v*) H_2O/D_2O solutions, using saturated $(CH_3)_4N^+Cl^-$ aqueous solution as external reference (43.54 ppm), 0.2 M cytidine and 0.22 M (dien)Pd(II).

Results

Kinetic Studies

Our preceding work has already revealed a secondary interaction at high pH, causing higher than usual absorbances [19]. Furthermore, a second, very slow signal was observed in the time-dependent study, following the faster Pd(II)–N3 interaction. Similar data were obtained using 1-methyl cytosine, which shows that the ribose moiety is not involved in this interaction. Furthermore no deamination was observed under the relatively mild reaction conditions.

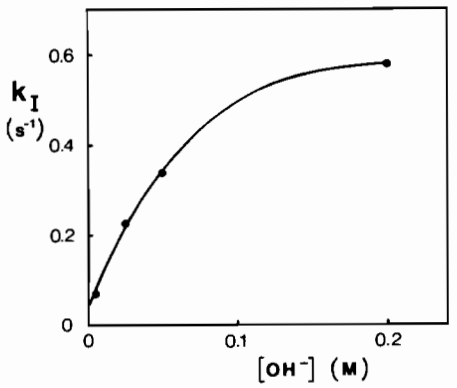


Fig. 1. Influence of OH^- concentration on k_I. T = 25 °C; μ = 0.2 M [cytidine] = 1.00×10^{-2} M; [(dien)Pd$(H_2O)^{2+}$] = 1.00×10^{-3} M.

pH-jump from pH = 6, where Pd(II)–N3 is the major product, to pH $\geqslant$ 11.7 produced two kinetic signals, easily separated one from the other. The first faster reaction produced an increase in absorbance (Reaction I) while the second, approximately 10^3 times slower, produced a decrease in absorbance (Reaction II).

For Reaction I, first order rate-constant, k_I, increases with OH^- towards a limiting value at high pH (Fig. 1). This behaviour is explained by a fast proton exchange equilibrium involving OH^-, followed by a rate-determining step. Kinetics were independent of excess Pd(II), cytidine or added Cl^- ions.

Reaction II was inhibited by increasing OH^- (Fig. 2). Furthermore, k_{II} increases with Cl^-, while Pd(II) and cytidine have no effect on the kinetics.

Scheme 1 describes mechanisms of both reactions and relates them to results at neutral pH. Addition of OH^- causes deprotonation of the NH_2 group (N4) of cytidine in the Pd(II)–N3 complex. This is

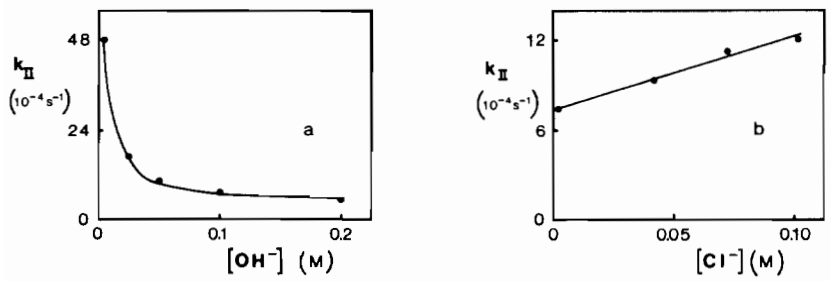
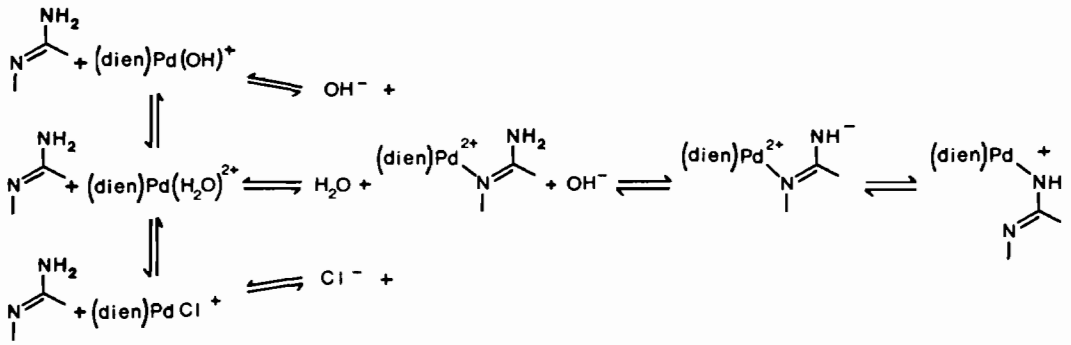


Fig. 2. Influence of OH^- and Cl^- concentrations on k_{II}. (a) [Cl^-] = 0; (b) [OH^-] = 0.10 M.



Scheme 1.

followed by slow intramolecular transfer of Pd(II) to the NH^- group in Reaction I (formation of Pd(II)–N4). This relatively fast reaction is followed by a return to equilibrium state where the main species are $(dien)Pd(OH)^+$, free cytidine and remaining (dien)-Pd–N4. This reaction is due to the high stability of the Pd–OH bond which causes partial dissociation of Pd–N3 and Pd–N4.

Interpretation of rate data uses the following simplified symbols: C = free cytidine; C_3^{2+} = Pd–N3 complex; $C_3H_{-1}^+$ = N4 deprotonated Pd–N3 complex; C_4^+ = Pd–N4 complex.

Reaction I

Reaction I can be described as follows:

$$C_3^{2+} + OH^- \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} C_3H_{-1}^+ \quad \text{fast} \tag{1}$$

$$C_3H_{-1}^+ \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} C_4^+ \quad \text{slow} \tag{2}$$

Using $K_1 = k_1/k_{-1}$, overall rate-constant k_I is given by:

$$k_I = \frac{k_2 K_1 [OH^-]}{1 + K_1 [OH^-]} + k_{-2} \tag{3}$$

Constants k_2 and K_1 have been determined by least-squares and presented in Table I.

Reaction II

Reaction II is represented by eqns. (4) to (8):

$$C_4^+ \underset{k_2}{\overset{k_{-2}}{\rightleftharpoons}} C_3H_{-1}^+ \tag{4}$$

$$C_3H_{-1}^+ \underset{k_1}{\overset{k_{-1}}{\rightleftharpoons}} C_3^{2+} + OH^- \tag{5}$$

$$C_3^{2+} + OH^- \underset{k_3}{\overset{k_{-3}}{\rightleftharpoons}} C + (dien)Pd(OH)^+ \tag{6}$$

TABLE I. Rate-parameters for the (dien)Pd(II)–cytidine System at High pH

K_1	16 M^{-1}
k_2	0.73 s^{-1}
k_{-2}	0.011 s^{-1}
K_2	66
k_3	0.023 M^{-1} s^{-1}
k_{-3}	0.18 M^{-1} s^{-1}
K_3	0.13[a]
k_4	2900 M^{-1} s^{-1} [b]
k_{-4}	0.017 s^{-1} (0.015)[b]
K_4	1.7×10^5 M^{-1}
k_5	69 M^{-1} s^{-1} (75)[b]
k_{-5}	0.23 M^{-1} s^{-1} (0.25)[b]
K_5	300[a]

[a] Determined by spectroscopy [19]. [b] Determined at pH = 7 [19].

$$C_3^{2+} + H_2O \underset{k_4}{\overset{k_{-4}}{\rightleftharpoons}} C + (dien)Pd(H_2O)^{2+} \tag{7}$$

$$C_3^{2+} + Cl^- \underset{k_5}{\overset{k_{-5}}{\rightleftharpoons}} C + (dien)PdCl^+ \tag{8}$$

Reaction (5) has been studied after the completion of Reaction I, by jumping the pH again, from high pH to low pH. This has been achieved by addition of appropriate amounts of $HClO_4$ or CH_3COOH to the reacting mixture before the beginning of the slow Reaction II. Under these limiting conditions reactions (6) to (8) are again negligible, because at low pH the final product at equilibrium is C_3^{2+} ((dien)Pd–N3) and, therefore $k_{II} = k_{-2}$. It's value, $k_{-2} = 0.011$ s^{-1}, is independent of final pH from 1.0 to 4.7.

Without this second pH jump, at high pH, results of Fig. 2 are obtained. Steps (4) and (5) are fast, as compared to the overall rate-constant k_{II}. Moreover, OH^- inhibits the reaction due to a decrease in C_3^{2+}

concentration with OH^-. Reactions (6) and (8) take account of the influence of OH^- and Cl^-, respectively.

This mechanism leads to a general expression for k_{II}:

$$k_{II} = \frac{k_{-3}[OH^-] + k_{-4} + k_{-5}[Cl^-]}{1 + K_1 + K_1K_2[OH^-]} + \left[\frac{k_3K_{OH}[OH^-] + K_wk_4 + K_wk_5K_{Cl}[Cl^-]}{K_{OH}[OH^-] + K_w(1 + K_{Cl}[Cl^-])}\right] \times [C] \quad (10)$$

where $K_{OH} = [(dien)Pd(OH)^+]/[OH^-][(dien)Pd(H_2O)^{2+}]$ and $K_{Cl} = [(dien)PdCl^+]/[Cl^-][(dien)Pd(H_2O)^{2+}]$.

These equilibria are fast as compared to the slow overall rate of Reaction II. Results obtained at different OH^- and Cl^- concentrations led to evaluation of the rate-constants. These are presented in Table I, together with those determined previously [19].

^1H NMR Results

Confirmation of the species proposed in Scheme 1 has been achieved by NMR studies. A series of ^1H-400 MHz spectra of the (dien)Pd(II)–cytidine system were taken in D_2O at different pD values. Typical spectra are shown in Fig. 3 and the chemical shifts of H5, H6 and H1′ protons are given in Table II, with population of the different species as a function of pD presented in Fig. 4.

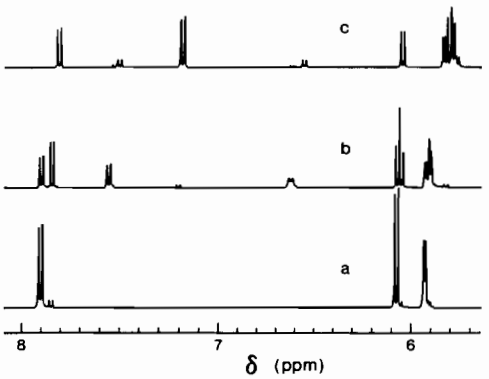


Fig. 3. ^1H NMR spectra of the (dien)Pd(II)–cytidine system at various pD values. (a) pD = 7.20; (b) pD = 10.17; (c) pD = 13.44.

The assignment of the signals was based on (i) order of appearance as a function of pD; (ii) relative intensities of the signals and (iii) coupling constants between protons H5–H6 (7.5–7.8 Hz) and protons H1′–H2′ (4.0–5.5 Hz). In neutral solution, the Pd–N3 complex (C_3^{2+}) is the main species, but because of the high Cl^- concentration a small fraction of cytidine remains uncomplexed and is also observed in the spectra.

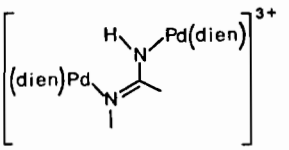
TABLE II. Assignment of ^1H NMR Signals to Different Species

pD	(ppm)[a,b]			Assignment[c]
	H6	H5	H1′	
7.20	7.898	6.068	5.925	C_3^{2+}
	7.843	6.048	5.901	C
10.17	7.896	6.066	5.922	C_3^{2+}
	7.840	6.046	5.900	C
	7.549	6.618	5.892	bi^{3+}
	7.200	5.818	5.883	C_4^+
13.44	7.350			C_3^{2+}
	7.806	6.040	5.832	C
	7.520	6.612		bi^{3+}
	7.180	5.802	5.783	C_4^+
	7.498	6.546	5.761	unknown

[a]All signals are doublets. [b]DSS as external reference. [c]Symbols definition in text.

As pD increases, the concentration of C_3^{2+} decreases while that of free cytidine increases, in agreement with Scheme 1.

Starting at pD ≃ 8, signals at 7.549 (H6), 6.618 (H5) and 5.892 ppm (H1′) were detected and indicate the presence of a new species. These doublets have been attributed to a binuclear complex, bi^{3+}, **2**, with sites N3 and deprotonated N4 of a cytidine molecule interacting with two (dien)Pd(II)'s as shown below.



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As the pD increases, the concentration of bi^{3+} increases and then decreases again at pD > 11. Signals corresponding to the Pd–N4 complex (C_4^+) are detected starting at pD ≃ 9 and its concentration increases with pD, according to Scheme 1. Dilution at constant pD leads to a dramatic decrease in the concentration of bi^{3+} as compared to C_4^+, which is in agreement with their different stoechiometry (2:1 *versus* 1:1).

Finally, at pD > 11, a minor product is present and was not further investigated. It results probably from substitution of two amino protons by two (dien)Pd(II) moieties.

The overall picture is given in Fig. 4 where the percentages of the different species, based on integration of H6 signals, is presented as a function of

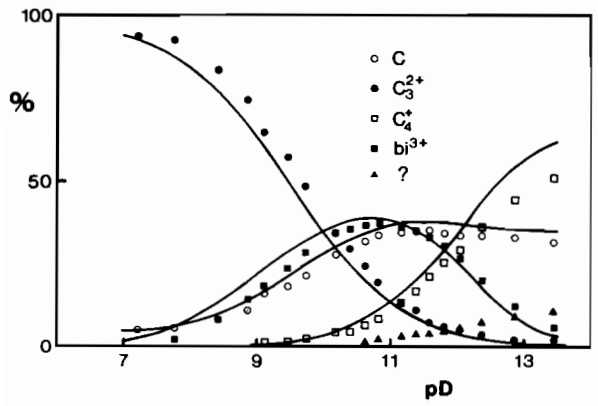


Fig. 4. Distribution curves of various species *vs.* pD. Points are determined from integration of H6 signals, curves are calculated.

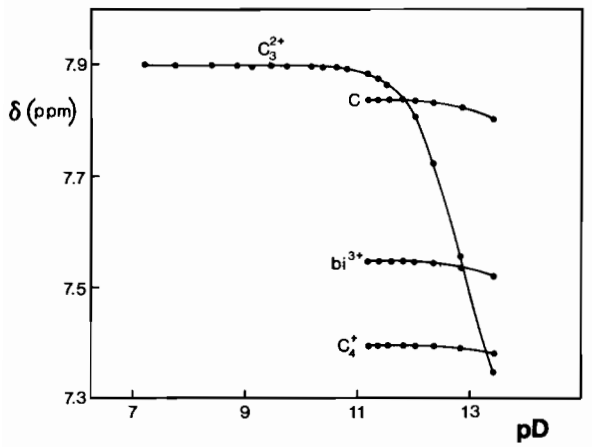


Fig. 5. Influence of pD on H6 chemical shifts.

pD. The H6 signal was chosen because of the absence of interference between different protons and peaks of different species.

Agreement between the experimental data and the calculated curves, based on equilibrium constants, derived from kinetic data at low concentrations is satisfactory. The binuclear complex has been accounted for by equation (11):

$$C_3H_{-1}^+ + (\text{dien})Pd(OH)^+ \overset{K_{bi}}{\rightleftharpoons} bi^{3+} + OH^- \qquad (11)$$

The slight discrepancies are due to the fact that NMR experiments were done in D_2O while data derived from kinetics in H_2O were used in the calculations. Furthermore, the minor unknown species was not included in the calculations.

Figure 5 shows the change of H6 chemical shifts with pD. While those of free cytidine (C), of binuclear complex (bi^{3+}) and of (dien)Pd–N4 (C_4^+) remain constant, H6 of (dien)Pd–N3 shows a decrease from 7.90 ppm at pD = 7.2 to 7.35 ppm at

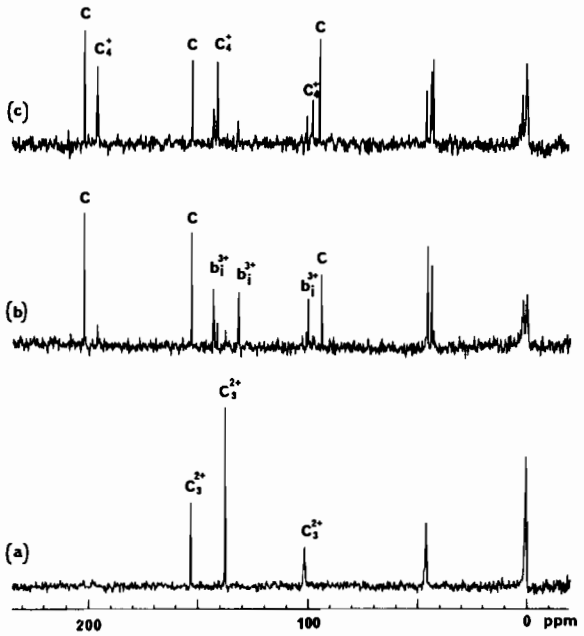


Fig. 6. ^{15}N NMR spectra of the (dien)Pd(II)–cytidine system with gated proton decoupling at different pH values: (a) pH = 6.6; (b) pH = 11.0; (c) pH = 12.1.

pD = 13.44. This is attributed to deprotonation of the NH_2 group of C_3^{2+}, leading to $C_3H_{-1}^+$.

In fact, at basic pH (>8), the amino protons of all species are rapidly exchanging with the protons of the medium, and individual N–H proton signals are not observed in H_2O solution.

However, at neutral pH, two separated signals are observed for the two amino protons of free cytidine or of the C_3^{2+} complex in H_2O. This indicates that both rotation about the C4–N4 bond and proton exchange rate are slow when compared to the chemical shift difference. The slow rotation about C4–N4 produces two non-equivalent environments for H5 in the bi^{3+} complex, that explains the broadening of the H5 doublet (Fig. 6).

^{13}C and ^{15}N NMR Results

The ^{13}C and ^{15}N data (Tables III and IV) confirm the ^1H results; examples of the ^{15}N spectra are shown in Fig. 6. The solution contains mainly the C_3^{2+} complex at neutral pH but when the pH is increased concentrations of C, bi^{3+} and then C_4^+ become more important. The chemical shifts of the different species remain practically unchanged within the pH range of 6.5 to 12.

The (dien)Pd(II) fixation at N3 (C_3^{2+}) induces a large upfield shift of C2 (–3.1 ppm), while the other carbons do not change significantly as it has been observed upon complex formation with (en)Pd(II) [3]. The presence of (dien)Pd(II) at N3 is best

TABLE III. ^{13}C Chemical Shifts[a] of Cytidine and their Derivatives in D_2O

	C2	C4	C5	C6	C1′	C2′	C3′	C4′	C5′
C[b]	158.52	167.20	97.34	142.67	91.20	70.50	75.00	84.92	62.00
C_3^{2+}[c]	155.45	166.60	96.80	143.13	91.68	70.36	75.06	85.20	61.81
bi^{3+}[d]	155.70	167.20	101.54	139.25	90.60	70.77	74.30	85.10	62.20
C_4^+[e]	159.59	171.80	102.27	136.80	89.96	70.91	74.09	84.79	62.34

[a] δ (ppm) from TMS (external reference dioxane/D_2O; δ = 67.40 ppm), 100.6 MHz. [b] Average values at pD = 6–12. [c] Average values at pD = 6–10. [d] Average values at pD = 10–12. [e] pD = 12.1.

TABLE IV. ^{15}N Chemical Shifts[a] for Cytidine and their Derivatives in D_2O/H_2O

	N1	N3	N(4)	NH(dien)	NH_2
C[b]	152.6	202.0	93.6		
C_3^{2+}[c]	152.8	137.5	101.3	45.9	0.0
bi^{3+}[d]	142.6	131.4	99.6	45.4; 43.4	1.5; −0.7
C_4^+[e]	141.3	196.2	96.8	42.3	−0.2

[a] δ(ppm) from NH_3 (external reference saturated aqueous (85:15 H_2O/D_2O) solution of $(CH_3)_4N^+Cl^-$, δ = 43.54 ppm, 40.62 MHz. [b] Average value for solutions of free cytidine and of cytidine–Pd(II) complexes at pH = 11.0 and 12.05. [c] pH = 6.6. [d] pH = 11.0. [e] pH = 12.1.

evidenced by the ^{15}N chemical shift; compared to free cytidine, the N3 and N4 nitrogens of C_3^{2+} are shifted by −64.5 and +7.7 ppm. These large chemical shift changes are comparable to those observed when N3 is protonated [20].

The substitution of one amino proton by (dien)-Pd(II) causes large chemical shifts of all atoms of the ring: N1 (−11.3 ppm), C2 (+1.09 ppm), N3 (−5.8 ppm). C4 (+4.64 ppm), C5 (+4.96 ppm) and C6 (−5.83 ppm). However, the amino nitrogen of C_4^+ is shifted downfield by only +3.2 ppm. This small shift change could be rationalized by assuming a near cancellation of paramagnetic and diamagnetic contributions to the nitrogen shielding [21].

The cumulative effect is observed on the ^{13}C and ^{15}N spectrum of the bi^{3+} complex. The chemical shifts changes of all the carbons (except C2) and of the nitrogens are practically the sums of those observed in C_3^{2+} and in C_4^+. Chemical shifts of N4 and C2 are more upfield shifted than predicted by perfect additivity. It is likely that shielding caused by Pd(II) at N4 is slightly different in bi^{3+} from that in C_4^+, which causes this effect.

Saturation Transfer Experiments

Slow exchange pathway between species is evidenced by the saturation transfer experiments performed on a solution containing the four species C, C_3^{2+}, bi^{3+} and C_4^+ at pH ~ 10.5. For example, upon the irradiation of the signal H6 of bi^{3+} the intensity of the H6 signal of C_4^+ decreases resulting from the exchange between these two species, and the intensity of the H5 and H1′ of bi^{3+} increases due to the nuclear Overhauser effect. The same results are obtained when the signals H5 of bi^{3+} or H6 of C_4^+ are saturated. The signals of C and C_3^{2+} are too close, and selective saturation, to establish the exchange between these two species, can not be performed. Upon the irradiation of the H6 signal of C, the signals of C_4^+ and bi^{3+} remain unchanged, confirming the absence of observable exchange between C and C_4^+ or bi^{3+}. Thus, on the narrow time scale of the saturation transfer experiment, only bi^{3+} and C_4^+ exchange fast enough to be evidenced; the exchange rate between the other species is much slower than the proton relaxation rate.

Discussion

The results of the present work evidence the interaction of (dien)Pd(II) with the deprotonated amino group of cytidine. Other metal ions are known to bind to this site, principally Hg(II) [22–28], Ru(III) [29, 30] and Ag(I) [31]. For CH_3Hg^+ it has been shown that under certain conditions both amino hydrogens can be substituted by the metal [28]. In the case of Pd(II) and Pt(II) complexes, only chelates, with a metal having two labile sites in *cis* configuration, have been shown to involve the exocyclic amino group [17, 18].

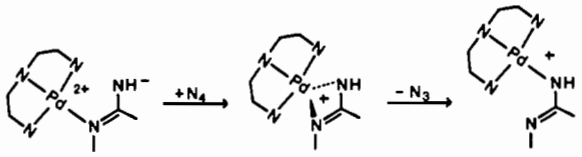
In free cytidine, the doublet of the amino group is delocalized in the cycle. Therefore, without its deprotonation, this site is of very low reactivity. The pK_a for deprotonation of the NH_2 group is about 14.8 [32], and, therefore, it remains protonated in the pH range studied.

The mechanism of formation of the Pd–N4 complex involves instead of cytidine, its Pd–N3 complex. The presence of the electrophilic Pd(II) at the N3 site increases acidity of the NH_2 group, similarly to the effect observed upon protonation of N3 [33]. The kinetic analysis led to a value of $K_1 = 16\ M^{-1}$, which corresponds to a pK_a of 12.8, an increase on acidity by a factor of 100. This enhances deprotonation at lower pH values, permitting subsequently binding of (dien)Pd(II) to this site.

Influence of pD on ^1H chemical shifts of the (dien)Pd–N3 complex (Fig. 3) is also in agreement with increased acidity of NH_2. This type of behaviour has also been noticed in the corresponding 1-methyl cytosine-CH_3Hg^+ system [25]. The pH-jump experiments, involving (dien)Pd–N3 as reactant, and a high resolution NMR study were necessitated in order to elucidate the overall mechanisms of the (dien)Pd(II)–cytidine system.

^1H, ^{13}C and ^{15}N NMR data confirm the sites of fixation of (dien)Pd(II) in the different species of Scheme 1 and evidence additional species at the higher concentrations used in these experiments.

Two possible mechanisms can account for the formation of the (dien)Pd–N4 complex (Reaction I). The first mechanism involves an intramolecular Pd(II)–transfer from N3 to N4 (Scheme 2). This process is related to the general mechanism of substitution of square-planar complexes. The NH^- group is conveniently located to serve as entering ligand. It can form a pentacoordinated transition-state which leads to the (dien)Pd–N4 complex by breaking of the Pd–N3 bond. The rate-constant for this process, $k_2 = 0.73\ s^{-1}$, is much higher than the rate-constant of decomposition of (dien)Pd–N3 ($k_{-3}[OH^-] \leqslant 0.036\ s^{-1}$; $k_{-4} = 0.017\ s^{-1}$; $k_{-5}[Cl^-] \leqslant 0.023\ s^{-1}$). Therefore, a mechanism which involves breaking of the Pd–N3 bond, followed by formation of the Pd–N4 bond, can be readily discarded.



Scheme 2.

The difference in rates between these two processes also explains the two reactions observed in the pH-jump experiments. Formation of Pd–N4, as first step, is kinetically favored in all cases, regardless of the final equilibrium conditions. Accordingly, even at high pH where the stable (dien)Pd(OH^+) is favored thermodynamically, first the fast intramolecular transfer is observed; it is then followed by a slow return to equilibrium, giving rise to Reaction II. However, when (dien)Pd(II) is mixed with cytidine at high pH, no reaction is observed, because of the high stability of the dominant species, (dien)Pd$(OH)^+$.

The existence of a binuclear species at higher concentration, as evidenced by NMR, leads to an alternate reaction path. It implies the formation of a binuclear species, with one (dien)Pd(II) each in N3 and N4 positions and, subsequently, dissociation of its Pd–N3 bond. This species can be formed by a bimolecular process between an NH^- of a (dien)Pd–N3 complex and (dien)Pd(II). However, this processes should be dependent on (dien)Pd(II) concentrations, both when formation or decomposition of the binuclear species is rate-determining. Obviously this is not the case under kinetic conditions and the intramolecular transfer is therefore preferred. This can be caused by the fact that (dien)Pd(OH^+), which is dominant at high pH, is of low reactivity, due to its poor leaving group, OH^-. At higher concentrations, when in fact significant concentrations of the binuclear species are present, this second reaction-path can contribute to the rates, provided that rate-constants of substitution of its Pd–N3 bond are much higher than those of (dien)Pd–N3.

Reaction II represents a sequence of reactions: Pd–N4 → Pd–N3 → Cytidine + (dien)Pd(II). Decomposition of (dien)Pd–N3 is in fact composed of slow substitution reactions by H_2O, OH^- and Cl^-, followed by fast redistribution of (dien)Pd(II) species according to thermodynamic stabilities. The main driving force for Reaction II is the great stability of (dien)Pd$(OH)^+$ at high pH, leading ultimately to dissociation of the cytidine complexes. The good agreement between values of the rate-constants of the present work (k_{-4}, k_5 and k_{-5}) and those obtained for the formation of (dien)Pd–N3 at pH = 7 [19] shows the coherence of the overall mechanism despite of the totally different equilibrium conditions in the two cases.

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