Sites of Palladium(II) Binding to Mixtures of Nucleoside Monophosphates[1]

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Abstract: In order to assess the individual nucleic base binding sites and relative binding strengths of several nucleic bases for antitumor Pt(II) compounds, we investigated much more rapidly reacting complexes of Pd(II). The addition of a 5'-phosphate group increases the extent of binding of dienPd^{2+} at N(7) over N(1) in purine nucleosides. The N(7)/N(1) molar ratio of dienPd^{2+} binding to adenosine, 2'/3'-AMP, and cyclic 3',5'-AMP is about 0.2, while for 5'-AMP it is 0.5 for the $ROPO_2H^-$ form and becomes 1.2 for the $ROPO_3^{2-}$ form. The last increase in ratio is coupled quantitatively to an acidification of 0.4 log unit of the phosphate deprotonation in the N(7)-bound complex compared to unbound 5'-AMP. In mixtures of nucleoside-5'-phosphates binding of dienPd^{2+} is selective and favors N(7) of GMP at all $5 < pH < 9$. For the other 5'-nucleotides the order is pH dependent. At pH 7 the order of dienPd^{2+}-binding strengths is N(7) of GMP > N(3) of TMP > N(3) of CMP > N(7) of AMP > N(1) of AMP. Application of the reactions of Pd(II) compounds as a thermodynamic reference for reactions of analogous Pt(II) compounds at equilibrium suggests that in many Pt(II) studies equilibrium was not achieved and reported stability constants for Pt(II) binding to nucleosides are too low.

Despite many studies conducted on binding of antitumor and related Pt(II) compounds to nucleic bases, it remains unclear whether the resulting complexes and inferred binding sites are under thermodynamic or kinetic control.[3] Features of results in the literature suggest that kinetic control is more frequent than commonly supposed. To form Pt(II) complexes, it is necessary either to heat the solutions or to allow them to stand for several days. The reported binding of *cis*-$(NH_3)_2Pt^{2+}$ to ribonucleosides, cytidine, adenosine, 1-methyladenosine, guanosine, and 1-methylguanosine at pH 6.5 with essentially equal stability constants of $\log K = 3.6 \pm 0.1$ has been ascribed to a lack of thermodynamic selectivity.[4] The constancy of the calculated "equilibrium" constants for such a diverse range of ligands suggests that kinetic rather than thermodynamic qualities are being measured. This specific topic is analyzed in the Discussion section.

In investigations aimed at understanding the binding sites of antitumor Pt(II) compounds to nucleic bases, we have employed Pd(II) compounds. Both metal ions possess similar ionic radii, prefer nitrogen rather than oxygen donor atoms, and form strongly tetragonal complexes, but those with Pd(II) react perhaps 10^5 times faster. Though Pd(II) remains a relatively slowly reacting metal ion, as demonstrated by the presence of both bound and unbound ligand peaks in NMR spectra, there is little doubt that the Pd(II) complexes are at equilibrium. Raising and lowering the pH, for example, yields an identical species distribution in the NMR spectrum. In this investigation we wished to avoid the complications produced by a special reaction between purine bases and enPd^{2+}.[5] With the terdentate diethylenetriamine (dien) in the compound dienPd^{2+}, only a single tetragonal Pd^{2+} coordination site is available for binding to nucleic bases. In this paper we use ^{1}H NMR spectra to elaborate the pH dependence of the binding sites of dienPd^{2+} to the ribonucleoside-5'phosphates, GMP, IMP, and AMP. With mixtures of nucleoside monophosphates we find that selective binding of dienPd^{2+} occurs, ruling out nearly equal stability constants.

Experimental Section

Nucleotides of the best quality available from Sigma Chemical Co. were used without further purification. Nitrates of dienPd$(D_2O)^{2+}$ and enPd$(D_2O)^{2+}$ were prepared as described previously.[6,7] Complexes were prepared in D_2O at 25 mM for ^{1}H NMR spectra and at 0.2 M for ^{13}C NMR spectra. The pH values are uncorrected for D_2O solvent. ^{1}H NMR spectra were recorded on a 90-MHz Varian EM-390 spectrometer at a probe temperature of 34 °C with *tert*-butyl alcohol as internal reference. ^{13}C NMR spectra were obtained on a Jeol PS-100/EC 100 FT spectrometer at 23.5 kG with dioxane as internal reference. Assignment of the H(8) peak in the purine bases was often aided by its exchange with solvent deuterium upon Pd^{2+} binding at N(7).[3]

Table I. H(8) Chemical Shifts in IMP and GMP and Complexes with dienPd^{2+} [a]

	IMP		GMP	
free ligand	$ROPO_3H^-$	$ROPO_3^{2-}$	$ROPO_3H^-$	$ROPO_3^{2-}$
B^-		7.20		6.83
BH_1	7.19	7.31	6.84	6.95
dienPd^{2+}				
M_7B		7.70		7.35
M_7BH_1	7.56	7.85	7.21	7.40
M_7BM_1	7.45	7.72	7.10	7.37

[a] In parts per million downfield from *tert*-butyl alcohol as internal reference.

Results

A consistent assignment of H(8) chemical shifts for free ligand and dienPd^{2+} complexes of IMP and GMP are shown in Table I. The H(2) chemical shifts of IMP are smaller and less diagnostic.[5] For both ligands the chemical shifts of the B^-, BH_1, M_7B, M_7BH_1, and M_7BM_1 species consistently occur 0.35–0.37 ppm upfield for GMP compared to those for IMP. (The subscripts identify the position on the purine ring at which a proton or metal ion binds.) In the free ligands deprotonation of the phosphate occurs with $pK_a = 6.2$. Ionization from N(1) gives the B^- species with $pK_a = 8.9$ in IMP and 9.4 in GMP.[3] The phosphate deprotonation in IMP and its complexes was also investigated by following the H(8) chemical shift over a range of pD values. For both the M_7BH_1 and M_7BM_1 complexes of IMP, the phosphate deprotonation occurs with $pK_a \simeq 5.8$ in D_2O. This value is about 0.5 log unit less than that of $pK_a \simeq 6.3$ found for free IMP in D_2O from the variation of the H(8) chemical shift. In comparison to the potentiometric titration curve of an equimolar solution of IMP and dienPd^{2+}, loss of the N(1) proton in M_7BH_1 to yield M_7B occurs in a higher pH (~8) range than phosphate deprotonation in the complex.

Because there is only one nucleic base hydrogen H(8) in guanosine and GMP, the effects of dienPd^{2+} complexation were studied by ^{13}C NMR. In comparison to free anionic guanosine at pH 11, the greatest chemical shift difference in the complex

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Table II. ^{1}H NMR Chemical Shifts of Nucleic Bases[a]

	pH 5	pH 9
CMP, ligand (H6)	6.77, 6.68	6.86, 6.77
dienPd (N3)	6.80, 6.71	6.96, 6.87
TMP, ligand (H6)	6.51	6.51
dienPd (N3)		6.32
GMP, ligand (H8)	6.84	6.89
dienPd (N7)	7.35	7.40
5'-AMP, ligand (H8, H2)	7.22, 6.94	7.34, 6.96
dienPd (N7)	7.80, 7.04	8.02, 7.03
dienPd (N1)	7.24, 7.36	7.37, 7.35
2'-/3'-AMP, ligand (H8, H2)	7.12, 6.96	7.09, 6.93
dienPd (N7)	7.68, 7.05	
dienPd (N1)	7.16, 7.37	7.16, 7.33
3'-5'-cAMP, ligand (H8, H2)	6.97, 6.97	
dienPd (N7)	7.51, 7.11	
dienPd (N1)	7.03, 7.37	

[a] In parts per million downfield from *tert*-butyl alcohol as internal reference.

with dienPd^{2+} at pH 9 is an upfield shift of C(2) of about 1.5 ppm (C(6) was not detected above noise in this complex). C(8) is shifted 0.4 ppm downfield in the complex. The results parallel those reported for reaction of dmenPd^{2+} with guanosine anion.[8] Both sets of results support coordination of Pd^{2+} at the deprotonated N(1) site.

In comparison to free ligand GMP at pH 4, the complex with equimolar dienPd^{2+} exhibits downfield ^{13}C NMR shifts of 0.6 ppm at C(2) and 1.1 ppm at C(8). This near reversal of the above results agrees with metalation at N(7) to give the complex M_7BH_1. As the pH is increased to 9 (the end point in the potentiometric titration), a mixture of at least three species in varying proportions is evident. Addition of a second equivalent of dienPd^{2+} at pH 9 yields a sharp spectrum with a single binuclear complex M_7BM_1. In the spectrum of this complex the ^{13}C peaks of the dien are doubled, indicating two different dienPd^{2+} environments.

With the ^{1}H NMR assignments in Table I and the ^{13}C NMR results, it is possible to follow the course of the complex species present in equimolar solutions of dienPd^{2+} and IMP or GMP as the pH is increased. At pH 4 the predominant complex is M_7BH_1. Phosphate deprotonation occurs in this complex with p$K_a \simeq 5.8$ (D_2O). From pH 5–9 even equimolar solutions contain, in addition to M_7BH_1 and M_7B, significant amounts of the binuclear complex M_7BM_1 and a corresponding amount of free ligand BH_1. An equimolar solution of inosine or guanosine and dienPd^{2+} also contains M_7BH_1 and M_7BM_1 in acidic and neutral solutions, but at pH 9 the sole complex is BM_1,[5] as demonstrated for guanosine by ^{13}C NMR in this study. The BM_1 complex occurs to a much lesser extent with IMP and GMP. Thus the presence of a 5'-phosphate group strongly favors dienPd^{2+} complexation at N(7) compared to N(1).

An equimolar mixture of the four 5'-mononucleotides, CMP, TMP, GMP, and AMP, displays six lines in the ^{1}H NMR spectrum more than 6.2 ppm downfield from *tert*-butyl alcohol due to resonances from carbon-bound protons on the nucleic bases indicated in Table II. CMP and AMP yield two resonances each, and TMP and GMP, one each. Nucleoside monophosphates undergo a deportonation from the phosphate group with p$K_a \simeq$ 6.2.[3] Table II contains ^{1}H NMR chemical shifts for species observed near pH 5 and also near pH 9. Because there is rapid proton exchange between acidic and basic forms, only a single peak occurs in the pH region of deprotonation. For this reason some of the chemical shifts recorded in Table II refer to more than one species. Examples include shifts for unbound nucleic bases TMP and GMP with a p$K_a \approx 9$ for heterocyclic ring deprotonations and complexes undergoing phosphate deprotonations in the pH 6 region such as GMP where the 7.35-ppm chemical shift is intermediate between the two values for "pure" M_7BH_1 complexes in Table I.

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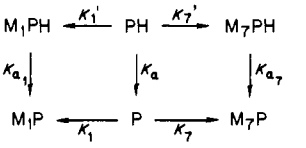
Scheme I



The effect of the addition of 1 equiv of dienPd^{2+} on the ^{1}H NMR spectrum of a solution containing equimolar amounts of the four 5'-mononucleotides depends upon the pH. Near pH 5 the resonance at 6.84 ppm due to H(8) of GMP is replaced by a new resonance at 7.35 ppm due to the M_7BH_1. A small amount of reaction with CMP is also evident (see below). A similar result is obtained near pH 7 except that the new peak appears at 7.46 ppm. Near pH 9 the free ligand peak of GMP also disappears, and the new peak appears at 7.40 ppm. In the dienPd^{2+} complex the proton at N(1) has been lost by this pH, and the predominant GMP species present is M_7B. A new feature that also appears at pH 9 is the partial disappearance of the TMP free ligand peak to give a new peak at 6.32 ppm due to metal ion substitution for the proton at N(3).

Addition of 1 equiv of enPd^{2+} to a solution of the four 5'-mononucleotides from pH 5 to 9 results in final disappearance of the GMP resonance. This disappearance suggests a enPd^{2+}-induced stacking of GMP, as reported earlier.[5] At the high pH end of the range appearance of a peak at 6.30 ppm indicates some reaction of TMP.

Results of the four 5'-nucleotides tested show that GMP reacts most strongly with both dienPd^{2+} and enPd^{2+} from pH 5 to 9. In order to learn the relative Pd^{2+}-binding capabilities of other nucleic bases, we investigated mixtures without GMP. Substitution of GMP by IMP in a mixture of four 5'-nucleotides reveals that IMP now reacts most strongly with both dienPd^{2+} and enPd^{2+} from pH 5 to 9. Peaks due to free ligand IMP disappear and in their place appear an array of new peaks associated with complex formation as described above and tabulated in Table I.

Experiments were performed with 5'-AMP in mixtures with pyrimidine 5'-nucleotides at pH 5, 7, and 9. Mole fractions were calculated from areas of peaks assigned as in Table II. In an equimolar mixture of CMP and AMP, at all three pH values, dienPd^{2+} binds to CMP with 0.6-mol fraction and to AMP with 0.4-mol fraction. In an equimolar mixture of TMP and AMP the binding is pH dependent due to the ionizable N(3) proton of TMP with pK_a = 10.0 in the free ligand. The mole fraction of dienPd^{2+} binding to TMP increases from about 0.3 at pH 5, through about 0.7 at pH 7, to 1.0 at pH 9. Additional experiments with AMP and the two pyrimidine nucleotides and dienPd^{2+} all present in equimolar amounts gave results consistent with those above at each pH value. At pH 5 CMP is the predominant ligand, by pH 7 TMP dominates, and at pH 9 TMP is virtually the only complexed ligand of the three. Thus in an equimolar mixture of AMP, CMP, and TMP with dienPd^{2+}, the AMP complex never becomes the dominant species at any pH 5–9.

Binary nucleotide mixtures of 5'-CMP and either the mixed isomers of 2'- and 3'-AMP (2'-/3'-AMP) or cyclic 3',5'-AMP were studied for their complexing capability for dienPd^{2+}. At all pH values from pH 5 to 9 5'-CMP is the strongest Pd^{2+} binder, reacting to the extent of about 0.7-mol fraction. From these experiments and from those in which 2'-/3'-AMP and cyclic AMP were the only nucleotides, the molar ratio of N(1)/N(7) binding by dienPd^{2+} is about 4 for the last two nucleotides independent of pH from pH 5 to 9. In contrast to these results, the N(1)/N(7) ratio for dienPd^{2+} binding to 5'-AMP decreases with increasing pH. Failure to recognize this dependence has resulted in mistaken identification of peaks in NMR spectra.

The molar ratio N(7)/N(1) of dienPd^{2+} binding to 5'-AMP depends upon the state of ionization of the phosphate group. It is possible to relate the increase in N(7)/N(1) binding with increasing pH to the decrease in phosphate pK_a produced by dienPd^{2+} binding at N(7) of 5'-AMP. In Scheme I we represent a protonated phosphate as PH and a deprotonated phosphate as P. The subscript on M identifies the nitrogen, N(1) or N(7), to

Table III. H(8) Chemical Shifts and N(7)/N(1) Ratios for dienPd^{2+} Binding to AMP

pH[a]	M_7B[b]	BM_1[b]	N(7)/N(1)	K_7/K_1	K_7'/K_1'
4.62	7.768	7.247			
4.89	7.779	7.247	0.54	1.26	0.54
5.46	7.819	7.253	0.59	1.21	0.51
5.85	7.867	7.269	0.70	1.25	0.53
6.45	7.953	7.311	0.93	1.26	0.54
7.06	8.002	7.349	1.12	1.24	0.53
7.79	8.013	7.360	1.19	1.22	0.52

[a] Meter readings uncorrected for D_2O solvent at 0.2 M $NaClO_4$. [b] H(8) chemical shift in parts per million downfield from *tert*-butyl alcohol as internal reference.

which the metal ion is bound (the phosphate group remains unbound).

Three phosphate acidity constants appear in the scheme: K_a for free ligand, K_{a1} for ligand with metal ion bound at N(1), and K_{a7} for ligand with metal ion bound at N(7). Four stability constants appear for metal ion binding at the nucleic base, the subscript denotes the nitrogen at which binding occurs, primes denote binding to ligand with a protonated phosphate, and the absence of primes indicates binding to ligand with deprotonated phosphate. The acidity and stability constants may be expressed in terms of concentrations in the usual way.

By observing the H(8) chemical shift, it is possible to follow its pH dependence for both the N(1)- and N(7)-metalated species in the same solution as base is added. The microscopic acidity constants K_{a1} and K_{a7} may be determined in this manner. Table III shows the H(8) chemical shifts as a function of pH. A least-squares fit to these points yields $pK_{a7} = 6.00 \pm 0.02$, $pK_{a1} = 6.37 \pm 0.05$. The limiting values of the H(8) chemical shifts are found to be: M_7PH, 7.76; M_7P, 8.02; M_1PH, 7.24; M_1P, 7.37 ppm. A small amount of free ligand appeared in the same solution, and from its H(8) chemical shift we calculate $pK_a = 6.40 \pm 0.07$ and the H(8) chemical shift limits: PH, 7.22; P, 7.34 ppm. The closeness of these values to the N(1)-metalated species and the small H(8) chemical shift range for both introduce the relatively large errors.

As anticipated, binding of dienPd^{2+} at N(1) of 5′-AMP produces little change in the pK_a for phosphate deprotonation elsewhere on the ligand, $pK_{a1} = 6.37$ and $pK_a = 6.40$. In contrast, dienPd^{2+} binding at N(7) results in an acidification of phosphate deprotonation by 0.40 log unit as $pK_{a7} = 6.00$. In combination with the two cycles in the equilibrium scheme, ratios of acidity constants may be used to compare relative binding at the deprotonated to protonated phosphate ligand for each nitrogen site. The stability constant ratio for binding at N(1) marginally favors the deprotonated over protonated phosphate ligand by $K_1/K_1' = K_{a1}/K_a$ = antilog 0.03 = 1.07. In contrast, the stability constant ratio for binding at N(7) markedly favors the deprotonated over protonated phosphate ligand by $K_7/K_7' = K_{a7}/K_a$ = antilog 0.40 = 2.5. The ratio of microscopic acidity constants for the N(7)- and N(1)-metalated complexes is related to stability constants in the above scheme: antilog 0.37 = 2.34 = $K_{a7}/K_{a1} = (K_7/K_1)/(K_7'/K_1')$.

On the same solutions on which the acidity constants were determined, integration of peaks in the ^1H NMR spectra allow determination of the molar ratio R of the sum of all N(7)-bound species to all N(1)-bound species. The results are also tabulated in the fourth column of Table III. For a complex with a metal ion at a given site, there is rapid exchange between phosphate protonated and deprotonated species. The N(7)/N(1) ratio trends from just above 0.5 at low pH, where the phosphate group is protonated, to about 1.2 at high pH, where the phosphate group is deprotonated. From the usual expressions for the equilibrium constants appearing in the above scheme we derive for the N(7)/N(1) molar ratio

$$R = \frac{[M_7PH] + [M_7P]}{[M_1PH] + [M_1P]} = \frac{K_7}{K_1}\,\frac{1 + (H^+)K_{a7}^{-1}}{1 + (H^+)K_{a1}^{-1}}$$

This equation describes the pH dependence of the ratio R through the region of phosphate deprotonation. The value for R appears in the fourth column of Table III for each pH listed in the first column. The results obtained above for pK_{a7} and pK_{a1} are incorporated, and the equation solved for the stability constant ratio K_7/K_1 corresponding to the N(7)/N(1) ratio for dienPd^{2+} binding to phosphate-deprotonated ligand. The calculated results tabulated in the fifth column of Table III are satisfactorily constant over the range of pH values, $K_7/K_1 = 1.24 \pm 0.02$. From the above equation this value is what R should attain at high pH where the phosphate group is deprotonated. Multiplication of the fifth column by K_{a1}/K_{a7} yields the stability constant ratio K_7'/K_1' corresponding to the N(7)/N(1) ratio for dienPd^{2+} binding to phosphate protonated ligand. This ratio is tabulated in the last column of Table III. Again the ratio is independent of pH and $K_7'/K_1' = 0.53 \pm 0.01$. This value is the limit R should reach at low pH where the phosphate group is protonated.

We are now able to check the ratios of stability constants obtained in the integrations against the ratio of acidity constants found from the H(8) chemical shifts in NMR spectra. To the equation at the end of the second to last paragraph, we add the results of this paragraph to obtain $2.34 = K_{a7}/K_{a1} = (K_7/K_1)/(K_7'/K_1') = 1.24/0.53 = 2.34$. The numerical agreement indicates that we have successfully coupled the H(8) chemical shift information for acidity constants with the areas under the peaks for stability constant ratios. This quantitative agreement provides strong support for the assignments of ^1H NMR peaks in Table II and in our previous studies.[5,6]

Discussion

Comparison of the H(8) chemical shift upon phosphate deprotonation in the free ligand BH_1 of IMP, GMP (Table I), and AMP reveals downfield shifts of 0.11 and 0.12 ppm. The direction of the shift opposes that usually observed for a deprotonation. Despite the "reverse" direction, significantly greater downfield shifts of 0.27–0.29 ppm occur upon phosphate deprotonation in the complexes M_7BH_1 and M_7BM_1. Due to the requirements of a cyclic system, this augmentation of about 0.16 ppm is also realized when metalation reactions are compared. Metalation at N(7) of the $ROPO_2H^-$ species of BH_1 to give M_7BH_1 yields a downfield shift of 0.37 ppm in IMP and GMP (Table I) and 0.35 ppm in inosine.[5] In contrast greater down field shifts of 0.50–0.54 ppm occur upon metalation of either phosphate-deprotonated ($ROPO_3^{2-}$) species BH_1 and B^- to give M_7BH_1 and M_7B, respectively, in both IMP and GMP (Table I).

A similar downfield chemical shift augmentation for H(8) also appears upon dienPd^{2+} binding at N(7) in complexes of 5′-AMP ($ROPO_3^{2-}$) in contrast to binding at N(7) in other adenosine ligands. The H(8) downfield shift upon coordination at N(7) of 5′-AMP in BM_1 is 0.15 ppm greater in $ROPO_3^{2-}$ than in $ROPO_3H^-$. For dienPd^{2+} coordination at B to give M_7B in $ROPO_3^{2-}$, the downfield shift of 0.68 ppm may be compared to H(8) chemical shifts upon similar coordination in the following ligands: ($ROPO_3H^-$)AMP, 0.54; 2′-/3′-AMP, 0.56; cyclic 3′,5′-AMP, 0.54 ppm.

Thus for all three purine 5′-nucleoside monophosphates investigated, GMP, IMP, and AMP, there is an additional downfield H(8) chemical shift evident on binding of dienPd^{2+} to N(7) of the dianionic phosphate form of the ligand, $ROPO_3^{2-}$, about 0.15 ppm greater than that found for binding at N(7) of other similar ligand species. These similar species include the $ROPO_2H^-$ form of the 5′-nucleoside monophosphate, nucleoside monophosphates other than 5′, and the corresponding nucleoside. The H(2) resonance of IMP and AMP lacks a similar augmentation in chemical shift.

The augmented H(8) downfield chemical shift appears to be related to the N(7)/N(1) mole ratio for dienPd^{2+} binding and to acidification of the phosphate deprotonation in the N(7)-bound complexes. In the case of the IMP complex the phosphate group deprotonates about 0.5 pH unit lower than in the free ligand. The ratio of N(7) to N(1) binding increases in the presence of a phosphate group. This effect is evident in equimolar complexes of dienPd^{2+} at pH 7. With IMP and GMP Pd^{2+} is predominantly

at N(7), while with the nucleosides inosine and guanosine it is mainly at N(1).

Coupling of the molar ratio of dienPd^{2+} binding at N(7) to N(1) of 5′-AMP to acidification of the phosphate deprotonation in the N(7) complex is described quantitatively at the end of the Results section. The N(7)/N(1) molar ratio increases from 0.5 to 1.2 upon deprotonation of the phosphate group, which is acidified in the N(7)-bound complex by 0.4 log unit compared to the case for the free ligand. These results may be compared to the N(7)/N(1) molar ratio of dienPd^{2+} binding to other adenine nucleotides. A similar high ratio of 1.3 is found for ADP and ATP from pH 4.5 to 8 with little effect of dienPd^{2+} binding at N(7) on the terminal phosphate pK_a.[9] Consistent with the high ratio, its pH independence, and lack of effect on phosphate pK_a, both $ROPO_3H^-$ and $ROPO_3^{2-}$ ionic forms of ADP and ATP display chemical shift augmentations similar to that of the $ROPO_3^{2-}$ form of AMP upon dienPd^{2+} binding at N(7).[9] Low N(7)/N(1) ratios of about 0.2 occur for 2′-/3′-AMP, cyclic 3′,5′-AMP, and adenosine.[6] The low values of about 0.2 for the N(7)/N(1) molar ratio may be considered to be the intrinsic ratio for dienPd^{2+} binding to the adenine base. The extent of raising of that ratio, first to 0.5 for AMP in the $ROPO_3H^-$ form and then more significantly to 1.2–1.3 for AMP in the $ROPO_3^{2-}$ form and ADP and ATP in both ionic forms, is perhaps an indication of the degree of hydrogen bonding to the 5′-phosphate group from a dien nitrogen coordinated to a Pd^{2+} bound at N(7). Outer-sphere complexes in which the 5′-phosphate group accepts a hydrogen bond from an amine ligand have been found in crystals.[10] This type of hydrogen bonding cannot occur in 2′-/3′-AMP or in cyclic 3′,5′-AMP or cannot occur when the metal ion is coordinated at N(1) of a purine base.

In discussing the binding sites of the nucleic base protion of mixtures of 5′-nucleoside monophosphates, it is convenient to use the first letter to identify the nucleotide and the number to locate the ring position. Thus the nucleotide stability order for protons bound to the heterocyclic rings is[3] T3 > G1 > I1 ≫ C3 > A1 > G7 > I7 > A7. By putting together the information contained in the results section, we obtain the following stability order for dienPd^{2+} binding to nucleoside monophosphates at each of the three pH values: pH 5, G7 ≫ C3 > A1 > A7 > T3; pH 7, G7 > T3 > C3 > A7 > A1; pH 9, G7 > T3 ≫ C3 > A7 > A1. The results at pH 5 largely correspond to the monoprotonated phosphate $ROPO_3H^-$, while those at pH 9 refer to the dianionic phosphate $ROPO_3^{2-}$. In comparison to the proton stability order, those for dienPd^{2+} binding exhibit a promotion of G7 and A7 sites.[3]

At all pH values from 5 to 9, dienPd^{2+} binds most strongly to GMP. The nucleic base portions of GMP, IMP, and TMP undergo deprotonations with pK_a = 9.4–10.0.[3] The elevation of T3 from the weakest binder at pH 5 to the second strongest at pH >7 results from dienPd^{2+} displacing the N(3) proton. A similar displacement occurs at the N(1) proton of guanosine and inosine,[5] but in GMP binding at N(7) predominates throughout the pH range, as already discussed.

The above nucleotide binding site stability orders found for dienPd^{2+} should be applicable to Pt^{2+} complexes at equilibrium. Failure to observe a similar order for Pt^{2+} complexes may be accounted for by the difficulty in reaching equilibrium. Pt^{2+} undergoes substitution reactions about 10^5 times slower than Pd^{2+}, which is still not rapid. It is expected that Pt^{2+} will have great difficulty in displacing protons from T3, U3, G1, and I1 sites in a reasonable time even at neutral pH. Furthermore, as mentioned above the N(7) sites of purines are promoted compared to N(1) in their Pd^{2+} (and Pt^{2+})-binding capabilities. Once Pt^{2+} has reacted at N(7), it will be strongly bound and essentially fixed even if binding at N(1) is thermodynamically favored.

Because of the dichotomy of two binding sites N(1) and N(7) in guanosine, inosine, and their nucleotides, interpretation of potentiometric titrations in terms of stability constants may be equivocal. An estimate of the expected stability constants at N(1) may be made from results of dienPd^{2+} and enPd^{2+} coordination to uridine and thymidine.[11] Similar to N(1) in purines, these nucleoside ligands possess a nitrogen bound hydrogen with pK_a = 9.2 and 9.8, respectively. The stability constant logarithms for binding of dienPd^{2+} are 8.1 and 8.3, respectively, and for binding of enPd^{2+}, 8.6 and 8.8.[11] For pH values well below pK_a where the predominant reaction is $Pd^{2+} + BH \rightleftharpoons PdB + H^+$, the average pH-dependent stability constants are $10^{-1.3}$ for dienPd^{2+} and $10^{-0.8}$ for enPd^{2+}. Since guanosine and inosine and their nucleotides possess a similar nitrogen bound hydrogen with a similar pK_a, their pH-dependent stability constants for Pd^{2+} binding at N(1) in the purine base portion should be comparable to those just described. This hypothesis is qualitatively supported by NMR results on these systems.[5]

We may utilize the results of the previous paragraph to estimate a minimum stability constant expected for binding of the platinum complex enPt^{2+} to guanosine. In the study mentioned in the introduction, a value of log K = 3.6 was derived at pH 6.5.[4] At this pH the equilibrium constant for binding of the palladium complex enPd^{2+} at N(1) is estimated to be $10^{-0.8}/10^{-6.5} = 10^{5.7}$. This value is already 2 orders of magnitude greater than the log K = 3.6 value. Two other considerations increase the disparity. Pt^{2+} binds to uridine at least 10 times more strongly than Pd^{2+}, and this difference should occur with other nucleic bases.[6] Finally, if metal ion binding occurs at N(7) rather than N(1), then bonding at N(7) must be greater than that just estimated for N(1). We conclude that the published value of log K = 3.6 for enPt^{2+} binding to guanosine at pH 6.5[4] is at least 3 orders of magnitude too small.

There are at least two explanations for the unreasonably low values of log K = 3.6 reported for aquated *cis*-$(NH_3)_2Pt^{2+}$ binding to nucleosides. Due to slow substitution about Pt^{2+}, the solutions may not have been at equilibrium. The equilibrium problem is compounded, moreover, by the reaction of *cis*-$(NH_3)_2Pt^{2+}$ to form, in neutral solution, a dihydroxo-bridged dimer, which is even more inert than the mononuclear compound. As we originally found for enPd$(H_2O)_2^{2+}$ in solution,[7] enPt$(H_2O)_2^{2+}$ also forms a dihydroxo-bridged dimer optimally through the species enPt$(H_2O)(OH^-)^+$. Since the pK_a values for water deprotonation in enPt$(H_2O)_2^{2+}$ are 5.8 and 7.6, dihydroxo-bridged dimer formation is especially favored in the pH 6.7 region.[7] This region is precisely where most binding studies of *cis*-$(NH_3)_2Pt^{2+}$ have been carried out. Thus there are two competing reactions, one to form complex and the other to form especially unreactive dihydroxo-bridged dimer. The greater the concentration of Pt^{2+}, the greater the prospect that most of the Pt^{2+} reacts to form dimer. In the studies referred to where log K = 3.6,[4] the Pt to ligand molar ratio was frequently greater than one, abetting dimer formation and reducing the amount of Pt^{2+} available for reaction with ligand. Dihydroxo-bridged dimer formation also affects interpretation of reactions with polynucleotides. Inaccessible nucleic base binding sites render the effective Pt to nucleic base mole ratio higher than analyzed, and instances of no reported reaction are probably due to the general inertness of Pt^{2+} and dimer formation. The reported lack of reactivity with DNA at pH 7 of *cis*-$(NH_3)_2Pt(H_2O)_2^{2+}$ compared to *cis*-$(NH_3)_2PtCl_2$[12] probably results from more ready dimerization of the aquo complex.

Reactions of Pd(II) compounds with nucleic bases serve as a thermodynamic reference for reactions of analogous Pt(II) compounds at equilibrium. Large differences between reaction products of analogous Pd(II) and Pt(II) compounds may be attributed to introduction of kinetic effects in the latter. Pt(II) does displace the N(3) proton from uridine and thymidine,[6] but displacement of the corresponding N(1) proton from guanosine and inosine and derivatives is diverted by prior coordination and kinetic fixation at N(7). With cis amine Pt(II) there is the additional impediment of inert dihydroxo-bridged dimer formation.

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