Complexes of (ethylenediamine)Pd(II) with Inosine, Guanosine, Adenosine and their Phosphates

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In order to elucidate the reactions of the relatively inert antitumor cis *diamine Pt(II) complexes, we have continued our investigations of the much more labile enPd(II). In its interaction with purine nucleosides and nucleotides enPd*$^{2+}$ *reacts in acidic solutions with N7 and in basic solutions with N1. In a wide pH region around neutral pH both N7 and N1 coordination occurs leading to possible polymeric complexes. Some of these complexes with mixed N7 and N1 coordination display unusual chemical shifts. For 5'-AMP this complex undergoes an acidification of the phosphate deprotonation by more than one log unit. For adenosine, the presence of chloride ion inhibits formation of complexes with unusual chemical shifts. Also with adenosine at high pH, the 6-amino group undergoes substitution of a proton by a metal ion, driven by formation of a binuclear dimer with each of two enPd*$^{2+}$ *coordinated to N1 of one adenine ring and to the 6-amino group of the other. Easy formation of 1:2 complexes of enPd*$^{2+}$ *and the 6-oxopurines in acidic solutions, where only N7 is coordinated, argues strongly against direct N7–O6 chelation as a significant bonding mode.*

Introduction

In this paper we continue reporting our investigations of $enPd^{2+}$ with nucleosides and their phosphates. It is a companion to our earlier paper on $enPd^{2+}$ complexes of uridine and cytidine [1]. We use the $enPd^{2+}$ as a model for antitumor *cis* diamine Pt(II) complexes [2–5]. Due to its likely rearrangement to the *trans* complex, it is not practical to use *cis* $(NH_3)_2Pd(II)$. The ethylenediamine ligand enforces a *cis* geometry. Because of the poor leaving group ability of Cl^- [5, 6] we employ the nitrate salt of $enPd(H_2O)_2^{2+}$. Some inhibitory properties of Cl^- that change reaction products are described in this paper. We provide a summary of the major complexes in $enPd^{2+}$ solutions of purine nucleosides and nucleotides.

In an earlier study of 5'-adenosine monophosphate (AMP) and inosine-5'-monophosphate (IMP) binding to planar dienPd(II) and enPd(II) we reported that in equimolar solutions with $enPd^{2+}$ both nucleoside monophosphates form complexes with an unexpectedly downfield shifted ribose HI' peak in proton magnetic resonance spectra [4]. We suggested that the complex was a polymer with both N1 and N7 coordinated to different $enPd^{2+}$. With respect to the binuclear complexes of $dienPd^{2+}$, M_7BM_1, where two different metal ions also coordinate to N1 and N7, the $enPd^{2+}$ complexes of both nucleoside monophosphates exhibit a 0.8 ppm downfield shift for the ribose HI'. In the same comparison the nucleic base H2 undergoes a 0.3 ppm upfield shift and H8 is shifted downfield. Similar results were found for deoxyinosine monophosphate [4]. In this paper we describe additional characteristics of the complex exhibiting the downfield shifted HI' resonance. We designate this complex generally X and specifically refer to its phosphate protonated and deprotonated forms as XH_p and X.

Experimental

All nucleosides and their phosphates were purchased from Sigma Chemical Company. Most experimental methods have already been described [1]. Adenosine substituted with deuterium at C8 was prepared by heating at high pH. Most NMR spectra were recorded on a Varian EM-390 spectrometer at 90 MHz and 34 °C. Though t-butyl alcohol was employed as an internal reference, all chemical shifts are reported downfield from DSS by adding 1.234 ppm. In most cases KNO_3 was added to control the ionic strength at 0.5 *M*. All experiments were performed in D_2O. The pH values are not corrected for D_2O [7].

Results

Selected results are reported as chemical shifts for nucleic base H8 and H2 and ribose H1' protons of assigned species in Table I. Complexes with equal amounts of $enPd^{2+}$ and ligand appear with greater mole fractions in equimolar solutions than complexes with a 1:2 $enPd^{2+}$ to ligand mole ratio which occur more commonly in solutions with excess ligand.

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TABLE I. Chemical Shifts of $enPd^{2+}$ Species.[a]

Species	H8	H2	H1′(J)	pH range
Inosine				
BH_1	8.30	8.19		$pK_a = 9.06$[b]
B^-	8.12	8.12		
M_7BH_1	8.63	8.24		2–4
$H_1B_7M_7BH_1$	8.65	8.24		2–5
B_1M_1B	8.12	8.29		8–12
5′-IMP				
BH_1H_p	8.42	8.20		$pK_a = 6.00$[b]
BH_1	8.54	8.20	6.12(5.3)	$pK_a = 9.27$[b]
B^-	8.40	8.14	6.12(5.7)	
$H_pH_1B_7M_7BH_1H_p$	8.78	8.20	6.08(3.7)	2
XH_p	8.82	7.95	6.91(2.4)	$pK_a = 5.91$,
X	9.09	7.95	6.91(2.3)	4–12
B_1M_1B	8.41	8.31	6.01(5.5)	8–12
5′-ITP				
$XH_p + X$	8.83	7.96	6.94(2.5)	4–11
Guanosine				
$H_1B_7M_7BH_1$	8.22		5.84(4.8)	2–4
B_1M_1B	7.87, 7.80		5.82(6.5), 5.71(6.5)	9–12
5′-GMP				
BH_1H_p	8.08		5.92(5.9)	$pK_a = 6.23$[b]
BH_1	8.19		5.92(5.4)	$pK_a = 9.73$[b]
B^-	8.10		5.92(6.1)	
$H_pH_1B_7M_7BH_1H_p$	8.45		5.88(4.0)	5–6
X	8.75		6.48(2.7)	9–12
Adenosine				
BH_1^+	8.53	8.44	6.14(5.1)	$pK_a = 3.89$[b]
B	8.30	8.21	6.16(6.0)	
B_1M_1B	8.32	8.74	5.98(5.6)	3–4
X	9.38	8.49	6.91(4.1)	1–4
Y	9.27, 9.25	8.81, 8.60	5.99(3.4)	1–4
$(MA)_2$	8.04, 8.03	8.48, 8.45	5.85(5.7), 5.83(5.7)	8–12
5′-AMP				
$^+BH_1H_p$	8.62	8.44		$pK_a = 4.05$[b]
BH_p	8.42	8.18		$pK_a = 6.23$[b]
B	8.56	8.21		
XH_p	9.45	8.50	6.95(2.3)	$pK_a = 5.16$,
X	9.85	8.48	6.92(2.5)	0–6
$H_pH_1B_7M_7BH_1H_p$	9.12	8.45	6.25(3.6)	0–1
5′-ATP				
$XH_p + X$	9.75	8.40	6.85(3.6)	0–8
2′,3′-AMP				
XH_p	9.46	8.52	6.97(3.8)	1–5

[a]At 34 °C in D_2O with 0.5 *M* KNO_3. [b]From Ref. 7.

Table I also lists the pH range over which each complex appears as a significant species in solutions with an $enPd^{2+}$ to ligand mole ratio corresponding to its stoichiometry. Though not all complexes are reported or identified in sometimes complicated spectra, the major part of the ligand mole fraction is included in Table I. In parentheses beside the H1′ chemical shift Table I also tabulates the ribose H1′–H2′ coupling constant, J. Subscripts on complexes designate binding sites for protons and $enPd^{2+}$. For example $HpH_1B_7M_7BH_1H_p$ refers to a 1:2 complex where both nucleoside phosphate ligands are coordinated to $enPd^{2+}$ at N7 and bear protons on H1 and the phosphate group.

In the pH 5–10 region equimolar solutions of $enPd^{2+}$ and inosine yield only broad spectra. Complex species with predominant N7 coordination occur at pH < 5, while at pH > 10 complexes with predominant N1 coordination take over. Assignments of H8 and H2 in the B_1M_1B complexes were made by heating the complexes at 60 °C for 21 hours to cause disappearance of the H8 peak [8]. In the pH 5–10 region both N1 and N7 coordinate to different $enPd^{2+}$ to give a polymeric complex presumably related to the X complex appearing with other ligands.

The complex X and the phosphate protonated complex XH_p are in rapid exchange and predominate in equimolar solutions of $enPd^{2+}$ and IMP over a wide range from pH 4–12. The tendency to form this 1:1 complex is so strong that it occurs (with other complexes) even in neutral solutions containing a 1:2 $enPd^{2+}$ to inosine mole ratio. Only at pH > 8 in 1:2 solutions does complex X give way to the B_1M_1B complex. As reviewed in the introduction, compared to other complexes, the X and XH_p complexes are characterized by a 0.8 ppm downfield shift for the ribose H1′ and a 0.3 ppm upfield shift for H2. With IMP, phosphate deprotonation of XH_p to give X yields an H8 downfield shift of 0.27 ppm. A nonlinear least squares analysis of this shift *versus* pH yields for the phosphate deprotonation from XH_p, $pK_a = 5.91 \pm 0.01$.

Complexes XH_p and X also occur with 5′-ITP. Due to the remoteness of the triphosphate group, H8 and other nucleoside nuclei are insensitive to its deprotonation. Chemical shifts of both the XH_p and X forms of ITP are similar to the XH_p form of IMP.

A similarity to inosine complex X was not observed with guanosine. Equimolar solutions of $enPd^{2+}$ and guanosine yield broad spectra. Solutions with a 1:2 mole ratio give 1:2 complexes as tabulated in Table I. Peaks in basic solutions for the B_1M_1B complex appear as doublets separated by 0.07–0.09 ppm. As discussed later, the doublets are due to isomer formation.

Although the downfield H1′ shift is only 0.6 instead of 0.8 ppm, 5′-GMP also yields a complex X (in absence of Cl^- electrode) in equimolar and basic solutions. The H1′–H2′ vicinal coupling constant is 2.7 Hz compared to the 2.3–2.5 Hz found in other XH_p and X monophosphate complexes. As indicated in Table I, for free ligands and solely N1 coordinated ligands, the coupling constant is greater than 5.1 Hz.

Solely N7 coordinated phosphate ligands yield intermediate coupling constant values of 3.6–3.7 Hz. The $H_1B_7M_7BH_1$ complex of guanosine yields a greater value of 4.8 Hz. Basic solutions with a 1:2 ratio of $enPd^{2+}$ and GMP give rise to 3 complexes yielding doublets for the H8 resonance from 8.6 to 8.8 ppm.

For adenosine, complex X exhibits a downfield H1′ shift of 1.0 ppm compared to other adenosine complexes, but the H1′–H2′ coupling constant is a relatively high 4.1 Hz, compared to 2.3–2.5 Hz found for all the nucleoside phosphate XH_p and X complexes. It is crucial for appearance of X complex with adenosine that Cl^- be absent, even adventitious Cl^- leaked from electrodes greatly complicates the spectrum at pH > 6. With $enPd^{2+}$, adenosine also forms in acid solutions an equimolar complex designated Y in which both the H8 and H2 peaks appear as doublets with separations of 0.02 and 0.21 ppm, respectively. Complex Y exhibits a relatively low H1′–H2′ coupling constant of 3.4 Hz. In basic solutions equimolar reagents yield a complex $(MA)_2$ in which all three peaks in Table I appears as doublets. The H8 and H2 peaks were assigned by deuterium substitution at C8 on the free ligand.

Equimolar solutions of $enPd^{2+}$ and either 5′-AMP or 2′,3′-AMP yield an XH_p complex indicating that the position of the phosphate group is not critical Nor is it necessary as an X complex is also formed with adenosine. Even though the nucleoside proton NMR peaks of the XH_p complexes of 2′,3′-AMP and 5′-AMP appear as singlets, the complexes display a multiplicity of en peaks.

Both the ribose H1′ chemical shift and H1′–H2′ coupling constant of the XH_p and X complexes of 5′-AMP are nearly identical to those of 5′-IMP. In contrast to IMP, however, for 5′-AMP a non-linear least squares treatment of the H8 chemical shift *versus* pH yields for the phosphate deprotonation from XH_p a $pK_a = 5.16 \pm 0.01$, a full log unit more acid than the free ligand value of 6.23. This acidity promotion may only be partly accounted for by introduction of positive charges on the adenine base as the $M_7BM_1H_p$ complex of $dienPd^{2+}$ exhibits $pK_a = 5.67$ [7]. Also in contrast to the ITP-IMP comparison, complexes XH_p and X for 5′-ATP exhibit an H8 chemical shift that is 75% of the way from the 5′-AMP XH_p to X shifts, rather than being virtually identical to the XH_p shift. This effect may be coupled to the low $pK_a = 5.16$ which suggests that in complex X the deprotonated phosphate of 5′-AMP serves as a hydrogen bond acceptor from another group in the complex.

Discussion

From the results of this research and our earlier detailed paper on $dienPd^{2+}$ binding to nucleosides and nucleotides [7], it is possible to formulate a general rule for metal ion binding to N7 of purine bases. There is a marked downfield shift of the H8 resonance on binding of either $dienPd^{2+}$ or $enPd^{2+}$ to N7. When Pd(II) binding has occurred at N7 the H8 chemical shift appears more downfield than 8.4 ppm for inosine, 8.6 ppm for IMP, 8.3 ppm for GMP, 8.88 ppm for adenosine, and 8.9 ppm for 5′-AMP. On this basis all the X and XH_p complexes in Table I, and the Y complex of adenosine, all bear an $enPd^{2+}$ at N7. Since the $(MA)_2$ complex of adenosine has lost an amino group proton, the generalization is not applicable to this complex.

For all ligands, complexes XH_p and X occur in solutions where N7 binding has already occurred and N1 binding is expected to be underway. The complexes are most favored in equimolar solutions. Since each $enPd^{2+}$ presents two ligand binding sites and each purine base two metal ion binding sites at N1 and N7, we seek arrangements that match the ligand and $enPd^{2+}$ potentialities. A dimer structure is sterically impossible. We can visualize polymers in which each $enPd^{2+}$ binds to N1 of one purine and to N7 of another $(B_7M_1)_n$, an $enPd^{2+}$ binds two purines either by N1 or N7 $(B_7M_7B_1M_1)_n$, or to a mixture of the two. Steric restrictions require that each of the two purines bound to a single $enPd^{2+}$ be approximately normal to the chelate plane; therefore the two ligands may be disposed either head-to-head or head-to-tail. The head-to-head arrangement suffers possible steric hindrance between C6 substituents (and with the 2-amino group in guanine) so that in a polymer a predominantly head-to-tail disposition may predominate.

In a head-to-tail arrangement of polymers H2 and H8 are in the vicinity of the C6 substituent. This disposition may account for the greater than 0.5 ppm greater downfield H8 and H2 shifts for XH_p and X complexes of adenosine and AMP (C_6-NH_2) than of IMP ($C_6 = 0$) when compared to their free ligands. In the $(B_7M_7B_1M_1)_n$ head-to-tail polymer only a C_2 axis relates the two purine rings on one $enPd^{2+}$, though they are chemical shift equivalent, the mirror image is not superimposable, and when *D*-ribose is also considered diastereomers result. Thus doublets are expected for each nucleoside proton in NMR spectra [1]. The carbon bound en protons form two A_2B_2 systems superimposed on one another.

In the $(B_7M_1)_n$ head-to-tail extended polymer the ligands are equivalent, but the mirror image of the coordination system is non-superimposable, with *D*-ribose disastereomers resulting, and again a doubling of nucleoside NMR peaks is expected. The carbon bound en protons form an ABCD system.

With a head-to-head arrangement of ligands a closed ring trimer may be built which appears sterically more acceptable in the alternating sequence $(-B_1-M_7B_1M_7B_1M_7-)$. The trimer possesses only a C_3 axis, the mirror image of the ring system is non-superim-

posable, with *D*-ribose diastereomers being formed, and once again a doubling of nucleoside NMR peaks is anticipated. The carbon bound en protons form an ABCD system.

All examples of likely structures reviewed in the last three paragraphs predict at least a doubling of nucleoside NMR peaks. Observed spectra are not so complicated as to suggest the presence of all three mentioned structures. A possible exception occurs in basic solutions of GMP where several doublets appear. Complex Y for adenosine yields one doublet for each base proton resonance as does complex X for deoxyinosine monophosphate [4]. Indeed the problem is to account for the presence of only singlet peaks for all XH_p and X complexes in Table I. Rapid free rotation about enPd–N bonds would average doublets to singlets, but this rotation in extended polymers and the trimer is expected to be slow. Either three is a cooperative succession of rapid rotations or there is insufficient resolution of the diastereomeric protons at 90 MHz. Even when the nucleoside proton resonances are singlets the carbon bound en protons often yield broad and multiplet peaks. The preceding analysis offers a natural explanation for complexity of the en proton NMR peaks.

The additional 2-NH_2 substituent probably accounts for the doublets appearing in the B_1M_1B complex only for guanosine in Table I. The H8 and H1 splittings for the guanosine B_1M_1B complex are by far the greatest such splittings reported in Table I. As for cytidine, where doubling also occurs in a similar complex, the coordinated ring nitrogen is flanked by amino and carbonyl oxygen groups. The last two groups may form two sets of hydrogen bonds in a head-to-tail complex which restricts rapid rotation [1]. With the other ligands the two hydrogen bonds about each $enPd^{2+}$ cannot occur and rapid rotation ensues.

For the high pH adenosine complex labeled $(MA)_2$ in Table I, compared to the free ligand and in contrast to all other complexes in Table I, there is a pronounced net upfield shift of the H8 nucleoside proton NMR peak. The H8 peak was identified by its exchange with deuterium in the free ligand. We attribute the H8 upfield shift to deprotonation and metalation of the 6-NH_2 group. The indicated $(MA)_2$ structure, where the symbol A designates an amino deprotonated base, is based on our recent characterization of a cytidine complex [1]. In equimolar solutions of $enPd^{2+}$ and cytidine the $(MA)_2$ complex begins to appear at pH 5 and is the major species in solution at $pH > 6$. In the $(MA)_2$ dimer, two cytidine ring anions bridge through N3 and a deprotonated amino N4 nitrogen two $enPd^{2+}$ to form an 8-membered ring [1, 9]. The N1 ring and N6 amino nitrogens of adenine are capable of forming a similar dimer structure. In the cytidine $(MA)_2$ complex the H6 chemical shift appears 0.7 ppm upfield from its position in the free base. In the adenosine complex recorded in Table I, compared to the free base, the H8 shift is 0.26 ppm upfield and the H2 shift 0.26 ppm downfield. The H2 downfield shift is consistent with $enPd^{2+}$ binding at N1 of the adenosine ring. The H8 upfield shift is then ascribed to accumulation of negative charge density on the 5-membered ring. Since models are lacking for a deprotonated and metalated 6-amino group, it is also possible that there is a simple MA complex where the $enPd^{2+}$ chelates between deprotonated N6 and the 5-membered ring N7 to form a strained chelate ring. In this case, however, we might expect a downfield shift of H8 and an upfield shift of H2 compared to the free ligand B, just the opposite of what is observed. For arguments from both the chemical shift directions and the analogy with cytidine we suggest the dimer $(MA)_2$ structure for the indicated adenosine complex in Table I. The doubling of the peaks is expected for either a head-to-tail (preferred) or head-to-head arrangement of the two adenosine rings [1].

There seems to have been a resurgence in an earlier but discredited idea that the amino (NH_2) group of adenine is a direct metal ion binding site. The effects of paramagnetic ions on the T_1 relaxation of ^1H have been interpreted to indicate Cu^{2+} binding [10], and of ^{13}C and ^{15}N to indicate Mn^{2+} binding at the 6-amino group [11]. Both investigations were conducted with large ligand excess and at a limited number of low pH values [10], or at pH 10 [11]. It has, however, been strongly argued that the adenine 6-amino group is not a metal ion binding site [12]. The group is not basic: even in the adenine trication all three protons are at other nitrogens and the 6-amino group remains unprotonated [13]. There is extensive electron delocalization from the amino group onto the rings. It is possible for the 6-amino group to be involved in indirect binding by hydrogen bond donation of a 6-NH_2 hydrogen to a water, Cl^- or other hydrogen bond acceptor located in the coordination sphere of a metal ion coordinated at N7 [12]. The 6-amino group can only become a direct metal ion binding site when the metal ion substitutes for one of the two amino protons to give a 6-imino group. In this study the strong nitrogen binder $enPd^{2+}$ only substitutes for an adenine amino hydrogen beginning at about pH 7. The much more weakly binding Cu^{2+} and Mn^{2+} are not expected to initiate this kind of binding at $pH < 10$ when in rapid exchange in the presence of donor atom competition from a large ligand excess. The T_1 effects of the paramagnetic ions may be explained by leakage of unpaired spin density to orbitals on carbon and nitrogen atoms [14, 15].

Though direct N7–O6 chelation has been proposed as an effective binding mode of antitumor *cis* diamine Pt(II) compounds, there is neither a single crystal structure nor any incontrovertible solution evidence to support this binding mode [4, 12]. Even the more labile $enPd^{2+}$ does not participate in direct

N7–O6 chelation. Easy formation of 1:2 $enPd^{2+}$–guanosine complexes indicate that any direct chelation in a single ligand must be weak [2]. In this research even equimolar acidic solutions of $enPd^{2+}$ and guanosine, GMP, IMP, or ITP contain more 1:2 than 1:1 complexes. This result suggests that the second stability constant is greater than the first. Under the acidic conditions used the binding is exclusively at N7. Because N7–O6 chelation with $enPd^{2+}$ demands a 1:1 complex, these results argue strongly against N7–O6 chelation being a significant interaction. Both Pd(II) and Pt(II) much prefer nitrogen or chloride to oxygen donor atoms. Direct N7–O6 chelation in the 6-oxopurines requires a strained geometry. Much more likely and observable is indirect chelation in which a metal ion bound at N7 contains in its coordination sphere a ligand such as water capable of donating a hydrogen bond to O6 [12].

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