Binding of *cis*- and *trans*-Dichlorodiammineplatinum(II) to Nucleosides. I. Location of the Binding Sites

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Abstract: *cis*-Dichlorodiammineplatinum(II) (I) is an effective antitumor agent in animals and man. The trans isomer II is ineffective. Since the cis configuration has the two neighboring leaving chloride groups, I may act as a bifunctional agent and II as a monofunctional agent. The interaction of I and II with purines, substituted purines, pyrimidines, and substituted pyrimidines in 0.1 *M* $NaClO_4$ at 37°, using uv spectrophotometry, is reported. It is possible to distinguish bidentate from monodentate binding, and to suggest the locations of the site of attack on nucleic acid components. The cis isomer forms a bidentate chelate with either 6-NH_2 + N-7 or 6-NH_2 + N-1 of adenosine, and 4-NH_2 + N-3 of cytidine. The trans isomer interacts monofunctionally at N-7,N-1 of adenosine and N-3 of cytidine. Both isomers bind monofunctionally to N-7 of guanosine and inosine. No evidence of binding of either isomer to uridine or thymidine is detected. We conclude that there is no monofunctional attack at the NH_2 group of cytidine, adenosine, or guanosine. The cis isomer binds bifunctionally at either N-1 + 6-NH_2 or N-7 + 6-NH_2 groups. This suggests that the amino groups of adenosine and cytidine will be occupied when they are sterically well disposed for bidentate chelation. Such sites are available in the organized structure of DNA.

The cis isomer of dichlorodiammineplatinum(II) shows a wide variety of biological activity[2a] strikingly similar to the action of the bifunctional alkylating agents (*e.g.*, nitrogen mustard).[2b] Thus the platinum salt causes filamentous growth in *E. coli* B[3] and induces prophage from lysogenic strains of bacteria.[4] It selectively inhibits DNA synthesis in human amnion AV_3 cells[5] and is effective against many tumors in animals and man.[6] Bacterial strains which are resistant to radiomimetic agents are also resistant to the cis isomer of this platinum salt.[7] By contrast, the trans isomer displays none of these biological actions up to a dose ten times greater than the most effective dose of the cis isomer. Although it is yet to be shown whether the cis isomer is carcinogenic, these facts suggest a parallel between the mode of action of the cis and trans platinum isomers and the classical bi- and monofunctional alkylating agents. Indeed, early tracer studies led to the suggestion that platinum complexes which forced filamentous growth in *E. coli* B attacked DNA,[8] and model studies, *in vitro*, have shown that both platinum isomers bind to DNA.[9] Recently, Roberts and Pascoe have been able to show that the cis isomer forms interstrand cross-links in DNA extracted from HeLa cells that have been treated with minimal doses of this isomer.[10] It is therefore of great interest to define sites on DNA which are open to attack by complexes of platinum(II), especially those sites on opposite strands which may be linked by a single molecule of the cis isomer.

This paper reports a study of the binding of *cis*- and *trans*-dichlorodiammineplatinum(II), referred to subsequently as I and II, respectively, to nucleosides. This is part of a program to locate the sites of attack on nucleic acids.

Since the platinum complexes I and II have only weak absorption in the ultraviolet[11] it has been possible using spectrophotometry to follow the reaction between I or II and the nucleosides up to a mole ratio of 10:1. In this way observation is made of binding of metal ions to sites on the aromatic ring of the purine and pyrimidine bases, but no information is obtained about possible binding to sugar residues. Each base provides a number of potential binding sites for metal ions on the aromatic ring. This fact has posed great difficulties for the understanding of the way in which other metal ions, such as copper(II) and monomethylmercury cation, bind to nucleosides.[12,13] Several techniques have been employed in an attempt to distinguish between possible sites of attack. Firstly, the pH dependence of the binding of I and II has been studied. Since the pK_a values of the ring nitrogen atoms are well documented,[13,14] predictions may be made of the variation over a wide pH range of the extent of binding to any given site. Simpson[13] has successfully applied this method to elucidate the positions of binding of CH_3Hg^+ to the ring nitrogen atoms of nucleosides. Secondly, a check of our conclusions has been obtained by observation of the binding of I and II to nucleosides with successive positions blocked with methyl groups. This method must be

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used with caution since in many cases methylation of ring nitrogen atoms significantly alters the pK_a values of the remaining unmethylated nitrogen atoms of the ring and it is very likely that the affinity of the nitrogen atoms for metal ions will likewise be significantly altered. Finally, advantage has been taken of the different stereochemical requirements of the isomers I and II.[15] Thus, because of the cis configuration of its two leaving groups, the chloride ions, I may bind two groups on one base, the latter acting as a bidentate ligand. However, with its leaving groups disposed trans about the metal ion, II will not bind more than one ligand atom per base. Therefore, by comparing the interaction of I and II with a base it has been possible to distinguish bidentate from monodentate binding. There is the possibility of complications arising from the bridging of one metal ion between two nucleosides. We have minimized this possibility in these experiments by working at platinum to ligand ratios greater than 1.0.

The principal object of this study is the location of binding sites of I and II on adenosine, guanosine, inosine, cytidine, uridine, and thymidine at equilibrium. The reactions were carried out in aqueous 0.1 *M* $NaClO_4$ at 37°. The criterion of the establishment of equilibrium was that the spectrum of the nucleosides had become invariant with time. In some cases this involved a time of several days. Under these conditions rapid hydrolysis of I and II takes place with the loss of both chloride ions.[16] Therefore the reacting species is the diaquo-, monoaquomonohydroxo-, or dihydroxodiammineplatinum(II).

Experimental Section

All purines and pyrimidines were obtained from Sigma Biochemicals and Miles Laboratories and used without further purification. The cis and trans isomers of $Pt^{II}(NH_3)_2Cl_2$ were prepared by Dr. J. Hoeschele as described elsewhere.[17,18a] Their purity was verified with chromatography and laser-Raman spectra.[18b]

All solutions were made up in 0.1 *M* $NaClO_4$ and maintained in the dark at 37° to avoid possible photoreactions of the platinum complexes. The spectra of the solutions were measured at room temperature using a 1-cm cell with a Cary Model 15 spectrophotometer. The solutions were equilibrated until no further change in absorbance occurred.

The pH dependence study was carried out by diluting 1 ml of 1 mol of each nucleoside to 10 ml with 0.7, 0.07, and 0.001 *M* $HClO_4$ and with 10^{-5}, 10^{-4}, 10^{-2}, and 10^{-1} *M* NaOH, giving 10^{-4} *M* nucleoside at each pH.

cis- and *trans*-$Pt(NH_3)_2Cl_2$ were dissolved in 0.1 *M* $NaClO_4$ to produce a solution of 10^{-3} *M*. Addition of equal volumes of nucleoside to the solution of platinum isomers allowed the reaction to proceed and produced solutions of pH values shown in Table I.

Table I

pH	No. of solution	pH	No. of solution
0.81	1	8.86	6
1.61	2	10.46	7
3.27	3	11.31	8
5.58	4	12.12	9
5.95	5		

The solutions are numbered to correspond with the curves in Figures 3–8.

Use of buffer solutions was avoided since all buffers are potential ligands for metal ions. The pH values of the original solutions 1, 2, and 3 and 7, 8, and 9 were quite stable but the pH of the remaining solutions had a tendency to drift toward a value of 6.3, the pK_a of carbonic acid, in spite of attempts to exclude carbon dioxide. The stability of the solutions with low and high pH values is due to the well-known "buffer capacity" of strong acids and alkalis.

The difference spectra were measured with four 1-cm cells, the sample beam having a cell containing the platinum isomer plus base in 0.1 *M* $NaClO_4$ and a cell containing 0.1 *M* $NaClO_4$ alone, whereas the reference beam contained two cells, one with base in 0.1 *M* $NaClO_4$ and the other cell with platinum isomers in 0.1 *M* $NaClO_4$ at the same pH of the sample solution.

pH Dependence of the Binding of I and II to Nucleosides. Adenosine has several potential ligand sites for the monodentate binding of metal ions, namely N-1, N-3, N-7, and the 6-amino group.[19] Furthermore, two possible sites for the bidentate location of a metal ion arise between N-1 and 6-amino and between N-7 and 6-amino. The pK_{a1} values of *cis*- and *trans*-$[Pt^{II}(NH_3)_2(H_2O)_2]^{2+}$ are 5.6 and 4.3, respectively, and the pK_{a2} values are 7.3 and 7.4, respectively.[20] The pK_a(N-1) of adenosine is 3.63, and no other nitrogen atoms are protonated over the pH range 0–14. The pH dependence of binding of different sites on the adenosine ring will depend upon whether $[Pt^{II}(NH_3)_2(H_2O)_2]^{2+}$, $[Pt^{II}(NH_3)_2(H_2O)(OH)]^{+}$, or $[Pt^{II}(NH_3)_2(OH)_2]$ predominates over the pH range considered and whether the ring nitrogen of adenosine is protonated or unprotonated in this pH range. The following types of reactions are expected when one ligand, either water or hydroxide ion, is replaced by a ring nitrogen. From pH 0 → pK_{a1} of platinum isomers

$$-NH^{+} + [Pt^{II}(NH_3)_2(H_2O_2)]^{2+} \longrightarrow [Pt^{II}(-N)(NH_3)_2(H_2O)]^{2+} + H_3O^{+} \quad (1)$$

$$-N + [Pt^{II}(NH_3)_2(H_2O)_2]^{2+} \longrightarrow [Pt^{II}(-N)(NH_3)_2(H_2O)]^{2+} + H_2O \quad (2)$$

From pH pK_{a1} → pK_{a2} of platinum isomers

$$-NH^{+} + [Pt^{II}(NH_3)_2(H_2O)(OH)]^{+} \longrightarrow [Pt^{II}(NH_3)_2(H_2O)(-N)]^{2+} + H_2O \quad (3)$$

$$-N + [Pt^{II}(NH_3)_2(H_2O)(OH)]^{+} \longrightarrow [Pt^{II}(NH_3)_2(H_2O)(-N)]^{2+} + OH^{-} \quad (4)$$

Above pH = pK_a of platinum isomers

$$-NH^{+} + [Pt^{II}(NH_3)_2(OH)_2] \longrightarrow [Pt^{II}(NH_3)_2(OH)(-N)]^{+} + H_2O \quad (5)$$

$$-N + [Pt^{II}(NH_3)_2(OH)_2] \longrightarrow [Pt^{II}(NH_3)_2(OH)(-N)]^{+} + OH^{-} \quad (6)$$

Clearly reaction 1 will be strongly suppressed at low pH whereas reactions 4 and 6 will be suppressed at high pH. The remaining reactions will be pH independent. Using these arguments a graph of the extent of monodentate binding at the several ring nitrogen atoms of adenosine as a function of pH has been plotted in Figure 1a. Both the cis and the trans isomers may bind in this way. However, there is open to the cis isomer an additional mode of binding which we call the bidentate mode, either between 6-amino and N-7 or between 6-amino and N-1. The pH dependence of this mode of binding is readily determined using similar arguments to those given in the preceding paragraph. The results are also plotted in Figure 1a. This argument has been repeated for the case of cytidine and guanosine and the conclusions are summarized in Figure 1b and 1c, respectively. The case of inosine can readily be obtained on inspection of Figure 1c by ignoring the line labeled 2-NH_2 and by using the pK_a values for inosine of 1.2 for N-7 and 8.9 for N-1.

Two assumptions have been made in the construction of Figure 1. In the first place it is assumed that the pK_a, for loss of a proton from coordinated water, of the product is about the same as the pK_a of the reactant. The data on the acid strengths of aquo complexes of this type are limited and are insufficient to permit generalizations on the variation of acidity with changes in ligands, either cis or trans, to the water group. It has been suggested that the pK_{a1} values of the diaquodiammineplatinum(II) isomers can be rationalized on the

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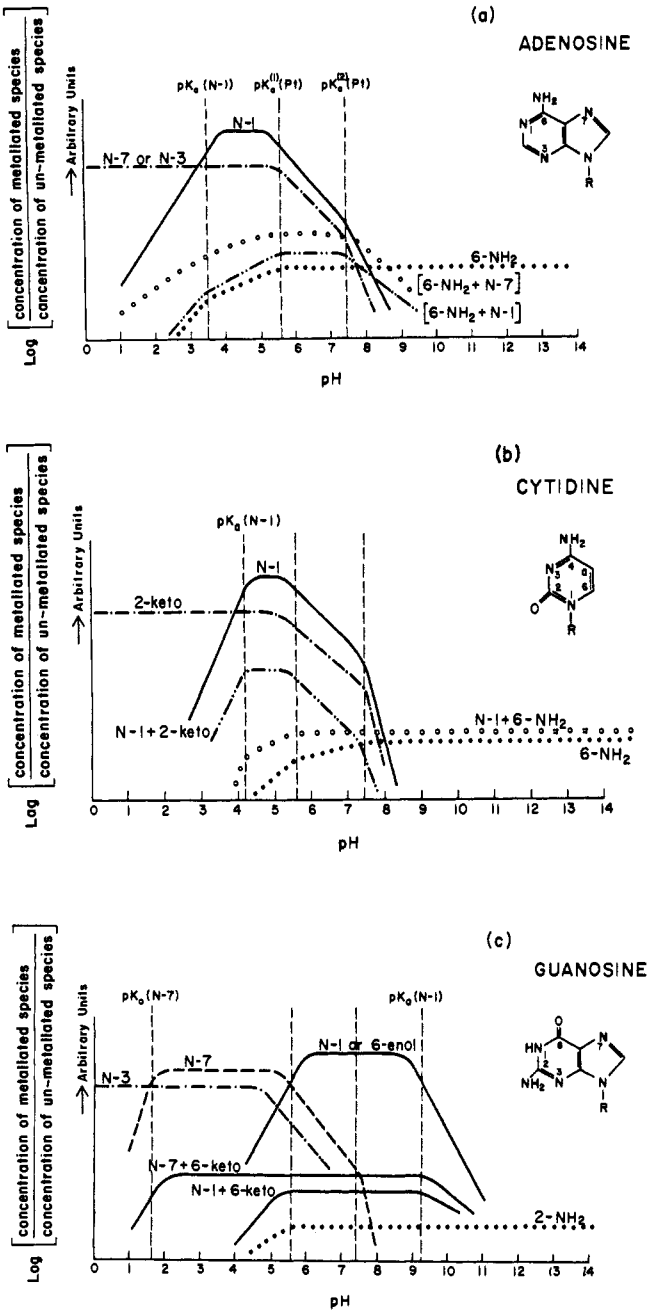

Figure 1. A plot of the extent of binding of I and II to sites on (a) adenosine, (b) cytidine, and (c) guanosine as a function of pH under equilibrium conditions in 0.1 M $NaClO_4$. The exact positions of the vertical dotted lines denoting the pK_{a1} and pK_{a2} values of diaquodiammineplatinum(II) depend upon the isomer. The diagram is shown with pK_{a1} and pK_{a2} values of 5.6 and 7.3, respectively, for the cis isomer. The lines would occur at pH 4.3 and 7.4 for the trans isomer. The order and relative displacement of the plateau within each plot are quite arbitrary. However, they have been ordered to correspond with the orders of the stability constants of CH_3Hg^+ on these sites (see ref 13). It is only qualitative variation with pH in the extent of binding to a site that is used to verify sites of platinum binding. Monodentate and bidentate sites of binding are plotted.

basis of the relative trans effect $NH_3 > H_2O$ making the Pt–O bond weaker acid for the first ionization constant.[21] The pK_{a2} values are then similar on account of the similar trans effects on NH_3 and OH^-. As ligands the ring nitrogen group of the purines and pyrimidines will be low in the trans effect series, close to pyridine and ammonia.

(21) For a discussion of this point, see F. Basolo and R. G. Pearson, "Mechanisms of Inorganic Reactions," Wiley, New York, N. Y., 1968, p 367.

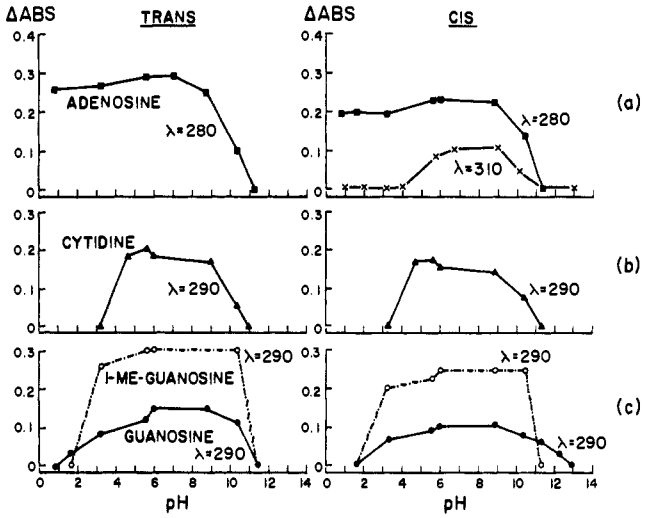

Figure 2. Summary of the experimental results of the pH dependence of the interaction of *cis*- and *trans*-$Pt^{II}(NH_3)_2Cl_2$ with nucleosides. The results of the difference spectra are plotted as the Δ absorbance (ΔA) at a given wavelength in a 1-cm path. The metal:base ratio was 10:1 in every case.

It is reasonable to conclude therefore that they will not have a sufficiently marked effect on the acidity constant of water coordinated to the metal to cause a gross alternation in the features of Figure 1.

Secondly, binding of platinum ion to amino groups of adenosine, cytidine, and guanosine has been considered and is assumed to be negligible unless a proton is lost in the process. We have no direct evidence in support of this assumption since the long time required for equilibrium to be reached has frustrated attempts to monitor proton release during the course of binding. However, there is quite extensive evidence that, for example, CH_3Hg^+ and Hg^{2+} binds the amino groups of the bases with loss of a proton at pH 7.[13,22] Silver(I) binds with the displacement of a proton above this pH.[23] The binding by these metal ions is weak and very high concentrations of metal ions are needed to detect it. Au(II) and Pt(IV) ions are sufficiently strongly polarizing to displace protons from ethylenediamine coordinated to them.[24] In the case of $Pt(en)_3^{4+}$ the pK_a values are 5.5, 9.7, and 6.7.[25] No pK_a values of any of the amino groups attached to bases or of ethylenediamine are known, but the pK_a of the former is expected to be several orders of magnitude lower than the pK_a of the latter. With this body of evidence, it is clear that we must consider the possibility of the binding of I and II to the amino groups. We further argue by analogy with the binding of CH_3Hg^+, Ag^+, and Hg^{2+} that the binding will be negligible at low pH, or equivalently, that binding is not important unless a proton is lost.

Results

Figure 1a shows that for adenosine, considering first monodentate binding, the only mode open to the trans isomer, it is possible to distinguish between the binding of the 6-amino group, which is the binding site favored at extremely high pH, binding to N-1 which rises to a maximum between pH 3.5 and pK_{a1} of the platinum isomer, and binding to N-1 and N-3, both of which are favored at low pH. This technique will not separate the last two possibilities. It is also clear from the figure that a bidentate mode of binding can be detected since firstly it is only possible with the cis isomer and secondly it rises to a maximum when the pH lies between the pK_{a1} and pK_{a2} of the platinum isomer. It is not possi-

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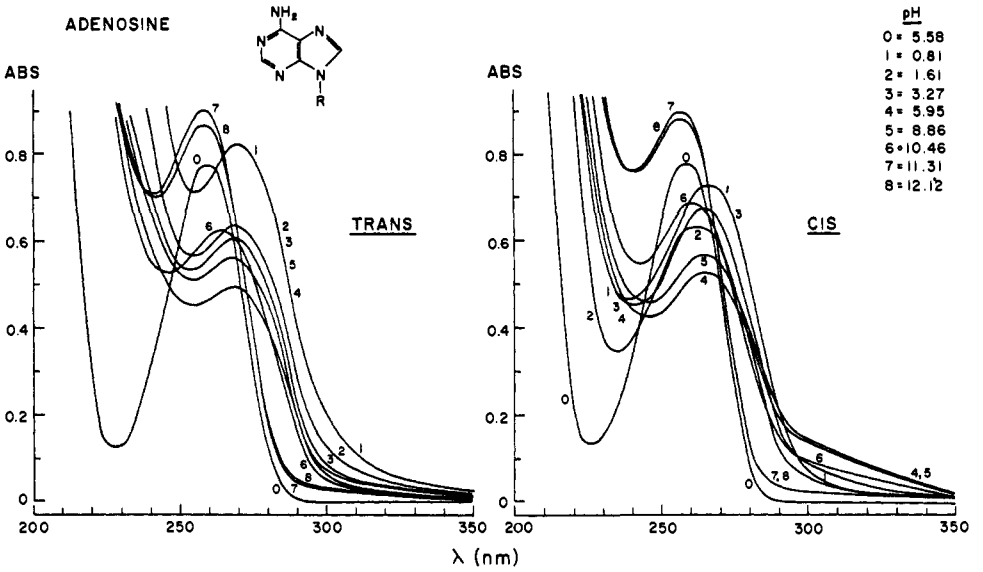

Figure 3. The pH dependence of the absorption spectrum of adenosine plus *cis*- and *trans*-$Pt^{II}(NH_3)_2Cl_2$. The absorbance values refer to a 1-cm path and the solutions had a 10:1 metal:base ratio, with a base concentration of 0.5×10^{-4} *M*.

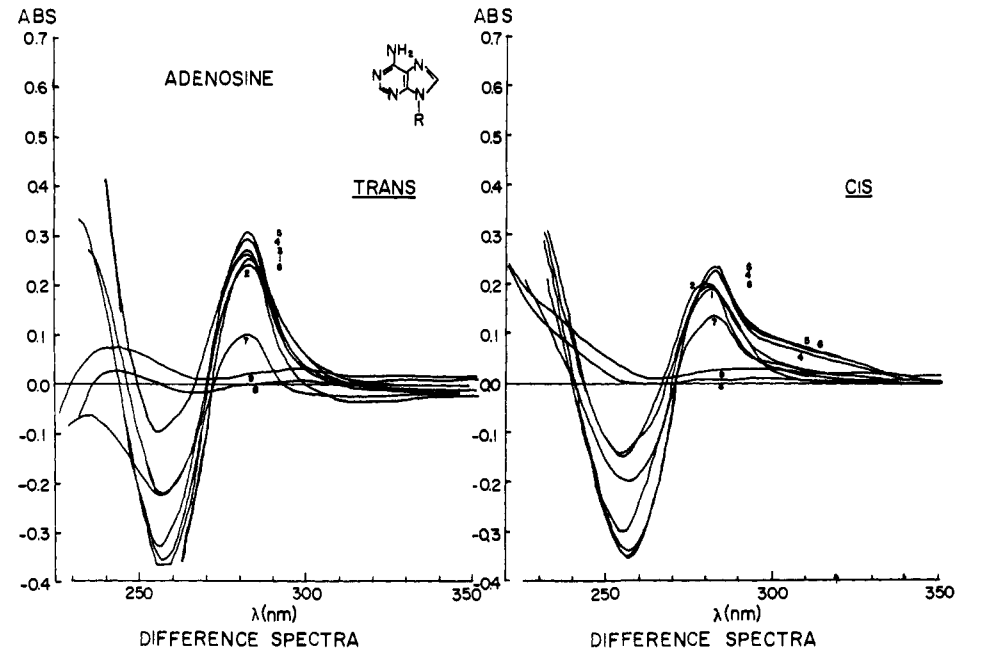

Figure 4. The difference spectra of adenosine with *cis*- and *trans*-$Pt^{II}(NH_3)_2Cl_2$ as a function of pH. The numbering of the curves in this and subsequent figures refers to the pH values given in the Experimental Section. The metal:base ratio was 10:1 in Figures 4–8.

ble, however, in this way to distinguish clearly between N-7 + 6-NH_2 and N-1 + 6-NH_2 bidentate sites.

The conclusions to be drawn from Figure 1b and c for cytidine, guanosine, and inosine run along parallel lines.

Figure 2 summarizes the results of the pH dependence studies of the binding of I and II to adenosine, cytidine, guanosine, and 1-methylguanosine. The metal:base ratio was 10:1 in each case, and the ΔA values refer to difference spectra.

Adenosine. The absorption spectra of adenosine equilibrated with I and II in 0.1 *M* $NaClO_4$ at 37° over a range of pH values are shown in Figure 3. The difference spectra, Figure 4, show clearly that the reaction of adenosine with I and II is negligible above pH 11. A comparison of Figures 1a and 2a reveals that there is no monodentate binding of the 6-amino group at this metal–ligand ratio. However, both isomers I and II bind adenosine below pH 1, Figure 2a, giving a slightly red-shifted spectrum of adenosine, curve 1 in Figure 3. This species must therefore arise from platinum monodentate binding either to N-7 or to N-3.

On raising the pH, the spectrum is markedly lowered in intensity and shifted to the blue by about 5 nm, Figure 3. This species, formed by both isomers, reaches a maximum concentration at pH 5.95, curve 4, and is therefore the result of monodentate binding to N-1. On increasing the pH further, the spectrum reverts toward that of free adenosine at the pH.

There is evidence of an additional species formed only by the cis isomer showing as a broad shoulder beyond 300 nm, Figure 3 and Figure 4. Since it is formed only

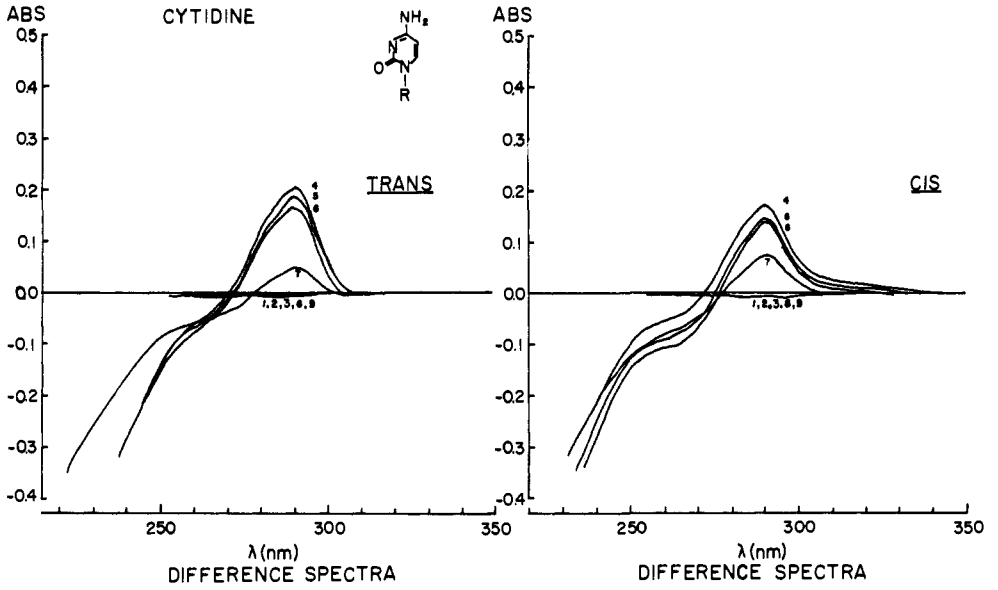

Figure 5. (a) The difference spectra of cytidine with *cis*- and *trans*-$Pt^{II}(NH_3)_2Cl_2$ as a function of pH.

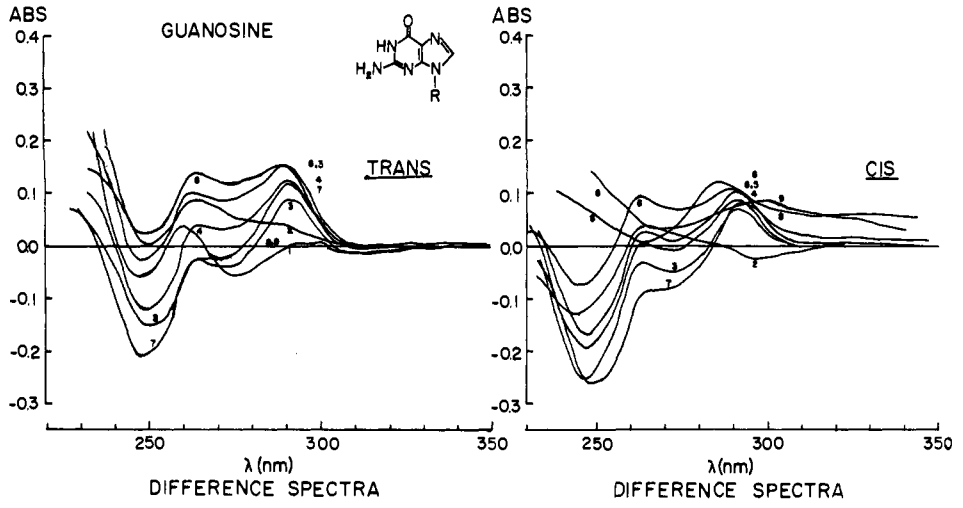

Figure 6. The difference spectra of guanosine with *cis*- and *trans*-$Pt^{II}(NH_3)_2Cl_2$ as a function of pH.

by the cis isomer, it is most likely a species arising from bidentate chelation. This is confirmed by its pH dependence, Figure 2a. The shoulder at 310 nm rises to a maximum between pH 6 and 8 and drops to zero above pH 11. Figure 1a shows that this is the behavior expected from bidentate chelation either to 6-NH_2 + N-7 or to 6-NH_2 + N-1. We cannot distinguish the alternatives at this stage.

Cytidine. The difference spectra for the pH dependence of the reaction between I and II and cytidine are plotted in Figure 5. Figure 2b shows that no binding takes place below pH 3 and above pH 10. Figure 1b allows us to conclude that neither the 4-NH_2 group nor the 2-keto group is bound by platinum in the monodentate mode. The binding which does occur peaks at pH 5–6, giving strong evidence in favor of binding to N-3.

Consideration has also been given to bidentate chelation between the N-3 and 2-keto groups, and between N-3 and 4-NH_2. It is clear from Figure 5 that the cis isomer does form a different complex from the trans and this is most likely bidentate bindng to either of these two groups.

Guanosine. The difference spectra of guanosine at various pH values are given in Figure 6. Considerable complexity is evident. A plot of the ΔA at 290 nm, a reasonably well-defined peak, is given in Figure 2c. There is very little binding above pH 11.0 showing that neither I nor II binds the 2-NH_2 group. The diminution in binding at low pH values points to the involvement of N-7 rather than N-3. Between pH 2 and 10 the trans isomer is successively bound to N-7 and N-1 (or to the oxygen atom when it is in the enol form). Figure 2c, trans, clearly shows a change in the types of binding between pH 5 and 6, the pH at which the binding site is expected to change from N-7 to N-1 according to Figure 1c. We note that measurements carried out at only one metal–base ratio do not reveal whether the base binds only one metal at a time. It is possible that, at metal:base ratios of 10:1, two sites of comparable binding constants will be occupied simultaneously.

Since the pH dependence of bidentate binding to N-7

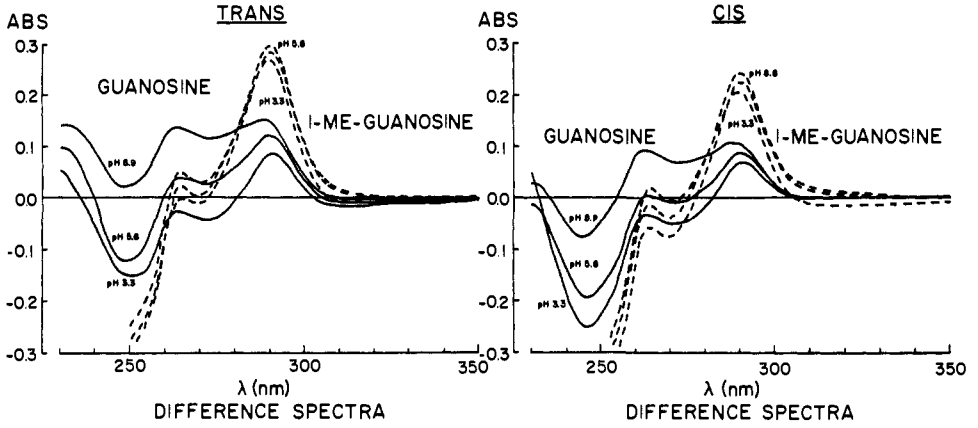

Figure 7. A comparison of the difference spectra of $Pt^{II}(NH_3)_2Cl_2$ isomers with guanosine (0.5×10^{-4} *M*) and 1-methylguanosine (1.1×10^{-4} *M*) as a function of pH. pH values are indicated in the figure.

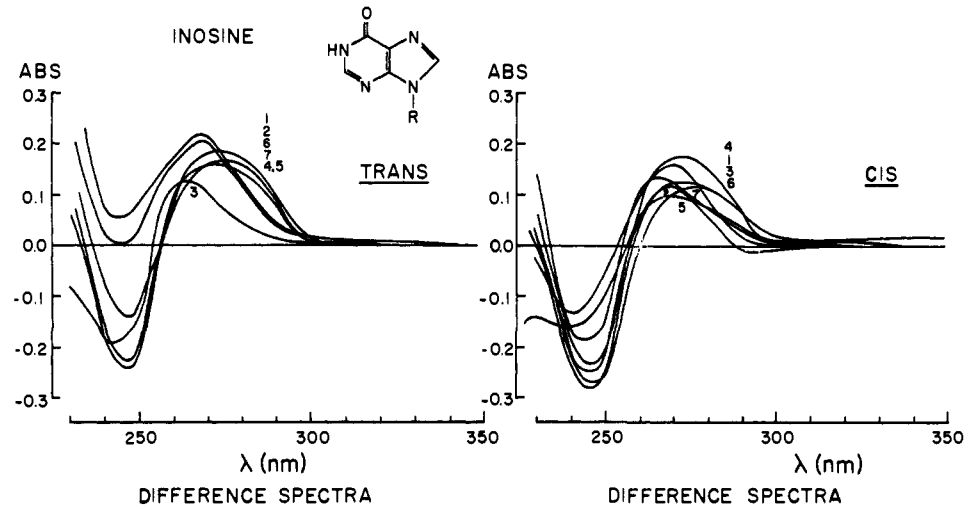

Figure 8. The difference spectra of inosine with *cis*- and *trans*-$Pt^{II}(NH_3)_2Cl_2$ as a function of pH.

+ 6-keto and N-1 + 6-keto is similar to that of monodentate binding to N-7 and N-1 (or 6-enol) no evidence of bidentate binding of I to such sites is available. The long-wavelength band for the cis isomer in Figure 6 cannot, at present, be assigned.

1-Methylguanosine. The binding of both I and II to this derivative of guanosine is extensive. The difference spectra at various pH values are compared with those of guanosine in Figure 7. The concentration of the species absorbing at 290 nm as a function of pH is plotted in Figure 2c. Since the N-1 site is now blocked with a methyl group, the sites of possible binding are the 2-NH_2 group, N-3, N-7, and the 6-keto group. The sharp decrease in binding at low pH eliminates the sites at N-3 and 6-keto, whereas the equally sharp drop above pH 11 eliminates the 2-NH_2 group. This confirms N-7 as a major site of binding. The pK_a of N-7 is ~2.4, compared with a value of 1.6 for the equivalent nitrogen atom of guanosine. This higher value accounts for the sharper fall, at low pH, in the concentration of the species metalated at N-7. The persistence of binding up to pH 11 reflects the remarkably high affinity of this site for metalation. This is confirmed by studies carried out as a function of metal: ligand ratio (see part II). No significant differences in the binding of I and II to 1-methylguanosine are apparent in Figure 7, suggesting that bidentate chelation between N-7 and the 6-keto group does not readily arise in spite of the high affinity of N-7 for platinum(II) and the proximity of the 6-keto group.

Inosine. The binding by inosine of I and II reveals a complex set of difference spectra as a function of pH, Figure 8. Since the peaks overlap considerably, it is not possible to follow the change in the concentration of any one species as a function of pH. However, using the interpretation of the guanosine spectrum as a guide, it is possible to arrive at a reasonable conclusion. The trans species binds to inosine, Figure 8, at pH 0.81 and 1.61, yielding a species with λ_{max} at 265 nm, but the intensity of this species drops at pH 3.27, curve 3, Figure 8, and a new species, λ_{max} 270 nm, has appeared at pH 5.58, curve 4, which persists until about pH 10, with a maximum close to pH 8.86, curve 6. The pK_a(N-7) of inosine is 1.2 and for (N-1), 8.9. Thus the species with λ_{max} 265 nm is metalated at N-7 and that with λ_{max} 270 nm at N-1. It may be noted that the difference spectra obtained with the trans isomer and inosine are closely similar to the difference spectra obtained with inosine and CH_3Hg^+.[13] The N-1 and N-7 sites are also those occupied by CH_3Hg^+.[13]

These conclusions are broadly confirmed by the reactions of I and II with 1-methylinosine and 7-methylinosine.

Uridine and Thymidine. No interaction of I and II

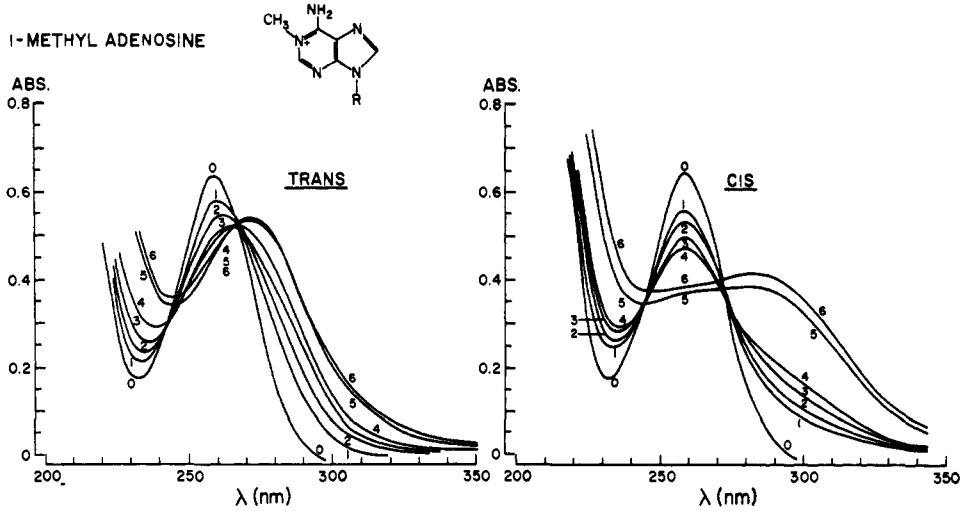

Figure 9. The absorption spectrum of 1-methyladenosine with *cis*- and *trans*-$Pt^{II}(NH_3)_2Cl_2$ as a function of the metal:base ratio (r). The base concentration was 5.32×10^{-5} M in a 1-cm path. The metal:base ratios for curves 1–6 are: 1, 1.5, 2, 3, 7.5, and 10.

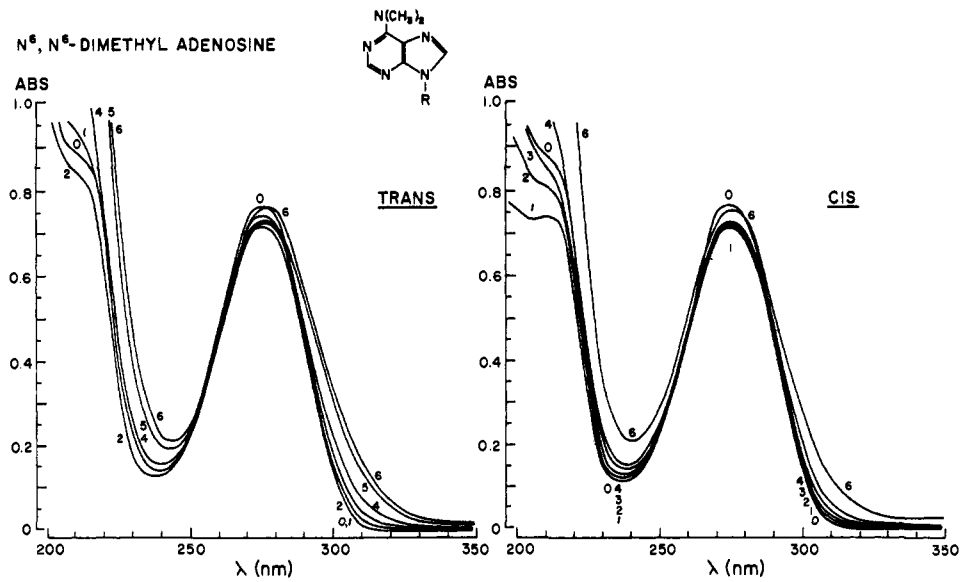

Figure 10. The absorption spectra of N^6,N^6-dimethyladenosine with *cis*- and *trans*-$Pt^{II}(NH_3)_2Cl_2$ as a function of metal:base ratio (r). The base concentration was 4.0×10^{-5} M in a 1-cm path. The metal:base ratio for curves 1–6 are: 1, 1.5, 2, 3, 7.5, and 10.

with uridine or with thymidine was detectable by spectrophotometry over the pH range 0–14.

Binding of I and II to Methylated Nucleosides. Methyladenosines. The interactions of I and II with 1-methyladenosine and N^6,N^6-dimethylaminopurine have been measured at pH 5.6 over a range of metal:ligand ratios of 1 to ~10; see Figures 9 and 10. The pK_a of 1-methyladenosine is 7.6, corresponding to the loss of a proton from the 6-amino group. Therefore, binding of a metal to the 6-amino group with loss of a proton will take place much more readily than to adenosine itself. This is seen in the spectrum of the binding of II, Figure 9. Although N-1, the major site of binding to adenosine at pH 5–6, is blocked, extensive binding to 1-methyladenosine is observed. However, this binding is different from that due to I, the cis isomer, which yields a species with a spectrum containing a broad maximum at 285 nm extending beyond 300 nm, remarkably similar to the shoulder seen in curves 4 and 5, Figure 3. This is to be expected since the pH dependence showed that the shoulder was due to bidentate binding either between 6-NH_2 and N-1 or between 6-NH_2 and N-7. The result with 1-methyladenosine leads to the conclusion that the mode of bidentate binding is between 6-NH_2 and N-7.

The extent of binding to N^6,N^6-dimethylaminopurine, Figure 10, is low compared to the extent of binding to adenosine. This is most likely brought about by steric crowding of the N-1 site by the methyl groups especially toward attack by a bulky metal complex. This result is consistent with the interpretation of the pH dependence data that at pH ~5.6 the major site occupied will be N-1 with negligible binding to N-3 and N-7.

Methylguanosine and Methylinosine. No reaction of I and II with 7-methylguanosine at pH 5.6 and at a metal:base ratio of 10:1 was detectable. The instability of 7-methylguanosine in alkaline solution hindered the study of the pH dependence of the binding. The pK_a(N-1) is 7.0 so that at pH 5–6 this site is blocked by a proton, and the N-7 site is blocked with the methyl group. On the other hand, 1-methylguanosine shows extensive reaction with both cis and trans isomers of

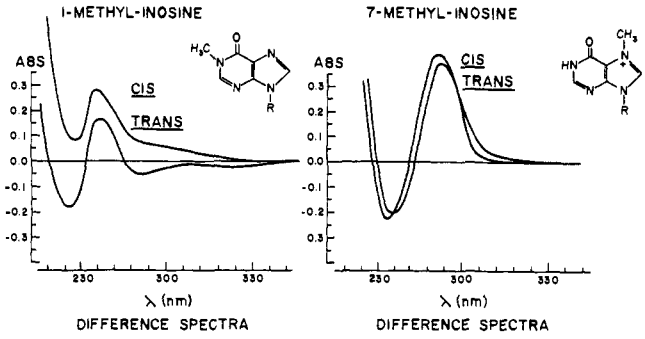

Figure 11. The difference spectra of 1-methylinosine and 7-methylinosine with *cis*- and *trans*-$Pt^{II}(NH_3)_2Cl_2$ at pH 3.27. The metal:base ratio was 10:1.

platinum. These observations confirm the conclusions of the pH dependence studies that N-7 is the main site occupied by metal with N-1 becoming an important metal binding site only above pH 6. Furthermore, the 6-keto group is not apparently a significant site of metalation.

Figure 11 shows the difference spectra of the reaction of I and II with 1-methylinosine and 7-methylinosine at a metal:base ratio of 10:1 at pH 3.27. The pH dependence showed that N-7 is the site occupied at very low pH, changing to N-1 as the pH is raised, the change-over point coming between pH 3 and 5. Figure 11 confirms that both N-1 and N-7 can be metalated.

3-Methylcytidine. No reaction was observed between I and II and 3-methylcytidine. This confirms the results from the pH dependence study that N-3 is the major binding site at pH 6.

Discussion

This work has demonstrated that both *cis*- and *trans*-$Pt^{II}(NH_3)_2Cl_2$ readily bind to the ring nitrogen atoms of the purines and pyrimidines with the loss of chloride ion and its replacement by the base as a ligand. The sites occupied are, broadly, those sites favorable to proton binding. In this respect, the binding of platinum(II) ions parallels the binding of other "b" metal cations, such as CH_3Hg^+,[13] Hg^{2+},[22] and Ag^+[23,26] ions. But the parallel is not perfect. For example, both cis and trans isomers bind to the N-3 of cytidine, a site with pK_a 4.2, but no binding to the 4-amino group is found even at metal–ligand ratios of 10:1 and at high pH. The amino group is a site not protonated over pH values accessible in aqueous solution. Guanosine also will bind I and II. The N-7 site, with pK_a 1.6, is the predominant site of attack, although N-1 is bound by platinum at higher pH values after removal of the proton. Here a chemical parallel with the nitrogen mustard alkylating agents arises since an important site of attack of the alkylating agents is the N-7 of guanosine.[27] Similarly inosine is bound at N-7, and, at higher pH, at N-1. No attack at the N-3 atoms of inosine or guanosine or at the 2-amino group of the latter has been detected in this work.

Although, at first sight, it is interesting that no attack of platinum at the 2-amino of guanosine or the 6-amino of cytidine has been observed, it should be noted that these groups are bound by CH_3Hg^+ only with a 100-fold excess of metal ion over that required to bind the sites of highest affinity, that is, N-3 of cytidine and N-7 of guanosine.[13] Because of the low solubility of I and II, it has not been possible to examine the mode of binding at these metal concentrations.

In none of the above results do clear-cut differences between the binding of the cis and the trans isomers arise and our conclusion is that guanosine and inosine are subject only to monofunctional attack by both I and II. This contrasts with the results of the binding experiments with adenosine and cytidine. In the former, both cis and trans isomers bind to the N-1 site, monofunctionally. This is the site of protonation of adenosine with pK_a 3.5. Some attack at N-7 with the trans isomer has been demonstrated although no previous evidence for attack at this site by other "b" metals exists. This is a site without a positively identified pK_a. On the other hand, the cis isomer binds bifunctionally between N-7 and the 6-amino group, most probably with the loss of a proton. This strongly suggests that the amino group of adenosine is a weak binding site which will be occupied particularly when there is a highly favorable entropy of chelation. This effect could become especially important in the binding of the cis isomer to sites which, though of low affinity toward monodentate binding of platinum(II), are sterically well disposed for bidentate chelation. Such sites are available in the organized structure of DNA. The effect on binding affinities of a suitable stereochemical disposition of sites on low monodentate affinity is explored in a later paper[28] using the dimer ApA in the stacked form as a model. It is already well known that the affinity of DNA for Hg(II) ions is a factor of ten greater than would be expected on the basis of the stability constants with the nucleosides alone.[13] Indeed the mercuration of primary amines compared to other sites appears to be greater in the polynucleotides than in the nucleosides.[13] Since these groups in DNA are well exposed to attack from the environment, it is important to examine this affinity for metal ions. In contrast, the site of bifunctional attack on cytidine is the N-3 and 2-keto group; the amino group is not attacked. It is clear, however, that the keto groups of guanosine, inosine, uridine, or thymidine are not strong platinum binding sites. The keto group has recently been implicated in the binding of Hg(II) ions by a recent structure determination of a complex formed between uracil and mercuric chloride.[29] More remarkable is the total absence of binding of either I or II to uridine or to thymidine since the N-3 site has a greater affinity for Hg(II) ions than any other site of nucleosides.[30] No obvious explanation can be put forward for this sharp difference.

Acknowledgments. The authors are grateful to Dr. R. J. P. Williams and Dr. J. Hoeschele for many stimulating discussions and suggestions. This work was supported by Grant No. CA-11349 from the National Cancer Institute.

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