Acid–Base and Metal-Ion-Binding Properties of the Quaternary [*cis*-$(NH_3)_2$Pt(dGuo)(dGMP)] Complex Formed Between *cis*-Diammineplatinum(II), 2′-Deoxyguanosine (dGuo), and 2′-Deoxyguanosine 5′-Monophosphate ($dGMP^{2-}$) in Aqueous Solution**

Helmut Sigel,* Bin Song, Gerda Oswald, and Bernhard Lippert*

Abstract: For the first time the acid–base properties of a quaternary Pt^{II} complex, [*cis*-$(NH_3)_2$Pt(dGuo)(dGMP)], are evaluated, as are the quinternary complexes resulting from its affinity for further metal ions. To this end the acid–base properties of $dGMP^{2-}$ (ref. [17]), dGuo, and of the quaternary complex resulting from their coordination to *cis*-$(NH_3)_2Pt^{2+}$ were determined by potentiometric pH titrations. Owing to the presence of the N7-coordinated *cis*-$(NH_3)_2Pt(dGuo)^{2+}$ moiety, the release of the proton from the $-P(O)_2(OH)^-$ group of [H{*cis*-$(NH_3)_2$Pt(dGuo)(dGMP)}]$^+$ is favored by $\Delta pK_a = 0.44$ compared with $H(dGMP)^-$ ($pK^H_{H(dGMP)} = 6.29$). The two H(N1) sites in the quaternary complex are on average acidified by $\Delta pK_a \approx 0.8$ compared with the acidity constants of the free ligands ($pK^H_{dGuo} = 9.24$; $pK^H_{dGMP} = 9.56$). A similar acidification is to be expected in DNA for intrastrand crosslinks by *cis*-$(NH_3)_2Pt^{2+}$ between two guanine residues; that is, at physiological pH (7.4) some deprotonation of H(N1) sites (ca. 6%) is already possible in principle. The stability constants of quinternary [M{*cis*-$(NH_3)_2$Pt(dGuo)(dGMP)}]$^{2+}$ complexes ($M = Mg^{2+}$, Cu^{2+}, Zn^{2+}) were also measured by potentiometric pH titrations. It is shown that the metal-ion-coordinating properties of the $-PO_3^{2-}$ group of the [*cis*-$(NH_3)_2$Pt(dGuo)(dGMP)] species are slightly *inhibited*, compared with the coordination tendency expected on the basis of its basicity. This inhibition is attributed to repulsion by the N7-coordinated Pt^{II}, which is somewhat more pronounced for M^{2+} than for H^+. However, it is moderate (about -0.2 log units), and therefore, with regard to DNA, one may conclude that *cis*-$(NH_3)_2Pt^{2+}$ coordinated to N7 of guanine residues affects the metal-ion-binding properties of the DNA phosphate backbone only slightly. This result is of further significance regarding individual nucleotides if these interact as substrates in enzymic reactions with different metal ions through their nucleobase as well as their phosphate residue.

Keywords: acid–base equilibria · DNA complexes · mixed-metal ion complexes · nucleotides · platinum · stability constants

1. Introduction

Cisplatin, *cis*-diamminedichloroplatinum(II) or [*cis*-$(NH_3)_2PtCl_2$], is a very effective anticancer drug and in wide clinical use against testicular and ovarian cancer.[1, 2] The *trans* isomer, [*trans*-$(NH_3)_2PtCl_2$], was found to be inactive.[3, 4] This might suggest a geometrical fit between cisplatin and its biological target in the tumor cell, which is generally accepted to be DNA;[5] primarily intrastrand crosslinks between adjacent guanine residues are formed involving platinum(II) binding to N7.[5, 6] These observations have fostered comprehensive studies on the properties of complexes formed with [*cis*-$(NH_3)_2Pt]^{2+}$ (see for example refs. [5–7]), [*trans*-$(NH_3)_2Pt]^{2+}$,[4, 8] and related compounds.[2]

Labile metal ions like Na^+ or Mg^{2+} affect the stability and conformational states of DNA and RNA,[9, 10] and enzymes that synthesize or cleave DNA or RNA invariably require metal ions,[9, 11–13] as do enzymes that utilize nucleotides as substrates.[12–14] This observation led to intensive research on

[*] Prof. Dr. H. Sigel, Dr. B. Song
Institut für Anorganische Chemie, Universität Basel
Spitalstr. 51, CH-4056 Basel (Switzerland)
Fax: (+41) 61-267-1017
E-mail: sigel@ubaclu.unibas.ch
Prof. Dr. B. Lippert, Dr. G. Oswald
Fachbereich Chemie, Universität Dortmund
Otto-Hahn-Strasse 6, D-44227 Dortmund (Germany)
Fax: (+49) 231-755-3797
E-mail: lippert@pop.uni-dortmund.de

[**] **Abbreviations and definitions:** $dGMP^{2-}$, 2′-deoxyguanosine 5′-monophosphate; dGuo, 2′-deoxyguanosine; dien, diethylene triamine = 1,4,7-triazaheptane; edta, 1,2-diaminoethane-*N*,*N*,*N*′,*N*′-tetraacetic acid; GMP^{2-}, guanosine 5′-monophosphate; M^{2+}, divalent metal ion; [Pt(dGuo)(dGMP)], [*cis*-$(NH_3)_2$Pt(dGuo)(dGMP)] (see also Figure 1); $R-PO_3^{2-}$, simple phosphate monoester or phosphonate ligand with R representing a noncoordinating residue (see also legend for Figure 3). Species given in the text without a charge either do not carry one or represent the species in general (i.e., independent from their protonation degree); which of the two versions applies is always clear from the context.

 © WILEY-VCH Verlag GmbH, D-69451 Weinheim, 1998 0947-6539/98/0406-1053 $ 17.50+.25/0

the metal-ion-binding properties of nucleotides and the constituents of nucleic acids.[2a, 15, 16] However, so far very little is known about the effect of nucleobase-bound Pt^{2+} on the acid–base[17, 18] and metal-ion-binding properties of other nearby sites, especially on the coordination of further kinetically labile metal ions[19] like Mg^{2+}, Cu^{2+}, or Zn^{2+} ($= M^{2+}$).

After having studied in detail[20] the coordinating properties of $dGMP^{2-}$ towards Mg^{2+}, Cu^{2+}, and Zn^{2+}, we are now in a position to report on the quaternary platinum(II) complex which has two N7-guanine sites beside two ammonias in its metal-ion coordination sphere, [*cis*-$(NH_3)_2$Pt(dGuo)(dGMP)] (Figure 1). We describe for the first time the stability of the

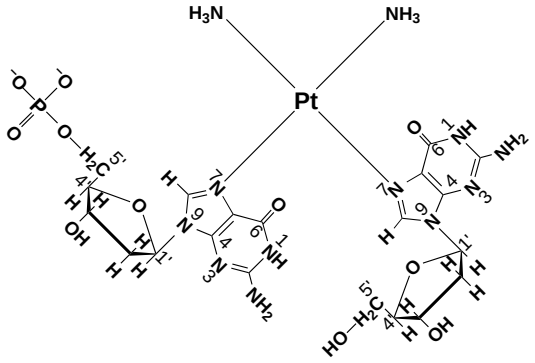


Figure 1. Formal structure of the quaternary [*cis*-$(NH_3)_2$Pt(dGuo)-(dGMP)] complex.

resulting quinternary complexes [M{*cis*-$(NH_3)_2$Pt(dGuo)-(dGMP)}]$^{2+}$ formed with Mg^{2+}, Cu^{2+}, or Zn^{2+}, and we evaluate the effect of the N7-coordinated [*cis*-$(NH_3)_2$Pt]$^{2+}$ unit on the metal-ion affinity of the phosphate group of the N7-bound $dGMP^{2-}$ (Figure 1).

The acid–base properties of dGuo were also determined and those[17, 20] of $dGMP^{2-}$ were taken into account to the extent necessary for a meaningful description of the effect of N7-coordinated [*cis*-$(NH_3)_2$Pt]$^{2+}$ on the H(N1) sites of dGuo and $dGMP^{2-}$ in [*cis*-$(NH_3)_2$Pt(dGuo)(dGMP)]. Conclusions regarding DNA and the effects of intrastrand crosslinks formed by [*cis*-$(NH_3)_2$Pt]$^{2+}$ as well as regarding nucleotide–metal ion systems involved in transphosphorylations are indicated.

2. Results and Discussion

2.1. Acidity constants of $H(dGuo)^+$, $H_2(dGMP)^{\pm}$, and [H{*cis*-$(NH_3)_2$Pt(dGuo)(dGMP)}]$^+$: 2′-Deoxyguanosine (dGuo) may accept a proton at N7 and release one from the H(N1) site;[21] hence, Equilibria (1) and (2) have to be considered.

$$H(dGuo)^+ \rightleftharpoons dGuo + H^+ \quad (1a)$$

$$K^{H}_{H(dGuo)} = [dGuo][H^+]/[H(dGuo)^+] \quad (1b)$$

$$dGuo \rightleftharpoons (dGuo - H)^- + H^+ \quad (2a)$$

$$K^{H}_{H(dGuo)} = [(dGuo - H)^-][H^+]/[dGuo] \quad (2b)$$

For the ribose moiety of guanosine it was shown[22] that $pK^{H}_{(Guo-H)} > 12.0$; hence, for 2′-deoxyguanosine it certainly also holds that $pK^{H}_{(dGuo-H)} > 12$, and therefore this deprotonation was not considered further. The results for Equilibria (1a) and (2a) are listed in Table 1; they agree excellently with an early[23] determination.[24]

For 2′-deoxyguanosine 5′-monophosphate ($dGMP^{2-}$) the situation is similar,[20] but in the pH range of relevance for this study a further proton binds to the $-PO_3^{2-}$ group. The resulting doubly protonated $H_2(dGMP)^{\pm}$ species releases its first proton from the H^+(N7) site ($pK^{H}_{H_2(dGMP)}$), giving $H(dGMP)^-$; next the $-P(O)_2(OH)^-$ residue is deprotonated ($pK^{H}_{H(dGMP)}$) leading to $dGMP^{2-}$, and finally the H(N1) site loses its proton (pK^{H}_{dGMP}) forming $(dGMP - H)^{3-}$. These acidity constants were determined recently[17, 20] under the conditions of the present study, and are listed under entry 2 in Table 1.[25]

Comparison of entries 1 and 2 of Table 1 reveals that the presence of the 5′-phosphate group enhances the basicity of N7 by $\Delta pK_a = 0.39 \pm 0.05$ and the one of $(N1)^-$ by $\Delta pK_a = 0.32 \pm 0.04$. Understandably, the charge effect appears to be slightly more pronounced on N7 than on $(N1)^-$ because in the *anti* conformation[21] N7 is somewhat closer to the phosphate group. These effects are of the order previously observed with related systems[26] and also close to the correction factor of $\Delta\Delta pK_a = 0.32$ needed in the next section in connection with the evaluation of a micro acidity constant for the effect of a $-PO_3^{2-}$ group (see ref. [29]).

Coordination of one dGuo and one $H_2(dGMP)^{\pm}$ species, each via N7, to [*cis*-$(NH_3)_2$Pt]$^{2+}$ gives the twofold protonated quaternary complex [H_2{*cis*-$(NH_3)_2$Pt(dGuo)(dGMP)}]$^{2+}$, abbreviated as [H_2{Pt(dGuo)(dGMP)}]$^{2+}$. A formal structure of the deprotonated complex is shown in Figure 1. The acidic [H_2{Pt(dGuo)(dGMP)}]$^{2+}$ species cannot accept more protons in the normal pH range, but it may in total release four protons. The first proton is probably released with a pK_a value similar to the one for $H_3(GMP)^+$ from its $-P(O)(OH)_2$ residue ($pK_a = 0.3 \pm 0.2$).[22] For the deprotonation of the resulting

Table 1. Negative logarithms of the acidity constants of $H(dGuo)^+$ and $H_2(dGMP)^{\pm}$ as well as of the quaternary [H{*cis*-$(NH_3)_2$Pt(dGuo)(dGMP)}]$^+$ complex (Figure 1), determined by potentiometric pH titrations in water at 25 °C and $I = 0.1$ M ($NaNO_3$).[a]

	Acid	$pK^{H}_{H(N7)}$ for H^+(N7)	$pK^{H}_{H(phosphate)}$ for $-OP(O)_2(OH)^-$	$pK^{H}_{H(N1)}$ for H(N1)
1	$H(dGuo)^+$	2.30 ± 0.04 [Eq. (1)]		9.24 ± 0.03 [Eq. (2)]
2	$H_2(dGMP)^{\pm}$	2.69 ± 0.03[b]	6.29 ± 0.01[b]	9.56 ± 0.02[b]
3	[H{Pt(dGuo)(dGMP)}]$^+$		5.85 ± 0.04 [Eq. (3)]	$8.20 \pm 0.03/9.05 \pm 0.10$ [Eqs. (4),(5)]

[a] So-called practical (or mixed) constants[25] are listed; see Section 4.3. The error limits given are *three times* the standard error of the mean value or the sum of the probable systematic errors, whichever is larger. [b] From refs. [17, 20].

© WILEY-VCH Verlag GmbH, D-69451 Weinheim, 1998
0947-6539/98/0406-1054 $ 17.50+.50/0 *Chem. Eur. J.* **1998**, *4*, No. 6

$[H\{Pt(dGuo)(dGMP)\}]^+$ species the Equilibria (3) – (5) may be written. In Equilibrium (3a) the $-P(O)_2(OH)^-$ group is

$$[H\{Pt(dGuo)(dGMP)\}]^+ \rightleftharpoons [Pt(dGuo)(dGMP)] + H^+ \quad (3a)$$

$$K^H_{[H\{Pt(dGuo)(dGMP)\}]} = \frac{[Pt(dGuo)(dGMP)][H^+]}{[H\{Pt(dGuo)(dGMP)\}^+]} \quad (3b)$$

$$[Pt(dGuo)(dGMP)] \rightleftharpoons [Pt(dGuo)(dGMP)-H]^- + H^+ \quad (4a)$$

$$K^H_{[Pt(dGuo)(dGMP)]} = \frac{[\{Pt(dGuo)(dGMP)-H\}^-][H^+]}{[Pt(dGuo)(dGMP)]} \quad (4b)$$

$$[Pt(dGuo)(dGMP)-H]^- \rightleftharpoons [Pt(dGuo)(dGMP)-2H]^{2-} + H^+ \quad (5a)$$

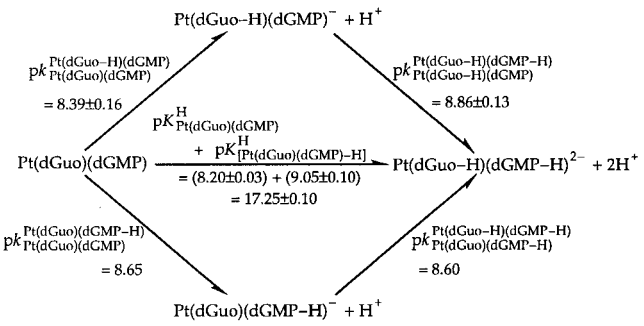
$$K^H_{[Pt(dGuo)(dGMP)-H]} = \frac{[\{Pt(dGuo)(dGMP)-2H\}^{2-}][H^+]}{[\{Pt(dGuo)(dGMP)-H\}^-]} \quad (5b)$$

deprotonated, whereas in reactions (4a) and (5a) the protons are released from the two H(N1) sites (Figure 1). The results of the potentiometric pH titrations are given in entry 3 of Table 1.

The result $pK^H_{[H\{Pt(dGuo)(dGMP)\}]} = 5.85 \pm 0.04$ shows unequivocally that this proton [Eq. (3)] is released from the monoprotonated phosphate group of the quaternary complex (Figure 1). The N7-coordinated $[cis\text{-}(NH_3)_2Pt(dGuo)]^{2+}$ unit acidifies $H(dGMP)^-$ by $\Delta pK_a = pK^H_{H(dGMP)} - pK^H_{[H\{Pt(dGuo)(dGMP)\}]} = (6.29 \pm 0.01) - (5.85 \pm 0.04) = 0.44 \pm 0.04$. The situation regarding the H(N1) sites is considerably less clearcut, though one may assume that the next proton [Eq. (4)] is mainly released from H(N1) of the N7-coordinated dGuo because the basicity of the $(N1)^-$ site is lower in dGuo than in $dGMP^{2-}$ (Table 1). Hence, the final proton [Eq. (5)] may be attributed mainly to the release from the H(N1) site of the N7-coordinated $dGMP^{2-}$. The average acidification by $[cis\text{-}(NH_3)_2Pt]^{2+}$ of the H(N1) sites in reactions (4) and (5) is given by $\Delta pK_a = {}^1/_2(pK^H_{dGuo} + pK^H_{dGMP}) - {}^1/_2(pK^H_{[Pt(dGuo)(dGMP)]} + pK^H_{[Pt(dGuo)(dGMP)-H]}) = {}^1/_2[(9.24 \pm 0.03) + (9.56 \pm 0.02)] - {}^1/_2[(8.20 \pm 0.03) + (9.05 \pm 0.10)] = 0.78 \pm 0.11$. This acidification at the nucleobase is considerably more pronounced than the one on the phosphate residue.

The above indications regarding the H(N1) deprotonations and the fact that the acidity constants $pK^H_{[Pt(dGuo)(dGMP)]}$ and $pK^H_{[Pt(dGuo)(dGMP)-H]}$ (Table 1, entry 3) are separated only by $\Delta pK_a = (9.05 \pm 0.10) - (8.20 \pm 0.03) = 0.85 \pm 0.10$, that is, that the corresponding buffer regions are overlapping, request a detailed evaluation.

2.2. Micro acidity constant scheme for $[cis\text{-}(NH_3)_2Pt(dGuo)(dGMP)]$: For a correct quantification of the intrinsic acidities of the H(N1) sites in $[cis\text{-}(NH_3)_2Pt(dGuo)(dGMP)]$ (Figure 1) it is necessary to calculate the corresponding micro acidity constants. Figure 2 summarizes the equilibrium scheme for this species following known routes,[22, 27, 28] defining the micro acidity constants (k) and giving their interrelation with the macro acidity constants (K). There are three independent equations, Equations (6a), (6b), and (6c), in Figure 2, but four unknown microconstants.[22, 27, 28] However, for $k^{[Pt(dGuo-H)(dGMP-H)]}_{[Pt(dGuo-H)(dGMP)]}$ a value may be estimated based on the



$$K^H_{Pt(dGuo)(dGMP)} = k^{Pt(dGuo-H)(dGMP)}_{Pt(dGuo)(dGMP)} + k^{Pt(dGuo)(dGMP-H)}_{Pt(dGuo)(dGMP)} \quad (6a)$$

$$\frac{1}{K^H_{[Pt(dGuo)(dGMP)-H]}} = \frac{1}{k^{Pt(dGuo-H)(dGMP-H)}_{Pt(dGuo-H)(dGMP)}} + \frac{1}{k^{Pt(dGuo-H)(dGMP-H)}_{Pt(dGuo)(dGMP-H)}} \quad (6b)$$

$$K^H_{Pt(dGuo)(dGMP)} \cdot K^H_{[Pt(dGuo)(dGMP)-H]} = k^{Pt(dGuo-H)(dGMP)}_{Pt(dGuo)(dGMP)} \cdot k^{Pt(dGuo-H)(dGMP-H)}_{Pt(dGuo-H)(dGMP)} = k^{Pt(dGuo)(dGMP-H)}_{Pt(dGuo)(dGMP)} \cdot k^{Pt(dGuo-H)(dGMP-H)}_{Pt(dGuo)(dGMP-H)} \quad (6c)$$

Figure 2. Equilibrium scheme for $[cis\text{-}(NH_3)_2Pt(dGuo)(dGMP)]$ defining the micro acidity constants (k) and showing their interrelation with the macro acidity constants (K) and the relationship between $[cis\text{-}(NH_3)_2Pt(dGuo-H)(dGMP)]^-$ and $[cis\text{-}(NH_3)_2Pt(dGuo)(dGMP-H)]^-$ and the other species present. In $[cis\text{-}(NH_3)_2Pt(dGuo-H)(dGMP)]^-$ the proton is released from H(N1) in dGuo, and in $[cis\text{-}(NH_3)_2Pt(dGuo)(dGMP-H)]^-$ from H(N1) in $dGMP^{2-}$; $[cis\text{-}(NH_3)_2Pt(dGuo)(dGMP)]$ is also often written as $[Pt(dGuo)(dGMP)]$. The arrows indicate the direction for which the acidity constants are defined. Use of the value estimated[29] for the microconstant $pk^{[Pt(dGuo-H)(dGMP-H)]}_{[Pt(dGuo-H)(dGMP)]}$ permits calculation of the other microconstants with Equations (6a), (6b), and (6c) (see also Section 2.2).

easily accessible (via statistical considerations)[17] micro acidity constant for the deprotonation of $[cis\text{-}(NH_3)_2Pt(dGMP-H)(dGMP)]^{3-}$ and by taking into account the difference in charge between this species and $[cis\text{-}(NH_3)_2Pt(dGuo-H)(dGMP)]^-$; this estimate[29] is given on the arrow at the right in the upper part of Figure 2. Now the other three microconstants can be calculated; the corresponding results are given on the arrows in the same figure.

These microconstants now permit the estimation of the ratio R of the (N1)-monodeprotonated species $[cis\text{-}(NH_3)_2Pt(dGuo-H)(dGMP)]^-$ and $[cis\text{-}(NH_3)_2Pt(dGuo)(dGMP-H)]^-$ (Figure 2) which are N1-deprotonated either at the N7-coordinated dGuo or at the likewise N7-bound $dGMP^{2-}$, respectively [Eq. (7)]. Evidently, $[cis\text{-}(NH_3)_2Pt(dGuo-H)(dGMP)]^-$ occurs at about 67% and the other

$$R = \frac{[cis\text{-}(NH_3)_2Pt(dGuo-H)(dGMP)^-]}{[cis\text{-}(NH_3)_2Pt(dGuo)(dGMP-H)^-]} = \frac{k^{Pt(dGuo-H)(dGMP)}_{Pt(dGuo)(dGMP)}}{k^{Pt(dGuo)(dGMP-H)}_{Pt(dGuo)(dGMP)}} = \frac{10^{-8.39}}{10^{-8.65}} = 10^{0.26} \approx \frac{2}{1} \approx \frac{67}{33} \quad (7)$$

(N1)-monodeprotonated species at about 33%. This estimate proves a) that both tautomeric forms of $[cis\text{-}(NH_3)_2Pt(dGuo)(dGMP)-H]^-$ occur simultaneously in appreciable amounts and b) that, as already tentatively concluded in Section 2.1, the species $[cis\text{-}(NH_3)_2Pt(dGuo-H)(dGMP)]^-$ dominates.

Of course, the four micro acidity constants given in Figure 2 now also enable us to estimate the acidification which

the various H(N1) sites experience individually owing to the N7-coordinated $[cis\text{-}(NH_3)_2Pt]^{2+}$ unit. For example, $\Delta pK_{a/dGuo} = pK^{H}_{dGuo} - pk^{[Pt(dGuo-H)(dGMP)]}_{[Pt(dGuo)(dGMP)]} = 9.24 - 8.39 = 0.85$, or $\Delta pK_{a/dGMP} = pK^{H}_{dGMP} - pk^{[Pt(dGuo-H)(dGMP-H)]}_{[Pt(dGuo-H)(dGMP)]} = 9.56 - 8.86 = 0.70$. Clearly, if this calculation is carried out for all four possibilities and averaged, one obtains the average acidification $\Delta pK_a = 0.78$ already given in Section 2.1. However, the individual results obtained by means of Figure 2 demonstrate that the acidification by $[cis\text{-}(NH_3)_2Pt]^{2+}$ of the various sites differs somewhat; this has also to be expected if $[cis\text{-}(NH_3)_2Pt]^{2+}$ forms an intrastrand crosslink between adjacent guanine residues in DNA.

2.3. Stability of the quinternary $[M\{cis\text{-}(NH_3)_2Pt(dGuo)\text{-}(dGMP)\}]^{2+}$ complexes: From previous studies[15, 22, 30] with purine-nucleoside 5′-monophosphates it is well known that a metal ion bound at the phosphate group may in addition interact with N7 of the purine moiety, and this has very recently[20] also been proven for the [M(dGMP)] complexes of Mg^{2+}, Cu^{2+}, and Zn^{2+}. This additional interaction gives rise to macrochelate formation, schematically expressed here in the intramolecular Equilibrium (8).

(8)

Of course, if the $[cis\text{-}(NH_3)_2Pt(dGuo)]^{2+}$ unit is N7-coordinated to $dGMP^{2-}$ to give the quaternary complex shown in Figure 1, macrochelate formation is not possible anymore. A further metal ion could coordinate now only to the still available $\text{-}PO_3^{2-}$ residue. The question in this case is: Does the repulsion between the two dipositively charged metal ions still allow the formation of such complexes, and if so, how stable are they?

The experimental data from such systems studied by potentiometric pH titrations can be completely described by taking into account the Equilibria (3a) and (9a), provided that

$$M^{2+} + [Pt(dGuo)(dGMP)] \rightleftharpoons [M\{Pt(dGuo)(dGMP)\}]^{2+} \quad (9a)$$

$$K^{M}_{[M\{Pt(dGuo)(dGMP)\}]} = \frac{[[M\{Pt(dGuo)(dGMP)\}]^{2+}]}{[M^{2+}][Pt(dGuo)(dGMP)]} \quad (9b)$$

evaluation of the data is not carried into the pH range where formation of hydroxo complexes occurs. The measured (exptl) stability constants according to Equation (9b) are listed in the second column of Table 2.

The simple fact that these stability constants can be measured proves that the complexes $[M\{cis\text{-}(NH_3)_2Pt(dGuo)(dGMP)\}]^{2+}$ form. How can these stability data be evaluated? In earlier studies[31, 32] a linear relationship was established between the logarithms of the stability constants of $M(R-PO_3)$ complexes, $\log K^{M}_{M(R-PO_3)}$, and the negative logarithms of the acidity constants of the corresponding monoprotonated $H(R-PO_3)^-$ species, $pK^{H}_{H(R-PO_3)}$, for several simple phosphate monoester ligands,[31] including methyl phosphate.[33] The points for complexes formed with phosphonates like methanephosphonate (abbreviated MeP^{2-}) or ethanephosphonate (EtP^{2-}) also fall on the same straight reference line for a given metal ion.[32] The corresponding straight-line equations for the complexes of Mg^{2+}, Cu^{2+}, or Zn^{2+} and $R-PO_3^{2-}$ ligands (where R is a residue unable to interact with M^{2+}) are given in Equations (10), (11) and (12), respectively.[32] The error limits of log stability constants calculated with given $pK^{H}_{H(R-PO_3)}$ values and Equations (10), (11), and (12) are ± 0.03, ± 0.06, and ± 0.06 log units (3σ),[22, 32] respectively, in the pK_a range 5–8 (aqueous solution; 25 °C; $I = 0.1$ M, $NaNO_3$).

Table 2. Stability constants, $\log K^{M}_{[M\{Pt(dGuo)(dGMP)\}]}$ [Eq. (9)],[a] determined by potentiometric pH titrations (exptl) for quinternary $[M\{cis\text{-}(NH_3)_2Pt(dGuo)(dGMP)\}]^{2+}$ complexes, and their comparison with the corresponding calculated (calcd)[b] stability constants based on the basicity of the phosphate group in $[cis\text{-}(NH_3)_2Pt(dGuo)(dGMP)]$ (see Figure 1), together with the resulting stability difference, $\log \Delta_{[M[Pt(dGuo)(dGMP)]}$ [Eq. (13)], for aqueous solutions at 25 °C and $I = 0.1$ M ($NaNO_3$).

M^{2+}	$\log K^{M}_{[M\{Pt(dGuo)(dGMP)\}]}$ exptl[a]	calcd[b]	$\log \Delta_{M[Pt(dGuo)(dGMP)]}$
Mg^{2+}	1.21 ± 0.04	1.49 ± 0.03	-0.28 ± 0.05
Cu^{2+}	2.60 ± 0.08	2.71 ± 0.06	-0.11 ± 0.10
Zn^{2+}	1.81 ± 0.06	2.00 ± 0.06	-0.19 ± 0.08

[a] The error limits (3σ; see footnote [a] of Table 1) of the derived data, in the present case for column 4, were calculated according to the error propagation after Gauss. [b] Calculated with $pK^{H}_{[H\{Pt(dGuo)(dGMP)\}]} = 5.85$ (Table 1) and the reference equations [see also Figure 3 and Eqs. (10), (11), and (12)[22, 32]].

$$\log K^{Mg}_{Mg(R-PO_3)} = (0.208 \pm 0.015) \cdot pK^{H}_{H(R-PO_3)} + (0.272 \pm 0.097) \quad (10)$$

$$\log K^{Cu}_{Cu(R-PO_3)} = (0.465 \pm 0.025) \cdot pK^{H}_{H(R-PO_3)} - (0.015 \pm 0.164) \quad (11)$$

$$\log K^{Zn}_{Zn(R-PO_3)} = (0.345 \pm 0.026) \cdot pK^{H}_{H(R-PO_3)} - (0.017 \pm 0.171) \quad (12)$$

The data points from the stability constants of the $[M\{cis\text{-}(NH_3)_2Pt(dGuo)(dGMP)\}]^{2+}$ complexes (Table 2, column 2) and the corresponding acidity constant $pK^{H}_{[H\{Pt(dGuo)(dGMP)\}]}$ (Table 1, entry 3) are shown in Figure 3 and are to be compared with the above-mentioned reference lines [Eqs. (10)–(12)] which reflect the stabilities of $M(R-PO_3)$ complexes solely determined by the basicity of the $\text{-}PO_3^{2-}$ group. As can clearly be seen, the data points for all three quinternary complexes fall below these lines, indicating an inhibitory effect.

The inhibition observed in Figure 3 may be quantified by applying $pK^{H}_{[H\{Pt(dGuo)(dGMP)\}]} = 5.85$ (Table 1) together with the straight-line equations [Eqs. (10)–(12)]. The resulting calculated (calcd) stability constants are the ones expected solely on the basis of the basicity of the $\text{-}PO_3^{2-}$ group of the quaternary platinum(II) complex (Table 2, column 3). Comparison of the measured and calculated constants according to Equation (13) furnishes the stability differences listed in the final column of Table 2; they correspond to the broken vertical lines in Figure 3.

$$\log \Delta_{[M[Pt(dGuo)(dGMP)]} = \log K^{M}_{[M\{Pt(dGuo)(dGMP)\}]} - \log K^{M}_{[M\{Pt(dGuo)(dGMP)\}]_{calcd}} \quad (13)$$

© WILEY-VCH Verlag GmbH, D-69451 Weinheim, 1998
0947-6539/98/0406-1056 $ 17.50+.50/0 Chem. Eur. J. 1998, 4, No. 6

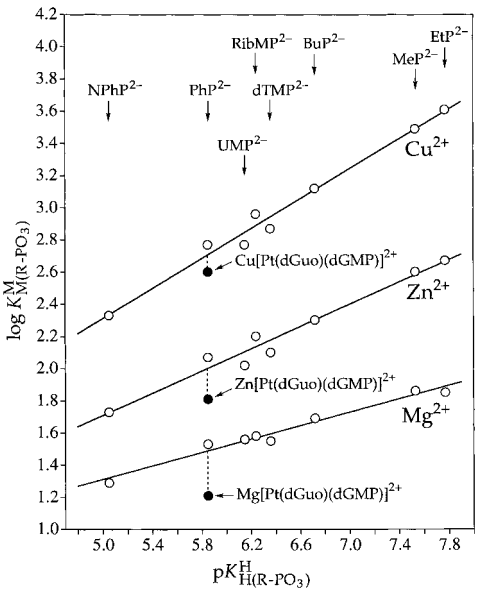


Figure 3. Comparison of the stability of several [M{*cis*-$(NH_3)_2$Pt-(dGuo)(dGMP)}]$^{2+}$ complexes (●) with the relationship between $\log K^{M}_{M(R-PO_3)}$ and $pK^{H}_{H(R-PO_3)}$ for the 1:1 complexes of Mg^{2+}, Zn^{2+}, and Cu^{2+} with some simple phosphate monoester or phosphonate ligands (R–PO_3^{2-}) (○): 4-nitrophenyl phosphate (NPhP^{2-}), phenyl phosphate (PhP^{2-}), uridine 5′-monophosphate (UMP^{2-}), D-ribose 5-monophosphate (RibMP^{2-}), thymidine [= 1-(2′-deoxy-β-D-ribofuranosyl)thymine] 5′-monophosphate (dTMP^{2-}), *n*-butyl phosphate (BuP^{2-}), methanephosphonate (MeP^{2-}), and ethanephosphonate (EtP^{2-}) (from left to right). The reference lines are drawn from Equations (10), (11), and (12). The points due to the equilibrium constants for the M^{2+}/[*cis*-$(NH_3)_2$Pt(dGuo)(dGMP)] systems (●) are based on the data given in Table 1 (entry 3) and Table 2 (column 2). The vertical broken lines emphasize the stability differences to the corresponding reference lines; these differences are equal to $\log \Delta_{[M[Pt(dGuo)(dGMP)]]}$ for the [M{*cis*-$(NH_3)_2$Pt(dGuo)(dGMP)}]$^{2+}$ complexes [Eq. (13)]. All the plotted equilibrium constant values refer to aqueous solutions at 25 °C and $I = 0.1$M ($NaNO_3$).

The negative stability differences mentioned (Table 2, column 4) reflect the repulsion between M^{2+} and the twofold positively charged platinum(II) located at N7 of dGMP^{2-}. Of course, this Pt^{2+} also has an effect on the deprotonation of the -$P(O)_2(OH)^-$ residue as discussed in Sections 2.1 and 2.2, but, as one might expect, this repulsive effect is somewhat larger on the binding of dipositively charged divalent metal ions than on that of the singly positively charged proton. Therefore, the data points for the quinternary complexes fall below the reference lines in Figure 3. However, it needs to be emphasized that, as our results prove, the affinity of the -PO_3^{2-} group in [*cis*-$(NH_3)_2$Pt(dGuo)(dGMP)] toward M^{2+} is still quite pronounced, since the inhibitory effect is very moderate (approximately −0.2 log unit; Table 2, column 4).

3. Conclusions

3.1. Possible biological relevance of guanine N(1) deprotonation as mediated by metal ions: The present results on the H(N1) acidification as a consequence of N7 coordination of a platinum(II) entity are in agreement with previous findings for model nucleobases,[34] nucleosides,[35] dinucleoside monophosphates,[36, 37] dinucleotides,[37, 38] and single-stranded deoxyoligonucleotides.[38, 39] For example, pK_a values as low as approximately 8 have been observed in cisplatin-modified DNA single strands.[39] Other metal ions behave similarly.[40, 41] The potential biological significance of this finding appears not to have received particular attention, either in the case of cisplatin or for other metals. We are well aware that cisplatin-related mutagenicity is generally ascribed to a structural distortion of DNA[42] rather than an electronic one, but it should be noted that even monofunctional [(dien)PtCl]Cl is a weak mutagen in the Ames test.[43] Its acidifying effect on guanine-(N1)H (p$K_a = 8.0$)[44] is virtually identical to that of cisplatin,[39] yet it does not structurally distort DNA.

As to the possible consequences of any base deprotonation process, relatively little is known. It is generally accepted that nucleobase ionization is somewhat suppressed in double-stranded DNA by the fact that the base is involved in H bonding,[45] yet that it is easily possible in single-stranded DNA. This difference is attributed to differences in solvent accessibility, but the fact that even double-stranded DNA is breathing is usually ignored. Possible consequences of nucleobase ionization such as loss of base-pairing specificity and base mispairing have not been considered to be biologically relevant to mutagenesis for many years, despite an early proposal by Lawley and Brookes in 1962.[46]

In model systems, loss of Watson–Crick base pairing between cytosine and N1-deprotonated guanine as well as guanine–guanine mispairing has been demonstrated, both in solution[47] and in the solid state.[34] Indeed, more recently evidence has been presented that supports the idea that ionized nucleobases can play a role in base mispairing and mutagenicity.[48] It has been proposed that a protein environment, specifically the active sites of polymerases,[49] might be intrinsically capable of stabilizing ionic states of nucleobases. On the other hand, it is clear that the concentration of an ionized species in such an environment can differ from that observed in a simple, aqueous medium.[48b] Irrespective of the question of whether a metal ion entity bound to DNA, be it platinum(II) or any other metal ion, can be accommodated in and processed by polymerases,[50] it is difficult to say whether a certain percentage of a deprotonated, N7-platinated guanine, as calculated for a single-strand DNA fragment or our model system,[51] is meaningful with respect to a biological effect.

Another aspect of interest also appears not to have been considered in the context of metal–DNA binding: If a metal ion at the N7 position of guanine acidifies the proton at N1, the probability of a proton transfer within the Watson–Crick base pair may become significant. The occurrence of spontaneous mutations have, among others, also been discussed in terms of a concerted proton transfer (from guanine-N1 to cytosine-N3, and from cytosine-N4 to guanine-O6) or a single proton transfer (from guanine-N1 to cytosine-N3), respectively.[52] Theoretical calculations of the energies of the various tautomeric forms of Watson–Crick guanine–cytosine base pairs suggest[52] that tautomerization of both bases by a double proton transfer could occur in one out of 10^6 to 10^9 base pairs; this is close to the occurrence of substitution mutations (with proofreading not considered).

3.2. Some further general considerations: Among the remarkable results of this study is the observation that a proton at the phosphate group of the quaternary [H{*cis*-$(NH_3)_2$Pt(dGuo)(dGMP)}]$^+$ complex (Figure 1) is only slightly acidified by the N7-coordinated [*cis*-$(NH_3)_2$Pt]$^{2+}$ unit ($\Delta pK_a \approx 0.4$). This contrasts with the more significant acidification (on average $\Delta pK_a \approx 0.8$) of the same platinum(II) unit on the H(N1) sites (see Section 2.1). As the overall charge of [*cis*-$(NH_3)_2$Pt(dGuo)(dGMP)] equals that of a DNA intrastrand crosslink unit formed with [*cis*-$(NH_3)_2$Pt]$^{2+}$ and two guanine residues, the present results are also meaningful for the effects of [*cis*-$(NH_3)_2$Pt]$^{2+}$ if bound to (single-stranded) DNA. Based on the observed acidification ($pK_a = 8.6$; average of the values in Figure 2)[53] one would expect that about 6% of the guanine residues, which carry a platinum(II) at N7, will be deprotonated at N1 under physiological conditions (pH 7.4).[53] As pointed out in Section 3.1, deprotonation is facilitated by an opening of the duplex giving access to the solvent (H_2O), a situation most likely relevant to DNA that is in an active state (transcription, replication). The present results further suggest, as indicated above, that the acidification of the H(N1) sites by platinum(II) turns these sites into better H donors, rendering them even more suitable for hydrogen bonding than the uncomplexed nucleobases. Of course, at the same time the acceptor properties of O(6) will be reduced. In any case, the electronic complementarity between guanine and cytosine residues will be disturbed.

A further important result regarding DNA is the observation that the formation of quinternary [M{*cis*-$(NH_3)_2$Pt(dGuo)(dGMP)}]$^{2+}$ complexes is only slightly inhibited; that is, the affinity of the -PO_3^{2-} group for divalent metal ions (Mg^{2+}, Cu^{2+}, Zn^{2+}) is only slightly affected by the platinum(II) coordinated at N7 of the same nucleotide unit. Consequently, one can expect that a nucleobase-bound platinum(II) in DNA will similarly affect the metal-ion-binding properties of the phosphate backbone only little.[54] Regarding the binding of K^+ or Mg^{2+}, this conclusion is important.

Finally, it may be emphasized that for nucleotide systems involved in transphosphorylations there is much evidence that in simple[55] as well as in enzymatic reactions[56] two (or even more) metal ions are involved. There is further evidence that in reactive intermediates a metal-ion – N7 interaction occurs not only in the metal-ion-promoted hydrolysis[55] of ATP but also in enzymatic reactions as proposed recently[57] for Zn^{2+}; similarly, adenosine N7 nitrogens are important in a Mg^{2+}-dependent ribozyme.[58] For such observations the results regarding the formation of the quinternary [M{*cis*-$(NH_3)_2$Pt(dGuo)(dGMP)}]$^{2+}$ complexes are meaningful because they prove that a phosphate residue of an individual nucleotide is only slightly affected in its metal-ion affinity if this same nucleotide is also undergoing a nucleobase – metal ion interaction (and vice versa); naturally, such diverse interactions allow the steric orientation of a substrate.

4. Experimental Section

4.1. Synthesis of [*cis*-$(NH_3)_2$Pt(dGuo)(dGMP)]: Na_2(dGMP)$\cdot 2H_2O$ (1.18 mmol) was added to a solution of [*cis*-$(NH_3)_2Pt(H_2O)_2](NO_3)_2$ (1.18 mmol) obtained from [*cis*-$(NH_3)_2PtCl_2$] and $AgNO_3$ (2 equiv) in water (15 mL) in the dark (3 h at 90 °C), and following filtration of AgCl. After 30 min at room temperature (reaction virtually complete, according to ^{1}H NMR spectroscopy), dGuo (1.18 mmol) was also added (pH of suspension 6.3), and after 2 d (pH then 6.4), an unidentified grayish precipitate was filtered off. The resulting colorless solution was brought to dryness by rotary evaporation (30 °C), redissolved in water and passed over Sephadex G10 (FPLC Pharmacia/LKB; UV detection 260 nm; eluent water). Pure [*cis*-$(NH_3)_2$Pt(dGuo)(dGMP)]$\cdot 5H_2O$ was obtained in low yield (4%). Anal. calcd (found) for $C_{20}H_{41}N_{12}O_{16}PPt$: C 25.78 (25.5); H 4.44 (5.4); N 18.04 (18.4). According to its IR spectrum (KBr) the product is free of NO_3^-. ^{1}H NMR (D_2O, pD 6.0, 0.01 M, TSP): $\delta = 8.60$ (s, H8, dGMP), 8.35 (s, H8, dGuo), 6.34 and 6.28 (t, H1′, dGMP, dGuo); H8 resonances 1:1, no indication of other species. Other fractions contained the product admixed with $NaNO_3$ and partly protonated, e.g. $H_{0.7}$[*cis*-$(NH_3)_2$Pt(dGuo)-(dGMP)]$(NO_3)_{0.7}\cdot 14\,NaNO_3\cdot H_2O$ (empirical formula based on elemental analysis data and potentiometric titration of HNO_3). These species proved very hygroscopic.

The composition of the [*cis*-$(NH_3)_2$Pt(dGuo)(dGMP)] complex was also confirmed by the potentiometric pH titrations described below: one equivalent of NaOH was needed in the pH range where -$P(O)_2(OH)^-$ groups are titrated and two equivalents of NaOH in the range where deprotonation of H(N1) sites occurs.

4.2. Materials for the titration experiments: The nitrate salts of Na^+, Mg^{2+}, Cu^{2+}, and Zn^{2+}, potassium hydrogenphthalate, the disodium salt of edta, HNO_3, and NaOH (Titrisol) (all pro analysi) were from Merck, Darmstadt (Germany). 2′-Deoxyguanosine was purchased from Sigma Chemical, St. Louis (MO, USA). The [*cis*-$(NH_3)_2$Pt(dGuo)(dGMP)] complex was prepared as described in Section 4.1.

The aqueous stock solutions of 2′-deoxyguanosine and of the complex were freshly prepared daily and the exact concentration was redetermined each time (see below); in the case of the complex the pH of the stock solution was adjusted with NaOH to about 8.4 prior to the determination of its concentration. All solutions were prepared with deionized, ultrapure (MILLI-Q185 PLUS, Millipore, 67120 Molsheim (France)), and CO_2-free water. The ligand concentration in the potentiometric pH titrations was always below 1 mM, which means that self-association is certainly negligible for these guanine derivatives.[22]

The titer of the NaOH used for the titrations was established with potassium hydrogenphthalate. The exact concentrations of the $M(NO_3)_2$ stock solutions were determined by potentiometric pH titration of their $M(edta)^{2-}$ complexes by measuring the proton equivalents liberated from $H(edta)^{3-}$ upon complex formation.

4.3. Potentiometric pH titrations: The pH titrations were carried out with a Metrohm E536 potentiograph equipped with an E665 dosimat and a 6.0202100(NB) combined macro glass electrode. The buffer solutions (pH 4.64, 7.00, 9.00, based on the NIST scale; for details see ref. [25]) used for calibration were also from Metrohm, Herisau (Switzerland). The direct pH-meter readings were used to calculate the acidity constants; that is, these constants are so-called practical, mixed, or Brønsted constants.[25] Their negative logarithms given for aqueous solutions at $I = 0.1$ M ($NaNO_3$) and 25 °C may be converted into the corresponding concentration constants by subtracting 0.02 from the listed pK_a values;[25] this conversion term contains both the junction potential of the glass electrode and the hydrogen ion activity.[25, 59] No conversion term is necessary for the stability constants of the metal-ion complexes; these are as usual concentration constants.

The ionic product of water (K_w) and the above-mentioned conversion term do not enter into the calculations because we evaluate the *differences* in NaOH consumption between solutions with and without ligand[25, 26] (see also below); this procedure also furnishes directly the concentration of the ligand.

4.4. Determination of the acidity constants: The constants $K^H_{H(dGuo)}$ [Eq. (1)] and K^H_{dGuo} [Eq. (2)] of $H(dGuo)^+$ were determined by titrating 25 mL of aqueous 6.0 mM HNO_3 ($I = 0.1$ M, $NaNO_3$; 25 °C) in the presence and absence of 0.93 mM dGuo under N_2 with 0.06 M NaOH (3 mL). The pH range (2.5 – 10.4) used for the calculations by employing the difference in NaOH consumption between the two titrations mentioned corresponded to about 61% (pH 2.5) neutralization for the equilibrium $H(dGuo)^+$/dGuo and 94% (pH 10.4) for the equilibrium dGuo/$(dGuo - H)^-$.

© WILEY-VCH Verlag GmbH, D-69451 Weinheim, 1998
0947-6539/98/0406-1058 $ 17.50+.50/0 *Chem. Eur. J.* **1998**, *4*, No. 6

All constants were calculated with an IBM compatible desk computer with a 80486 processor (connected to an Epson Stylus 1000 printer and a Hewlett–Packard 7475A plotter) by a curve-fit procedure using a Newton–Gauss nonlinear least-squares program.

The monoprotonated quaternary [*cis*-$(NH_3)_2$Pt(dGuo)(dGMP)] complex is abbreviated in the following as [H{Pt(dGuo)(dGMP)}]$^+$; its acidity constants $K^{H}_{[H\{Pt(dGuo)(dGMP)\}]}$, $K^{H}_{[Pt(dGuo)(dGMP)]}$, and $K^{H}_{[Pt(dGuo)(dGMP)-H]}$ [Eqs. (3)–(5)] were determined by titrating 25 mL of aqueous 0.4 mM HNO_3 and $NaNO_3$ ($I = 0.1$ M, 25 °C) in the presence and absence of 0.2–0.3 mM [Pt(dGuo)(dGMP)] under N_2 with 0.02 M NaOH (2 mL). The pH range used for the calculations (based on the differences between the two titrations) corresponded to about 1% neutralization for the equilibrium [H{Pt(dGuo)(dGMP)}]$^+$/[Pt(dGuo)(dGMP)] and about 90% neutralization for [Pt(dGuo)(dGMP) – H]$^-$/[Pt(dGuo)(dGMP) – 2H]$^{2-}$. The results given in Table 1 are the averages of 16 and 9 independent pairs of titrations for the various acidity constants of H(dGuo)$^+$ and [H{Pt(dGuo)-(dGMP)}]$^+$, respectively.

4.5. Determination of the stability constants: As only small amounts of [Pt(dGuo)(dGMP)] were available, the solutions used for the determination of the acidity constants (see Section 4.4) were reused; HNO_3 was added again, along with $M(NO_3)_2$, and then the titrations were repeated with NaOH to determine the stability constants of the [M{Pt(dGuo)(dGMP)}]$^{2+}$ complexes. In some cases, after such a titration, another portion of HNO_3 was added to the same solution and the titration with NaOH repeated. Of course, the various dilutions of the solutions were considered in the calculations. The [Pt(dGuo)(dGMP)]/M^{2+} ratios were: for Mg^{2+} 1:211, 1:172, 1:106, for Cu^{2+} 1:14, 1:11, 1:7, and for Zn^{2+} 1:70, 1:35. A consequence of this procedure is that the ionic strength was sometimes slightly greater than 0.1 M; the most extreme case occurred in an experiment with Mg^{2+}, where I reached a value of 0.16 M.

For the [M{Pt(dGuo)(dGMP)}]$^{2+}$ complexes the stability constant $K^{M}_{[M\{Pt(dGuo)(dGMP)\}]}$ [Eq. (9)] was computed by taking into account the species H^+, [H{Pt(dGuo)(dGMP)}]$^+$, [Pt(dGuo)(dGMP)], M^{2+}, and [M{Pt(dGuo)(dGMP)}]$^{2+}$.[60] The experimental data were evaluated every 0.1 pH unit from about 3% complex formation to a neutralization degree of about 90% (Mg^{2+}) or to the beginning of the hydrolysis of M(aq)$^{2+}$ (Cu^{2+}, Zn^{2+}). The values calculated individually under the given restrictions for $\log K^{M}_{[M\{Pt(dGuo)(dGMP)\}]}$ showed no dependence on pH (Mg^{2+}, pH range about 4.9–6.1; Cu^{2+}, 4.3–5.1; Zn^{2+}, 4.4–5.6) or on the excess amount of M^{2+}. The results given in Section 2.3 for the Mg^{2+}, Cu^{2+}, and Zn^{2+} complexes are always the averages of 3 independent pairs of titrations.

Acknowledgments: The competent technical assistance of Rita Baumbusch in the preparation of this manuscript is gratefully acknowledged. This study was supported by the Swiss National Science Foundation (H.S.), the Deutsche Forschungsgemeinschaft (B.L.), and the Fonds der Chemischen Industrie (B.L.). This research is also part of the COST D8 programme, and in this context received support from the Swiss Federal Office for Education and Science (H.S.).

Received: December 16, 1997 [F938]

[1] For a recent update on the clinical status, see: *Platinum and Other Metal Coordination Compounds in Cancer Chemotherapy* (Eds.: H. M. Pinedo, J. M. Schornagel), Plenum, New York, **1996**, pp. 1–357.

[2] Recent research on this and related topics is summarized in: a) *Interactions of Metal Ions with Nucleotides, Nucleic Acids, and Their Constituents* (Eds.: A. Sigel, H. Sigel), in *Met. Ions Biol. Syst., Vol 32*, M. Dekker, New York, Basel, Hong Kong, **1996**, pp. 1–814; b) *Probing of Nucleic Acids by Metal Ion Complexes of Small Molecules* (Eds.: A. Sigel, H. Sigel), in *ibid., Vol. 33*, **1996**, pp. 1–678.

[3] B. Rosenberg, L. Van Camp, E. B. Grimley, A. J. Thomson, *J. Biol. Chem.* **1967**, *242*, 1347–1352.

[4] N. Farrell, *Met. Ions Biol. Syst*. **1996**, *32*, 603–639; see ref. [2a].

[5] J. P. Whitehead, S. J. Lippard, *Met. Ions Biol. Syst*. **1996**, 32, 687–726; see ref. [2a].

[6] a) P. M. Takahara, A. C. Rosenzweig, C. A. Frederick, S. J. Lippard, *Nature* **1995**, *377*, 649–652; b) P. M. Takahara, C. A. Frederick, S. J. Lippard, *J. Am. Chem. Soc.* **1996**, *118*, 12309–12321.

[7] a) J. Reedijk, *Chem. Commun.* **1996**, 801–806; b) M. J. Bloemink, J. Reedijk, *Met. Ions Biol. Syst.* **1996**, *32*, 641–685; see ref. [2a].

[8] B. Lippert, *Met. Ions Biol. Syst.* **1996**, *33*, 105–141; see ref. [2b].

[9] a) S. J. Lippard, J. M. Berg, *Principles of Bioinorganic Chemistry*, University Science Books, Mill Valley, CA, **1994**; b) J. J. R. Fraústo da Silva, R. J. P. Williams, *The Biological Chemistry of the Elements*, Clarendon, Oxford, **1991**.

[10] a) C. Klevickis, C. M. Grisham, *Met. Ions Biol. Syst.* **1996**, *32*, 1–26; see ref. [2a]; b) K. Aoki, *ibid.* **1996**, *32*, 91–134; see ref. [2a]; c) E. Sletten, N. Å. Frøystein, *ibid.* **1996**, *32*, 397–418; see ref. [2a]; d) V. G. Bregadze, *ibid.* **1996**, *32*, 419–451; see ref. [2a]; e) A. M. Pyle, *ibid.* **1996**, *32*, 479–520; see ref. [2a]; f) M. Sabat, B. Lippert, *ibid.* **1996**, *33*, 143–176; see ref. [2b].

[11] T. Steitz, S. J. Smerdon, J. Jäger, C. M. Joyce, *Science* **1994**, *266*, 2022–2025.

[12] J. E. Coleman, D. P. Giedroc, *Met. Ions Biol. Syst.* **1989**, *25*, 171–234.

[13] A. S. Mildvan, *Magnesium* **1987**, *6*, 28–33.

[14] K. M. Downey, A. G. So, *Met. Ions Biol. Syst*. **1989**, *25*, 1–30.

[15] a) H. Sigel, *Chem. Soc. Rev.* **1993**, *22*, 255–267; b) H. Sigel, *ACS Symp. Ser.* **1989**, 402, 159–204.

[16] H. Sigel, B. Song, *Met. Ions Biol. Syst.* **1996**, *32*, 135–205; see ref. [2a].

[17] B. Song, G. Oswald, M. Bastian, H. Sigel, B. Lippert, *Metal-Based Drugs* **1996**, *3*, 131–141.

[18] H. Sigel, B. Lippert, *Pure Appl. Chem.* **1998**, in press.

[19] B. Song, G. Feldmann, M. Bastian, B. Lippert, H. Sigel, *Inorg. Chim. Acta* **1995**, *235*, 99–109.

[20] B. Song, H. Sigel, *Inorg. Chem.* **1998**, *37*, 2066–2069.

[21] R. B. Martin, Y. H. Mariam, *Met. Ions Biol. Syst.* **1979**, *8*, 57–124.

[22] H. Sigel, S. S. Massoud, N. A. Corfù, *J. Am. Chem. Soc.*, **1994**, *116*, 2958–2971.

[23] H. Reinert, R. Weiss, *Hoppe–Seyler's Z. Physiol. Chem.* **1969**, *350*, 1321–1326.

[24] *IUPAC Stability Constants Database*, *Release 2, Version 2.60* (compiled by L. D. Pettit, H. K. J. Powell), Academic Software, Timble, Otley (W. Yorks, U.K.), **1994**.

[25] H. Sigel, A. D. Zuberbühler, O. Yamauchi, *Anal. Chim. Acta* **1991**, *255*, 63–72.

[26] M. Bastian, H. Sigel, *J. Coord. Chem.* **1991**, *23*, 137–154.

[27] R. B. Martin, *Met. Ions Biol. Syst.* **1979**, *9*, 1–39.

[28] B. Song, R. K. O. Sigel, H. Sigel, *Chem. Eur. J.* **1997**, *3*, 29–33.

[29] A value for $pk^{Pt(dGuo-H)(dGMP-H)}_{Pt(dGuo-H)(dGMP)}$ may be estimated from the micro acidity constant $pk^{cis\text{-}(NH_3)_2Pt(dGMP-H)_2}_{cis\text{-}(NH_3)_2Pt(dGMP-H)(dGMP)} = 9.18 \pm 0.04$ determined earlier.[17] However, in doing so one has to take into account that the species [*cis*-$(NH_3)_2$Pt(dGuo – H)(dGMP)]$^-$ and [*cis*-$(NH_3)_2$Pt-(dGMP – H)(dGMP)]$^{3-}$ differ by two charge units due to the additional presence of a -PO_3^{2-} group in the latter species. This charge effect can be estimated by calculating the average acidification effect of [*cis*-$(NH_3)_2$Pt]$^{2+}$ in the two mentioned complexes ompared with the situation in the free ligands: a) for [*cis*-$(NH_3)_2$Pt(dGMP)$_2$]$^{2-}$, $\Delta pK_{a/i} = K^{H}_{dGMP} - {}^1/_2(pK^{H}_{[cis\text{-}(NH_3)_2Pt(dGMP)_2]} + pK^{H}_{[cis\text{-}(NH_3)_2Pt(dGMP-H)(dGMP)]} = (9.56 \pm 0.02) - {}^1/_2[(8.73 \pm 0.04) + (9.48 \pm 0.04)] = 0.455 \pm 0.06$; b) for [*cis*-$(NH_3)_2$Pt(dGuo)(dGMP)], $\Delta pK_{a/ii} = {}^1/_2(pK^{H}_{dGuo} + pK^{H}_{dGMP}) - {}^1/_2(pK^{H}_{[cis\text{-}(NH_3)_2Pt(dGuo)(dGMP)]} + K^{H}_{[cis\text{-}(NH_3)_2Pt(dGuo)(dGMP)-H]}) = {}^1/_2[(9.24 \pm 0.03) + (9.56 \pm 0.02)] - {}^1/_2[(8.20 \pm 0.03) + (9.05 \pm 0.10)] = 0.775 \pm 0.11$. Hence, the additional -PO_3^{2-} group has a retarding effect of $\Delta\Delta pK_a = \Delta pK_{a/ii} - \Delta pK_{a/i} = 0.32 \pm 0.125$, a result which corresponds well with the observations made in Section 2.1, and this value now needs to be deducted from the micro acidity constant given above to yield $pk^{Pt(dGuo-H)(dGMP-H)}_{Pt(dGuo-H)(dGMP)} = pk^{cis\text{-}(NH_3)_2Pt(dGMP-H)_2}_{cis\text{-}(NH_3)_2Pt(dGMP-H)(dGMP)} - \Delta\Delta pK_a = (9.18 \pm 0.04) - (0.32 \pm 0.125) = 8.86 \pm 0.13$.

[30] a) H. Sigel, S. S. Massoud, R. Tribolet, *J. Am. Chem. Soc.* **1988**, *110*, 6857–6865; b) M. D. Reily, T. W. Hambley, L. G. Marzilli, *J. Am. Chem. Soc.* **1988**, *110*, 2999–3007.

[31] S. S. Massoud, H. Sigel, *Inorg. Chem.* **1988**, *27*, 1447–1453.

[32] H. Sigel, D. Chen, N. A. Corfù, F. Gregáň, A. Holý, M. Strašák, *Helv. Chim. Acta* **1992**, *75*, 2634–2656.

[33] A. Saha, N. Saha, L.-n. Ji, J. Zhao, F. Gregáň, S. A. A. Sajadi, B. Song, H. Sigel, *J. Biol. Inorg. Chem.* **1996**, *1*, 231–238.

[34] G. Schröder, B. Lippert, M. Sabat, C. J. L. Lock, R. Faggiani, B. Song, H. Sigel, *J. Chem. Soc. Dalton Trans.* **1995**, 3767–3775 and references therein.

[35] D. Lemaire, M.-H. Fouchet, J. Kozelka, *J. Inorg. Biochem.* **1994**, *53*, 261 – 271.
[36] J. C. Chottard, J. P. Girault, G. Chottard, J. Y. Lallemand, D. Mansuy, *J. Am. Chem. Soc.* **1980**, *102*, 5565 – 5572.
[37] J.-P. Girault, G. Chottard, J.-Y. Lallemand, J.-C. Chottard, *Biochemistry* **1982**, *21*, 1352 – 1356.
[38] M. J. Bloemink, R. J. Heetebrij, K. Inagaki, Y. Kidani, J. Reedijk, *Inorg. Chem.* **1992**, *31*, 4656 – 4661 and references therein.
[39] J. P. Caradonna, S. J. Lippard, *Inorg. Chem.* **1988**, *27*, 1454 – 1466 and references therein.
[40] a) M. J. Clarke, H. Taube, *J. Am. Chem. Soc.* **1974**, *96*, 5413 – 5419; b) M. E. Kastner, K. F. Coffey, M. J. Clarke, S. E. Edmonds, K. Eriks, *ibid.* **1981**, *103*, 5747 – 5752.
[41] H. Sigel, *J. Am. Chem. Soc.* **1975**, *97*, 3209 – 3214.
[42] For a detailed discussion of this topic and in particular of that of AG vs. GG adducts, see: a) D. Burnouf, M. Daune, R. P. P. Fuchs, *Proc. Natl. Acad. Sci. U.S.A.* **1987**, *84*, 3758 – 3762; b) D. Burnouf, C. Gauthier, J.-C. Chottard, R. P. P. Fuchs, *ibid.* **1990**, *87*, 6087 – 6091; c) L. J. N. Bradley, K. J. Yarema, S. J. Lippard, J. M. Essigman, *Biochemistry* **1993**, *32*, 982 – 988; d) M.-H. Fouchet, E. Guittet, J. A. H. Cognet, J. Kozelka, C. Gauthier, M. Le Bret, K. Zimmermann, J.-C. Chottard, *J. Biol. Inorg. Chem.* **1997**, *2*, 83 – 92.
[43] L. Lecointe, J. P. Macquet, J. L. Butour, C. Paoletti, *Mutat. Res.* **1977**, *48*, 139 – 144.
[44] N. P. Johnson, J. P. Macquet, J. L. Wiebers, B. Monsarrat, *Nucl. Acids Res.* **1982**, *10*, 5255 – 5271.
[45] J. W. Drake, R. H. Baltz, *Annu. Rev. Biochem.* **1976**, *45*, 11 – 37.
[46] P. D. Lawley, P. Brookes, *J. Mol. Biol.* **1962**, *4*, 216 – 219.
[47] B. Lippert, *J. Am. Chem. Soc.* **1981**, *103*, 5691 – 5697.
[48] a) H. Yu, R. Eritja, L. B. Bloom, M. F. Goodman, *J. Biol. Chem.* **1993**, *268*, 15935 – 15943; b) M. F. Goodman, *Nature* **1995**, *378*, 237 – 238; c) L. C. Sowers, M. F. Goodman, R. Eritja, B. Kaplan, G. V. Fazakerley, *J. Mol. Biol.* **1989**, *205*, 437 – 447.
[49] A. Warshel, J. Åqvist, S. Creighton, *Proc. Natl. Acad. Sci. U.S.A.* **1989**, *86*, 5820 – 5824.
[50] In vitro experiments on base-pairing fidelity suggest that metalated DNA templates can be processed; see, e.g.: a) E. T. Snow, L.-S. Xu, *Biochemistry* **1991**, *30*, 11238 – 11245; b) M. A. Sirover, L. A. Loeb, *Science* **1976**, *194*, 1434 – 1436.
[51] See Section 3.2 and footnote [53].
[52] J. Florián, J. Leszczyński, *J. Am. Chem. Soc.* **1996**, *118*, 3010 – 3017 and references therein.
[53] It needs to be emphasized that the total charge of -2 of the two guanine nucleotide units involved in a DNA intrastrand crosslink is compensated by the N7-coordination of $[cis\text{-}(NH_3)_2Pt]^{2+}$; in other words, $[cis\text{-}(NH_3)_2Pt(dGuo)(dGMP)]$ has exactly the same overall charge, but the negative fraction is located on a single $\text{-}PO_3^{2-}$ residue. However, if anything, this charge concentration has a more retarding effect on the release of the proton from the H(N1) site. If one considers further that it is generally assumed that approximately two-thirds of the negative charges of DNA are counterbalanced by metal cations, the estimate of $pK_a \approx 8.6$ is a conservative one. Application of this average value to a DNA intrastrand crosslink unit formed with $[cis\text{-}(NH_3)_2Pt]^{2+}$ and two guanine residues, gives the micro acidity constants $pk_{a/1} = 8.3$ and $pk_{a/2} = 8.9$. These two values represent the *statistical* separation of the two pk_a values, which in practice could be larger but not smaller (compare, for example, with the situation in Table 1). Hence, if our above calculation were based on $pk_a = 8.3$, we would conclude that 11% of H(N1) sites have already lost their proton. In other words, from $pK_{a/average} = 8.6$ the given estimate of 6% is again confirmed to be a conservative one. Certainly, one could argue that even in our conservative estimate things such as the orientation of the two guanine residues (probably head-to-tail in our complex, whereas in the DNA intrastrand crosslink their orientation is head-to-head, and there is also some strain because Pt^{2+} is not in the plane of the guanine residues[6]) have not yet been compensated for. However, accepting such (unconvincing) arguments and also taking into account a possibly reduced intrinsic polarity one might force the issue to the utmost reasonable point and apply a value of $pk_a = 8.9$ (which could also mean $pK_{a/average} = 9.2$; see above): even then one has to conclude that a 3% deprotonation of the H(N1) sites still results. This means that N7 coordination of cisplatin to guanine residues leads unavoidably to some deprotonated $(N1)^-$ sites. This conclusion is also in accord with $pK_a \approx 8$ given[39] for cisplatin-modified DNA single strands.
[54] It is the relative effect, i.e. $\log \Delta \approx -0.2$ (Table 2), which is addressed here and which needs to be subtracted from the absolute stability constants, which are of course different for a singly negatively charged phosphate bridge in DNA and the $\text{-}PO_3^{2-}$ residue considered in our complex.
[55] a) H. Sigel, *Coord. Chem. Rev.* **1990**, *100*, 453 – 539; b) H. Sigel, *Inorg. Chim. Acta* **1992**, *198 – 200*, 1 – 11; c) H. Sigel, *Pure Appl. Chem.* **1998**, in press.
[56] N. Sträter, W. N. Lipscomb, T. Klabunde, B. Krebs, *Angew. Chem.* **1996**, *108*, 2158 – 2190; *Angew. Chem. Int. Ed. Engl.* **1996**, *35*, 2024 – 2055.
[57] F. Y.-H. Wu, W.-J. Huang, R. B. Sinclair, L. Powers, *J. Biol. Chem.* **1992**, *267*, 25560 – 25567.
[58] D.-J. Fu, L. W. McLaughlin, *Biochemistry* **1992**, *31*, 10941 – 10949.
[59] H. M. Irving, M. G. Miles, L. D. Pettit, *Anal. Chim. Acta* **1967**, *38*, 475 – 488.
[60] R. Griesser, H. Sigel, *Inorg. Chem.* **1970**, *9*, 1238 – 1243.

© WILEY-VCH Verlag GmbH, D-69451 Weinheim, 1998
0947-6539/98/0406-1060 $ 17.50+.50/0 *Chem. Eur. J.* **1998**, *4*, No. 6