Inorg. Chem. **2003**, *42*, 3475–3482

Acid–Base and Metal Ion Binding Properties of Guanylyl(3′→5′)guanosine (GpG^-) and 2′-Deoxyguanylyl(3′→5′)-2′-deoxyguanosine [$d(GpG)^-$] in Aqueous Solution§

Carla P. Da Costa and Helmut Sigel*

Department of Chemistry, Inorganic Chemistry, University of Basel, Spitalstrasse 51, CH-4056 Basel, Switzerland

Received November 14, 2002

The acidity constants of guanylyl(3′→5′)guanosine (GpG^-) and 2′-deoxyguanylyl(3′→5′)-2′-deoxyguanosine [$d(GpG)^-$] for the deprotonation of their (N1)H sites were measured by potentiometric pH titrations in aqueous solution (25 °C; I = 0.1 M, $NaNO_3$). The same method was used for the determination of the stability constants of the 1:1 complexes formed between Mg^{2+}, Ni^{2+}, or Cd^{2+} (= M^{2+}) and $(GG–H)^{2-}$, and in the case of Mg^{2+} also of $(GG–2H)^{3-}$, where GG^- = GpG^- or $d(GpG)^-$. The stability constants of the $M(GG)^+$ complexes were estimated. The acidity constants of the $H(dGuo)^+$ and dGuo species (dGuo = 2′-deoxyguanosine) and the stability constants of the corresponding $M(dGuo)^{2+}$ and $M(dGuo–H)^+$ complexes were also measured. Comparison of these and related data allows the conclusion that N7 of the 5′G unit in GG^- is somewhat more basic than the one in the 3′G moiety; the same holds for the $(N1)^-$ sites. On the basis of comparisons with the stability constants measured for the dGuo complexes, it is concluded that M^{2+} binding of the GG dinucleoside monophosphates occurs predominantly in a mono-site fashion, meaning that macrochelate formation is not very pronounced. Indeed, it was a surprise to find that the stabilities of the complexes of dGuo or $(dGuo–H)^-$ and the corresponding ones derived from GG^- are so similar. Consequently, it is suggested that in the $M(GG)^+$ and M(GG–H) complexes the metal ion is mainly located at N7 of the 5′G unit since this is the more basic site allowing also an outer-sphere interaction with the C6 carbonyl oxygen and because this coordination mode is also favorable for an electrostatic interaction with the negatively charged phosphodiester bridge. It is further suggested that Mg^{2+} binding (which is rather weak compared to that of Ni^{2+} and Cd^{2+}) occurs mainly in an outer-sphere mode, and on the basis of the so-called *Stability Ruler* it is concluded that the binding properties of Zn^{2+} to the GG species are similar to those of Ni^{2+} and Cd^{2+}.

1. Introduction

Divalent metal ions are inextricably involved in defining the structure and function of DNA and RNA molecules.[1−3] In nucleoside monophosphates the terminal, 2-fold negatively charged phosphate group is the primary binding site for the metal ions important in life processes.[4,5] However, along a nucleic acid chain, the singly negatively charged phosphodiester linkage is for many metal ions no longer the dominating binding site, partly because only one negative charge is present instead of the two in nucleoside monophosphates and also because the phosphodiester bridge is much less basic than a monophosphate−monoester residue. Of course, practically any metal ion may interact electrostatically with the phosphodiester backbone by charge neutraliza-

* To whom correspondence should be addressed. Fax: ++41-61-267 1017. E-mail: Helmut.Sigel@unibas.ch.

§ Further abbreviations (see also Figure 1): $5′AMP^{2-}$, adenosine 5′-monophosphate; $3′dGMP^{2-}$, 2′-deoxyguanosine 3′-monophosphate; $5′dGMP^{2-}$, 2′-deoxyguanosine 5′-monophosphate; dGuo, 2′-deoxyguanosine; GG, GpG and/or d(GpG); $3′GMP^{2-}$, guanosine 3′-monophosphate; $5′GMP^{2-}$, guanosine 5′-monophosphate; Guo, guanosine; I, ionic strength; K_a, general acidity constant; M^{2+}, general divalent metal ion. Species which are given in the text without a charge either do not carry one or represent the species in general (i.e., independent of their protonation degree); which of the two versions applies is always clear from the context. Expressions like $(GG−H)^{2-}$ should be read as "GG minus H" meaning that the dinucleoside monophosphate GG^- has lost a proton from one of its (N1)H sites.

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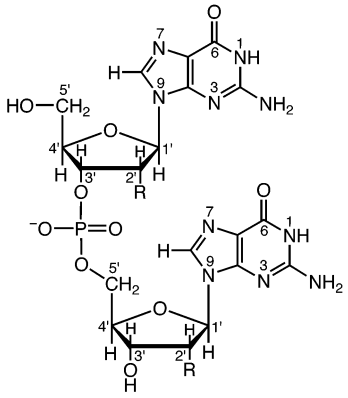
10.1021/ic020672l CCC: $25.00
Published on Web 05/07/2003



d(GpG)⁻: R = H
GpG⁻: R = OH

Figure 1. Chemical structures of the two dinucleoside monophosphates considered in this study: guanylyl(3′→5′)guanosine (GpG^-) and 2′-deoxyguanylyl(3′→5′)-2′-deoxyguanosine [$d(GpG)^-$].

tion,[2] yet, it is mostly the binding to nucleobases that confers selectivity and specificity to metal ion coordination in oligonucleotides and nucleic acids.[1,6,7]

In double-stranded nucleic acids with Watson−Crick base-pairing some of the potential binding sites of nucleobases able to interact with metal ions are occupied in hydrogen bonding to the complementary strand.[8] This is the case for N3 and O2 of the cytosine residue. The carbonyl oxygens of uracil or thymine do not have a high affinity for metal ions, and O4 of these nucleobases is also involved in base-pairing interactions as are the N1/(N1)H groups of the adenine and guanine moieties. However, the N7 sites of guanine and adenine residues are still free, and they constitute two of the preferred metal ion binding sites in the major groove of DNA.[1,9] A higher basicity of the guanine N7 as compared to the adenine N7[10,11] and a favorable electrostatic potential[12,13] favor guanine N7 somewhat over adenine N7 for metal ion coordination.[5,14,15] This was the main reason why we decided to begin our studies of dinucleotides or more precisely dinucleoside monophosphates and their interactions with divalent metal ions with guanylyl(3′→5′)guanosine (GpG^-) and 2′-deoxyguanylyl(3′→5′)-2′-deoxyguanosine [$d(GpG)^-$] [GG^- = GpG^- and/or $d(GpG)^-$] (see Figure 1).

In fact, GG dinucleoside monophosphates have already been in the center of studies, especially in those focusing on the interaction with *cis*-$(NH_3)_2PtCl_2$ (e.g., refs 16−19). This compound, commonly known as cisplatin, is a successful antitumor drug routinely used in the clinic against several kinds of cancer.[20,21] The antitumor action of cisplatin is attributed to its binding to DNA,[17] which occurs preferentially to the N7 sites of two consecutive guanines[17] causing a distortion of the double helix[22] that subsequently leads to apoptosis.[21]

Studies have dealt with the structural characteristics of *cis*-$(NH_3)_2Pt^{2+}$ bound to GG dinucleoside monophosphates or dinucleotides (e.g., refs 19,23) but little is known[24−26] about the properties of the dinucleoside monophosphates alone, i.e., their acid−base properties or their binding affinities toward labile metal ions. Early works[27−30] had concentrated on the release of protons from the protonated nucleobases in the acidic pH range[27] and the intramolecular stacking equilibria, stacking being more pronounced in the monoprotonated $H(GpG)^{\pm}$ species (in which one of the two guanine residues carries a proton at N7) than in the GpG^- form which exists in the neutral pH range.[29] There is also a study[31] dealing with the effect of divalent metal ions on the conformation of GpG^- and related dinucleoside monophosphates. However, no quantitative data have been provided on the stability of any of these complexes.

In the present study, which extends our experience with guanine derivatives,[32−36] the deprotonation properties of the (N1)H sites in GpG^- and $d(GpG)^-$ are compared with those of guanosine and 2′-deoxyguanosine, and the metal ion binding characteristics of these ligands are quantified for Mg^{2+}, Ni^{2+}, and Cd^{2+}.

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2. Experimental Section

2.1. Materials. Four different lots of the sodium salt of 2′-deoxyguanylyl(3′→5′)-2′-deoxyguanosine [d(GpG)⁻] and two different lots of the triethylammonium salt of guanylyl(3′→5′)-guanosine (GpG⁻) were purchased from Sigma Chemical Co., St. Louis, MO. A third lot of GpG⁻, this time the sodium salt, was obtained from Jena BioScience GmbH, Jena, Germany. During the studies no differences between the various lots were detected. 2′-Deoxyguanosine was from Fluka Chemie AG, Buchs, Switzerland. HNO_3, NaOH (Titrisol), the nitrate salts of Na^+, Mg^{2+}, Ni^{2+}, and Cd^{2+}, the disodium salt of EDTA, and potassium hydrogen phthalate (all pro analysis) were obtained from Merck KGaA, Darmstadt, Germany.

All solutions were prepared with deionized, ultrapure (MILLI-Q 185 PLUS; from Millipore S.A., 67120 Molsheim, France) and CO_2-free water. The aqueous stock solutions of the ligands were freshly prepared daily, and their exact concentration was newly determined each time by titrations with NaOH. The titer of the NaOH used for the titrations was established with potassium hydrogen phthalate, and the concentrations of the M^{2+} stock solutions were determined via their EDTA complexes by measuring the proton equivalents liberated from $H(EDTA)^{3-}$ upon complex formation.

The triethylammonium ion present in two of the GpG⁻ lots must be considered an impurity since it also has acid−base properties. However, it was possible to evaluate the potentiometric pH titrations of GpG by taking into account in the curve-fitting procedure triethylamine and its triethylammonium ion by keeping fixed in the calculations the known[25] acidity constant, $pK_a = 10.76$ ($I = 0.1$ M), of the latter. Indeed, an excellent fit of the experimental data resulted, and the acidity constants obtained for GpG⁻ with the triethylammonium and the sodium salts were identical within the error limits.

2.2. Potentiometric pH Titrations. The potentiometric pH titrations were carried out with a Metrohm E536 potentiograph equipped with a Metrohm E655 or E665 dosimat and a 6.0222.100 combined double-junction macro glass electrode from Metrohm AG, Herisau, Switzerland.

The buffer solutions (pH 4.00, 7.00, and 9.00 based on the scale of the U.S. National Institute of Standards and Technology (NIST)) used for the pH calibrations were also from Metrohm AG. An additional buffer solution with a pH of 9.98 (25 °C; based on the NIST scale), purchased from Merck KGaA, Darmstadt, Germany, was also used. The direct pH meter readings were applied in the calculations of the acidity constants,[37,38] i.e., these are so-called practical, mixed or Brønsted constants.[38] Their negative logarithms given for aqueous solution 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.[38] This conversion term contains both the junction potential of the glass electrode and the hydrogen ion activity.[38,39]

It should be emphasized that the ionic product of water (K_w) and the mentioned conversion term do not enter into the calculation procedures because the differences in NaOH consumption between solutions with and without ligand are evaluated (see also below; for further details refs 37 and 38 may be consulted). No conversion term is necessary for the stability constants of the metal ion complexes; these are as usual concentration constants. The results showed no dependence on the excess amount of M^{2+} or the ligand concentration used in the experiments, which means that the stability of the 1:1 complexes was measured (see below).

2.3. Determination of Equilibrium Constants Involving d(GpG). For the determination of the acidity constants $K^{H}_{d(GpG)}$ and $K^{H}_{d(GpG-H)}$ (eqs 5 and 6) of d(GpG)⁻, 25 mL of aqueous 1.6×10^{-3} mM HNO_3 (25 °C; $I = 0.1$ M, $NaNO_3$) was titrated under N_2 in the presence and absence of 0.14 or 0.17 mM d(GpG) with up to 3 mL of 0.01 M NaOH. Two further experiments were performed with a ligand concentration of 0.07 mM, and in this case 3.5 mL of 4 mM NaOH was used. The data were evaluated with a curve-fitting procedure using a Newton−Gauss nonlinear least-squares program by employing every 0.1 pH unit the difference in NaOH consumption between the two mentioned titrations, i.e., with and without ligand. The two acidity constants were calculated within the pH range 8.0−10.7, corresponding to about 4% (initial) neutralization for the equilibrium $d(GpG)^-/d(GpG-H)^{2-}$ and about 66% (final) for $d(GpG-H)^{2-}/d(GpG-2H)^{3-}$. The final results are the averages of 10 independent pairs of titrations for $K^{H}_{d(GpG)}$ and nine for $K^{H}_{d(GpG-H)}$.

At the end of each titration a small volume (ca. 1 mL) of 0.03 M HNO_3 was added in order to bring the solution to its initial pH (ca. 5), and then a similar small volume of $M(NO_3)_2$ was added; thereafter the titration was repeated. These titrations in the presence of M^{2+} (with and without ligand) were evaluated for the determination of the stability constants $K^{M}_{M[d(GpG-H)]}$ for Mg^{2+}, Ni^{2+}, and Cd^{2+} (eq 13), and $K^{M}_{M[d(GpG-2H)]}$ for Mg^{2+} (eq 14). The total volume of the titration solutions was ca. 30 mL with $I \simeq 0.09$ M for Ni^{2+} and Cd^{2+}, and 0.12 M for Mg^{2+}. The ligand concentration varied between 0.11 and 0.14 mM, except in one case in the presence of Cd^{2+} where it was 0.057 mM. The ligand-to-metal ratios were 1:173, 1:87.7, and 1:84 for Mg^{2+}; 1:12.9, 1:12.4, and 1:11.9 for Ni^{2+}; and 1:24.7, 1:23.4, and 1:12.9 for Cd^{2+}.

The calculations were done in two different ways; i.e., (i) by ignoring and (ii) by taking into account the existence of a $M[d(GpG)]^+$ complex. For the first case the data were evaluated every 0.1 pH unit in the accessible pH range, the upper limit being determined by the hydrolysis of $M(aq)^{2+}$, by considering the species H^+, $d(GpG)^-$, $d(GpG-H)^{2-}$, $d(GpG-2H)^{3-}$, and M[d(GpG−H)], plus $M[d(GpG-2H)]^-$ for the Mg^{2+} system. In the second case, in addition the species $H_2[d(GpG)]^+$, $H[d(GpG)]^{\pm}$, and $M[d(GpG)]^+$ were taken into account. Neither the acidity constants $K^{H}_{H_2[d(GpG)]}$ and $K^{H}_{H[d(GpG)]}$ nor the stability constant $K^{M}_{M[d(GpG)]}$ was determined now due to the scarcity of the ligand, but they were either taken from the literature[27,28] or estimated (see also section 3.4); these values were then kept constant in the calculations for $K^{M}_{M[d(GpG-H)]}$ and $K^{M}_{M[d(GpG-2H)]}$. It should be emphasized that the fitting procedure of the experimental data was equally satisfactory in both instances.

Three independent titration pairs were evaluated for each metal ion system. Representative examples for the employed pH ranges are 7.0−10.0, 6.4−7.7, and 6.5−7.8 for the Mg^{2+}, Ni^{2+}, and Cd^{2+} systems, respectively, corresponding to variations in the formation degrees of about 3.2−0.3% for $Mg[d(GpG)]^+$ (if considered), 0.1−14% for Mg[dGpG−H)], 0−20% for $Mg[d(GpG-2H)]^-$; 10.4−8.5% for $Ni[d(GpG)]^+$, 1−17% for Ni[d(GpG−H)]; and 15.2−11.9% for $Cd[d(GpG)]^+$ and 1.3−20.7% for Cd[d(GpG−H)]. The formation degrees of M[d(GpG−H)] and $Mg[d(GpG-2H)]^-$ remained practically unchanged whether or not $M[d(GpG)]^+$ was taken into account (see Table 3).

2.4. Determination of Equilibrium Constants Involving GpG. With the two lots of the triethylammonium salt of GpG⁻, the acidity

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constants K^{H}_{GpG} and $K^{H}_{(GpG-H)}$ (eqs 5 and 6) of GpG^- were determined by titrating 25 mL of 1.6×10^{-3} mM HNO_3 (25 °C; $I = 0.1$ M, $NaNO_3$) in the presence and absence of 0.24 mM GpG with about 4 mL of 9.7 mM NaOH or 0.25 mM GpG with up to 3 mL of 12.4 mM NaOH. The GpG stock solutions of the commercial product were relatively acidic and therefore adjusted to pH about 7.2. The experimental data sets were evaluated with the curve-fitting procedure mentioned in section 2.3 for the determination of the acidity constants of $d(GpG)^-$, but the acidity constant of the triethylammonium ion, $pK_a = 10.76$ ($I = 0.1$ M),[25] was additionally taken into account by keeping its value fixed.

Two further titration pairs were recorded in the corresponding way with the sodium salt of GpG^-. This time the GpG concentration was 0.048 mM and 3 mL of 9 mM NaOH was used for the titrations. The acidity constants were calculated in the way described in section 2.3.

The final results for the acidity constants K^{H}_{GpG} and $K^{H}_{(GpG-H)}$ are the averages of 5 independent titration pairs evaluated within the pH range 7.6−10.5 corresponding to a neutralization degree of about 2% (initial) for the equilibrium $GpG^-/(GpG-H)^{2-}$ and about 55% (final) for the equilibrium $(GpG-H)^{2-}/(GpG-2H)^{3-}$.

At the end of the titrations of the sodium salt of GpG^-, the solution was reacidified to a pH of about 5 with a small amount of 0.03 M HNO_3 and then an equally small amount of $Mg(NO_3)_2$ or $Cd(NO_3)_2$ was added. By repeating the titrations with and without ligand, the stability constants $K^{Mg}_{Mg(GpG-H)}$, $K^{Mg}_{Mg(GpG-2H)}$, and $K^{Cd}_{Cd(GpG-H)}$ were determined. The total volume under titration was close to 30 mL with concentrations of 8.54 mM for Mg^{2+} and 1.18 mM for Cd^{2+}, and a GpG concentration of about 0.040 mM. The ionic strength was about 0.11 M ($NaNO_3$). The experimental data were evaluated in the same way as described in section 2.3 for the systems with d(GpG); i.e., by (i) ignoring and (ii) considering the formation of a $M(GpG)^+$ species. The curve-fitting procedure was done in the pH range 7.7−9.9 for Mg^{2+} and 7.0−7.8 for Cd^{2+}; this corresponds to variations in the complex formation degrees of about 2.3−0.3%, 0.6−13.6%, and 0−12.9% for $Mg(GpG)^+$, Mg(GpG−H), and $Mg(GpG-2H)^-$, respectively; and 6.1−5.4% and 2.0−11.2% for $Cd(GpG)^+$ and Cd(GpG−H), respectively. The formation degrees for the N1-deprotonated species remained practically unchanged whether or not $M(GpG)^+$ was taken into account. The estimated stability constants, $K^{M}_{M(GpG)}$, were the same as used for the $M[d(GpG)]^+$ systems (see Table 3). The results calculated for the stability constants of the M(GpG−H) and Mg(GpG−2H)$^-$ complexes should be considered as estimates since, due to the lack of compound, only one independent titration pair could be performed for each metal ion system.

2.5. Determination of Equilibrium Constants Involving dGuo. For the determination of the acidity constants $K^{H}_{H(dGuo)}$ and K^{H}_{dGuo} (eqs 1 and 2) of $H(dGuo)^+$, 25 mL of aqueous 5 mM HNO_3 (25 °C; $I = 0.1$ M, $NaNO_3$) were titrated in the presence and absence of 0.93 mM dGuo under N_2 with 3 mL of 0.06 M NaOH. The acidity constants were evaluated within the pH range 2.5−10.7 corresponding to a neutralization degree for the two equilibria of about 59−100% for $H(dGuo)^+$/dGuo and 0−97% for dGuo/$(dGuo-H)^-$. The final results are the average of 8 independent pairs of titrations; they are identical within the error limits with those previously published.[33,34]

The stability constants $K^{M}_{M(dGuo)}$ and $K^{M}_{M(dGuo-H)}$ (eqs 7 and 8) of the complexes formed with Mg^{2+} and Cd^{2+} were determined under the conditions described above for the acidity constants except that $NaNO_3$ was partly or fully replaced by $M(NO_3)_2$. The dGuo-to-metal ratios were 1:35.8 with Mg^{2+} and 1:17.9 or 1:14.3 with Cd^{2+}. For the dGuo/Mg^{2+} systems the data were evaluated from a pH of about 3.1−9.9 with complex formation degrees of around 6.0 via 7 to 1.1% for $Mg(dGuo)^{2+}$ and 0−19% for $Mg(dGuo-H)^+$. The buffer depression for the $Mg(dGuo)^{2+}$ complex was very small, i.e., $\Delta pK_a = 0.03$ only. For this reason the errors in the various calculations for $K^{Mg}_{Mg(dGuo)}$ were very large and the final (averaged) value should be regarded as an estimation. However, once this average for $K^{Mg}_{Mg(dGuo)}$ was obtained, the experimental data were reevaluated by keeping this value constant and then $K^{Mg}_{Mg(dGuo-H)}$ was calculated. The agreement for the individual results of $K^{Mg}_{Mg(dGuo-H)}$ was excellent; however, the large error of $K^{M}_{M(dGuo)}$ was also considered in the error limit given for $K^{Mg}_{Mg(dGuo-H)}$. For the latter complex the buffer depression was quite significant, with $\Delta pK_a = 0.11$. The results for each of the two constants are the averages of 8 independent titration pairs.

The experimental data sets of the dGuo/Cd^{2+} system were evaluated within the pH range of about 2.8−7.9 corresponding to a complex formation degree of about 29 via 36 to 21% for $Cd(dGuo)^{2+}$ and about 0−39% for $Cd(dGuo-H)^+$. In the titrations with Cd^{2+}, the buffer depressions were about 0.19 and 1.39 pK_a units for $K^{Cd}_{Cd(dGuo)}$ and $K^{Cd}_{Cd(dGuo-H)}$, respectively; hence, no difficulty in the evaluation of the constants occurred.

The experimental data for the Mg^{2+} and Cd^{2+} systems were evaluated with the curve-fitting procedure by considering every 0.1 pH unit the concentration of H^+, $H(dGuo)^+$, dGuo, $(dGuo-H)^-$, $M(dGuo)^{2+}$, and $M(dGuo-H)^+$.

3. Results and Discussion

The dinucleoside monophosphates considered in this study are known[40−42] to undergo aggregate formation via self-association by nucleobase stacking and guanine−guanine hydrogen bonding.[41] However, with the concentrations used in this work for GpG and d(GpG), i.e., below 2.5×10^{-4} M, no self-association is expected.[40] This also holds for the measurements with dGuo.[43−45] Hence, the following results refer in all instances to the monomeric species.

3.1. Acidity Constants of the Protonated Ligands. For reasons of comparison we also needed the acidity constants of guanosine (Guo) and 2′-deoxyguanosine (dGuo). The former ones had been measured previously in this laboratory,[14,46] but the ones for $H(dGuo)^+$ were determined now by potentiometric pH titrations. These nucleosides may be protonated at N7, and they may also release a proton from their (N1)H site.[10] Consequently, the following two deprotonation equilibria need to be considered (G = Guo and dGuo):

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

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

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

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

The dinucleoside monophosphates [GG^- = GpG^- or $d(GpG)^-$] can be protonated at the phosphodiester bridge,

(40) Savoie, R.; Klump, H.; Peticolas, W. L. *Biopolymers* **1978**, *17*, 1335−1345.
(41) Walmsley, J. A.; Schneider, M. L.; Farmer, P. J.; Cave, J. R.; Toth, C. R.; Wilson, R. M. *J. Biomol. Struct. Dyn.* **1992**, *10*, 619−638.
(42) Ghana, R.; Walss, C.; Walmsley, J. A. *J. Biomol. Struct. Dyn.* **1996**, *14*, 101−110.

but only in strongly acidic solution because the pK_a value of such a site is expected[47,48] to be below 1, and therefore this reaction is not considered further in the present study. However, a N7 site is clearly more basic,[10] and therefore a 2-fold protonation at these sites is expected to occur and to give rise to $H_2(GG)^+$ species. The release of these protons is quantified by equilibria 3 and 4:

$$H_2(GG)^+ \rightleftharpoons H(GG)^{\pm} + H^+ \quad (3a)$$

$$K^H_{H_2(GG)} = [H(GG)^{\pm}][H^+]/[H_2(GG)^+] \quad (3b)$$

$$H(GG)^{\pm} \rightleftharpoons GG^- + H^+ \quad (4a)$$

$$K^H_{H(GG)} = [GG^-][H^+]/[H(GG)^{\pm}] \quad (4b)$$

A further release of protons is possible from the (N1)H sites of the GG^- species (see Figure 1) as described by equilibria 5 and 6:

$$GG^- \rightleftharpoons (GG-H)^{2-} + H^+ \quad (5a)$$

$$K^H_{GG} = [(GG-H)^{2-}][H^+]/[GG^-] \quad (5b)$$

$$(GG-H)^{2-} \rightleftharpoons (GG-2H)^{3-} + H^+ \quad (6a)$$

$$K^H_{(GG-H)} = [(GG-2H)^{3-}][H^+]/[(GG-H)^{2-}] \quad (6b)$$

All these acidity constants were determined by potentiometric pH titrations and are listed in Table 1 together with the acidity constants of several related species.[49,50]

It should be emphasized that because of the scarcity of the GG compounds we could measure only the acidity constants due to equilibria 5 and 6. The values for $H_2(GpG)^+$ (eqs 3 and 4) were taken from the literature (Table 1, entry 1),[27,28] and those for $H_2[d(GpG)]^+$ are estimates (see Table 1, entry 2). Therefore, it is comforting to see that the acidity constants given in the same earlier work[28] for $H(Guo)^+$ and $H_2(3'GMP)^{\pm}$ agree within the error limits with those given in Table 1; only the value[28] for $H_2(5'GMP)^{\pm}$, i.e., $pK^H_{H_2(5'GMP)} = 2.34$, is somewhat lower than the one of entry 7 in Table 1. On the other hand, the pK_a values[28] for the release of a proton from the (N1)H sites in 3′GMP and 5′GMP are again in excellent agreement with those in Table 1.

However, it also needs to be emphasized that the pK_a values given in ref 28 for the deprotonation of the (N1)H sites of GpG^-, i.e., $pK^H_{GpG} = 9.16$ and $pK^H_{(GpG-H)} = 9.76$, are not correct. These values are based on spectrophotometric measurements which were analyzed[28] "for a single pK process (which) gave $pK_{app} = 9.46$". By assuming that in this process actually two protons were released and by applying the statistical separation of ±0.3 to the pK_{app} value, the above-mentioned two acidity constants were obtained.[28] These two acidity constants differ significantly from our results given in Table 1 in column 4 of entry 1. By comparing our pK^H_{GpG} value (9.34 ± 0.07) with the pK_{app} value (9.46 ± 0.04) of ref 28 one is led to conclude that in the spectrophotometric experiments[28] only the first of the two (N1)H sites in GpG^- was studied. Clearly, there is no doubt that the results given in Table 1 in column 4 of entry 1, and this also applies for the corresponding values of entry 2, regarding equilibria 5 and 6, are correct; this is also confirmed by the comparisons discussed below.

Table 1. Negative Logarithms of the Acidity Constants for the Deprotonation of the $(N7)H^+$ and (N1)H Sites in $H_2(GpG)^+$ and $H_2[d(GpG)]^+$, Together with Some Related Data, as Determined by Potentiometric pH Titrations[a] in Aqueous Solution (25 °C; I = 0.1 M, $NaNO_3$)[b]

		pK_a of the sites	
no.	acids	$(N7)H^+$	(N1)H
1	$H_2(GpG)^+$	1.49 ± 0.03/2.51 ± 0.03[c]	9.34 ± 0.07/10.38 ± 0.10[d]
2	$H_2[d(GpG)]^+$	1.69 ± 0.10/2.71 ± 0.10[e]	9.37 ± 0.03/10.39 ± 0.07[d]
3[f]	$H(Guo)^+$	2.11 ± 0.04	9.22 ± 0.02
4[d,g]	$H(dGuo)^+$	2.34 ± 0.03	9.25 ± 0.02
5[h]	$H_2(3'GMP)^{\pm}$	2.12 ± 0.04	9.35 ± 0.02
6[h]	$H_2(3'dGMP)^{\pm}$	2.29 ± 0.04	9.45 ± 0.03
7[f]	$H_2(5'GMP)^{\pm}$	2.48 ± 0.04	9.49 ± 0.02
8[i]	$H_2(5'dGMP)^{\pm}$	2.69 ± 0.03	9.56 ± 0.02

[a] The only exceptions are the values in column 3 of entries 1 and 2 (see below). [b] The error limits given are for all values measured in our own laboratory *3 times* the standard error of the mean value or the sum of the probable systematic errors, whichever is larger. [c] From Table 1 of ref 28; in ref 27 exactly the same values are given for I = 0.5 M. [d] Determined in this study. [e] Estimated values; the average ($\Delta pK_a = 0.20$) of the differences between the 2′-deoxyguanosine and guanosine compounds (entries 3−8) was added to the values given in column 3 of entry 1. The given errors are also estimates. [f] From refs 14 and 46. [g] The present values are in excellent accord with previous determinations;[33,34] i.e., $pK^H_{H(dGuo)} = 2.30 \pm 0.04$ and $pK^H_{dGuo} = 9.24 \pm 0.03$. [h] Sigel, H.; Song, B.; Zhao, J. Results to be published. [i] From refs 49 and 50.

3.2. Further Considerations on the Acid−Base Properties of GpG^- and $d(GpG)^-$. It is interesting to observe that the addition of a 2-fold negatively charged phosphate group to the 3′ site of Guo or dGuo enhances the basicity of the $(N1)^-$ site only little (cf. entries 3 and 4 with 5 and 6 of Table 1); i.e., by $\Delta pK_a = 0.13 \pm 0.03$ and 0.20 ± 0.04, respectively. Furthermore, the pK_a values of the $3'GMP^{2-}$ and $3'dGMP^{2-}$ species (entries 5 and 6) are very similar to the first pK_a value of the corresponding GpG^- and $d(GpG)^-$ species (entries 1 and 2), indicating that the difference in charge between $3'(d)GMP^{2-}$ and GG^- has only little influence on the release of the first proton from the GpG^- and $d(GpG)^-$ species. This contrasts with the corresponding comparison of $5'GMP^{2-}$ and $5'dGMP^{2-}$ with the dinucleoside monophosphates; in these two cases increases of $\Delta pK_a = pK^H_{5'GMP} - pK^H_{GpG} = (9.49 \pm 0.02) - (9.34 \pm 0.07) = 0.15 \pm 0.07$ and analogously of 0.19 ± 0.04 (entries 1 and 7 and 2 and 8) are observed, respectively. These comparisons

(43) Scheller, K. H.; Hofstetter, F.; Mitchell, P. R.; Prijs, B.; Sigel, H. *J. Am. Chem. Soc.* **1981**, *103*, 247−260.
(44) (a) Corfù, N. A.; Tribolet, R.; Sigel, H. *Eur. J. Biochem.* **1990**, *191*, 721−735. (b) Corfù, N. A.; Sigel, H. *Eur. J. Biochem.* **1991**, *199*, 659−669.
(45) Yamauchi, O.; Odani, A.; Masuda, H.; Sigel, H. *Met. Ions Biol. Syst.* **1996**, *32*, 207−270.
(46) Da Costa, C. P.; Sigel, H. *Inorg. Chem.* **2000**, *39*, 5985−5993.
(47) Since the N7 sites of purines are protonated under these conditions, repulsion is expected and therefore $pK_a < 1$. This is in accord with the measured value $pK^H_{H_3(5'AMP)} = 0.4 \pm 0.2$ (cf. refs 14 and 48) and the estimated one $pK^H_{H_3(5'GMP)} = 0.3 \pm 0.2$ (see ref 14).
(48) Tribolet, R.; Sigel, H. *Eur. J. Biochem.* **1987**, *163*, 353−363.
(49) Song, B.; Oswald, G.; Bastian, M.; Sigel, H.; Lippert, B. *Metal-Based Drugs* **1996**, *3*, 131−141.
(50) Song, B.; Sigel, H. *Inorg. Chem.* **1998**, *37*, 2066−2069.

indicate that the release of the first proton in GG^- occurs preferably from the (N1)H site of the 3′G unit and the release of the second proton from the (N1)H site of the 5′G unit (see Figure 1), indicating that $(N1)^-$ of the latter is seemingly about 1 pK unit more basic. However, with regard to the last-mentioned conclusion, great care needs to be exercised because the pK_a values of the two (N1)H sites (despite Δ pK_a = 1.0) clearly overlap, and therefore micro acidity constants[10,51] need to be determined to quantify the intrinsic acid−base properties of these two sites. This aim could be achieved by studying, e.g., the acid−base properties of 7-methylguanylyl(3′→5′)guanosine and of guanylyl(3′→5′)-7-methylguanosine (for details regarding micro constant schemes see ref 10).

As mentioned, due to the scarcity of the two GGs the release of the protons from the $(N7)H^+$ sites could not be measured, but the pK_a values for $H_2(GpG)^+$ are available,[28] and those for $H_2[d(GpG)]^+$ could be estimated (Table 1, entries 1 and 2, column 3). It is interesting to compare in this context the difference Δ p$K_{a/N1}$ = p$K^H_{(GpG-H)}$ − pK^H_{GpG} = (10.38 ± 0.10) − (9.34 ± 0.07) = 1.04 ± 0.12 for the release of the (N1)-protons with that for Δ p$K_{a/N7}$ = p$K^H_{H(GpG)}$ − p$K^H_{H_2(GpG)}$ = (2.51 ± 0.03) − (1.49 ± 0.03) = 1.02 ± 0.04, which is due to the release of the (N7)-protons. The fact that these differences are identical within their error limits confirms the validity of the acidity constants involved because one expects that the extent of the $(N1)H/(N1)^-$ interaction corresponds to that of the $(N7)H^+$/N7 interaction since the distances are comparable and the differences in charge are the same (0/±1).

There is one further interesting comparison, namely, that the pK_a of $H_2(5'GMP)^\pm$ (2.48 ± 0.04; Table 1, entry 7) is within its error limits identical with the release of the final (N7)-proton (pK_a = 2.51 ± 0.03; entry 1) of $H(GpG)^\pm$; the same is true for the corresponding comparison between the 2′-deoxy compounds (Table 1, entries 2 and 8, column 3). Note, the pK_a values of the $(N7)H^+$ site in the 3′(d)GMPs (entries 5 and 6) are lower. Therefore, this comparison indicates that N7 of the 5′G unit is the somewhat more basic site in the two dinucleoside monophosphates (Figure 1). This conclusion agrees with an earlier one.[27] Of course, also in this instance the pK_a values of the two $(N7)H^+$ sites overlap and, indeed, micro acidity constants have been given[27] to account for the intrinsic basicity of the (N7) sites in GpG^-. On the basis of these data[27] one calculates that the $(N7)H^+$/5′G tautomer preponderates with approximately 63% over the 37% of the $(N7)H^+$/3′G tautomer in $H(GpG)^\pm$.

To conclude, both the $(N1)^-$ and the N7 sites of the 5′G unit are somewhat more basic than the corresponding sites of the 3′G part.

3.3. Stability Constants of $M(dGuo)^{2+}$ and $M(dGuo-H)^+$ Complexes. Since the stability constants of those metal ion complexes of the GG^- species in which none of the (N1)H sites was deprotonated could not be measured due to the scarcity of the dinucleoside monophosphates, we determined by potentiometric pH titration the corresponding stability constants for the complexes formed between Mg^{2+} or Cd^{2+} (=M^{2+}) and 2′-deoxyguanosine (dGuo).

Table 2. Logarithms of the Stability Constants of Some $M(dGuo)^{2+}$ and $M(dGuo-H)^+$ Complexes (Eqs 7 and 8) as Determined by Potentiometric pH Titrations in Aqueous Solution, Together with the Negative Logarithms of the Acidity Constants of the $M(dGuo)^{2+}$ Species (Eqs 9 and 10) and the Extent of the Acidification at the (N1)H Site Caused by the Bound M^{2+} (Eq 11) (25 °C; I = 0.1 M, $NaNO_3$)[a,b]

M^{2+}	log $K^M_{M(dGuo)}$	log $K^M_{M(dGuo-H)}$	p$K^H_{M(dGuo)}$	Δ p$K_{a/N1/dGuo}$
Mg^{2+}	0.35 ± 0.25	0.94 ± 0.14	8.66 ± 0.29	0.59 ± 0.29
Ni^{2+}	1.53 ± 0.09	3.20 ± 0.18	7.58 ± 0.20	1.67 ± 0.20
Cd^{2+}	1.53 ± 0.07	3.15 ± 0.03	7.63 ± 0.08	1.62 ± 0.08

[a] For the error limits see footnote *b* of Table 1; the error limits of the derived data were calculated according to the error propagation after Gauss. [b] The values for the Ni^{2+} system are from ref 34.

The neutral dGuo interacts via N7 with divalent metal ions to give $M(dGuo)^{2+}$ complexes,[34] and the (N1)-deprotonated ligand reacts to yield the $M(dGuo-H)^+$ species. Only these two kinds of complexes form, since the experiments involving metal ions were carried out at a high M^{2+}:dGuo ratio (see section 2.5). Consequently, the experimental data of the potentiometric pH titrations could be fully explained by taking into account equilibria 1 and 2 as well as the following two complex-forming equilibria 7 and 8, as long as the evaluation of the data was not carried into the pH range where hydroxo complex formation occurs.

$$M^{2+} + G \rightleftharpoons M(G)^{2+} \quad (7a)$$

$$K^M_{M(G)} = [M(G)^{2+}]/([M^{2+}][G]) \quad (7b)$$

$$M^{2+} + (G-H)^- \rightleftharpoons M(G-H)^+ \quad (8a)$$

$$K^M_{M(G-H)} = [M(G-H)^+]/([M^{2+}][(G-H)^-]) \quad (8b)$$

Of course, the complex $M(G)^{2+}$ formed according to equilibrium 7 may lose a proton from its (N1)H site to give $M(G-H)^+$ according to equilibrium 9. The corresponding acidity constant, $K^H_{M(G)}$, may be calculated[52] with eq 10.

$$M(G)^{2+} \rightleftharpoons M(G-H)^+ + H^+ \quad (9a)$$

$$K^H_{M(G)} = [M(G-H)^+][H^+]/[M(G)^{2+}] \quad (9b)$$

$$pK^H_{M(G)} = pK^H_G + \log K^M_{M(G)} - \log K^M_{M(G-H)} \quad (10)$$

The results determined for the mentioned systems are summarized in Table 2 together with the corresponding values for the Ni^{2+}/dGuo system taken from our earlier work.[34] The error limits of some of the constants are rather large; this is the consequence of the low stability of these complexes which gives rise only to a small buffer depression (see section 2.5). It may be added that the stabilities of the corresponding $M(Guo)^{2+}$ and $M(Guo-H)^+$ complexes are expected to be the same within the error limits for the Guo and dGuo ligands; that this is the case has previously been shown[34] for the corresponding Cu^{2+} systems.

The acidification of the (N1)H site of dGuo caused by Mg^{2+}, Ni^{2+}, or Cd^{2+} coordinated to N7 is quite remarkable,

(51) Song, B.; Sigel, R. K. O.; Sigel, H. *Chem. Eur. J.* **1997**, *3*, 29−33.

(52) Sigel, H. *Eur. J. Biochem.* **1968**, *3*, 530−537.

Table 3. Stability Constants of Some M^{2+} Complexes Formed in Aqueous Solution with d(GpG) or GpG (Eqs 12−14) as Determined[a] by Potentiometric pH Titrations in Aqueous Solution, Together with the Negative Logarithms of the Acidity Constants of the $M(GG)^+$ and M(GG−H) Species (Eqs 15−18) and the Extent of the Acidification of the (N1)H Sites by M^{2+} Complexation (25 °C; $I \approx 0.1$ M, $NaNO_3$)[b]

no.[c]	GG	M^{2+}	$\log K^{M}_{M(GG)}$[a]	$\log K^{M}_{M(GG-H)}$	$pK^{H}_{M(GG)}$	$\Delta pK_{a1/GG}$	$\log K^{M}_{M(GG-2H)}$	$pK^{H}_{M(GG-H)}$	$\Delta pK_{a2/GG}$
1a	d(GpG)	Mg^{2+}	0.45 ± 0.4	1.43 ± 0.11	8.4 ± 0.4	1.0 ± 0.4	2.02 ± 0.05	9.80 ± 0.14	0.59 ± 0.16
b			ignored	1.35 ± 0.15			1.97 ± 0.05	9.77 ± 0.17	0.62 ± 0.18
2a		Ni^{2+}	1.9 ± 0.2	3.87 ± 0.15	7.40 ± 0.25	1.97 ± 0.25			
b			ignored	3.79 ± 0.15					
3a		Cd^{2+}	1.75 ± 0.3	3.56 ± 0.07	7.56 ± 0.31	1.81 ± 0.31			
b			ignored	3.50 ± 0.10					
4a	GpG	Mg^{2+}	0.45 ± 0.4	1.53 ± 0.15	8.3 ± 0.4	1.0 ± 0.4	2.0 ± 0.2	9.9 ± 0.3	0.5 ± 0.3
b			ignored	1.48 ± 0.15			1.9 ± 0.2	10.0 ± 0.3	0.4 ± 0.3
5a		Cd^{2+}	1.75 ± 0.3	3.6 ± 0.2	7.5 ± 0.4	1.8 ± 0.4			
b			ignored	3.5 ± 0.2					

[a] The values given in column 4 were not measured but estimated on the basis of the known stabilities of the corresponding M^{2+} complexes formed with dGuo (Table 1) and H[3′/5′(d)GMP]⁻ species (see text in section 3.4). [b] For the error limits see footnote *a* of Table 2. [c] The values in entries 1b, 2b, and 3b have been calculated without considering the formation of $M[d(GpG)]^+$ complexes, while entries 1a, 2a, and 3a give for each metal ion system the result of the calculations where this species was considered and its stability constant (see footnote *a*) was kept fixed in the calculation procedure for the other stability constants. The analogous comments hold for entries 4 and 5 (see also sections 2.3 and 2.4).

as the results in the last column on the right in Table 2 demonstrate. These values are the differences defined by eq 11:

$$\Delta\, pK_{a/N1/dGuo} = pK^{H}_{dGuo} - pK^{H}_{M(dGuo)} \qquad (11)$$

In accord with the lower stability of the Mg^{2+} complexes also the acidification by this metal ion is smaller. The very similar behavior of the Ni^{2+} and Cd^{2+} systems is expected according to the *Stability Ruler* defined by Martin.[53] In agreement herewith is also the $Pb(Guo)^{2+}$ complex somewhat less stable ($\log K^{Pb}_{Pb(Guo)} = 1.25 \pm 0.17$)[46,54] than the $Ni(dGuo)^{2+}$ and $Cd(dGuo)^{2+}$ species, whereas for $Zn(dGuo)^{2+}$ a similar stability is expected.

3.4. Stabilities of Complexes Formed with the Dinucleoside Monophosphates. Since the M^{2+} complexes with the GG^- ligands are also not very stable, it was again necessary to work with a relatively large excess of M^{2+} compared to the concentration of the GGs to obtain a high enough formation degree of the complexes (sections 2.3 and 2.4). Therefore, the upper limit of the pH that could be evaluated in the experiments was determined by the formation of M^{2+} hydroxo complexes; this pH became in all cases evident from the titrations carried out in the absence of ligand (see sections 2.3 and 2.4). By taking into account the acidity constants defined by equilibria 3−6 and by considering in addition the following complex formation reactions, the data of the potentiometric pH titrations could be perfectly explained:

$$M^{2+} + GG^- \rightleftharpoons M(GG)^+ \qquad (12a)$$

$$K^{M}_{M(GG)} = [M(GG)^+]/([M^{2+}][GG^-]) \qquad (12b)$$

$$M^{2+} + (GG-H)^{2-} \rightleftharpoons M(GG-H) \qquad (13a)$$

$$K^{M}_{M(GG-H)} = [M(GG-H)]/([M^{2+}][(GG-H)^{2-}]) \qquad (13b)$$

$$M^{2+} + (GG-2H)^{3-} \rightleftharpoons M(GG-2H)^- \qquad (14a)$$

$$K^{M}_{M(GG-2H)} = [M(GG-2H)^-]/([M^{2+}][(GG-2H)^{3-}]) \qquad (14b)$$

Of course, one may also consider the two deprotonation reactions 15 and 16,

$$M(GG)^+ \rightleftharpoons M(GG-H) + H^+ \qquad (15a)$$

$$K^{H}_{M(GG)} = [M(GG-H)][H^+]/[M(GG)^+] \qquad (15b)$$

$$M(GG-H) \rightleftharpoons M(GG-2H)^- + H^+ \qquad (16a)$$

$$K^{H}_{M(GG-H)} = [M(GG-2H)^-][H^+]/[M(GG-H)] \qquad (16b)$$

which are interlinked with equilibria 12−14 by the following two equations:

$$pK^{H}_{M(GG)} = pK^{H}_{GG} + \log K^{M}_{M(GG)} - \log K^{M}_{M(GG-H)} \qquad (17)$$

$$pK^{H}_{M(GG-H)} = pK^{H}_{(GG-H)} + \log K^{M}_{M(GG-H)} - \log K^{M}_{M(GG-2H)} \qquad (18)$$

However, equilibria 14 and 16 are of relevance in the present study only for the Mg^{2+} systems; with Ni^{2+} and Cd^{2+} the formation of the hydroxo complexes occurs before the onset of equilibria 14 and 16.

The stability constants defined by equilibrium 12 could not be measured due to the scarcity of the two GGs which prevented us from working at the low pH necessary to determine these constants for the various $M(GG)^+$ systems. However, on the basis of the stability constants of the $M(dGuo)^{2+}$ complexes (Table 2) and those formed between M^{2+} and the H(3′GMP)⁻ (cf. ref 55), H(3′dGMP)⁻ (cf. ref 55), and H(5′GMP)⁻ (cf. ref 5) species, values for the stability of the $M(GG)^+$ complexes could be estimated; these values are listed in column 4 of Table 3, and they were taken into account in the calculations for the other values listed under entries "a" in Table 3.

To demonstrate that the constants listed under entries "a" in Table 3 are valid results despite the above-mentioned

(53) (a) Martin, R. B. *Met. Ions Biol. Syst.* **1986**, *20*, 21−65. (b) Martin, R. B. In *Molecular Biology and Biotechnology*; Meyers, R. A., Ed.; VCH Publishers: New York; 1995; pp 83−86. (c) Martin, R. B. In *Encyclopedia of Molecular Biology and Molecular Medicine*; Meyers, R. A., Ed.; VCH: Weinheim, 1996; Vol. 1, pp 125−134.
(54) Sigel, H.; Da Costa, C. P.; Martin, R. B. *Coord. Chem. Rev.* **2001**, *219−221*, 435−461.
(55) Sigel, H.; Song, B.; Zhao, J. Results to be published.

estimates, we have also evaluated our potentiometric pH titration data by considering only equilibria 5 and 6 as well as 13 for the systems with Ni^{2+} and Cd^{2+}. In the case of the Mg^{2+} systems in addition equilibrium 14 was taken into account as already mentioned above. This means that, in this evaluation of the experimental data, the formation of $M(GG)^+$ complexes was ignored, yet this simplified model (sections 2.3 and 2.4) still satisfied the experimental data very well because the formation degree of the $M(GG)^+$ species is always low and not dependent on the H^+ concentration at pH >5. Indeed, the stability constants determined in this way for the M(GG−H) and Mg(GG−2H)$^-$ complexes, which are listed under entries "b" in Table 3, are within the error limits identical with those determined by the more sophisticated model, though they are in all instances somewhat lower, i.e., by 0.05−0.1 log unit in the maximum (Table 3, column 5). To conclude, for any future work the values listed in the "a" entries of Table 3 should be used.

4. Structural Considerations and Conclusions

A comparison of the stability constants given in Table 2 (columns 2 and 3) for the $M(dGuo)^{2+}$ and $M(dGuo-H)^+$ complexes with those listed in Table 3 (columns 4 and 5) for the $M(GG)^+$ and M(GG−H) species indicates that the constants are of a similar order, which means that metal ion binding to the guanine residue(s) is the stability-determining factor. Since N7 of the 5′G unit is somewhat more basic than the one of the 3′G unit in the GG species (see Figure 1), we conclude that M^{2+} is preferably coordinated to the N7 site of the 5′G; this binding mode also allows an outersphere interaction with the C6 carbonyl oxygen[14] as well as a maximal electrostatic interaction with the negatively charged phosphate bridge and possibly even macrochelate formation to a certain extent as it has been suggested before for a Pt(II) complex of a related dinucleoside monophosphate[56,57] and as it is well-known to occur with M(5′GMP) (cf. refs 5 and 14) and related complexes.[5,15,58]

In a previous study[34] of more simple guanine derivatives it was shown that (N7)-coordinated divalent metal ions acidify the (N1)H site in the following decreasing order: Cu^{2+} ($\Delta pK_a = 2.2 \pm 0.3$) > Ni^{2+} (1.7 ± 0.15) > Pt^{2+} (1.4 ± 0.1) ∼ Pd^{2+} (1.4) (see also ref 36). The value due to the formation of the Ni[d(GpG−H)] species with $\Delta pK_{a1/GG} = 1.97 \pm 0.25$ (Table 3, column 7) fits within its error limits well into this picture, giving no hint for the formation of significant amounts of intramolecular chelates involving both N7 sites of d(GpG), but rather suggesting largely binding at a single site. Furthermore, the same $\Delta pK_{a1/GG}$ value (=1.8 ± 0.3) resulting from the formation of the Cd[d(GpG−H)] complex is in accord with the already mentioned *Stability Ruler*.[53] This *Ruler* also predicts similar stabilities for the Zn^{2+} complexes as observed for the Ni^{2+} and Cd^{2+} ones. Indeed, that Zn^{2+} has a significant affinity toward the N7 sites of guanine residues is confirmed by a very recent crystal structure determination of a short DNA duplex where Zn^{2+} ions interact with the terminal guanine residue.[8] The possible biological relevance of guanine (N1)H deprotonation as mediated by metal ions has been discussed.[33]

The results given in Tables 2 and 3 for the Mg^{2+} complexes confirm the general experience[24−26] that the affinity of this metal ion toward N sites is not very pronounced, and in a series of complexes formed with benzimidazole-type ligands (=1,3-dideazapurines) it was concluded[59] that outersphere interactions dominate because the stability of the complexes depends only little on the basicity of the N sites. This conclusion about the Mg^{2+} complexes is also in agreement with results for sterically hindered benzimidazole-type ligands where significant inhibiting effects on the stability of the complexes formed with Ni^{2+}, Zn^{2+}, and Cd^{2+} are observed, whereas the stability of the Mg^{2+} complexes remains practically unaffected.[60] In a recent theoretical study,[61] in accord with a crystal structure,[62] it was also concluded that hydrated Mg^{2+} ions prefer to reside near the N7/O6 sites of guanines, though some monodentate binding to N7 of pentahydrated Mg^{2+} might also occur, again as suggested by a theoretical study[63] and a crystallographic structure.[64] We conclude that Mg^{2+} most likely coordinates predominantly in an outersphere manner to guanine sites but that some direct coordination to N7 is also possible.

Acknowledgment. The competent technical assistance of Mrs. Rita Baumbusch and Mrs. Astrid Sigel in the preparation of this manuscript and a research grant from the Swiss National Science Foundation, which made this study possible, are gratefully acknowledged.

IC020672L

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