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Metal-Ion-Coordinating Properties of the Dinucleotide 2′-Deoxyguanylyl(5′→3′)-2′-deoxy-5′-guanylate ($d(pGpG)^{3-}$): Isomeric Equilibria Including Macrochelated Complexes Relevant for Nucleic Acids**

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Abstract: The interaction between divalent metal ions and nucleic acids is well known, yet knowledge about the strength of binding of labile metal ions at the various sites is very scarce. We have therefore studied the stabilities of complexes formed between the nucleic acid model d(pGpG) and the essential metal ions Mg^{2+} and Zn^{2+} as well as with the generally toxic ions Cd^{2+} and Pb^{2+} by potentiometric pH titrations; all four ions are of relevance in ribozyme chemistry. A comparison of the present results with earlier data obtained for $M(pUpU)^-$ complexes allows the conclusion that phosphate-bound Mg^{2+} and Cd^{2+} form macrochelates by interaction with N7, whereas the also phosphate-coordinated Pb^{2+} forms a 10-membered chelate with the neighboring phosphate diester bridge. Zn^{2+} forms both types of chelates with formation degrees of about 91% and 2.4% for $Zn[d(pGpG)]^-_{cl/N7}$ and $Zn[d(pGpG)]^-_{cl/PO}$, respectively; the open form with Zn^{2+} bound only to the terminal phosphate group, $Zn[d(pGpG)]^-_{op}$, amounts to about 6.8%. The various intramolecular equilibria have also been quantified for the other metal ions. Zn^{2+}, Cu^{2+}, and Cd^{2+} also form macrochelates in the monoprotonated M[H;d(pGpG)] species (the proton being at the terminal phosphate group), that is, the metal ion at N7 interacts to some extent with the $P(O)_2(OH)^-$ group. Thus, this study demonstrates that the coordinating properties of the various metal ions toward a pGpG unit in a nucleic acid differ: Mg^{2+}, Zn^{2+}, and Cd^{2+} have a significant tendency to bridge the distance between N7 and the phosphate group of a (d)GMP unit, although to various extents, whereas Pb^{2+} (and possibly Ca^{2+}) prefer a pure phosphate coordination.

Keywords: bioinorganic chemistry · chelates · DNA metal ion complexes · nucleotides · RNA metal ion complexes

1. Introduction

The DNA fragment d(pGpG), and especially its interaction with the anticancer drug Cisplatin (*cis*-$[(NH_3)_2PtCl_2]$), has been studied relatively often[1] due to the accumulation of evidence that the antitumor action of Cisplatin results from the preferential binding of *cis*-$(NH_3)_2Pt^{2+}$ to the N7 sites of two consecutive guanines in DNA.[2] This kind of binding

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[**] Abbreviations and definitions (see also Figure 1): $dGMP^{2-}$: 2′-deoxyguanosine 5′-monophosphate; dGuo: 2′-deoxyguanosine; $d(GpG)^-$: 2′-deoxyguanylyl(3′→5′)-2′-deoxyguanosine; $d(pGpG)^{3-}$: see Figure 1; en: ethylenediamine; GMP^{2-}: guanosine 5′-monophosphate; GpG^-: guanylyl(3′→5′)guanosine; Guo: guanosine; *I*: ionic strength; K_a: general acidity constant; M^{2+}: divalent metal ion; $pGpG^{3-}$: guanylyl(5′→3′)-5′-guanylate; $pUpU^{3-}$: see Figure 1; $R\text{-}PO_3^{2-}$: simple phosphate monoester or phosphonate ligand with R representing a non-interacting residue. Species written without a charge either do not carry one or represent the species in general [i.e., independent of their protonation degree, e.g., d(pGpG)]; which of the two possibilities applies is always clear from the context. In formulas such as M[H;d(pGpG)], the H^+ ion and $d(pGpG)^{3-}$ are separated by a semicolon to facilitate reading, yet they appear within the same square brackets to indicate that the proton is at the ligand without defining its location. A formula like $d(pGpG-H)^{4-}$ means that the compound has lost a further proton and is to be read as $d(pGpG)^{3-}$ *minus* H^+.

causes distortion of the double helix,[3] stalling of the polymerases,[4] and subsequently leads to apoptosis.[1] Naturally, the success of Cisplatin as a drug has led to further studies of d(pGpG) (Figure 1)[5,6] with Cisplatin derivatives such as

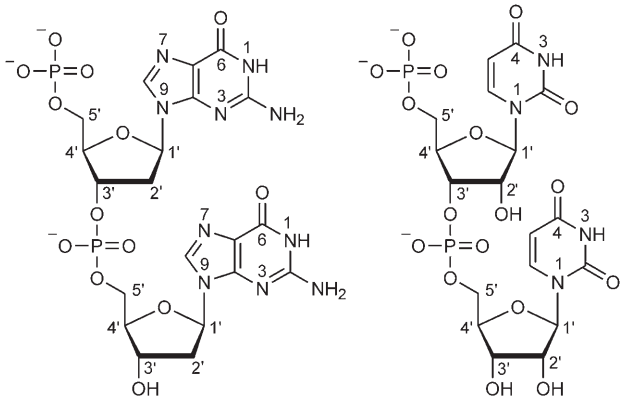

Figure 1. Chemical structure of the trianion of 2′-deoxyguanylyl(5′→3′)-2′-deoxy-5′-guanylic acid, that is, 2′-deoxyguanylyl(5′→3′)-2′-deoxy-5′-guanylate, abbreviated as $d(pGpG)^{3-}$, and also known[5] as 2′-deoxy[5′-phosphate-guanylyl-(3′→5′)-guanosine]. In addition, the structure of uridylyl-(5′→3′)-5′-uridylate ($pUpU^{3-}$) is shown; this dinucleotide is used for comparisons in the present study. The two nucleoside units in each dinucleotide are shown in both structures in their predominant *anti* conformation.[6]

(en)Pt^{2+},[7] [*cis*-$(NH_3)_2$Pt(4-methylpyridine)Cl]$^+$,[8] or unsymmetrically substituted derivatives of *cis*-$(NH_3)_2Pt^{2+}$.[1,9] More recently other active antitumor complexes such as [(2*R*)-aminomethylpyrrolidine](1,1-cyclobutanedicarboxylato)platinum(II)[10] or tetrakis(μ-carboxylato)dirhodium(II,II)[11] and their interaction with d(pGpG) have also received attention.

Surprisingly, there are hardly any studies of d(pGpG) and kinetically labile metal ions of biological significance. One early study,[12] which considered the co-precipitation of deoxynucleotides with calcium phosphate, showed that d(pGpG) precipitates best among the 14 dinucleotides investigated. The dinucleoside monophosphate d(GpG) is far more effective than d(pGpG) in the formation of G-tetrads and extended superstructures in the presence of Na^+ or K^+.[5] However, for both compounds K^+ induces more stable structures than Na^+. In studies of d(pGpG) with actinomycin D[13] or its effect on human immunodeficiency virus type 1 integrase[14] the role of metal ions remains unclear.

Considering the wide use of the d(pGpG) fragment in rather diverse studies, as indicated above, and its relevance as a model ligand for nucleic acids in general, including ribozymes,[15,16] it is astonishing to find that its detailed acid–base properties have only very recently been determined[17] and its coordination chemistry with labile metal ions of biological relevance has so far not been studied at all.[18] This is especially surprising in view of the available N7 sites as they play a prominent role in DNA[1,6] and RNA[6,15,19] as metal-ion-binding locations. For these reasons, and also for the ones outlined below, we have now studied these properties of d(pGpG). It is important to note in this context that d(pG) and pG derivatives behave identically toward metal ions as far as binding to the phosphate group or the nucleobase moiety is concerned.[20–22] This means that the 2′-hydroxy group has no direct effect on the metal-ion-coordinating properties in the physiological pH range as the 2′-OH/3′-OH unit of the ribose residue has a pK_a of about 12.5.[23] A very small indirect effect due to the slightly altered acid–base properties between oxy and deoxy derivatives[17,22] is possible, but this is strictly a relative one and is cancelled out if the small pK_a differences are taken into account.[24] This fact is important because it means that the results described in this study apply in the physiological pH range for all single-stranded nucleic acids with GMP units, be they DNA or RNA.

Until now very little quantitative information concerning the binding strength of metal ions to the individual coordinating sites (atoms) of a nucleic acid has been available.[22,25,26] Evidently, the most frequently repeated individual site is the phosphate diester bridge, -O-$P(O)_2^-$-O-, in which the two terminal oxygen atoms together carry one negative charge. It is difficult to measure the metal ion affinity of such a site in a polymer or even in an oligonucleotide such as $UpUpU^{2-}$ directly as the primary protons of phosphate residues are released with pK_a values of about 1 (or below),[17] and thus no competition for binding occurs between a metal ion and a proton within the experimentally accessible pH range, which includes the physiological one. Recently, we have succeeded in measuring the metal ion affinity of such a phosphate–diester unit in an indirect way by using a dinucleotide containing both a terminal 5′-phosphate group and a 5′→3′-phosphate diester bridge, that is, $pUpU^{3-}$.[27] The terminal phosphate group is the main binding site in such a dinucleotide because of its twofold negative charge. However, chelate formation with the neighboring phosphate diester bridge is at least theoretically possible and therefore information can be gained about the metal ion affinity of this second site. It is important to note that the distance between the two phosphate groups in such a dinucleotide can adapt to the range of distances found in complex nucleic acid structures, therefore the result[27] that some metal ions form chelates (Zn^{2+}, Pb^{2+}) and others not (Mg^{2+}, Cd^{2+}) is also relevant for RNA and DNA polymers. These four metal ions were selected for the present study because the necessary information about their phosphate interaction is available[27] and all four play a role in ribozyme chemistry.

After the phosphate diester bridge the second most important binding site for metal ions is N7 of the guanine residue,[6,15,19] which lies in the major groove of nucleic acids.[6] Therefore, to learn something about the metal-ion-binding properties of this site in nucleic acids, we selected the dinucleotide model d(pGpG) (Figure 1). A metal ion coordinated at its 5′-terminal phosphate group may form a macrochelate with N7 of the corresponding guanine residue, as is known from studies with GMP^{2-} [28] and $dGMP^{2-}$,[29] or inter-

act further with the phosphate diester bridge, as is known from the work with $pUpU^{3-}$.[27] This means one has to consider a priori the complex species shown in Equilibrium (1), where no distinction is made between inner-sphere and outer-sphere binding:

$$M^{2+} + d(pGpG)^{3-} \overset{K^{M}_{M[d(pGpG)]_{op}}}{\rightleftharpoons} M[d(pGpG)]^{-}_{op} \begin{matrix} \overset{K_{I/N7}}{\diagup} M[d(pGpG)]^{-}_{cl/N7} \\ \underset{K_{I/PO}}{\diagdown} M[d(pGpG)]^{-}_{cl/PO} \end{matrix} \quad (1)$$

In this equilibrium $M[d(pGpG)]^{-}_{op}$ designates the "open" complex in which the metal ion is only bound to the terminal phosphate group, and the macrochelated or "closed" isomers involving also the guanine-N7 site or the phosphate–diester bridge are termed $M[d(pGpG)]^{-}_{cl/N7}$ and $M[d(pGpG)]^{-}_{cl/PO}$, respectively. A simultaneous binding of the 5′-phosphate-coordinated metal ion to both N7 sites or to the N7 site of the 5′ end and the neighboring phosphate diester bridge is not possible for steric reasons. As proven in this study, all three complex isomers considered in Equilibrium (1) occur with Zn^{2+}, whereas Mg^{2+} and Cd^{2+} form, along with the open species, only the macrochelate involving N7, and Pb^{2+} one with the phosphate diester bridge. The same type of interactions are expected to occur with nucleic acids.

2. Results and Discussion

2.1. Definition of the equilibrium constants and corresponding results: The ligand concentrations in the experiments of this study (0.15 mM; see Section 4.3 below) are such that self-association is negligibly small, that is, more than 99% of the d(pGpG) species are present in their monomeric forms.[17] Hence, the following results refer in all instances to the monomeric species.

The dinucleotide $d(pGpG)^{3-}$ (Figure 1) can accept three protons at its phosphate groups and two more at the N7 sites of the two guanine residues to give $H_5[d(pGpG)]^{2+}$. In addition, the two (N1)H protons can be released under basic conditions. The two primary phosphate protons are released with $pK_a \leq 1$ and one of the $(N7)H^{+}$ units is deprotonated with $pK_a = 2.4 \pm 0.2$.[17] Thus, these three acid–base equilibria play no role in the pH range of our experiments. Consequently, the first protonated species that needs to be taken into account is $H_2[d(pGpG)]^{-}$, where one proton is at the 5′-phosphate group and the other one at an N7 site. This means the following deprotonation reactions [Eq. (2)–(4)] are relevant:

$$H_2[d(pGpG)]^{-} \rightleftharpoons H[d(pGpG)]^{2-} + H^{+} \quad (2a)$$

$$K^{H}_{H_2[d(pGpG)]} = [H[d(pGpG)]^{2-}][H^{+}]/[H_2[d(pGpG)]^{-}] \quad (2b)$$

$$H[d(pGpG)]^{2-} \rightleftharpoons d(pGpG)^{3-} + H^{+} \quad (3a)$$

$$K^{H}_{H[d(pGpG)]} = [d(pGpG)^{3-}][H^{+}]/[H[d(pGpG)]^{2-}] \quad (3b)$$

$$d(pGpG)^{3-} \rightleftharpoons d(pGpG{-}H)^{4-} + H^{+} \quad (4a)$$

$$K^{H}_{d(pGpG)} = [d(pGpG{-}H)^{4-}][H^{+}]/[d(pGpG)^{3-}] \quad (4b)$$

In Equilibrium (2a) the second $(N7)H^{+}$ site is deprotonated ($pK^{H}_{H_2[d(pGpG)]} = 2.98 \pm 0.13$), and this is followed by the reaction in Equilibrium (3a) where the proton of the $P(O)_2(OH)^{-}$ group is lost ($pK^{H}_{H[d(pGpG)]} = 6.56 \pm 0.03$).[17] The resulting $d(pGpG)^{3-}$ species is the most relevant one for complex formation in the physiological pH range. The first of the two (N1)H sites of the guanine residues is ionized with $pK^{H}_{d(pGpG)} = 9.54 \pm 0.08$ [Eq. (4)].[17] The $M[d(pGpG{-}H)]^{2-}$ complex could only be studied in the case of Mg^{2+} as with all other metal ions this is not possible due to hydrolysis of a coordinated water molecule (see Section 4.3). The pH range where the second (N1)H unit is also deprotonated and the $d(pGpG{-}2H)^{5-}$ species therefore becomes relevant ($pK^{H}_{d(pGpG-H)} = 10.11 \pm 0.14$)[17] was not reached in the present study.

The question now arises as to which species need to be considered for metal-ion binding. It follows from the information summarized in the preceding paragraph that in the $H[d(pGpG)]^{2-}$ species the proton is located at the 5′-phosphate end and that the N7 sites are free. As a consequence, complex formation with $H[d(pGpG)]^{2-}$ without the loss of a proton needs to be considered because metal-ion binding solely to a N7 unit is known.[22] It is evident that $d(pGpG)^{3-}$ with a free PO_3^{2-} group will also form stable complexes, and this also holds for the (N1)H-deprotonated species. Indeed, by taking into account the acidity constants defined by Equilibria (2)–(4) and by considering in addition the following complex formation reactions [Eq. (5)–(7)], the experimental data of the potentiometric pH titrations can be explained perfectly.

$$M^{2+} + H[d(pGpG)]^{2-} \rightleftharpoons M[H;d(pGpG)] \quad (5a)$$

$$K^{M}_{M[H;d(pGpG)]} = [M[H;d(pGpG)]]/([M^{2+}][H[d(pGpG)]^{2-}]) \quad (5b)$$

$$M^{2+} + d(pGpG)^{3-} \rightleftharpoons M[d(pGpG)]^{-} \quad (6a)$$

$$K^{M}_{M[d(pGpG)]} = [M[d(pGpG)]^{-}]/([M^{2+}][d(pGpG)^{3-}]) \quad (6b)$$

$$M^{2+} + d(pGpG{-}H)^{4-} \rightleftharpoons M[d(pGpG{-}H)]^{2-} \quad (7a)$$

$$K^{M}_{M[d(pGpG-H)]} = [M[d(pGpG{-}H)]^{2-}]/([M^{2+}][d(pGpG{-}H)^{4-}]) \quad (7b)$$

Complex formation according to Equilibrium (7a) could only be studied with Mg^{2+}. With all other metal ions (Zn^{2+}, Cd^{2+}, and Pb^{2+}) only the stability constants for Equilibria (5a) and (6a) could be determined due to hydrolysis

reactions of these metal ions. The pH where hydrolysis occurs was evident in all cases from the titrations carried out in the absence of ligand (see Section 4.3). The stability constants of the four M^{2+}/d(pGpG) systems studied are listed in columns 2, 3, and 4 of Table 1.

2.2. Acidifications due to metal ion coordination: From a careful consideration of the equilibria discussed so far, it is evident that the complex $M[d(pGpG)]^-$ can not only be formed according to Equilibrium (6a) but also according to the following deprotonation reaction (8):

$$M[H;d(pGpG)] \rightleftharpoons M[d(pGpG)]^- + H^+ \quad (8a)$$

$$K^H_{M[H;d(pGpG)]} = [M[d(pGpG)]^-][H^+]/[M[H;d(pGpG)]] \quad (8b)$$

Similarly, the species $M[d(pGpG-H)]^{2-}$ can originate according to either Equilibrium (7a) or Equilibrium (9a):

$$M[d(pGpG)]^- \rightleftharpoons M[d(pGpG-H)]^{2-} + H^+ \quad (9a)$$

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

However, the acidity constants defined by Equations (8b) and (9b) are connected with the other equilibrium constants already mentioned by Equations (10) and (11), respectively:

$$pK^H_{M[H;d(pGpG)]} = pK^H_{H[d(pGpG)]} + \log K^M_{M[H;d(pGpG)]} - \log K^M_{M[d(pGpG)]} \quad (10)$$

$$pK^H_{M[d(pGpG)]} = pK^H_{d(pGpG)} + \log K^M_{M[d(pGpG)]} - \log K^M_{M[d(pGpG-H)]} \quad (11)$$

In this way the acidity constants for Equilibria (8a) and (9a) can be calculated and are listed in columns 5 and 6 of Table 1, respectively.

The acidity difference according to Equation (12) describes the acidification of the phosphate-bound proton in $H[d(pGpG)]^{2-}$ upon metal-ion binding. These metal-ion-initiated acidifications are considerable (Table 1, column 7) as all $pK^H_{M[H;d(pGpG)]}$ values are between 5 and 5.5 (Table 1, column 5), that is, more than one pK unit below the $pK^H_{H[d(pGpG)]}$ value of 6.56 ± 0.03. At the same time all $pK^H_{M[H;d(pGpG)]}$ values are more than two pK units above the $pK^H_{H_2[d(pGpG)]}$ value of 2.98 ± 0.13. These results confirm unequivocally the principal structure of the M[H;d(pGpG)] complexes indicated in Section 2.1, with the metal residing at N7 and the proton at the 5′-phosphate group (see also Section 2.3).

$$\Delta pK_{a/PO} = pK^H_{H[d(pGpG)]} - pK^H_{M[H;d(pGpG)]} \quad (12)$$

A Mg^{2+} ion coordinated at the PO_3^{2-} group of $d(pGpG)^{3-}$ also achieves an acidification of (N1)H in the $Mg[d(pGpG)]^-$ complex, as defined by Equation (13):

$$\Delta pK_{a/N1} = pK^H_{d(pGpG)} - pK^H_{M[d(pGpG)]} \quad (13)$$

This acidification of about half a pK unit is much less pronounced (Table 1, column 8) than that of $\Delta pK_{a/PO}$. The $pK^H_{Mg[d(pGpG)]}$ value of 9.0 (Table 1, column 6) is clearly above the physiological pH range. On the other hand, about 10% of the total amount of (N1)H-deprotonated $Mg[d(pGpG-H)]^{2-}$ species already exist at pH 8. Hence, by clustering more Mg^{2+} ions around such a nucleotide unit, as is known to occur with ribozymes,[30–33] and by further involving the guanine residue,[19] it is possible to shift the deprotonation of the (N1)H site into the physiological pH range.

2.3. Solution structures of the M[H;d(pGpG)] complexes: Because the analysis of potentiometric pH titrations yields only the amount and distribution of the species of a net charge type, for example, of M[H;d(pGpG)], further information is required to locate the binding sites of the proton and the metal ion. In the preceding section we have seen, based on comparisons of acidity constants, that in the M[H;d(pGpG)] complexes the metal ion is at N7 and that the proton is part of the terminal $P(O)_2(OH)^-$ group. However, it is known that a metal ion bound to N7 of a purine system in a nucleotide complex may form macrochelates by also interacting with the $P(O)_2(OH)^-$ residue.[34] Hence, the following intramolecular Equilibrium (14) between the open (op) and chelated or closed (cl) isomers needs to be considered:

$$M[H;d(pGpG)]_{op} \rightleftharpoons M[H;d(pGpG)]_{cl} \quad (14)$$

Table 1. Stability constants of some M^{2+} complexes formed with d(pGpG) [Eqs. (5)–(7)], as determined by potentiometric pH titrations in aqueous solution, together with the negative logarithms of the acidity constants of the M[H;d(pGpG)] and $M[d(pGpG)]^-$ species [Eqs. (8)–(11)][a] and the extent of acidification at the $P(O)_2(OH)^-$ [Eq. (12)] and (N1)H [Eq. (13)] sites upon M^{2+} complexation (25 °C; $I = 0.1$ M, $NaNO_3$).[b]

M^{2+}	$\log K^M_{M[H;d(pGpG)]}$ [Eq. (5)]	$\log K^M_{M[d(pGpG)]}$ [Eq. (6)]	$\log K^M_{M[d(pGpG-H)]}$ [Eq. (7)]	$pK^H_{M[H;d(pGpG)]}$ [Eqs. (8), (10)]	$pK^H_{M[d(pGpG)]}$ [Eqs. (9), (11)]	$\Delta pK_{a/PO}$ [Eq. (12)]	$\Delta pK_{a/N1}$ [Eq. (13)]
Mg^{2+}	1.17 ± 0.10	2.43 ± 0.06	2.96 ± 0.08	5.30 ± 0.12	9.01 ± 0.13	1.26 ± 0.12	0.53 ± 0.15
Zn^{2+}	2.30 ± 0.07	3.66 ± 0.05		5.20 ± 0.09		1.36 ± 0.09	
Cd^{2+}	2.44 ± 0.07	4.01 ± 0.06		4.99 ± 0.10		1.57 ± 0.10	
Pb^{2+}	3.05 ± 0.20	4.14 ± 0.10		5.47 ± 0.23		1.09 ± 0.23	

[a] The acidity constants of the dinucleotide[17] are $pK^H_{H_2[d(pGpG)]} = 2.98 \pm 0.13$ [(N7)H$^+$; Eq. (2)], $pK^H_{H[d(pGpG)]} = 6.56 \pm 0.03$ [$P(O)_2(OH)^-$; Eq. (3)], $pK^H_{d(pGpG)} = 9.54 \pm 0.08$ [(N1)H; Eq. (4)], and $pK^H_{d(pGpG-H)} = 10.11 \pm 0.14$ [(N1)H];[b] these values are in part needed for the calculations based on Equations (10)–(13). [b] The error limits given are three times the standard error of the mean value (3σ) or the sum of the probable systematic errors, whichever is larger. The error limits of the derived data, in the present case for columns 5–8, were calculated according to the error propagation according to Gauss.

Because any kind of chelate formation must lead to an enhanced complex stability,[26–28,35] it is necessary to determine the stability of the open isomer in Equilibrium (14). This stability constant cannot be measured directly, although estimates can be made based on the stability of $M(dGuo)^{2+}$ complexes. The stability constants for the $M(dGuo)^{2+}$ complexes[36–38] listed in column 3 of Table 2 need to be corrected for the charge effect that a divalent metal ion at N7 experiences due to the negatively charged $P(O)_2(OH)^-$ group. This charge effect amounts to 0.40 ± 0.15 log units, as determined in various other cases where the distances between the positive and negative charges are of a comparable size.[39,40] In other words, the indicated correction leads to an estimate of the stability constants of the open forms of the phosphate-monoprotonated $M(H;dGMP)^+$ complexes with M^{2+} at N7; these values are listed in column 4 of Table 2.

One could argue that the small basicity difference between N7 in dGuo and N7 in $H(dGMP)^-$ also needs to be considered. However, we have ignored this effect (it amounts at the most to 0.1 log units; see footnote [i] of Table 2) because it is within the given error limits and also because the effect of the bridging phosphate group, which carries a single negative charge, is possibly slightly overestimated by the following value: the charge effect that an M^{2+} ion bound at the phosphate group in $M(pUpU)^-$ experiences due to the negatively charged phosphate bridge is well defined by 0.24 ± 0.04 log units.[27] The charge effect of the same bridge on M^{2+} at N7 is not known, but because there are also eight bonds between the relevant atoms (see Figure 1), one may assume that it is close to 0.24 log units as well (possibly a bit smaller because the considered atoms are on opposite sites of the ribose "plane"). Hence, by adding 0.24 ± 0.04 to the values listed in column 4 of Table 2, one obtains the estimated stability constants given in column 5 for the open isomers of the M[H;d(pGpG)] species. Now we are in a position to compare the measured stability constants of the M[H;d(pGpG)] complexes with the estimated ones due to the open isomers according to Equation (15):

$$\log \Delta_{M/H;d(pGpG)} = \log K^{M}_{M[H;d(pGpG)]} - \log K^{M}_{M[H;d(pGpG)]_{op}} \qquad (15)$$

The corresponding results are listed in the final column of Table 2. Despite the large error limits (3σ values are given), it is clear that in a number of cases an enhanced stability is definitely observed, which means that macrochelate formation according to Equilibrium (14) occurs. Of course, one could argue that not only macrochelate formation of the N7-bound metal ion with the $P(O)_2(OH)^-$ group occurs, but that also (N7)M(N7) macrochelates form. In this case the closed species in Equilibrium (14) would encompass both isomers. Indeed, that the (N7)M(N7) isomers exist to some degree cannot be excluded completely, although it seems to us that these must be minor species if the results given below for $M(H;dGMP)^+$ as well as the overall properties of the M^{2+}/d(pGpG) systems are considered. Indeed, phosphate-involving macrochelate formation was also confirmed for the $M[d(pGpG)]^-$ complexes discussed in Sections 2.4 and 2.5, and no hint of (N7)M(N7) chelates was obtained in studies[22] of GpG and d(GpG) systems involving divalent metal ions of the present kind.

Table 2. Stability-constant comparisons[a] for the M[H;d(pGpG)] complexes between the measured stability constants ($K^{M}_{M[H;d(pGpG)]}$),[b] which encompass all isomers of Equilibrium (14), and the estimated ones for the open $M[H;d(pGpG)]_{op}$ isomers ($K^{M}_{M[H;d(pGpG)]_{op}}$)[c] that are based on the stabilities of the $M(dGuo)^{2+}$ complexes (column 3) corrected for the charge effects of the terminal $P(O)_2(OH)^-$ group ($K^{M}_{M(H;dGMP)_{op}}$)[d] and the bridging PO_2^- unit to give the values listed in column 5 ($K^{M}_{M[H;d(pGpG)]_{op}}$) (aqueous solution; 25 °C; $I = 0.1$ M, $NaNO_3$).

M^{2+}	$\log K^{M}_{M[H;d(pGpG)]}$ [Eq. (5)][b]	$\log K^{M}_{M(dGuo)}$	$\log K^{M}_{M(H;dGMP)_{op}}$ [d]	$\log K^{M}_{M[H;d(pGpG)]_{op}}$ [e]	$\log \Delta_{M/H;d(pGpG)}$ [Eq. (15)]
Mg^{2+}	1.17 ± 0.10	0.35 ± 0.25[f]	0.75 ± 0.29	0.99 ± 0.29	0.18 ± 0.31
Cu^{2+}	3.3 ± 0.3[j]	2.12 ± 0.14[g]	2.52 ± 0.21	2.76 ± 0.21	0.54 ± 0.37
Zn^{2+}	2.30 ± 0.07	1.16 ± 0.11[h]	1.56 ± 0.19	1.80 ± 0.19	0.50 ± 0.20
Cd^{2+}	2.44 ± 0.07	1.53 ± 0.07[f]	1.93 ± 0.17	2.17 ± 0.17	0.27 ± 0.18
Pb^{2+}	3.05 ± 0.20	1.31 ± 0.18[i]	1.71 ± 0.23	1.95 ± 0.23	0.10 ± 0.30

[a] For the error limits see footnote [b] of Table 1. [b] These values are taken from column 2 of Table 1. [c] See Section 2.3. [d] The charge effect of the $P(O)_2(OH)^-$ group on a metal ion coordinated at N7 amounts to 0.40 ± 0.15 log units (see Section 2.3) and therefore the resulting complex stabilities refer to the open isomers of the $M(H;dGMP)^+$ species. [e] The additional charge effect of the bridging $P(O)_2^-$ unit amounts to 0.24 ± 0.04 log units (see Section 2.3). [f] Taken from ref. [22]. [g] Taken from ref. [36]. [h] Based on the measured[37] stability difference between the $Cd(Guo)^{2+}$ [37b] and $Zn(Guo)^{2+}$ [37a,b] complexes, that is, $\log K^{Cd}_{Cd(Guo)} - \log K^{Zn}_{Zn(Guo)} = (1.17 \pm 0.06) - (0.80 \pm 0.06) = 0.37 \pm 0.08$, which is expected to be the same for the $M(dGuo)^{2+}$ species, one obtains for the stability of the $Zn(dGuo)^{2+}$ complex $\log K^{Zn}_{Zn(dGuo)} = \log K^{Cd}_{Cd(dGuo)} - (0.37 \pm 0.08) = (1.53 \pm 0.07) - (0.37 \pm 0.08) = 1.16 \pm 0.11$. [i] An estimate based on the stability of $Pb(Guo)^{2+}$, $\log K^{Pb}_{Pb(Guo)} = 1.25 \pm 0.17$,[38] and the basicity difference[17] of N7 (i.e., $\Delta pK_a = pK^{H}_{H(dGuo)} - pK^{H}_{H(Guo)} = (2.30 \pm 0.04) - (2.11 \pm 0.04) = 0.19 \pm 0.06$, which gives with the slope $m = 0.32$ (average of m for Cu^{2+}, Zn^{2+}, and Cd^{2+})[24] for the $\log K^{Pb}_{PbL}$ versus pK^{H}_{HL} plot a stability increase of 0.06 ± 0.06 log units), gives for the stability constant of $Pb(dGuo)^{2+}$, $\log K^{Pb}_{Pb(dGuo)} = (1.25 \pm 0.17) + (0.06 \pm 0.06) = 1.31 \pm 0.18$. [j] This value is an estimate based on $\log K^{Cu}_{Cu[H;d(pGpG)]_{op}} + \log \Delta_{Zn/H;d(pGpG)} = (2.76 \pm 0.21) + (0.50 \pm 0.20) = 3.3 \pm 0.3$.

Following previous methods,[27,35,38] the calculation of $\log \Delta_{M/H;d(pGpG)}$ values [Eq. (15); Table 2, column 6] allows us to define the position of the intramolecular Equilibrium (14); that is, to calculate values for the dimension-less equilibrium constant $K^{*}_{I/H}$ according to Equation (16):

$$K^{*}_{I/H} = \frac{[M[H;d(pGpG)]_{cl}]}{[M[H;d(pGpG)]_{op}]} \qquad (16a)$$

$$= \frac{K^{M}_{M[H;d(pGpG)]}}{K^{M}_{M[H;d(pGpG)]_{op}}} - 1 \qquad (16b)$$

$$= 10^{\log \Delta_{M[H;d(pGpG)]}} - 1 \qquad (16c)$$

Once $K^{*}_{I/H}$ is known, the formation degree or the percentage of the macrochelated or closed species in Equilibri-

um (14) follows from Equation (17):

$$\% \, M[H;d(pGpG)]_{cl} = 100 \cdot K^*_{I/H}/(1 + K^*_{I/H}) \quad (17)$$

These formation degrees are listed in column 4 of Table 3.

Despite the large error limits it is evident that several of these metal ions are able to form macrochelates (possibly in part in an outer-sphere manner). In the case of Mg^{2+} and Pb^{2+} these values also encompass a formation degree of zero, thereby indicating that with these two metal ions, especially in the case of Pb^{2+}, most likely only small amounts, if any, of macrochelates are present according to Equilibrium (14). Indeed, Pb^{2+} is a special case, as is also evident from the results described in Sections 2.4 and 2.5.

Table 3. Extent of macrochelate formation in M[H;d(pGpG)] complexes [Eq. (14)] as calculated from the stability enhancement $\log \Delta_{M/H;d(pGpG)}$ [Eq. (15)] and quantified by the dimensionless equilibrium constant $K^*_{I/H}$ [Eq. (16)] and the percentage of the macrochelated isomers $M[H;d(pGpG)]_{cl}$ [Eq. (17)] in aqueous solution (25 °C; $I = 0.1$ M, $NaNO_3$).[a]

M^{2+}	$\log \Delta_{M/H;d(pGpG)}$	$K^*_{I/H}$	% $M[H;d(pGpG)]_{cl}$
Mg^{2+}	0.2 ± 0.3[b]	~0.58	~37 (~0/68)[c]
Cu^{2+}	0.55 ± 0.35[b]	2.55 (0.58/6.94)[d]	72 (37/87)[d]
Zn^{2+}	0.50 ± 0.20	2.16 ± 1.45	68 ± 15
Cd^{2+}	0.27 ± 0.18	0.86 ± 0.77	46 ± 22
Pb^{2+}	0.1 ± 0.3	~0.26	~21 (~0/60)[c]

[a] For the error limits see footnote [b] of Table 1. The values in the second column are from the final column in Table 2. [b] These values from Table 2 are rounded to the nearest 0.00/0.05 log units. [c] These estimates can be zero within their error limits; therefore, the upper and lower limits are given in parentheses. [d] The upper and lower limits which do *not* encompass zero are given in parentheses.

Interestingly, the constants listed in column 4 of Table 2 for the open $M(H;dGMP)^+_{op}$ isomer now allow an interpretation of values published earlier[29] for the stabilities of the $M(H;dGMP)^+$ complexes with Cu^{2+} and Zn^{2+}. One obtains:

$$\log \Delta_{Cu/H;dGMP} = \log K^{Cu}_{Cu(H;dGMP)} - \log K^{Cu}_{Cu(H;dGMP)_{op}} \quad (18a)$$

$$= (2.81 \pm 0.06) - (2.52 \pm 0.21) = 0.29 \pm 0.22 \quad (18b)$$

$$\log \Delta_{Zn/H;dGMP} = \log K^{Zn}_{Zn(H;dGMP)} - \log K^{Zn}_{Zn(H;dGMP)_{op}} \quad (19a)$$

$$= (1.76 \pm 0.06) - (1.56 \pm 0.19) = 0.20 \pm 0.20 \quad (19b)$$

Application of these $\log \Delta_{M/H;dGMP}$ values (error limit: 3σ) to the evaluation procedure defined by Equations (16) and (17) yields $K^*_{I/H} = 0.95$ for the $Cu(H;dGMP)^+$ system and a formation degree of approximately 50% for the macrochelate. The corresponding value for the $Zn(H;dGMP)^+$ system is $K^*_{I/H} = 0.58$, that is, about 40% formation of $Zn(H;dGMP)^+_{cl}$.

In conclusion, in spite of all the shortcomings the results of this section prove that metal ions like Cu^{2+}, Zn^{2+}, and Cd^{2+} may span the distance from N7 of a guanine residue to a terminal oxygen atom of the corresponding bridging phosphate group in nucleic acids and thus give rise to macrochelate formation. It should be noted that the charges occurring in the discussed systems correspond to those within a nucleic acid strand.

2.4. Proof of an enhanced stability of the $M[d(pGpG)]^-$ complexes: The primary metal-ion-binding site of $d(pGpG)^{3-}$ is clearly the phosphate group at the 5′ end (see Figure 1). Hence, it is necessary to define the stability of such a pure PO_3^{2-}/M^{2+} interaction because any additional interaction, either with the neighboring phosphate diester bridge or the N7 unit of a guanine residue, as indicated in Equilibrium (1), must lead to an increased complex stability.[27,28,35] This can be done by applying the previously defined straight-line correlations,[39] which are based on $\log K^M_{M(R\text{-}PO_3)}$ versus $pK^H_{H(R\text{-}PO_3)}$ plots for simple phosphate monoesters[41] and phosphonates.[39] These ligands are abbreviated as $R\text{-}PO_3^{2-}$, where R represents a noncoordinating residue. The parameters for these straight-line equations, that is, the slopes m and the intercepts b with the y axis, as defined by Equation (20), have been tabulated before.[27,39] Hence, with a known pK_a value for the deprotonation of a $P(O)_2(OH)^-$ group the expected stability constant can be calculated for any phosphate-M^{2+} complex.

$$\log K^M_{M(R\text{-}PO_3)} = m \cdot pK^H_{H(R\text{-}PO_3)} + b \quad (20)$$

Plots of $\log K^M_{M(R\text{-}PO_3)}$ versus $pK^H_{H(R\text{-}PO_3)}$, according to Equation (20), are shown in Figure 2 for the 1:1 complexes of Mg^{2+}, Zn^{2+}, and Pb^{2+}, with the (empty) data points of the eight simple ligand systems[39,41] used for the determination of the straight reference lines.[27,39] The data points due to the $M[d(pGpG)]^-$ complexes and also those due to the $M(pUpU)^-$ species, which have been included in Figure 2 for comparison, are clearly above their reference lines, thus proving an increased complex stability for all cases. The stability increase observed varies considerably from metal ion to metal ion. The increase is larger for $Zn[d(pGpG)]^-$ than for $Mg[d(pGpG)]^-$, but in both cases the stability enhancements are more pronounced than those of the corresponding $M(pUpU)^-$ species, thereby indicating that the two dinucleotides behave differently. This contrasts with the observations made for Pb^{2+}, where the complexes with both dinucleotides are also considerably more stable than expected for a sole phosphate binding, although the stability enhancements of more than one log unit each are approximately of the same order.

A quantitative evaluation of the situation seen in Figure 2 is possible by applying the mentioned straight-line parameters together with $pK^H_{H[d(pGpG)]} = 6.56 = pK^H_{H(R\text{-}PO_3)}$ to the straight-line Equation (20). The results of these calculations (Table 4, column 3) represent the stability constants of $M(R\text{-}PO_3)$ complexes in which no additional interaction occurs, that is, the metal ion is coordinated only to a phosphate group that has the basicity of the terminal phosphate

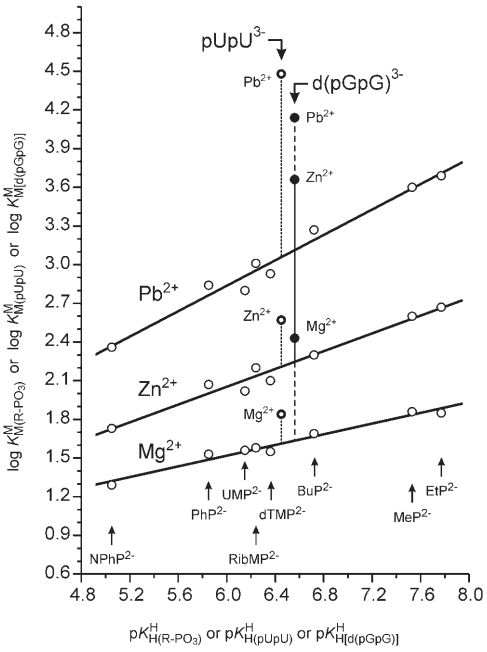

Figure 2. Evidence for an enhanced stability of the M[d(pGpG)]$^-$ complexes of Mg^{2+}, Zn^{2+}, and Pb^{2+}; for comparison the data points for the corresponding M(pUpU)$^-$ systems are also given. The enhanced complex stability follows from the relationship between $\log K^{M}_{M(R\text{-}PO_3)}$ and $pK^{H}_{H(R\text{-}PO_3)}$ for M(R-PO$_3$) complexes of some simple phosphate monoester and phosphonate ligands (R-PO$_3^{2-}$): (from left to right) 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-}). The least-squares lines [Eq. (20)] are drawn through the corresponding eight data sets[39,41] according to the straight-line parameters listed in Table 3 of reference [27]. The data points due to the M^{2+}/H^+/d(pGpG)$^{3-}$ systems are based on the constants listed in Table 1, and those for the corresponding pUpU^{3-} systems are from reference [27]. The vertical full (Zn^{2+}) and broken (Pb^{2+}, Mg^{2+}) lines emphasize the stability differences from the reference lines, $\log \Delta_{M/d(pGpG)}$, as defined in Equation (21) (see also Table 4, column 4). The vertical dotted lines describe the corresponding situation for the pUpU^{3-} systems. All plotted equilibrium constants refer to aqueous solutions at 25 °C and $I = 0.1$ M ($NaNO_3$).

$$\log \Delta_{M/d(pGpG)} = \log K^{M}_{M[d(pGpG)]} - \log K^{M}_{M(R\text{-}PO_3)} \quad (21)$$

What is the basis for such enhanced affinities? The stability increases of the various M^{2+} complexes with d(pGpG)$^{3-}$ vary significantly. They are smallest for Mg^{2+} and largest for Zn^{2+} and Cd^{2+} (the latter two being identical within their error limits). This contrasts with the previously investigated pUpU^{3-} complexes of Mg^{2+}, Mn^{2+}, and Cd^{2+}, where the corresponding stability enhancements are identical within their error limits.[27] Considering the different coordinating properties[42] of these three metal ions, this must mean, and this was concluded previously,[27] that the increased stability with pUpU^{3-} is simply due to a charge effect on going from M(R-PO$_3$) to M(pUpU)$^-$. In other words, the metal ion coordinated to the terminal phosphate group in pUpU^{3-} "feels" the presence of the negative charge located on the neighboring phosphate diester bridge.

This charge effect is represented by the average of the $\log \Delta_{M/pUpU}$ values for the Mg^{2+}, Mn^{2+}, and Cd^{2+} systems ($\log \Delta_{M/pUpU/charge} = 0.24 \pm 0.04$).[27] Of course, the charge effect of the bridging phosphate diester unit is expected to be the same in pUpU^{3-} and d(pGpG)$^{3-}$. Hence, any further stability increase observed for M[d(pGpG)]$^-$ complexes must be attributed to an *additional* interaction of the metal ion already coordinated to the terminal phosphate group of d(pGpG)$^{3-}$. This further increase is defined by Equation (22):

$$\log \Delta^{*}_{M/d(pGpG)/cor} = \log \Delta_{M/d(pGpG)} - \log \Delta_{M/pUpU/charge} \quad (22a)$$

$$= \log \Delta_{M/d(pGpG)} - (0.24 \pm 0.04) \quad (22b)$$

The corresponding results are listed in column 5 of Table 4.

It is revealing to compare these corrected stability enhancements for the M[d(pGpG)]$^-$ complexes with those observed[27] for the M(pUpU)$^-$ species (the latter values are given in the final column of Table 4). In this comparison it must be kept in mind that the uracil residues do not participate in metal-ion binding in the M(pUpU)$^-$ complexes[27] and that therefore any increased complex stability must be attributed to an interaction of the phosphate-coordinated metal ion with the neighboring phosphate diester bridge, which gives rise to the formation of a 10-membered chelate. Hence, from the comparison of the data in columns 5 and 6 in Table 4 it follows that in the Mg^{2+} and Cd^{2+} complexes of d(pGpG)$^{3-}$ a guanine-N7 interaction must take place; the same is true for Zn[d(pGpG)]$^-$, but in this case some interaction with the phosphate diester bridge is also to be expected, as the value for $\log \Delta^{*}_{Zn/pUpU}$ re-

Table 4. Comparison of the stability constants for the M[d(pGpG)]$^-$ complexes between the measured stability constants [Eq. (6)] and the calculated stability constants for M(R-PO$_3$) species, based on the reference-line Equation (20) and the basicity of the terminal phosphate group of d(pGpG)$^{3-}$ ($pK^{H}_{H[d(pGpG)]} = 6.56$), together with the stability differences $\log \Delta_{M/d(pGpG)}$ [Eq. (21)] as well as the corrected stability enhancements $\log \Delta^{*}_{M/d(pGpG)/cor}$ as defined by Equation (22); the corresponding values for the M(pUpU)$^-$ complexes are given for comparison (25 °C; $I = 0.1$ M, $NaNO_3$).[a]

M^{2+}	$\log K^{M}_{M[d(pGpG)]}$	$\log K^{M}_{M(R\text{-}PO_3)}$	$\log \Delta_{M/d(pGpG)}$	$\log \Delta^{*}_{M/d(pGpG)/cor}$	$\log \Delta^{*}_{M/pUpU/cor}$
Mg^{2+}	2.43 ± 0.06	1.64 ± 0.03	0.79 ± 0.07	0.55 ± 0.08	−0.01 ± 0.06
Zn^{2+}	3.66 ± 0.05	2.25 ± 0.06	1.41 ± 0.08	1.17 ± 0.09	0.13 ± 0.08
Cd^{2+}	4.01 ± 0.06	2.56 ± 0.05	1.45 ± 0.08	1.21 ± 0.09	−0.01 ± 0.06
Pb^{2+}	4.14 ± 0.10	3.11 ± 0.08	1.03 ± 0.13	0.79 ± 0.14	1.16 ± 0.26

[a] For the error limits see footnote [b] of Table 1. The values in the second column are from column 3 in Table 1 and those in the final column are from Table 5 in reference [27].

group in d(pGpG)$^{3-}$. Comparison of these data with the measured stability constants (Table 4, column 2) according to Equation (21) yields enhanced stabilities for all four complexes studied.

veals. From a comparison of the data for $Pb[d(pGpG)]^-$ and $Pb(pUpU)^-$ it follows that here the corrected stability enhancements overlap within the error limits and that therefore in both cases only the mentioned 10-membered chelates involving the two phosphates are formed.

To conclude, for Mg^{2+} and Cd^{2+} the upper pathway of Equilibrium (1), which leads to the formation of $M[d(pGpG)]^-_{cl/N7}$ macrochelates, operates, whereas in the case of Pb^{2+} only the lower pathway, which gives the 10-membered $Pb[d(pGpG)]^-_{cl/PO}$ chelate, is of relevance. The truly interesting situation occurs with $Zn[d(pGpG)]^-$ because here all species shown in Equilibrium (1) are formed.

2.5. The extent of chelate formation in the M[d(pGpG)]$^-$ complexes: The charge-corrected stability enhancements $\log \Delta^*_{M/d(pGpG)/cor}$, as discussed in the preceding section and listed in column 5 of Table 4, reflect all additional interactions that a metal ion coordinated to the terminal phosphate group in $d(pGpG)^{3-}$ experiences according to Equilibrium (1). In other words, it encompasses the total (tot) amounts of all chelated complexes. Hence, the definition given in Equation (23) holds:

$$[M[d(pGpG)]^-_{cl/tot}] = [M[d(pGpG)]^-_{cl/N7}] + [M[d(pGpG)]^-_{cl/PO}] \quad (23)$$

Therefore, in analogy to Equations (16) and (17), and in agreement with Equilibrium (1), one can define Equations (24) and (25):

$$K_{I/tot} = \frac{[M[d(pGpG)]^-_{cl/tot}]}{[M[d(pGpG)]^-_{op}]} = \frac{[M[d(pGpG)]^-_{cl/N7}] + [M[d(pGpG)]^-_{cl/PO}]}{[M[d(pGpG)]^-_{op}]} \quad (24a)$$

$$= 10^{\log \Delta^*_{M/d(pGpG)/cor}} - 1 \quad (24b)$$

$$\%\, M[d(pGpG)]^-_{cl/tot} = 100 \cdot K_{I/tot}/(1 + K_{I/tot}) \quad (25)$$

Of course, knowledge of $\%\, M[d(pGpG)]^-_{cl/tot}$ also allows us to calculate the formation degree of the open species, $\%\, M[d(pGpG)]^-_{op}$, by subtracting the former from 100%. The results for $K_{I/tot}$, $\%\, M[d(pGpG)]^-_{cl/tot}$, and $\%\, M[d(pGpG)]^-_{op}$ are listed in Table 5 in columns 3, 4, and 5, respectively.

On the basis of Equilibrium (1), the experimentally accessible overall stability constant, as defined in Equation (6b), may be rewritten[39,43] as Equation (26), which includes the definitions in Equations (27)–(29):

$$K^M_{M[d(pGpG)]} = \frac{[M[d(pGpG)]^-]}{[M^{2+}][d(pGpG)^{3-}]} \quad (6b)$$

$$= \frac{[M[d(pGpG)]^-_{op}] + [M[d(pGpG)]^-_{cl/N7}] + [M[d(pGpG)]^-_{cl/PO}]}{[M^{2+}][d(pGpG)^{3-}]} \quad (26a)$$

$$= K^M_{M[d(pGpG)]_{op}} + K_{I/N7} \cdot K^M_{M[d(pGpG)]_{op}} + K_{I/PO} \cdot K^M_{M[d(pGpG)]_{op}} \quad (26b)$$

$$= K^M_{M[d(pGpG)]_{op}} (1 + K_{I/N7} + K_{I/PO}) \quad (26c)$$

$$K^M_{M[d(pGpG)]_{op}} = \frac{[M[d(pGpG)]^-_{op}]}{[M^{2+}][d(pGpG)^{3-}]} \quad (27)$$

$$K_{I/N7} = [M[d(pGpG)]^-_{cl/N7}]/[M[d(pGpG)]^-_{op}] \quad (28)$$

$$K_{I/PO} = [M[d(pGpG)]^-_{cl/PO}]/[M[d(pGpG)]^-_{op}] \quad (29)$$

Equation (30) then follows from Equations (24) and (26):

$$K_{I/tot} = K_{I/N7} + K_{I/PO} \quad (30)$$

Of course, if one of the closed isomers, for example, $M[d(pGpG)]^-_{cl/PO}$ [Eq. (1)] is not formed, $K_{I/PO}$ [Eq. (29)] becomes zero and Equation (30) reduces to $K_{I/tot} = K_{I/N7}$, that is, to a two-isomer problem, as discussed in Section 2.3.

If three isomers are formed according to Equilibrium (1), $K_{I/tot}$ and the concentration fractions of $M[d(pGpG)]^-_{cl/tot}$ and $M[d(pGpG)]^-_{op}$ can still be determined [Eqs. (24) and (25)] as shown above (Table 5, columns 3–5). It can be assumed that the $K_{I/PO}$ values of the $M[d(pUpU)]^-_{cl/PO}$ species[27] represent the stabilities of the $M[d(pGpG)]^-_{cl/PO}$ isomers well because both 10-membered chelates contain a structurally identical phosphate backbone. Thus, $K_{I/N7}$ can be calculated

Table 5. Charge-corrected stability enhancements, $\log \Delta^*_{M/d(pGpG)/cor}$, for the formation of the isomeric species $M[d(pGpG)]^-_{op}$, $M[d(pGpG)]^-_{cl/N7}$, and $M[d(pGpG)]^-_{cl/PO}$ [see Eq. (1)], together with the percentages in which the isomers occur in aqueous solution at 25 °C and $I = 0.1$ M ($NaNO_3$).[a]

M^{2+}	$\log \Delta^*_{M/d(pGpG)/cor}$ [Eq. (22)]	$K_{I/tot}$ [Eqs. (24, 30)][b]	$\%\, M[d(pGpG)]^-_{cl/tot}$ [Eq. (25)][b]	$\%\, M[d(pGpG)]^-_{op}$ [Eqs. (1, 24a)][c]	$K_{I/N7}$ [Eqs. (28, 30)]	$K_{I/PO}$ [Eqs. (29,30)]	$\%\, M[d(pGpG)]^-_{cl/N7}$ [Eq. (28)]	$\%\, M[d(pGpG)]^-_{cl/PO}$ [Eq. (29)]
Mg^{2+}	0.55 ± 0.08	2.55 ± 0.65	71.8 ± 5.2	28.2 ± 5.2	2.6 ± 0.7[d]		72 ± 5.5[d]	
Zn^{2+}	1.17 ± 0.09	13.791 ± 3.065	93.24 ± 1.40	6.76 ± 1.40	13.44 ± 3.08[e]	0.35 ± 0.25[f]	91 ± 2.5[g]	2.4 ± 1.8[g]
Cd^{2+}	1.21 ± 0.09	15.22 ± 3.36	93.8 ± 1.3	6.2 ± 1.3	15.2 ± 3.4[d]		94 ± 1.5[d]	
Pb^{2+}	0.79 ± 0.14	5.17 ± 1.99	83.8 ± 5.2	16.2 ± 5.2		5.2 ± 2.0[d]		84 ± 5.5[d]

[a] For the error limits see footnote [b] of Table 1. The values in the second column are from column 5 in Table 4. [b] These values follow from the given equations. [c] These values follow from $100 - \%\, M[d(pGpG)]^-_{cl/tot}$. [d] Rounded values from columns 3 and 4. [e] Calculated from $K_{I/tot}$ and $K_{I/PO}$ according to Equation (30). [f] This value refers to $Zn(pUpU)^-$ and is taken from reference [27]; see also Section 2.5. [g] $\%\, M[d(pGpG)]_{cl/PO}$ was calculated from $K_{I/PO}$ and $\%\, M[d(pGpG)]^-_{op}$ [Eq. (29)]. The value for $\%\, M[pGpG)]_{cl/N7}$ follows from the difference $\%\, M[d(pGpG)]^-_{cl/tot} - \%\, M[d(pGpG)]^-_{cl/PO}$; it may also be calculated from Equation (28) with $K_{I/N7}$ and $\%\, M[d(pGpG)]^-_{op}$. The result is the same for both calculation methods, but the error limits are understandably larger for the second method.

from Equation (30), and hence the formation degrees of all of the isomers become known (Table 5).

By comparison with the properties of $M(pUpU)^-$ species it was concluded in the last two paragraphs of Section 2.4 that for $Mg[d(pGpG)]^-$ and $Cd[d(pGpG)]^-$ only the upper pathway of Equilibrium (1) operates and that in these two cases the increased complex stabilities are completely due to macrochelate formation with N7, that is, only the species $M[d(pGpG)]^-_{cl/N7}$ form. Therefore, the rounded values calculated for "tot" are repeated in columns 6 and 8 of Table 5 under the heading $K_{I/N7}$ and % $M[d(pGpG)]^-_{cl/N7}$. The formation degrees of the macrochelated complexes of Mg^{2+} and Cd^{2+} with about 72% and 94% in this dinucleotide are quite large. However, for Mg(dGMP) it is also known that a macrochelate with a formation degree of about 40% exists;[29] similarly, for Cd(GMP) a formation degree of about 84% is reached for the macrochelate.[44,45] All these results prove that both metal ions, in the case of Mg^{2+} possibly partly in an outer-sphere manner,[28] are able to bridge the distance between a phosphate group and an N7 site in a GMP unit. This is of relevance for the interaction of these two metal ions with nucleic acids.

The affinity of Pb^{2+} toward N7 of a purine residue is known to be small,[38] and, indeed, at the end of Section 2.4 it was concluded that the enhanced complex stability of $Pb[d(pGpG)]^-$ is solely due to an interaction of the phosphate-bound Pb^{2+} with the neighboring phosphate diester bridge. Therefore, the results in columns 3 and 4 of Table 5 are repeated in rounded form in columns 7 and 9, respectively. The formation degree of 84 ± 6% of this 10-membered chelate is, within the error limits, the same as that observed previously for $Pb(pUpU)^-$ (93 ± 4%).[27] These results are in agreement with the known high affinity of Pb^{2+} for O-donor sites.[42]

Zn^{2+} is known to be a chameleon-type metal ion,[46] and, indeed, all isomers of Equilibrium (1) occur for $Zn[d(pGpG)]^-$ (see end of Section 2.4 and Table 5). Although its formation degree of about 2.4% is small, it is clear that Zn^{2+} can form the 10-membered $Zn[d(pGpG)]^-_{cl/PO}$ chelate. The domination of the $Zn[d(pGpG)]^-_{cl/N7}$ species, with about 91%, is in agreement with the large formation degree of the N7-macrochelate formed by Zn(dGMP), that is, $Zn(dGMP)_{cl/N7}$ amounts to 86 ± 3%. It should be mentioned that a reduction of the local intrinsic dielectric constant, as can occur in the folds of nucleic acids,[33] will favor the $Zn[d(pGpG)]^-_{cl/PO}$ species, which is formed by an ionic-type interaction.

3. Conclusions

This study of $M[d(pGpG)]^-$ complexes and comparison of their properties with those of $M(pUpU)^-$ species has revealed the individual binding properties of the various metal ions to nucleic acids. Mg^{2+}, Zn^{2+}, and Cd^{2+} are able to span the N7–phosphate distance in a GMP unit, and correspondingly they all form the $M[d(pGpG)]^-_{cl/N7}$ isomer. These results are corroborated by the observations made with the monoprotonated M[H;d(pGpG)] species. Note that in the latter complexes an interaction of the N7 coordinated metal ion occurs with the $P(O)_2(OH)^-$ residue and in this residue the two terminal oxygen atoms carry together one negative charge unit. This means that this situation corresponds to that of a phosphate diester bridge, as occurs in nucleic acids. Hence, metal-ion bridging of a GMP unit in nucleic acids is expected to occur to some extent with the mentioned metal ions.

For Pb^{2+}, the 10-membered chelate bridging two neighboring phosphate residues is an important species, and this is most likely[26] also true for Ca^{2+}. Zn^{2+} can form both types of isomers, with a preference for the N7 one [Eq. (1)] and Mn^{2+} is expected to behave similarly and to also form both isomers. The intensity of the guanine–N7 interaction decreases in the order $(Cu^{2+}) > Cd^{2+} \sim Zn^{2+} > Mg^{2+}$ (Sections 2.3 and 2.5), in agreement with theoretical calculations[47] and other experimental data.[28,44]

That metal ions are needed for many reactions of nucleic acids, especially ribozymes, is well known.[33] It is also known that different metal ions have different effects.[48] However, small dinucleotides may also need metal ions for their biological interplay. For example, the cyclic dimer of GMP, c-di-GMP, plays a critical role in bacterial cell signaling and is thereby hydrolyzed to pGpG and finally to GMP by phosphodiesterases;[49] these hydrolysis reactions are strongly Mg^{2+}-dependent.

4. Experimental Section

4.1. Materials: The synthesis of the trisodium salt of $d(pGpG)^{3-}$ was described recently[17] and the same batch of this compound was used here. The sources of the other materials and reagents have also been given previously and the $M(NO_3)_2$ and NaOH stock solutions were prepared as before.[27]

The aqueous stock solutions of d(pGpG) were freshly prepared daily and the pH of the solutions was adjusted close to 8.0 with sodium hydroxide. The exact concentration of the ligand solutions was determined in each experiment by evaluation of the corresponding titration pair, that is, the differences in NaOH consumption between solutions with and without ligand (see below).

4.2. Potentiometric pH titrations: The pH titrations were carried out with the reported equipment, which was calibrated as described.[27] The given acidity constants (25 °C; $I=0.1$ M, $NaNO_3$) are so-called practical, mixed, or Brønsted constants,[27,50] which may be converted into the corresponding concentration constants by subtracting 0.02 from the listed pK_a values.[50] The stability constants of the complexes presented are, as usual, concentration constants.

4.3. Determination of the equilibrium constants: The acidity constants of $H_2[d(pGpG)]^-$ were determined by titrating 30 mL of aqueous 0.5 mM HNO_3 (25 °C; $I=0.1$ M, $NaNO_3$) under N_2 with up to 3.5 mL of 0.02 M NaOH in the presence and absence of 0.15 mM $d(pGpG)^{3-}$.[17]

Because only small amounts of d(pGpG) were available to us, at the end of each of the above titrations a small volume (about 0.8 mL) of 0.1 M HNO_3 was added to the solutions to give an initial pH of about 3.3. A further comparatively small volume of a solution of $M(NO_3)_2$ ($M^{2+}=Zn^{2+}$, Cd^{2+}) was subsequently added and the titrations were repeated. The total volume of these solutions was about 35 mL, with an ionic strength of between 0.095 and 0.10 M. The stability constants of the Mg^{2+}

and Pb^{2+} systems were determined under the same conditions as the acidity constants, but $NaNO_3$ was partly (Pb^{2+}) or fully (Mg^{2+}) replaced by $M(NO_3)_2$ (25 °C; $I = 0.1$ M). The metal-to-ligand ratios in the various titrations were close to 222:1 for Mg^{2+}, 41:1 and 27:1 for Zn^{2+}, 27:1 and 24:1 for Cd^{2+}, and 2.5:1 for Pb^{2+}. The calculated stability constants for the M^{2+} complexes, where two different metal ion concentrations were employed, showed no dependence on the excess of M^{2+} used. Equally importantly, the fitting procedures of the experimental data gave no indication of the formation of $M_2[H;d(pGpG)]^{2+}$ or other 2:1 M^{2+}:ligand species.

The stability constants of the various complexes were calculated as described by curve-fitting procedures using a Newton–Gauss non-linear least-squares program.[27] The titration data were evaluated every 0.1 pH units in the accessible pH range, the upper limit being determined by the hydrolysis of $M(aq)^{2+}$, which was evident from the titrations without ligand. Representative examples for the pH ranges employed are 3.6–8.0 for the Mg^{2+} and 3.3–5.9 for the Zn^{2+}, Cd^{2+}, and Pb^{2+} systems. The final results for the stability constants are the averages of two independent titrations in the case of the Zn^{2+} and Cd^{2+} systems, whereas for Mg^{2+} and Pb^{2+} only one titration could be performed due to the lack of ligand.

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