A stability concept for metal ion coordination to single-stranded nucleic acids and affinities of individual sites

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Abstract: The three-dimensional architecture and function of nucleic acids strongly depend on the presence of metal ions, among other factors. Given the negative charge of the phosphate-sugar backbone, positively charged species, mostly metal ions, are necessary for compensation. However, these ions also allow and induce folding of complicated RNA structures. Furthermore, metal ions bind to specific sites, stabilizing local motifs and positioning themselves correctly to aid (or even enable) a catalytic mechanism, as, for example, in ribozymes. Many nucleic acids thereby exhibit large differences in folding and activity depending not only on the concentration but also on the kind of metal ion involved. As a consequence, understanding the role of metal ions in nucleic adds requires knowing not only the exact positioning and coordination sphere of each specifically bound metal ion but also its intrinsic site affinity. However, the quantification of metal ion affinities toward certain sites in a single-stranded (though folded) nucleic acid is a demanding task, and few experimental data exist. In this Account, we present a new tool for estimating the binding affinity of a given metal ion, based on its ligating sites within the nucleic acid. To this end, we have summarized the available affinity constants of Mg2+, Ca2+, Mn2+, Cu2+, Zn2+, Cd2+, and Pb2+ for binding to nucleobase residues, as well as to mono- and dinucleotides. We have also estimated for these ions the stability constants for coordinating the phosphodiester bridge. In this way, stability increments for each ligand site are obtained, and a dear selectivity of the ligating atoms, as well as their discrimination by different metal ions, can thus be recognized. On the basis of these data, we propose a concept that allows one to estimate the intrinsic stabilities of nucleic acid-binding pockets for these metal ions. For example, the presence of a phosphate group has a much larger influence on the overall affinity of Mg2+, Ca2+, or Mn2+ compared with, for example, that of Cd2+ or Zn2+. In the case of Cd2+ and Zn2+, the guanine N7 position is the strongest intrinsic binding site. By adding up the individual increments like building blocks, one derives an estimate not only for the overall stability of a given coordination sphere but also for the most stable complex if an excess of ligating atoms is available in a binding pocket saturating the coordination sphere of the metal ion. Hence, this empirical concept of adding up known intrinsic stabilities, like building blocks, to an estimated overall stability will help in understanding the accelerating or inhibiting effects of different metal ions in ribozymes and DNAzymes.

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A Stability Concept for Metal Ion Coordination to Single-Stranded Nucleic Acids

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CONSPECTUS

The three-dimensional architecture and function of nucleic acids strongly depends, among other factors, on the presence of metal ions. Most importantly, metal ions, needed to compensate the negative charge of the phosphate-sugar backbone, allow and induce folding of complicated RNA structures. On the other hand, metal ions bind to specific sites to stabilize local motifs and to be positioned correctly to aid in, or even enable, the catalytic mechanism of, e.g., ribozymes. Many nucleic acids thereby exhibit large differences with regard to folding and activity based not only on the concentration but also on the nature of the metal ion applied. As a consequence, to understand the role of metal ions in nucleic acids, it is not only necessary to know the exact positioning and coordination sphere of each specifically bound metal ion, but also its intrinsic site affinity. However, the quantification of metal ion affinities to certain sites in a single-stranded (though folded) nucleic acid is a demanding task and only a few experimental data exist. In this Account we present a new tool to estimate the binding affinity of a given metal ion based on its coordinating and ligating sites within the nucleic acid: To this end we

have summarized the available affinity constants of Mg^{2+}, Ca^{2+}, Mn^{2+}, Cu^{2+}, Zn^{2+}, Cd^{2+} or Pb^{2+} for their binding to nucleobase residues, mono- and dinucleotides, and we have also estimated those of the phosphodiester bridge. In this way stability increments for each liganding site are obtained and a clear selectivity of the ligating atoms as well as their discrimination by different metal ions can thus be recognized. Based on these data we propose a concept that allows to estimate the intrinsic stabilities of nucleic acid-binding pockets for the mentioned metal ions. For example, the presence of a phosphate group has a much larger influence on the overall affinity of Mg^{2+}, Ca^{2+}, or Mn^{2+} compared to, e.g., Cd^{2+} or Zn^{2+}. In the case of the latter two metal ions, the guanine N7 position is the strongest intrinsic binding site. By adding up the individual increments like building blocks, one receives an estimate not only for the overall stability of a given coordination sphere, but also for the most stable complex if an excess of ligating atoms is available in a binding pocket saturating the coordination sphere of the metal ion. Hence, the here described empirical concept of adding up known intrinsic stabilities, like building blocks, to an estimated overall stability will help to understand the accelerating or inhibiting effects of different metal ions in ribozymes and DNAzymes.

1. Introduction

Nucleic acids are macromolecules carrying a negative charge due to their phosphate diester backbone. Consequently, the nucleotide units present require an equal amount of cations. These are mostly Na^+, K^+ and Mg^{2+}, though some other divalent metal ions or positively charged organic residues may also be present. The "intensity" of the interaction between metal ions and nucleic acids can be described by adsorption isotherms which provide kind of "averaged" binding constants.[1] Yet such "average" affinities are not very helpful because, e.g., Mg^{2+} may be directly involved in the three-dimensional folding of a nucleic acid and in ribozymes it may participate in the reaction process;[2,3] hence, such metal ions are bound to distinct sites but their affinities have hardly been quantified.

It was Szent-Györgyi who (to the best of our knowledge) first pointed out more than 50 years ago the relevance of nucleobase-metal ion interactions by proposing a Mg^{2+}-chelate of ATP (Figure 1A).[4]

Figure 1

This structure contains severe shortcomings:[5] (i) At the physiological pH of about 7.5, especially if a metal ion is coordinated, the triphosphate group no longer carries any protons. (ii) For the present more important: $(C6)NH_2$ of the adenine residue does not bind metal ions. This group has no basic but rather acidic properties, i.e., with pK_a about 17 it may release a proton.[6] (iii) Of a more subtle nature: Binding of Mg^{2+} to N7 does not occur significantly innersphere but rather outersphere, i.e., a coordinated H_2O forms a hydrogen bond to N7.[5,7] A more realistic coordination pattern is shown in Figure 1B,C.[5,7,8] Nevertheless, Szent-Györgyi's structure[4] had a tremendous influence because it triggered the interest of biochemists and coordination chemists in the metal ion-binding properties of nucleotides,[8,9] and thus indirectly also in nucleic acids.

Evidently, nucleobase residues must be involved in the formation of metal ion-binding pockets as they occur, e.g., in ribozymes. Therefore, we propose an empirical stability concept for metal ion binding to single-stranded nucleic acids and summarize first the M^{2+} affinities of nucleobases towards Mg^{2+}, Ca^{2+}, Mn^{2+}, Cu^{2+}, Zn^{2+}, Cd^{2+}, and Pb^{2+} as they follow from measurements with nucleosides. This allows to develop affinity sequences for the nucleobases shown in Figure 2. Next, the M^{2+} affinity of

Figure 2

the phosphodiester bridge was estimated and integrated into the sequences. The stability increments obtained in this way were thereafter complemented by data obtained from the M^{2+}-binding properties of dinucleotides. These results are then extrapolated to obtain estimates of the stability constants for binding pockets in nucleic acids.

2. Metal Ion-Affinity Sequences for Nucleobase Residues

The nucleotide units of DNA contain mostly adenine, guanine, cytosine, and thymine. RNA has the same residues except that thymine is replaced by the closely related uracil. The two purine and the cytosine residues contain imidazole- and pyridine-type nitrogens well suited for M^{2+} binding (Figure 2). There is nothing the like in uracil or thymine as long as (N3)H is not deprotonated and this requires exceptional conditions at physiological pH.[10] Hence, uracil and thymine offer commonly only their carbonyl groups which bind M^{2+} very weakly with the assistance of a primary binding site.[10,11]

As a consequence we need to consider the M^{2+}-binding properties of the adenine, guanine, and cytosine residues. These are best quantified as their nucleosides (Ns) according to equilibrium 1:

$$M^{2+} + Ns \rightleftharpoons M(Ns)^{2+} \quad (1a)$$

$$K^{M}_{M(Ns)} = [M(Ns)^{2+}]/([M^{2+}][Ns]) \quad (1b)$$

Table 1 summarizes the results, mostly obtained via potentiometric pH titrations.[12–18]

Table 1

The nucleoside-complex stability decreases in the order Guo > Cyt > Ado for all seven M^{2+} considered. The problem is that these complexes are formed with neutral nucleosides, i.e., the effect that the negative charge of the phosphodiester bridge, RO-P(O)$_2^-$-OR', exercises on M^{2+} coordinated at a nucleobase residue is not considered. A correction is possible by using as mimics phosphate-monoprotonated nucleoside 5'-monophosphate complexes with M^{2+} at the nucleobase residue, $(M{\cdot}NMP{\cdot}H)^+$ (eq 2):

$$M^{2+} + NMP{\cdot}H^- \rightleftharpoons (M{\cdot}NMP{\cdot}H)^+ \quad (2a)$$

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

Consequently, the stability constants of the $M(Ns)^{2+}$ complexes (Table 1) need to be corrected (i) for the small difference in basicity between the Ns and $(NMP\cdot H)^{-}$ species,[7] and (ii) for the charge effect of the phosphodiester bridge. The latter is well represented by the RO-$P(O)_2^-$-OH residue in the $(M\cdot NMP\cdot H)^{+}$ complexes and amounts to 0.40±0.15 log unit.[19,20] The corrected values for the $M(Ns)^{2+}$ complexes representing now the stabilities of the "open" $(M\cdot NMP\cdot H)^{+}$ species are listed in Table 2; note, no macrochelate formation involving the monoprotonated phosphate group is considered.[15]

Table 2

The so-called micro stability constants (*k*) of Table 2 quantify the M^{2+} affinities of nucleobases and the affinity sequences summarized in Figure 3 (lower part; black values) evolve from these constants. Though the absolute sizes of the values differ for the various M^{2+}, the order within the sequences remains the same. However, the affinity change from site to site may differ much depending on the M^{2+} involved, thus indicating selectivity.

3. Inclusion of the Phosphodiester Bridge into the Affinity Sequences for Nucleic Acids

Unfortunately no stability constants of relevant phosphodiesters are known. Therefore we estimated stability constants for the M^{2+} considered here in three ways:

(i) As the charge of a phosphate diester, $(RO)_2PO_2^-$, corresponds to that of formate, HCO_2^-, and acetate, $CH_3CO_2^-$, their complex-stability constants[21,22] gave one data series.

(ii) Next, we extrapolated the known $\log K^{M}_{M(R\text{-}PO_3)}$ versus $pK^{H}_{H(R\text{-}PO_3)}$ straight-line plots[8,11,18,20] which hold for phosphate monoesters, $R\text{-}PO_3^{2-}$, in the pK_a range from about 4.5 to 8, to $pK_a = 1$, the approximate acidity constant[23] of a phosphate diester, giving another set of constants.

(iii) Finally, we used the stability differences between complexes formed with diphosphate monoesters ($R\text{-}DP^{3-}$) and their monoprotonated form, $\log K^{M}_{M(R\text{-}DP)} - \log K^{M}_{M(H;R\text{-}DP)}$.[24] These logarithmic differences reflect the effect of the proton and were subtracted from the stability constants,

$\log K^{M}_{M(R\text{-}PO_3)}$ (valid for pK_a = 6.2),[8,11,18,20] of phosphate monoesters.

The three sets of stability constants turned out to be rather similar for a given M^{2+} allowing us to average the values (though in part weighted). The estimated log stability constant for $M[(RO)_2PO_2]^+$ of Mg^{2+} is $\log K^{Mg}_{Mg[(RO)_2PO_2]} = 0.45$; for the other M^{2+} they are: 0.4 (Ca^{2+}), 0.7 (Mn^{2+}), 1.0 (Cu^{2+}), 0.7 (Zn^{2+}), 0.8 (Cd^{2+}), and 0.9 (Pb^{2+}) (25° C; I = 0.1M). That these values are reasonable is confirmed by a previous estimate for $Pb[ROP(O)_2OH]^+$ ($\log K^{Pb}_{Pb[ROP(O)_2OH]} = 0.7 \pm 0.4$)[16] and also by the stability constant measured[25] for the Ni^{2+} complex of monoprotonated D-ribose 5-monophosphate, log $K^{Ni}_{Ni(H;RibMP)} = 0.7$, because the stability of Ni^{2+}-phosphate complexes is commonly similar to those with Mn^{2+} and Zn^{2+}.[9]

However, there is another point: Compared to the individual nucleobases present in a nucleic acid, the phosphodiester bridge occurs in excess because with each nucleobase a diester bridge is connected. If one assumes, to make matters easy to handle, that the four main nucleobases of RNA and DNA occur in about equal amounts, then the anionic phosphodiester bridge has a 4-fold excess. Application of this statistical factor leads to eq 3:

$$\log k = \log K^{M}_{M[(RO)_2PO_2]} + 0.6 \qquad (3)$$

The resulting micro stability constants quantifying the affinity of the phosphodiester bridge, in combination with the stability data in Table 2, provide the individual log affinity constants of the various sites as given in Figure 3 (upper part). In the lower part the affinity sequences for each M^{2+} are

Figure 3

given for the five nucleobases and the single-charged phosphodiester bridge: For Mg^{2+}, Ca^{2+}, and Mn^{2+} this negatively charged phosphate bridge has a higher affinity than any of the nucleobases. For Zn^{2+} the affinity of the guanine and the phosphate unit are relatively similar, whereas e.g., Cu^{2+} has a much higher affinity towards guanine and cytosine. This selectivity is of high relevance for the properties of nucleic acids in the presence of metal ions and it explains, e.g., why Cu^{2+} penetrates into the double helix of DNA in contrast to Mg^{2+}.[26]

4. Neighboring Phosphate Units Stabilize Metal Ion Binding!

So far we considered only the M^{2+} affinity of individual binding sites. However, experience[7,8,27] teaches that nucleotide units may interact with M^{2+} via more than just one coordinating atom (Figure 1). If neighboring nucleotide units are present, this should even be more true, but so far quantitative studies only exist for two dinucleotides (Figure 4).[9,15,23]

Figure 4

Because the uracil residue has no remarkable M^{2+} affinity,[24,28] as long as (N3)H is not deprotonated,[10] one expects M^{2+} binding to $pUpU^{3-}$ at the terminal phosphate group with the potential to form 10-membered chelates involving the neighboring phosphodiester bridge. Since any additional interaction of the terminal phosphate-coordinated M^{2+} must be reflected in an increased complex stability[29] it is best to insert the results[23] for the M^{2+}/$pUpU^{3-}$ systems into straight-line plots of $\log K^{M}_{M(R\text{-}PO_3)}$ versus $pK^{H}_{H(R\text{-}PO_3)}$, which hold for simple phosphate monoester and phosphonate ligands.[8,11,20,23]

Figure 5

In Figure 5 the negatively charged $M(pUpU)^-$ complexes for Mg^{2+}, Zn^{2+}, and Pb^{2+}show an increased stability compared to their straight reference lines representing uncharged $M(R\text{-}PO_3)$ species. With the known parameters for the straight-line plots, the expected stabilities based on $pK^{H}_{H(pUpU)}$ = 6.44±0.02 can be calculated.[23] These values are listed in Table 3 (column 3) together with the observed

Table 3

stability enhancements (column 4) as defined in eq 4, where $(d)pNpN^{3-}$ represents any dinucleotide:

$$\log \Delta_{M/(d)pNpN} = \log K^{M}_{M(d)pNpN} - \log K^{M}_{M(R\text{-}PO_3)} \qquad (4)$$

These differences are all positive but those for the Mg^{2+}, Mn^{2+}, and Cd^{2+} complexes are identical within their error limits. Considering that the binding affinities toward liganding sites differ considerably[7,21,22] for these three metal ions (column 2), the present result can only mean that this stability enhancement, on average $\log \Delta_{M/pUpU/charge} = 0.24\pm0.04$,[23] is the reflection of the charge at the neighboring phosphodiester unit, which M^{2+} coordinated to the terminal phosphate group "feels". Hence, only

$Zn(pUpU)^-$ and $Pb(pUpU)^-$ are additionally stabilized (Table 3, column 4).

A stability enhancement beyond 0.24 log unit must result from an additional interaction,[29] i.e., chelate formation according to equilibrium 5 (op = open; cl = closed/chelated):

$$M[(d)pNpN]^-_{op} \rightleftharpoons M[(d)pNpN]^-_{cl} \quad (5)$$

This extra stability enhancement is generally defined by eq 6a, and for $pUpU^{3-}$ by eq 6b:

$$\log \Delta^*_{M/(d)pNpN} = \log \Delta_{M/(d)pNpN} - \log \Delta_{M/(d)pUpU/charge} \quad (6a)$$

$$\log \Delta^*_{M/pUpU} = \log \Delta_{M/pUpU} - (0.24 \pm 0.04) \quad (6b)$$

The position of the intramolecular equilibrium 5 (see ref 23) is given by the dimensionless constant K_I of eq 7,

$$K_I = [M[(d)pNpN]^-_{cl}]/[M[(d)pNpN]^-_{op}] = 10^{\log\Delta^*} - 1 \quad (7)$$

and the formation degree of the closed species by eq 8:

$$\% \; M[(d)pNpN]^-_{cl} = 100 \cdot K_I/(1 + K_I) \quad (8)$$

Formation degrees of about 26 and 93% for the 10-membered chelates follow from these considerations for $Zn(pUpU)^-$ and $Pb(pUpU)^-$ (Table 3, lower part). Among the five systems for which data exist[23] only these two can with certainty form a chelate between two neighboring phosphate groups. For the Mg^{2+}, Mn^{2+}, and Cd^{2+} complexes the formation degrees are zero within the error limits, yet, due to these errors small amounts (up to 15%)[23] of the chelated species could still exist.

5. Studies of a Dinucleotide Allowing Macrochelate Formation

Macrochelate formation of guanosine 5'-phosphates involving N7 is wellknown[7–9] and therefore also expected for the dinucleotide $d(pGpG)^{3-}$ (Figure 4) as well as within a general GG sequence. Indeed, increased complex stabilities for the $M[d(pGpG)]^-$ complexes of Mg^{2+}, Zn^{2+}, and Pb^{2+} are observed (Figure 5).[15] Comparison of the experimentally obtained stability constants for the four systems studied (Table 4, column 2; defined in analogy to eq 1) with the values calculated for the $M(R\text{-}PO_3)$ complexes

Table 4

(column 3; based on $pK^H_{H[d(pGpG)]} = 6.56 \pm 0.03$)[15] gives according to equation 4 the stability differences

listed in column 4. Correction of these values for the charge effect (eq 6) results in the stability enhancements (column 5). These values are significantly higher for the complexes of Mg^{2+}, Zn^{2+}, and Cd^{2+} compared to those of the corresponding $M(pUpU)^-$ species (column 6), except for $Pb[d(pGpG)]^-$ which shows a lower stability enhancement.[15]

From columns 5 and 6 of Table 4 it follows that Mg^{2+} and Cd^{2+} in $M[d(pGpG)]^-$ form only macrochelates with N7 (see also below) and no indication for the formation of the 10-membered chelate with the neighboring phosphodiester bridge exists. Pb^{2+} only forms the latter chelate, whereas for $Zn[d(GpG)]^-$ the equilibria depicted in Figure 6 exist.[15] The formation degrees of the various

Figure 6

species were calculated by applying known procedures;[20] the results[15] are summarized in Table 5 together with estimations for $Ca[d(pGpG)]^-$. However, regarding the $M[d(pGpG)]^-_{cl/N7}$ formation, the

Table 5

above view is a simplification because two N7 positions of the two pre-oriented guanine residues (via stacking) in $M[d(pGpG)]^-$ are present (see also Figure 6, legend).[27,30] This is in accord with a comparison of the data summarized in Table 6.[31]

Table 6

The stability enhancements $\log \Delta^*_{M/d(pGpG)}$ of the $M[d(pGpG)]^-$ complexes are on average by about 0.3 log unit larger than the ones determined for M(dGMP), $\log \Delta_{M/dGMP}$ (Table 6, column 4). Since the possibility of intranucleotide macrochelate formation of the phosphate-coordinated M^{2+} with guanine-N7 (possibly involving (C6)O in an outersphere manner)[9] is identical in both complexes, the additional stability increase by 0.3 log unit of $M[d(pGpG)]^-$ means that a further interaction must occur with the neighboring nucleotide. Self-stacking of guanine residues is wellknown (e.g.,[7]) and thus, may also be expected in $M[d(pGpG)]^-$ as it occurs in GpG^- (see in ref 30). Clearly, this leads to an orientation of the $d(pGpG)^{3-}$ ligand, giving thus rise to a second N7, possibly even (C6)O, interaction (Figure 7)[32,33]. Indeed, similar interactions are found with Mg^{2+} in an x-ray structure of a large

Figure 7

ribosomal subunit.[32] In other words, an additional interaction of the M^{2+} coordinated at the terminal phosphate group in $M[d(pGpG)]^-$ occurs involving in total three or even four sites.

To summarize, the stability enhancements of column 4 in Table 6 hold for a single intranucleotide macrochelate formation within $M[d(pGpG)]^-$. Instead, the larger values of column 2 apply for a three-/four-point interaction involving the neighboring GMP unit (see Figure 7). If chelate formation in nucleic acids is considered, in both instances the charge effect of 0.24±0.04 needs to be added (Table 3, lower part). A so-called three-/four-point interaction thereby denotes a coordination pattern involving one phosphate and two N7 sites as well as possibly one (C6)O as fourth liganding atom, irrespective if mediated by a water molecule or not.

The mentioned orientation of the guanines due to stacking within $M[d(pGpG)]^-$ is also responsible for the reduced stability of $Pb[d(pGpG)]^-$ compared to $Pb(pUpU)^-$ (Table 4, columns 5,6). In the latter case the dinucleotide can freely rotate around the bridging phosphodiester and thus adjusts easily to the steric requirements for the formation of the 10-membered chelate. With $d(pGpG)^{3-}$ the same kind of Pb^{2+} binding is inhibited because the intramolecular stack needs first to be "broken" or at least reduced to allow formation of the 10-membered chelate. This is a nice example how metal ion coordination may enforce a structural change in a nucleic acid.

6. Application of the Summarized Stability Increments to Nucleic Acids

In many nucleic acids a metal ion is coordinated to a phosphodiester bridge (e.g., Figure 7) and we propose now, that if one wants to consider, e.g., Mg^{2+} binding to a phosphodiester unit and a nucleobase, one needs to add up the two increments of 1.05 for initial phosphate binding (Figure 3) and 0.79 for the nucleobase coordination (Table 4, column 4) resulting in a three-/four-point interaction, giving the intrinsic stability constant for a pGpG site of 1.8 log units. However, a hexacoordinating Mg^{2+} still has two to three further binding sites. For statistical reasons and steric constraints we estimate that the stability increment is about 0.5 log units smaller (i.e., about 3/5 from 0.79). This gives an overall micro stability constant of 2.3 log units, which agrees perfectly with recent experimental

data:[34] The different Mg^{2+}-binding sites present in branch-domain 6 of a group II intron ribozyme vary in their affinities between 2.14±0.03 and 2.38±0.06. This variation possibly indicates that the coordination sphere of Mg^{2+} is not always completely filled by RNA-binding sites.

Similarly, the stability increments for Cd^{2+} would be 1.4 (phosphate unit; Figure 3) plus 1.45 (nucleobase; Table 4, column 4), giving a micro stability constant of about 2.85 log units for a three-/four-point interaction. If further coordination sites are used, another approximately 0.85 log units (i.e., about 3/5 from 1.45) may be added giving in total an intrinsic stability constant of about 3.7 log units for a fully coordinated Cd^{2+} in a nucleic acid. These values agree closely with recent experimental results obtained for a group II intron ribozyme:[35] The log stability constants for the binding of four Cd^{2+} to specific regions within domain 5 range from 2.80±0.12 to 3.6±0.2.[35]

Should an experimental value be smaller than the sum of the increments considered here, this is an indication that not all coordination sites of M^{2+} are filled by ligating sites of the nucleic acid. In addition: (i) We estimate that the approximate error limit for the sum of the stability increments for a given binding pocket is about ±0.3 log unit. (ii) Less important, the values used are partly a combination of ribose and 2'-deoxyribose data because no other stability constants are available. However, we believe that this shortcoming is of relatively little influence and only on the order of about ±0.1 log unit (see Table 4 in ref 27). Hence, the estimated values for a DNA-binding pocket may actually be enlarged and those for an RNA pocket reduced by about 0.1 log unit. This difference can be attributed to the higher hydrophobicity of DNA compared with RNA.[27]

Unfortunately, the known stability increments that need to be added to the stability constant due to the M^{2+}-phosphodiester interaction (Figure 3) are limited to Mg^{2+}, Zn^{2+}, and Cd^{2+} (Table 4, column 4). However, it may be noted that the stability enhancement, $\log \Delta_{Mg/d(pGpG)} = 0.79\pm0.07$ is very close to $\log k = 0.76\pm0.29$ estimated for the guanine-N7/Mg^{2+} interaction (Table 2, column 2, and Figure 3) (note, these two values have different dimensions). The values for Zn^{2+} also agree roughly within their error limits, i.e., $\log \Delta_{Zn/d(pGpG)} = 1.41\pm0.08$ (Table 4, column 4) versus $\log k = 1.66\pm0.19$ (Figure 3 and Table 2, column 2). The agreement of the Cd^{2+} values is poorer but still not totally off, i.e., log

$\Delta_{Cd/d(pGpG)}$ = 1.45±0.08 (Table 4, column 4) versus log k = 2.04±0.17 (Figure 3 and Table 2); hence, in the absence of log $\Delta_{M/d(pGpG)}$ values we recommend to apply the affinity values given in Figure 3.[36]

For example, for Mn^{2+} binding to a phosphodiester unit together with a three-/four-point interaction involving a GG unit one obtains approximately 2.35 log units [1.3 (phosphate) + 1.05 (guanine); Figure 3] for the intrinsic micro stability constant and for a more saturated coordination sphere (plus 0.65; i.e., 3/5 from 1.05) 3.0 log units. Should only a single macrochelate form, the value would be about 2.1 log units (2.35–0.3; 0.3 log unit being the difference following from columns 2 versus 4 in Table 6).

7. Mg^{2+} Binding in the Ribosome. Some Statistical Considerations

The large ribosomal subunit of *Haloarcula morismortui* was analyzed at a resolution of 2.4 Å with respect to its metal ion-binding sites.[32] This subunit comprises 3045 nucleotides and thus the same number of negative charges. In total 88 Na^+/K^+ could be identified and 116 Mg^{2+} were localized in the structure determination. This means, 320 negative charges are neutralized corresponding to about 10.5% of the phosphodiester-bridge charges. Furthermore, the 116 Mg^{2+} ions identified represent only a small fraction (7.6%) of the divalent metal ions needed for charge compensation; i.e., the overwhelming part of these ions is only very loosely bound and not fixed in a certain site. However, the relatively tightly bound cations are crucial for the structural stability and reactivity of the RNA.

Only two of the 116 localized Mg^{2+} in this RNA subunit are exclusively protein-bound, but 106 interact with the phosphates in accord with the prediction of Figure 3. From the in total 116 Mg^{2+} 26 form an innersphere 10-membered chelate with a neighboring phosphate bridge. This corresponds to about 22% which is somewhat above the upper limit of 15% estimated for aqueous solution (Section 4). However, in such a complicated RNA fold as the ribosome, hydrogen bonding and stacking between the building blocks add steric restraints and rigidity to the backbone, increasing the chance of Mg^{2+} binding to two (or more) phosphates. The basic affinity of these 26 Mg^{2+} to two neighboring phosphate units amounts to about log k = 1.4, as deduced from the above established increments [1.05

(phosphate; Figure 3) + 0.24 (charge; Table 3) + 0.1 (chelate)].

Interestingly, 50 Mg^{2+} are bound to at least one phosphate moiety and any one N7 position. Out of these are 30 contacts of an intranucleotide type involving guanine residues. Based on the 106 phosphate-bound Mg^{2+} this corresponds to a formation degree of 28% for macrochelates. This is astonishingly close to the 31±7% found[27] in solution for $Mg(GMP)_{cl/N7}$. Again, the stability of such intramolecular macrochelates (two-point interaction) can now be estimated to be about log k = 1.55 [1.05 (phosphate; Figure 3) + 0.79 (Table 4) – 0.3 (terminating sentence of Section 6)]. Any further interaction with the RNA will lead to an increased stability, as is expected for 10 Mg^{2+} exhibiting a three-point macrochelation (Figure 7) giving the estimated affinity constant log k = 1.85 [1.05 (phosphate; Figure 3) + 0.79 (Table 4; column 4)].

There are also 22 intranucleotide macrochelates involving an adenine residue but none contains N3 of a cytosine residue. This is understandable because in the more stable[17] *anti* conformation N3 of cytosine points away from a metal ion at the phosphodiester bridge. Interestingly, the ratio of 50 Mg^{2+} at gua-N7 to 22 Mg^{2+} at ade-N7 equals 2.3, which is very close to the ratio of the corresponding stability constants of 2.5 [$10^{0.75}$:$10^{0.35}$ (Figure 3)].

The carbonyl oxygens of the pyrimidine nucleobases can only be considered as minor binding sites: no innersphere coordination of cytosine-O2 to Mg^{2+} could be detected, and only one to uracil-O2 and three to uracil-O4. If one also takes outersphere coordination into account, uracil-O4 remains the major binding site among the pyrimidine carbonyls. 25 Mg^{2+} coordinate through a water molecule to uracil-O4, six to uracil-O2, and nine to cytosine-O2.

8. Conclusions and Outlook

In this Account we have first summarized the available quantitative information regarding M^{2+} binding to nucleobase residues. Next, we estimated stability constants for M^{2+} coordination to the phosphodiester bridge and by taking into account the simultaneous binding of a metal ion to various sites as it can occur in dinucleotides, we developed an empirical concept that allows to estimate

intrinsic (or micro) stability constants for "cavities" formed by nucleic acids.

Comparison with the few available experimental data allowed to verify the concept which will be helpful to predict stability constants for given micro environments within large nucleic acids. However, the outlined concept will need adjustments and fine tuning, e.g., by actually measuring the various M^{2+} affinities of phosphate diesters and by studying complex stabilities of dinucleotides like $pGpU^{3-}$, $pUpG^{3-}$, $pGpA^{3-}$, and $pApG^{3-}$. Yet, from the examples given, it is evident that the concept of adding up stability increments works and provides the intrinsic (or micro) stability constant of a certain cavity in a nucleic acid with a reasonable accuracy.

Overall, the concept allows conclusions about which metal ions preferably bind at which sites of a nucleic acid, and in which cases the coordination sphere of a metal ion bound to a nucleic acid is only partially saturated. Finally, the concept should be helpful in improving our understanding of the interactions and equilibria between metal ions and single stranded nucleic acids, and consequently especially towards RNAs and ribozymes.

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BIOGRAPHICAL INFORMATION

Roland K. O. Sigel is Associate Professor at the University of Zürich (Institute of Inorganic Chemistry). He received his Ph.D. (with distinction) from the University of Dortmund, Germany (Bernhard Lippert). After nearly three years at Columbia University, New York (Anna Marie Pyle), he was appointed as Assistant Professor (2003–2008) at the University of Zürich endowed with a *Förderungsprofessur* from the Swiss National Science Foundation. He is the recipient of the 2008 EuroBIC Medal and also of the Werner Award (2009) of the Swiss Chemical Society. His research interests focus on the structure and metal ion-binding properties of large ribozymes (group II introns), riboswitches, and also DNA.

Helmut Sigel, Emeritus Professor at the University of Basel (Department of Chemistry, Inorganic Chemistry), endowed with numerous honors, has a longstanding interest in metal ion-nucleotide interactions.

Both authors have formerly edited together with Astrid Sigel the series *Metal Ions in Biological Systems* and since 2006 they are editing the *Metal Ions in Life Sciences* series, now published by the Royal Society of Chemistry (Cambridge, UK).

FOOTNOTES

*Both authors contributed equally to this Account and correspondence may be addressed to either of them. In fact, father and son had a lot of fun in the writing process and learned much from each other. Emails: <roland.sigel@aci.uzh.ch> <helmut.sigel@unibas.ch>

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REFERENCES

1 Bregadze, V. G. Metal Ion Interactions with DNA: Considerations on Structure, Stability, and Effects from Metal Ion Binding. *Met. Ions Biol. Syst.* **1996**, *32*, 419–451.

2 Sigel, R. K. O.; Pyle, A. M. Alternative Roles for Metal Ions in Enzyme Catalysis and the Implication for Ribozyme Chemistry. *Chem. Rev.* **2007**, *107*, 97–113.

3 Sigel, R. K. O. Group II Intron Ribozymes and Metal Ions - A Delicate Relationship. *Eur. J. Inorg. Chem.* **2005**, *12*, 2281–2292.

4 Szent-Györgyi, A. Mechanochemical Coupling in Muscle. In *Enzymes: Units of Biological Structure and Function*; Gaebler, O. H., Ed.; Academic Press: New York, 1956; pp 393–397.

5 Sigel, H. Isomeric Equilibria in Complexes of Adenosine 5'-Triphosphate with Divalent Metal Ions - Solution Structures of $M(ATP)^{2-}$ Complexes. *Eur. J. Biochem.* **1987**, *165*, 65–72.

6 Lippert, B. Alterations of Nucleobase pK_a Values Upon Metal Coordination: Origins and Consequences. *Prog. Inorg. Chem.* **2005**, *54*, 385–443.

7 Sigel, H.; Griesser, R. Nucleoside 5'-Triphosphates: Self-association, Acid-Base, and Metal Ion-Binding Properties in Solution. *Chem. Soc. Rev.* **2005**, *34*, 875–900.

8 Sigel, H.; Song, B. Solution Structures of Nucleotide-Metal Ion Complexes. Isomeric Equilibria. *Met. Ions Biol. Syst.* **1996**, *32*, 135–206.

9 Sigel, R. K. O.; Sigel, H. Complex Formation of Nickel(II) and Related Metal Ions with Sugar Residues, Nucleobases, Phosphates, Nucleotides, and Nucleic Acids. *Met. Ions Life Sci.* **2007**, *2*, 109–180.

10 Knobloch, B.; Linert, W.; Sigel, H. Metal Ion-Binding Properties of (N3)-Deprotonated Uridine, Thymidine, and Related Pyrimidine Nucleosides in Aqueous Solution. *Proc. Natl. Acad. Sci. USA* **2005**, *102*, 7459–7464.

11 Sigel, H.; Kapinos, L. E. Quantification of Isomeric Equilibria for Metal Ion Complexes Formed in Solution by Phosphate or Phosphonate Ligands with a Weakly Coordinating Second Site. *Coord. Chem. Rev.* **2000**, *200–202*, 563–594.

12 Da Costa, C. P.; Sigel, H. 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. *Inorg. Chem.* **2003**, *42*, 3475–3482.

13 Kapinos, L. E.; Song, B.; Sigel, H. Acid-Base and Metal Ion-Coordinating Properties of Benzimidazole and Derivatives (=1,3-Dideazapurines) in Aqueous Solution: Interrelation Between Complex Stability and Ligand Basicity. *Chem. Eur. J.* **1999**, *5*, 1794–1802.

14 Song, B.; Zhao, J.; Griesser, R.; Meiser, C.; Sigel, H.; Lippert, B. Effects of (N7)-Coordinated

Nickel(II), Copper(II), or Platinum(II) on the Acid-Base Properties of Guanine Derivatives and Other Related Purines. *Chem. Eur. J.* **1999**, *5*, 2374–2387.

15 Knobloch, B.; Sigel, H.; Okruszek, A.; Sigel, R. K. O. 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. *Chem. Eur. J.* **2007**, *13*, 1804–1814.

16 Da Costa, C. P.; Sigel, H. Lead(II)-Binding Properties of the 5'-Monophosphates of Adenosine (AMP^{2-}), Inosine (IMP^{2-}), and Guanosine (GMP^{2-}) in Aqueous Solution. Evidence for Nucleobase-Lead(II) Interactions. *Inorg. Chem.* **2000**, *39*, 5985–5993.

17 Knobloch, B.; Sigel, H. A Quantitative Appraisal of the Ambivalent Metal Ion-Binding Properties of Cytidine in Aqueous Solution and an Estimation of the *Anti-Syn* Energy Barrier of Cytidine Derivatives. *J. Biol. Inorg. Chem.* **2004**, *9*, 365–373.

18 Da Costa, C. P.; Sigel, H. Stabilities of Complexes Formed Between Lead(II) and Simple Phosphonate or Phosphate Monoester Ligands Including some Pyrimidine-Nucleoside 5'-Monophosphates (CMP^{2-}, UMP^{2-}, $dTMP^{2-}$). *J. Biol. Inorg. Chem.* **1999**, *4*, 508–514.

19 Bastian, M.; Sigel, H. Stability and Structure of Binary and Ternary Metal Ion Complexes of Orotidinate 5'-Monophosphate (OMP^{3-}) in Aqueous Solution. *J. Coord. Chem.* **1991**, *23*, 137–154.

20 Sigel, H.; Chen, D.; Corfù, N. A.; Gregáň, F.; Holý, A.; Strášak, M. Metal Ion-Coordinating Properties of Various Phosphonate Derivatives, Including 9-[2-(Phosphonylmethoxy)ethyl]adenine (PMEA), an Adenosine Monophosphate (AMP) Analogue with Antiviral Properties. *Helv. Chim. Acta* **1992**, *75*, 2634–2656.

21 *IUPAC Stability Constants Database*; Release 5, Version 5.16; compiled by Pettit, L. D. and Powell, H. K. J.; Academic Software: Timble, Otley, West Yorkshire, U.K., 2001

22 *NIST Critically Selected Stability Constants of Metal Complexes* **2001**, Reference Database 46, Version 6.4 (data collected and selected by Smith, R. M. and Martell, A. E.), US Department of Commerce, National Institute of Standards and Technology, Gaithersburg, MD, USA.

23 Knobloch, B.; Suliga, D.; Okruszek, A.; Sigel, R. K. O. Acid-Base and Metal Ion-Binding Properties of the RNA-Dinucleotide Uridylyl-(5'→3')-[5']uridylate ($pUpU^{3-}$). *Chem. Eur. J.* **2005**, *11*, 4163-4170.

24 Sajadi, S. A. A.; Song, B.; Gregáň, F.; Sigel, H. Acid-Base and Metal Ion-Coordinating Properties of Pyrimidine-Nucleoside 5'-Diphosphates (CDP, UDP, dTDP) and of Several Simple Diphosphate Monoesters. Establishment of Relations between Complex Stability and Diphosphate Basicity. *Inorg. Chem.* **1999**, *38*, 439–448.

25 Thomas, J. C.; Frey, C. M.; Stuehr, J. E. Interactions of Divalent Metal Ions with Inorganic and Nucleoside Phosphates. 7. Kinetics of the Ni(II)-P_i, -RibP, and -CMP Systems. *Inorg. Chem.* **1980**, *19*, 501–504.

26 Sigel, H. Catalase and Peroxidase Activity of Cu^{2+} Complexes. *Angew. Chem. Internat. Edit.* **1969**, *8*, 167–177.

27 Freisinger, E.; Sigel, R. K. O. From Nucleotides to Ribozymes – A Comparison of Their Metal Ion-Binding Properties. *Coord. Chem. Rev.* **2007**, *251*, 1834–1851.

28 Massoud, S. S.; Sigel, H. Metal Ion-Coordinating Properties of Pyrimidine-Nucleoside 5'-Monophosphates (CMP, UMP, TMP) and of Simple Phosphate Monoesters, Including D-Ribose 5-Monophosphate. Establishment of Relations Between Complex Stability and Phosphate Basicity. *Inorg. Chem.* **1988**, *27*, 1447–1453.

29 Sigel, H.; Massoud, S. S.; Corfù, N. A. Comparison of the Extent of Macrochelate Formation in Complexes of Divalent Metal Ions with Guanosine (GMP^{2-}), Inosine (IMP^{2-}), and Adenosine 5'-Monophosphate (AMP^{2-}). The Crucial Role of N7 Basicity in Metal Ion-Nucleic Base Recognition. *J. Am. Chem. Soc.* **1994**, *116*, 2958–2971.

30 Knobloch, B.; Okruszek, A.; Sigel, H. Inosylyl(3'→5')inosine (IpI^-). Acid-Base and Metal Ion-Binding Properties of a Dinucleotide Monophosphate in Aqueous Solution. *Inorg. Chem.* **2008**, *47*, 2641–2648.

31 Song, B.; Sigel, H. Metal Ion-Coordinating Properties of 2'-Deoxyguanosine 5'-Monophosphate

(dGMP^{2-}) in Aqueous Solution. Quantification of Macrochelate Formation. *Inorg. Chem.* **1998**, *37*, 2066–2069.

32 Klein, D. J.; Moore, P. B.; Steitz, T. A. The Contribution of Metal Ions to the Structural Stability of the Large Ribosomal Subunit. *RNA* **2004**, *10*, 1366–1379.

33 Koradi, R.; Billeter, M.; Wüthrich, K. MOLMOL: A Program for Display and Analysis of Macromolecular Structures. *J. Mol. Graphics* **1996**, *14*, 29–32 & 51–55.

34 Erat, M. C.; Sigel, R. K. O. Determination of the Intrinsic Affinities of Multiple Site-Specific Mg^{2+} Ions Coordinated to Domain 6 of a Group II Intron Ribozyme. *Inorg. Chem.* **2007**, *46*, 11224–11234.

35 Knobloch, B.; Erat, M. C.; Sigel, R. K. O. Cadmium(II) Binding to the Catalytic Core Domains of Group II Intron Ribozymes. *Results to be published.*

36 There is one more point: At least in those instances where the M^{2+} affinity of gua-N7(O6) is larger than of PO_2^- (Figure 3), one might want to consider statistical effects because less sites become available than in a "free" M^{2+}; e.g., 5/6 or 4/6 depending on the participation of O6. This would give with 5/6 (in accord with crystal structures; see ref 29) for Zn^{2+} log k = 1.38±0.19 and for Cd^{2+} with 4/6 (see ref 29) log k = 1.36±0.17, improving the agreement with the stability enhancement log Δ considerably. -- Furthermore, the proposed adding up of log stability increments is simply based on intuition (in contrast to the procedure based on Table 4) because it actually means that the microconstants of the individual binding sites are multiplied with each other. This approach differs from the recently described quantification of the chelate effect.[37] However, the present "multiplication" appears to be justified because it compensates for the chelate effect; i.e., once M^{2+} is coordinated to one site in a nucleic acid, other potential binding sites are spatially close, thus facilitating their entrance into the M^{2+}-coordination sphere.

37 Sigel, H.; Operschall, B. P.; Griesser, R. Xanthosine 5'-Monophosphate (XMP). Acid-Base and Metal-Ion-Binding Properties of a Chameleon-like Nucleotide. *Chem.Soc. Rev.* **2009**, *38*, DOI: 10.1039/b902181g.

Legends for the Figures

FIGURE 1. A: Quadridentate Mg^{2+} coordination to H_2ATP^{2-}, as suggested by Szent-Györgyi[4] in 1956 (redrawn). **B, C**: Tentative and simplified structure for macrochelated innersphere (B) and outersphere (B) $M(ATP)^{2-}$ isomers (reproduced by permission of the Federation of European Biochemical Societies (FEBS) from *Eur. J. Biochem.*, ref 5). Note, the terms innersphere and outersphere are used here solely with regard to M^{2+}-N7 coordination. The type of phosphate coordination may vary depending on the M^{2+} involved.[5,7,8] For example, evidence exists (see in ref 7) that in $Mg(ATP)^{2-}$ phosphate binding occurs as a mixture of β,γ-bidentate and α,β,γ-tridentate complexation.

FIGURE 2. Structures of the nucleobases (R = H) occurring in nucleic acids and in their nucleosides (R = ribose; for dThd R = 2'-deoxyribose).

FIGURE 3. Upper part: Individual M^{2+} affinities for the various binding sites within nucleotide residues in single-stranded nucleic acids based on the data of Table 2 (rounded values) and eq 3. **Lower part:** M^{2+}-affinity sequences for single-stranded nucleic acids with the phosphodiester groups highlighted in red. "~" means the complex-stability difference is below 0.2 log unit, ">" indicates a difference larger than 0.2 log unit, and ">>" a stability difference of more than 0.5 log unit.

FIGURE 4. Structures of the trianions of uridylyl-(5'→3')-5'-uridylate ($pUpU^{3-}$) and 2'-deoxyguanylyl(5'→3')-2'-deoxy-5'-guanylate ($d(pGpG)^{3-}$) with the two nucleoside units in each dinucleotide in the predominant *anti* conformation.

FIGURE 5. Evidence for an enhanced stability of the $M(pUpU)^{-}$ and $M[d(pGpG)]^{-}$ complexes of Mg^{2+}, Zn^{2+}, and Pb^{2+}, based on $\log K^{M}_{M(R\text{-}PO_3)}$ versus $pK^{H}_{H(R\text{-}PO_3)}$ plots for $M(R\text{-}PO_3)$ complexes where

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 are drawn through the corresponding eight data sets;[20,28] the straight-line parameters are listed in refs 11, 20, and 23. The data for the $M^{2+}/H^+/pUpU^{3-}$ and $M^{2+}/H^+/d(pGpG)^{3-}$ systems are taken from refs 23 and 15. The vertical dotted lines emphasize the stability differences to the reference lines, log $\Delta_{M/pUpU}$ (defined in eq 4), for $M(pUpU)^-$ complexes. The vertical full (Zn^{2+}) and broken (Pb^{2+}, Mg^{2+}) lines describe the situation for the $M[d(pGpG)]^-$ complexes. All plotted constants refer to aqueous solutions at 25°C and I = 0.1 M ($NaNO_3$). This is an altered version of Figure 2 in ref 15 (*Chem. Eur. J.* 2007); reproduced with permission; copyright (2007) Wiley-VCH, Weinheim, Germany.

FIGURE 6. In the equilibrium scheme $M[d(pGpG)]^-_{op}$ designates the "open" complex in which M^{2+} is only bound to the terminal phosphate group. The "closed" or macrochelated isomers involving either the phosphodiester bridge or the N7 sites are termed $M[d(pGpG)]^-_{cl/O}$ or $M[d(pGpG)]^-_{cl/N7}$. Note, in contrast to our previous conclusion (ref 15), we are now convinced (see text in connection with Table 6) that due to self-stacking within $M[d(pGpG)]^-$ the dinucleotide is preorientated[27,30] allowing thus an interaction of M^{2+} coordinated at the terminal phosphate group with both N7 sites (see also Figure 7). Hence, additional equilibria exist and $M[d(pGpG)]^-_{cl/N7}$ represents all isomers containing N7 in two-, three-, or four-point interactions (cf. the crystal structure studies in Section 7). Clearly, the analytical concentration of $M[d(pGpG)]^-$, determined in the experiments,[15] encompasses the sum of all isomeric species in the equilibrium scheme. Evidently, $K^M_{M[d(pGpG)]op} = [M[d(pGpG)]^-_{op}]/([M^{2+}][d(pGpG)^{3-}])$.

FIGURE 7. Three- and four-point interaction of a M^{2+} with two consecutive guanines as is known for Mg^{2+} in the ribosome.[32] Mg^{2+} is innersphere coordinated to a phosphodiester-oxygen and five water molecules. Hydrogen bonding, i.e., outersphere coordination, is observed to the two N7 atoms (broken

lines), thus forming a three-point interaction. A third water molecule forms a hydrogen bond to (C6)O (dotted line) resulting in a four-point interaction (prepared with the MOLMOL[33] program and the PDB file 1S72).[32]

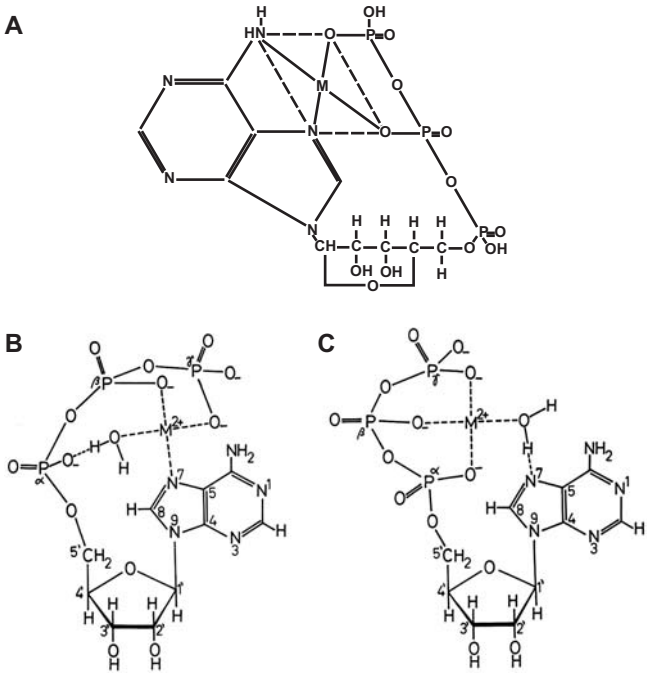


Figure 1

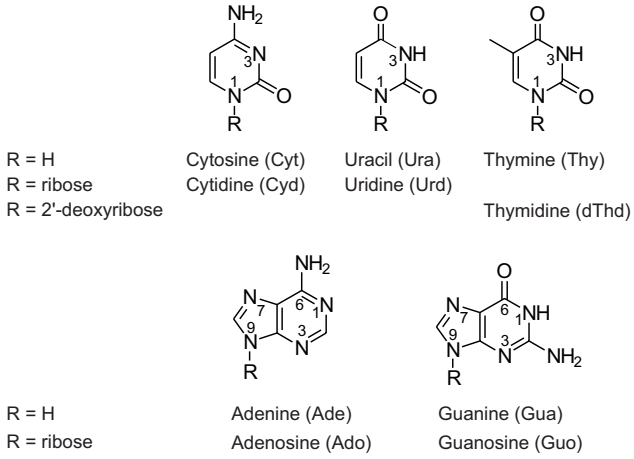
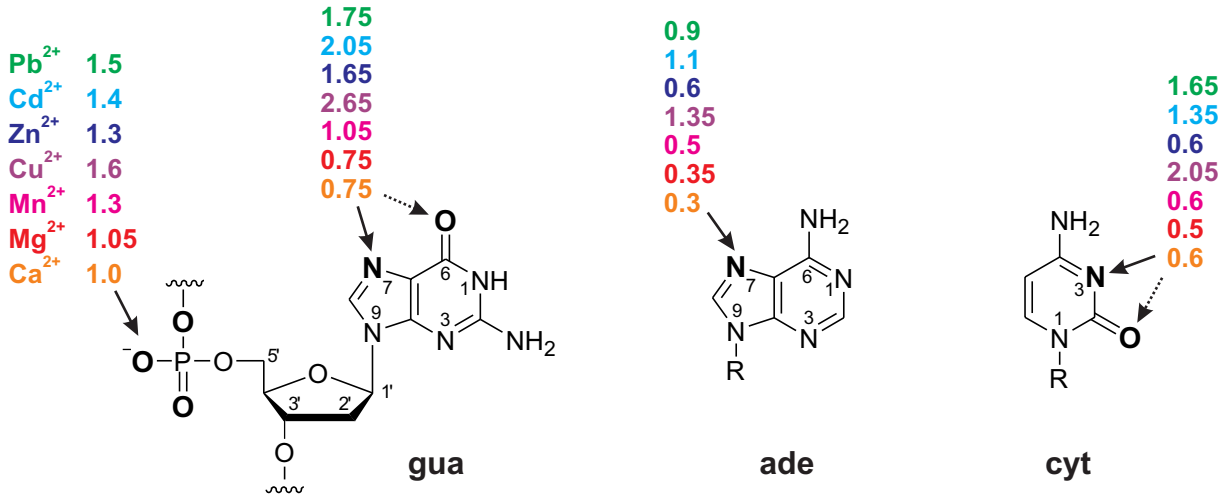

NH2
O
NH
O
R
R = H
R = ribose
R = 2'-deoxyribose
Cytosine (Cyt)
Cytidine (Cyd)
Uracil (Ura)
Uridine (Urd)
Thymine (Thy)
Thymidine (dThd)
Adenine (Ade)
Adenosine (Ado)
Guanine (Gua)
Guanosine (Guo)

Figure 2



Ca^{2+}:	PO_2^- (log *k* = 1.0)	>	gua-N7(O6) (0.75)	~	cyt-N3(O2) (0.6)	>	ade (0.3)	>	ura	~	thy
Mg^{2+}:	PO_2^- (log *k* = 1.05)	>	gua-N7(O6) (0.75)	>	cyt-N3(O2) (0.5)	~	ade (0.35)	>	ura	~	thy
Mn^{2+}:	PO_2^- (log *k* = 1.3)	>	gua-N7(O6) (1.05)	>	cyt-N3(O2) (0.6)	~	ade (0.5)	>	ura	~	thy
Cu^{2+}:	gua-N7(O6) (log *k* = 2.65)	>>	cyt-N3(02) (2.05)	>	PO_2^- (1.6)	>	ade (1.35)	>	ura	~	thy
Zn^{2+}:	gua-N7(O6) (log *k* = 1.65)	>	PO_2^- (1.3)	>>	cyt-N3(O2) (0.6)	~	ade (0.6)	>	ura	~	thy
Cd^{2+}:	gua-N7(O6) (log *k* = 2.05)	>>	PO_2^- (1.4)	~	cyt-N3(O2) (1.35)	>	ade (1.1)	>	ura	~	thy
Pb^{2+}:	gua-N7(O6) (log *k* = 1.75)	~	cyt-N3(O2) (1.65)	~	PO_2^- (1.5)	>>	ade (0.9)	>	ura	~	thy

Figure 3

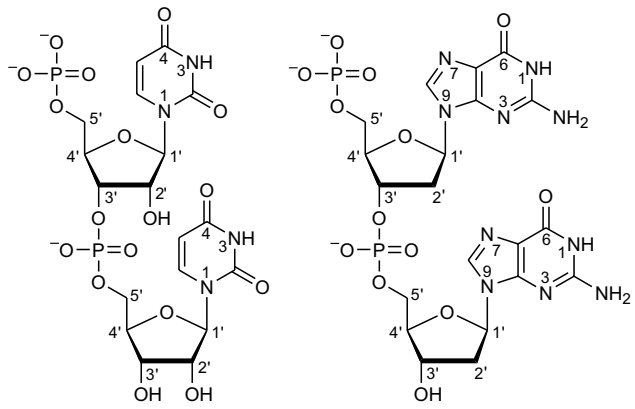
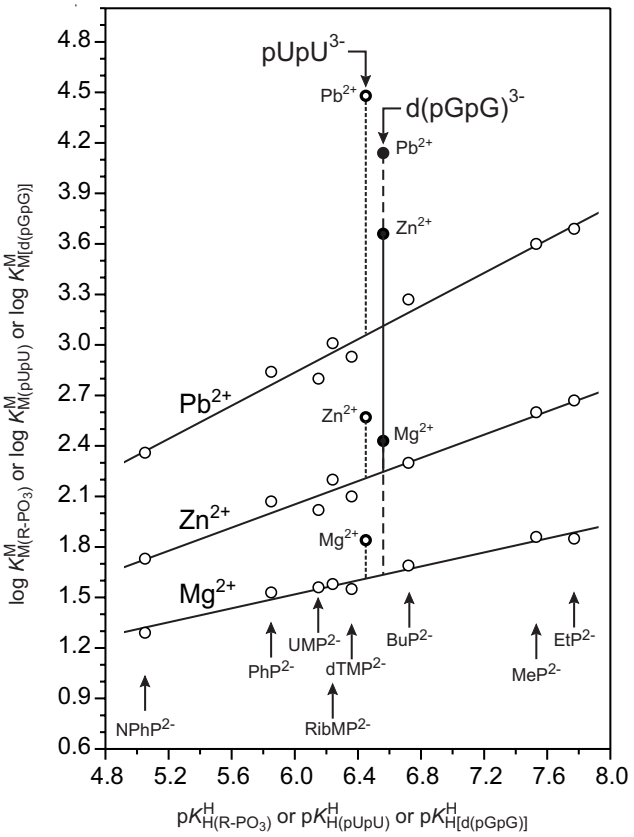


Figure 4


pUpU3-
Pb2+
d(pGpG)3-
Pb2+
Zn2+
Pb2+
Zn2+
Mg2+
Zn2+
Mg2+
Mg2+
UMP2-
BuP2-
EtP2-
PhP2-
dTMP2-
MeP2-
NPhP2-
RibMP2-
log KM M(R-PO3) or log KM M(pUpU) or log KM M[d(pGpG)]
pKH H(R-PO3) or pKH H(pUpU) or pKH H[d(pGpG)]
4.8
4.5
4.2
3.9
3.6
3.3
3.0
2.7
2.4
2.1
1.8
1.5
1.2
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7.6
8.0

Figure 5

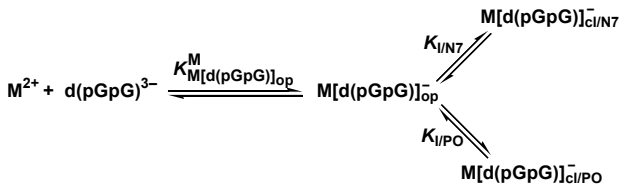

M[d(pGpG)]⁻cl/N7
K l/N7
M2+ + d(pGpG)3−
K M M[d(pGpG)]op
M[d(pGpG)]⁻op
K l/PO
M[d(pGpG)]⁻cl/PO

Figure 6

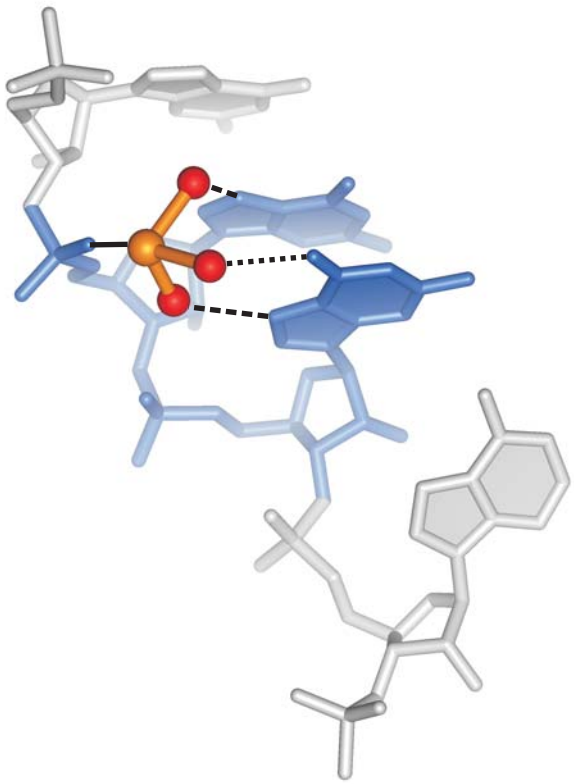
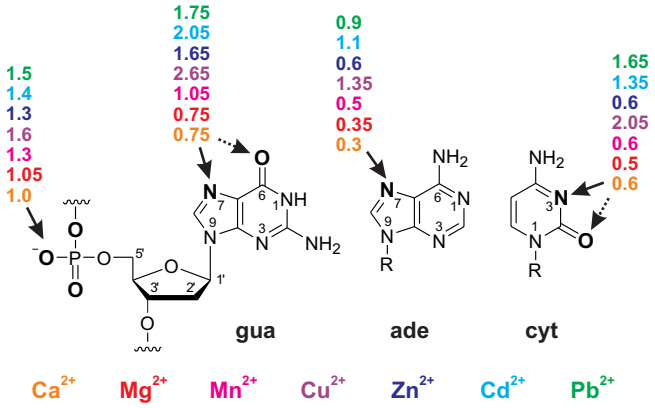


Figure 7



Graphic for Conspectus

Table 1. Metal Ion Affinity of Nucleobase Residues in Nucleosides[a]

M^{2+}	Guo[b]	Cyd[h]	Ado[j]
Mg^{2+}	0.35±0.25[c]	0.12±0.04	–0.06±0.15
Ca^{2+}	0.35±0.26[d]	0.18±0.06	–0.12±0.12
Mn^{2+}	0.57±0.2[d]	0.19±0.08	0.04±0.09
Cu^{2+}	2.12±0.14[e]	1.56±0.06	0.84±0.04
Zn^{2+}	1.16±0.11[f]	0.20±0.11	0.15±0.04
Cd^{2+}	1.53±0.07[c]	0.91±0.07	0.64±0.03
Pb^{2+}	1.25±0.17[g]	1.20±0.07[i]	0.4±0.3[g]

[a] Log stability constants according to eq 1 (aqueous solution; 25°C; I = 0.1 M, $NaNO_3$). The error limits correspond to three times the standard error of the mean value. [b] Except for $Pb(Guo)^{2+}$, values for 2'-deoxyguanosine (dGuo) are listed as these are more widely available. Based on $pK_a = pK^{H}_{H(dGuo)} - pK^{H}_{H(Guo)} \approx 0.2$,[12] and on log K versus pK_a slopes,[13] we estimate that the stability constants for the $M(Guo)^{2+}$ complexes are at most by 0.1 log unit smaller. [c] From ref 12. [d] Estimate based on the log K versus pK_a straight-line plot[13] for benzimidazoles by also considering the other listed values. [e] From ref 14. [f] Estimate from ref 15. [g] From ref 16. [h] From ref 17. [i] From ref 18. [j] Kapinos, L. E.; Sigel, H; results to be published.

Table 2. Logarithms of the Stability Constants for the "Open" $(M{\cdot}NMP{\cdot}H)^{+}$ complexes (Eq 2)[a,b]

M^{2+}	$(M{\cdot}GMP{\cdot}H)^{+}$	$(M{\cdot}CMP{\cdot}H)^{+}$	$(M{\cdot}AMP{\cdot}H)^{+}$
Mg^{2+}	0.76±0.29	0.52±0.16	0.35±0.21
Ca^{2+}	0.75±0.30	0.58±0.16	0.28±0.19
Mn^{2+}	1.04±0.25	0.60±0.17	0.48±0.17
Cu^{2+}	2.66±0.21	2.03±0.16	1.33±0.16
Zn^{2+}	1.66±0.19	0.62±0.19	0.62±0.16
Cd^{2+}	2.04±0.17	1.33±0.17	1.11±0.15
Pb^{2+}	1.77±0.23	1.64±0.17	0.9±0.35

[a] See text in Section 2. [b] The error limits of these derived data were calculated according to the error propagation after Gauss taking into account the error limits of Table 1 plus ±0.15 log unit (see text).

Table 3. Stability Constant Comparisons for $M(pUpU)^-$ with $M(R\text{-}PO_3)$ Complexes and the Stability Enhancements Defined by Eq 4[a,b]

M^{2+}	$\log K^{M}_{M(pUpU)}$	$\log K^{M}_{M(R\text{-}PO_3)}$	$\log \Delta_{M/pUpU}$
Mg^{2+}	1.84±0.04	1.61±0.03	0.23±0.05[†]
Mn^{2+}	2.49±0.05	2.22±0.05	0.27±0.17[†]
Zn^{2+}	2.57±0.03	2.20±0.06	0.37±0.07
Cd^{2+}	2.75±0.03	2.52±0.05	0.23±0.05[†]
Pb^{2+}	4.45±0.25	3.05±0.08	1.40±0.26

[†] average: $\log \Delta_{M/pUpU/charge} = 0.24\pm0.04$

Zn^{2+}: $\log \Delta^* = 0.13\pm0.08$ (eq 6) -- % $Zn(pUpU)^-_{cl} = 26\pm14\%$ (eq 8)

Pb^{2+}: $\log \Delta^* = 1.16\pm0.26$ (eq 6) -- % $Pb(pUpU)^-_{cl} = 93\pm4\%$ (eq 8)

[a] All data are from ref 23 (aqueous solution; 25ºC; I = 0.1 M, $NaNO_3$). [b] The error limits are three times the standard error of the mean value (3σ); those of the derived data (column 4) were calculated according to the error propagation after Gauss.

Table 4. Stability Constant Comparisons for Some $M[d(pGpG)]^-$ Complexes[a]

M^{2+}	log $K^{M}_{M[d(pGpG)]}$	log $K^{M}_{M(R\text{-}PO_3)}$	log $\Delta_{M/d(pGpG)}$	log Δ*	log Δ*
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±0.08	0.13±0.08
Cd^{2+}	4.01±0.06	2.56±0.05	1.45±0.08	1.21±0.08	–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] All data are from ref 15 (aqueous solution; 25ºC; I = 0.1 M, $NaNO_3$); those of column 6 follow according to eq 6b from the values listed in Table 3 (column 4). For the error limits see footnote '*b*' of Table 3. This table is adapted from Table 4 in ref 15 by permission; copyright (2007) Wiley-VCH, Weinheim, Germany.

Table 5. Percentages of Isomeric Species Formed in Intramolecular Equilibria with $M[d(pGpG)]^-$ Complexes[a]

M^{2+}	% $M[d(pGpG)]^-_{op}$	$M[d(pGpG)]^-_{cl/N7}$	%$M[d(pGpG)]^-_{cl/PO}$
Mg^{2+}	28±5	72±5.5	
Ca^{2+}	~39[b]	~47[b]	~14[b]
Zn^{2+}	6.8±1.4	91±2.5	2.4±1.8
Cd^{2+}	6±2	94±1.5	
Pb^{2+}	16±5		84±6

[a] The data for Mg^{2+}, Zn^{2+}, Cd^{2+}, and Pb^{2+} are from ref 15 (aqueous solution; 25°C; I = 0.1 M, $NaNO_3$); see also Figure 6 and text, and for the error limits footnote '*b*' of Table 3. [b] Estimates based on the following reasonings: The ionic radius of Ca^{2+} is only slightly smaller than the one of Pb^{2+} and both metal ions have a very adaptable coordination sphere. Therefore one may expect that Ca^{2+} also bridges to some extent the two neighboring phosphate groups forming the 10-membered chelate. In the calculations it is assumed that the connected stability enhancement (as kind of a lower limit) corresponds to that observed for Zn^{2+}, i.e., $\log \Delta^*_{Ca/pUpU} = 0.13\pm0.08$ (cf. Table 3, lower part). The stability enhancement due to the formation of the N7-macrochelate with Ca^{2+} is assumed to be about one half of that observed for Mg^{2+}: This assumption is based on the complexes formed with AMP^{2-}, IMP^{2-} and GMP^{2-} (see Tables 7 and 9 in ref 8); hence, $\log \Delta_{Ca/N7} = (0.55\pm0.08)\cdot(1/2) = 0.28\pm0.08$. Therefore, one obtains overall $\log \Delta^*_{Ca/d(pGpG)} = (0.13\pm0.08) + (0.28\pm0.08) = 0.41\pm0.11$ and thus[15] $K_{I/tot} = 1.57$; with $K_{I/PO} = 0.35$ [from Zn(pUpU)][23] finally $K_{I/N7} = 1.22$ follows. Note, $K_{I/tot} = K_{I/N7} + K_{I/PO}$ (cf. ref 15) in accord with Figure 6.

Table 6. Comparison of Stability Enhancements and Formation Degrees of the Macrochelated Species Involving N7 for $M[d(pGpG)]^-$ and M(dGMP) Complexes[a]

M^{2+}	$\log \Delta^*_{M/d(pGpG)}$	% $M[d(pGpG)]^-_{cl/N7}$	$\log \Delta_{M/dGMP}$	% $M(dGMP)_{cl/N7}$
Mg^{2+}	0.55±0.08	72±5.5	0.23±0.05	41±7
Zn^{2+}	1.16±0.09[b]	91±2.5	0.84±0.08	86±3
Cd^{2+}	1.21±0.09	94±1.5	0.92±0.11[c]	88±3

[a] The values in columns 2 and 3 are from Tables 4 (column 5) and 5 (column 3). The M(dGMP) data are from ref 31 (aqueous solution; 25°C; I = 0.1 M, $NaNO_3$). Regarding the error limits see footnote '*b*' of Table 3. [b] This value is corrected for the interaction of Zn^{2+} with the neighboring phosphodiester bridge.[27] [c] Estimate from ref 27.