# 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

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The stability constants of the 1:1 complexes formed between $Pb^{2+}$ and the nucleosides (Ns), adenosine and guanosine, as well as between the nucleotides ($NMP^{2-}$), $AMP^{2-}$, $IMP^{2-}$, and $GMP^{2-}$, were determined by potentiometric pH titrations in aqueous solution (25 °C; $I = 0.1$ M, $NaNO_3$). Based on previously established log $K^{Pb}_{Pb(R-PO_3)}$ versus p$K^{H}_{H(R-PO_3)}$ straight-line plots ($R-PO_3^{2-}$ = simple phosphate monoester or phosphonate ligands where R is a noninteracting site), it is shown that the Pb(IMP) and Pb(GMP) complexes are more stable than is expected on the basis of the basicity of the phosphate group of $IMP^{2-}$ and $GMP^{2-}$. This means that macrochelates are formed, where the phosphate-coordinated $Pb^{2+}$ also interacts with N7 of the nucleobase residue. In contrast, the stability of the Pb(AMP) complex is governed by the basicity of the $AMP^{2-}$ phosphate group. These results agree with the observations made for the $Pb(Ns)^{2+}$ complexes: $Pb(adenosine)^{2+}$ is very unstable in contrast to $Pb(guanosine)^{2+}$, the stability of which is very similar to the one of $Pb(cytidine)^{2+}$ studied previously. The stability constants of the $Pb(Ns)^{2+}$ complexes also allowed an evaluation of the structure in solution of the monoprotonated $Pb(H;NMP)^{+}$ complexes, the stabilities of which were also determined. We were able to show that the proton is located at the phosphate group and $Pb^{2+}$ at the N7/(C6)O site of $H(GMP)^{-}$; in the case of $H(AMP)^{-}$ $Pb^{2+}$ is probably about equally distributed between the adenine residue and the monoprotonated phosphate group. On the basis of the stability constants of these complexes and their structures in solution, it is possible to provide a series which reflects the decreasing affinity for $Pb^{2+}$ of nucleobase residues in single-stranded nucleic acids: guanine ≃ cytosine > (hypoxanthine) > adenine > uracil ≃ thymine. The $Pb^{2+}$ affinity of the phosphodiester linkage, $-PO_3^{-}-$, is similar to the one of the adenine residue, but is expected to be more significant due to its larger abundance. The relevance of these results for lead-activated ribozymes is briefly discussed.

## 1. Introduction

Lead has been recognized as a toxic element for many centuries.[1] It produces a variety of adverse effects in mammals:[1,2] it acts on the central and peripheral nervous system, induces inflammatory response,[3] modulates immune functions,[3,4] has genetic effects,[5] and influences the homeostasis of essential metal ions.[6] However, the molecular mechanisms underlying lead toxicity are still not well understood, though it is evident that Pb(II)−nucleic acid interactions may be critical for the toxic action of this metal ion. In fact it is clear that, e.g., Pb(II) ions are efficient in RNA depolymerization.[7] Indeed, the Pb(II)-induced hydrolysis of RNA is much studied,[8,9] and so-called leadzymes, the activity of which depends on the presence of $Pb^{2+}$, exist.[10−12]

Considering that hardly any information exists on the interaction between $Pb^{2+}$ and nucleotides or related ligands,[13−15] we initiated corresponding research and reported recently on the stability of binary complexes formed between $Pb^{2+}$ and

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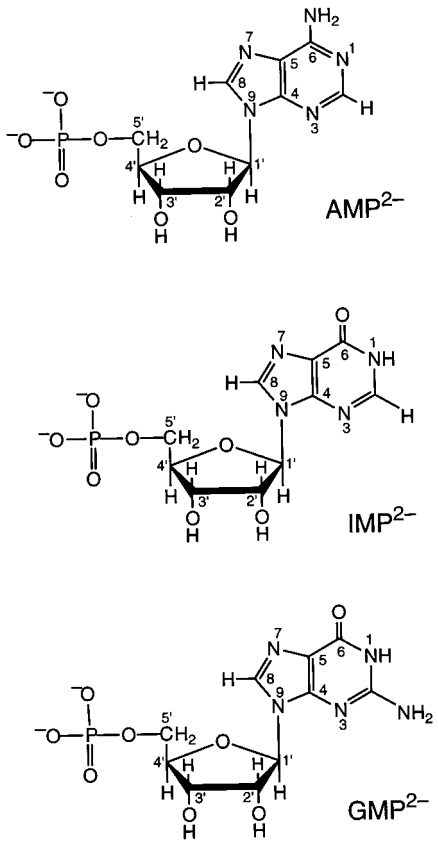
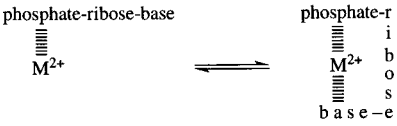



**Figure 1.** Chemical structures of adenosine 5′-monophosphate ($AMP^{2-}$), inosine 5′-monophosphate ($IMP^{2-}$), and guanosine 5′-monophosphate ($GMP^{2-}$) in their dominating anti conformation.[18,19]

simple phosph(on)ate ligands.[16] Now we present our results on the stability and structure of the complexes formed between $Pb^{2+}$ and $AMP^{2-}$, $IMP^{2-}$, or $GMP^{2-}$ (Figure 1)[17−19] in aqueous solution. The primary binding site for $Pb^{2+}$, which determines to a very large part the stability of the complexes, is the phosphate group of the nucleotides.[16] However, previously it has been shown for several metal ions[20,21] such as $Mn^{2+}$, $Cu^{2+}$, $Zn^{2+}$, and $Cd^{2+}$ that they interact also with N7 of the purine nucleobase residue[22] of the nucleotides forming macrochelates[20,21] according to equilibrium 1:




(1)

Indeed, in several studies dealing with leadzymes[10,12] or $Pb^{2+}$-mediated RNA cleavages[9,23] as well as for $Pb^{2+}$−DNA interactions[24] evidence is provided that $Pb^{2+}$ not only interacts with phosphate groups but also with nitrogen and oxygen atoms of nucleobases. Hence, we attempted to quantify in addition the stability of the $Pb^{2+}$ complexes formed with adenosine (Ado) and guanosine (Guo). Previously we had shown[16] that the stability of the Pb(UMP) and Pb(dTMP) complexes is solely determined by the basicity of the phosphate group and that the uracil and thymine moieties are not involved in metal ion binding. This is also true for Pb(CMP),[16] since the anti conformation of $CMP^{2-}$ dominates in solution[25] and a phosphate-coordinated metal ion thus can not reach the N3/(C2)O site of the cytosine residue. On the other hand, the affinity of $Pb^{2+}$ is somewhat more pronounced for the cytosine moiety than for the monoprotonated phosphate group as was shown[16] for Pb(H;CMP)$^{+}$ where the proton is at the phosphate group and $Pb^{2+}$ mainly at the N3/(C2)O site. Therefore, the main question for the present was, Do the purine nucleotides $AMP^{2-}$, $IMP^{2-}$, and $GMP^{2-}$ behave like simple phosphate esters or does $Pb^{2+}$ form a macrochelate with the nucleobase residue?

## 2. Experimental Section

**2.1. Materials.** Adenosine 5′-monophosphoric acid and the disodium salt of guanosine 5′-monophosphate were from Serva Feinbiochemica GmbH, Heidelberg, Germany. The disodium salt of inosine 5′-monophosphate, adenosine, and guanosine were from Sigma Chemical Co., St. Louis, MO. The other reagents were the same as used previously.[16] All solutions were prepared with deionized, ultrapure (MILLI-Q 185 PLUS, from Millipore S. A., 67120 Molsheim, France), and $CO_2$-free water.

The aqueous stock solutions of the ligands were freshly prepared daily, and their exact concentration was newly determined each time by titrations with NaOH. The titer of the NaOH used for the titrations was established with potassium hydrogen phthalate and that of the $Pb^{2+}$ stock solutions with EDTA.

**2.2. Potentiometric pH Titrations.** These were carried out with the same equipment and in exactly the same way as described recently.[16] In the titrations of adenosine and guanosine, due to the high $Pb^{2+}$ concentrations needed in the experiments, an interference of $Pb^{2+}$ with the $Cl^{-}$ ions from the electrolyte of the electrode occurred. To overcome this problem, we used separate electrodes, i.e., a pH measuring electrode (Metrohm 6.0133.100) in combination with an Ag/AgCl reference electrode (Metrohm 6.0726.100) where the outer part was filled with a saturated $KNO_3$ solution. In this way no further interactions were observed and reproducible, high-quality titration curves were obtained. We are grateful to Metrohm AG, Herisau, Switzerland, for providing this information.

The determined acidity constants are so-called practical, mixed, or Brønsted constants.[26] Their negative logarithms given for aqueous solution at $I = 0.1$ M ($NaNO_3$) and 25 °C may be converted into the corresponding concentration constants by subtracting 0.02 from the

(15) *Joint Expert Speciation System (JESS)*, version 6.0; joint venture by Murray, K., and May, P. M.; Division of Water Technology, CSIR, Pretoria, South Africa, and School of Mathematical and Physical Sciences, Murdoch University, Murdoch, Western Australia, 1999.

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(17) Abbreviations and definitions. Ado, adenosine; $AMP^{2-}$, adenosine 5′-monophosphate; $CMP^{2-}$, cytidine 5′-monophosphate; Cyd, cytidine; $dTMP^{2-}$, thymidine 5′-monophosphate; $GMP^{2-}$, guanosine 5′-monophosphate; Guo, guanosine; *I*, ionic strength; $IMP^{2-}$, inosine 5′-monophosphate; Ino, inosine; $M^{2+}$, general divalent metal ion; $NMP^{2-}$, nucleoside 5′-monophosphate; Ns, nucleoside; $R-PO_3^{2-}$, simple phosphate monoester or phosphonate ligand with R representing a noncoordinating residue (see also legend of Figure 3); $UDP^{3-}$, uridine 5′-diphosphate; $UMP^{2-}$, uridine 5′-monophosphate. Species written without a charge either do not carry one or represent the species in general (i.e., independent of their protonation degree); which of the two possibilities applies is always clear from the context. In formulas such as Pb(H;NMP)$^{+}$, $H^{+}$ and $NMP^{2-}$ are separated by a semicolon to facilitate reading, yet they appear within the same parentheses to indicate that the proton is at the ligand without defining its location (see sections 3.3−3.5).

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listed p$K_a$ values;[26] this conversion term contains both the junction potential of the glass electrode and the hydrogen ion activity.[26,27] The ionic product of water ($K_w$) and the conversion term do not enter into our calculation procedures because we evaluate the *differences* in NaOH consumption between a pair of solutions; i.e., a solution with and one without ligand are always titrated (see also below; for further details refs 26 and 28 may be consulted). The stability constants presented are, as usual, concentration constants.

**2.3. Determination of Equilibrium Constants of the Nucleoside Systems.** The acidity constants $K^{H}_{H(Guo)}$ and $K^{H}_{Guo}$ of $H(Guo)^+$ and Guo (eqs 2 and 3), respectively, were determined by titrating 25 mL of aqueous 6.6 mM $HNO_3$ (25 °C; $I = 0.1$ M, $NaNO_3$) in the presence and absence of 0.93 mM Guo under $N_2$ with 3 mL of 0.06 M NaOH. The constants were calculated with a curve-fitting procedure using a Newton−Gauss nonlinear least-squares program by employing every 0.1 pH unit the difference in NaOH consumption between the two mentioned titrations within the pH range 2.9−10.5 corresponding to about 86% (initial) neutralization for the equilibrium $H(Guo)^+$/Guo and about 95% (final) for Guo/$(Guo-H)^-$. The final results are the averages of six independent pairs of titrations; they were within their error limit identical with the previously measured values.[20]

The stability constant $K^{Pb}_{Pb(Guo)}$ (eq 7) of the $Pb(Guo)^{2+}$ complex was determined under the conditions given above for the acidity constants, but $NaNO_3$ was partly or fully replaced by $Pb(NO_3)_2$ (25 °C; $I = 0.1$ M). The ratios Guo:$Pb^{2+}$ were 1:35.8 and 1:28.7. The experimental data were collected every 0.1 pH unit from the lowest accessible pH (about 2.2) to the beginning of the hydrolysis of $Pb(aq)^{2+}$ (at pH about 4.4); the latter was evident from the titrations in the absence of ligand. The calculations were done with the same curve-fitting procedure mentioned above for the acidity constants, and in this way an *apparent* acidity constant p$K_a'$ was obtained, from which the stability constant was calculated as described previously.[29] The buffer depression[29] was with $\Delta pK_a$ about 0.2 quite significant. The individual results showed no dependence on the excess of $Pb^{2+}$ used in the experiments. The final result of the stability constant is the average of 5 independent pairs of titrations.

The acidity constant $K^{H}_{H(Ado)}$ (eq 2) was obtained by titrating 50 mL of aqueous 2.4 mM $HNO_3$ in the presence and absence of 0.6 mM Ado with 2 mL of 0.06 M NaOH. Otherwise the conditions were identical with those given above for Guo and also the same evaluation procedure was employed by using the pH range from about 2.8 to 5.6 for the calculations; this range corresponds to a neutralization between about 13% and 99% for the equilibrium $H(Ado)^+$/Ado. The final result is the average of seven independent pairs of titrations and it is within its error limits identical with the value determined previously.[19]

The stability constant $K^{Pb}_{Pb(Ado)}$ (eq 7) of the $Pb(Ado)^{2+}$ complex was determined under the same conditions used for the acidity constant of $H(Ado)^+$, but part or all of $NaNO_3$ was replaced by $Pb(NO_3)_2$ to give Ado:$Pb^{2+}$ ratios of 1:55.6 and 1:50. The determination of this stability constant was 2-fold hampered: (i) Due to the hydrolysis of $Pb(aq)^{2+}$ only the pH range from about 3.2 to 4.5 was experimentally accessible. (ii) The buffer depression (see ref 29) $\Delta pK_a$ equalled only about 0.04 and was thus very small. Consequently, the error in the calculations for $K^{Pb}_{Pb(Ado)}$ was very large and the result, which is the average of five independent pairs of titrations, can only be considered as an estimate.

**2.4. Determination of the Equilibrium Constants of the Nucleotide Systems.** The acidity constants $K^{H}_{H_2(GMP)}$ and $K^{H}_{H(GMP)}$ of $H_2(GMP)^{\pm}$ (eqs 4 and 5) were determined by titrating under $N_2$ 50 mL of aqueous 3 mM $HNO_3$ (25 °C; $I = 0.1$ M, $NaNO_3$) in the presence and absence of 0.6 mM $GMP^{2-}$ (the stock solution was adjusted to pH 8.3) with 2.5 mL of 0.06 M NaOH. The constants were calculated with the above-mentioned curve-fitting procedure in the pH range from 3.2 to 7.0; this means the neutralization degree reached from about 84% to 100% and from 3% to 84% for the equilibria $H_2(GMP)^{\pm}$/$H(GMP)^-$ and $H(GMP)^-$/$GMP^{2-}$, respectively. The results from the seven pairs of titrations made now are within their error limits identical with the previous results.[20]

The stability constants $K^{Pb}_{Pb(H;GMP)}$ and $K^{Pb}_{Pb(GMP)}$ of the $Pb(H;GMP)^+$ and Pb(GMP) complexes (eqs 10 and 11) were determined under the same conditions, but part of $NaNO_3$ was replaced by $Pb(NO_3)_2$ ($I = 0.1$ M); the GMP:$Pb^{2+}$ ratios were 1:27.8, 1:13.9, and 1:6.9. The constants were calculated from the experimentally accessible pH range 2.6−5.0 [beginning of the hydrolysis of $Pb(aq)^{2+}$] by taking into account the species $H^+$, $H_2(GMP)^{\pm}$, $H(GMP)^-$, $GMP^{2-}$, $Pb^{2+}$, $Pb(H;GMP)^+$, and Pb(GMP). The formation degrees for $Pb(H;GMP)^+$ and Pb(GMP) varied between about 7−40% and 2−30%, respectively. The final results are the averages of seven independent pairs of titrations.

The experimental conditions for the titrations with IMP were the same as given above for the GMP systems, but now the acidity constants $K^{H}_{H(IMP)}$ and $K^{H}_{IMP}$ of $H(IMP)^-$ were determined (eqs 5 and 6). The pH range from 4.5 to 9.1 was evaluated which corresponds to an initial neutralization degree of 2% for the equilibrium $H(IMP)^-$/$IMP^{2-}$ and of 56% (final) for $IMP^{2-}$/$(IMP-H)^{3-}$. The results from six independent pairs of titrations are within the error limits identical with the previous ones.[20] The 11 pairs of titrations in the presence of $Pb^{2+}$ were also made as given above; this also applies to the calculations, but the constant $K^{Pb}_{Pb(H;IMP)}$ for the $Pb(H;IMP)^+$ complex could only be estimated.

The experimental conditions as well as the evaluations for the determination of the equilibrium constants for the AMP systems ([AMP] = 0.6 mM) with and without $Pb^{2+}$ were identical with those used previously[16] for the corresponding CMP systems, except that now AMP:$Pb^{2+}$ ratios of 1:27.8, 1:11, 1:8.3, and 1:5 were used. The evaluations of the eight titration pairs for the acidity constants $K^{H}_{H_2(AMP)}$ and $K^{H}_{H(AMP)}$ for $H_2(AMP)^{\pm}$ (eqs 4 and 5) encompassed the pH range 3.3−7.9 which corresponds to an initial neutralization degree of 23% for the equilibrium $H_2(AMP)^{\pm}$/$H(AMP)^-$ and to a final one of 98% for $H(AMP)^-$/$AMP^{2-}$; the average results were within their error limits identical with the previous ones.[22] The $Pb^{2+}$/AMP titrations could only be evaluated in the pH range 3.2−4.6, which corresponds to formation degrees of about 1−9.5% for $Pb(H;AMP)^+$ and 0.5−7.5% for Pb(AMP) because of the formation of a precipitate which clearly occurred before the onset of the hydrolysis of $Pb(aq)^{2+}$. The final results for the stability constants, $K^{Pb}_{Pb(H;AMP)}$ and $K^{Pb}_{Pb(AMP)}$ (eqs 10 and 11), are the averages of 10 pairs of titrations.

## 3. Results and Discussion

All potentiometric pH titrations ($I = 0.1$ M, $NaNO_3$; 25 °C) were carried out at ligand concentrations below 1 mM, usually at 0.6 mM. Under these conditions self-stacking of the nucleosides and the NMPs of Figure 1 is negligible.[20] Hence, all the results presented below apply to monomeric species.

**3.1. Acidity Constants of the Protonated Nucleosides and Nucleotides.** The nucleosides (Ns) adenosine (Ado) and guanosine (Guo) can both accept a proton at the purine ring, Ado at N1 and Guo at N7.[20] Consequently, the following equilibrium needs to be considered for both nucleosides:

$$H(Ns)^+ \rightleftharpoons Ns + H^+ \qquad (2a)$$

$$K^{H}_{H(Ns)} = [Ns][H^+]/[H(Ns)^+] \qquad (2b)$$

The neutral guanosine may loose a further proton from the (N1)H site, giving rise to equilibrium 3a:

$$Guo \rightleftharpoons (Guo-H)^- + H^+ \qquad (3a)$$

$$K^{H}_{Guo} = [(Guo-H)^-][H^+]/[Guo] \qquad (3b)$$

Clearly, the nucleoside 5′-monophosphates ($NMP^{2-}$) shown in Figure 1 can bind three protons, two at the phosphate group and one at the purine moiety. The first proton is released from

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**Table 1.** Negative Logarithms of the Acidity Constants (Eqs 2−6)][a] of the Monoprotonated Nucleosides, $H(Ns)^+$, and the Twofold Protonated Nucleoside 5′-Monophosphates, $H_2(NMP)^{\pm}$, Considered in This Study and as Determined by Potentiometric pH Titrations in Aqueous Solution at 25 °C and $I = 0.1$ M ($NaNO_3$)[b,c]

| acid | $pK^H_{H(Ns)}$ or $pK^H_{H_2(NMP)}$ (eqs 2, 4) | $pK^H_{H(NMP)}$ (eq 5) | $pK^H_{Ns}$ or $pK^H_{NMP}$ (eqs 3, 6) |
|---|---|---|---|
| $H(Ado)^+$ | $3.61 \pm 0.03$[d] | | |
| $H(Ino)^+$ | $(1.06 \pm 0.06)$[20,30] | | $(8.76 \pm 0.03)$[20] |
| $H(Guo)^+$ | $2.11 \pm 0.04$ | | $9.22 \pm 0.02$[e] |
| $H_2(AMP)^{\pm}$ | $3.84 \pm 0.02$[d] | $6.21 \pm 0.01$ | |
| $H_2(IMP)^{\pm}$ | $(1.30 \pm 0.10)$[20] | $6.22 \pm 0.01$ | $9.02 \pm 0.02$ |
| $H_2(GMP)^{\pm}$ | $2.48 \pm 0.04$ | $6.25 \pm 0.02$ | $(9.49 \pm 0.02)$[20] |

[a] So-called practical (or mixed) acidity constants[26] are listed; see section 2.2. [b] The error limits are *3 times* the standard error of the mean value ($3\sigma$) or the sum of the probable systematic errors, whichever is larger. [c] The values in parentheses are given for comparison; they are taken from the indicated references. All the other acidity constants have been redetermined during this work (see sections 2.3 and 2.4); the results were identical within their error limits with the published values which therefore are again listed above; they are taken from refs 19 (Ado), 20 (Guo, IMP, GMP) and 22 (AMP). [d] These values refer to the deprotonation of the $(N1)H^+$ site of the adenine residue; all the other values in this column refer to the deprotonation of a $(N7)H^+$ site. [e] This error limit was enlarged from ±0.01 in ref 20 to ±0.02 to be in accord with the experiments carried out now.

the $-P(O)(OH)_2$ group at a rather low pH ($pK_a \leq 0.5$)[20] and is not of relevance for the present study. The second proton (eq 4) is released from the $(N1)H^+$ site of $H_2(AMP)^{\pm}$ or the $(N7)H^+$ site of $H_2(IMP)^{\pm}$ and $H_2(GMP)^{\pm}$ followed by the proton from the $-P(O)_2(OH)^-$ group (eq 5). Finally, $IMP^{2-}$ and $GMP^{2-}$ may be deprotonated at their (N1)H site leading to the $(NMP-H)^{3-}$ species (eq 6). Hence, the following three equilibria are relevant for this work:

$$H_2(NMP)^{\pm} \rightleftharpoons H(NMP)^- + H^+ \quad (4a)$$

$$K^H_{H_2(NMP)} = [H(NMP)^-][H^+]/[H_2(NMP)^{\pm}] \quad (4b)$$

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

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

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

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

We have measured by potentiometric pH titrations the pertinent acidity constants which all agreed within their error limits with the values published earlier by our group.[19−22,26,30] The corresponding results are listed in Table 1, and the site attributions of the protons as described above are confirmed by these data.

**3.2. Stability Constants of $Pb^{2+}$ Complexes Formed with Nucleosides.** Various metal ions (such as $Cu^{2+}$, $Ni^{2+}$) can form complexes with adenosine[31,32] and guanosine.[33] Therefore, we also attempted to measure stability constants of the corresponding $Pb(Ns)^{2+}$ complexes. Since the stability of these complexes is expected to be low, a large excess of $Pb^{2+}$, compared to Ns, was used in the experiments (see section 2.3). Indeed, the experimental data could be fully explained by taking into account equilibria 2a and 7a as long as the evaluation of the

$$Pb^{2+} + Ns \rightleftharpoons Pb(Ns)^{2+} \quad (7a)$$

$$K^{Pb}_{Pb(Ns)} = [Pb(Ns)^{2+}]/([Pb^{2+}][Ns]) \quad (7b)$$

data was not carried into the pH range where hydrolysis of $Pb(aq)^{2+}$ occurs (see section 2.3).

The stability constants given below for the $Pb(Ado)^{2+}$ and $Pb(Guo)^{2+}$ complexes (eq 7) are the average of five independent experiments for each system; they refer to aqueous solutions at 25 °C and $I = 0.1$ M (for the error limits see footnote *b* in Table 1):

$$\log K^{Pb}_{Pb(Ado)} = 0.4 \pm 0.3 \quad (8)$$

$$\log K^{Pb}_{Pb(Guo)} = 1.25 \pm 0.17 \quad (9)$$

The determination of $K^{Pb}_{Pb(Guo)}$ (eq 9) was straightforward, whereas $K^{Pb}_{Pb(Ado)}$ (eq 8) is only an estimate because the measurements were hampered not only by the hydrolysis of $Pb(aq)^{2+}$, but also by the instability of $Pb(Ado)^{2+}$ which leads to a very low buffer depression ($\Delta pK_a$) between titrations of the Ado and the Ado/$Pb^{2+}$ systems (see section 2.3). Indeed, in an early attempt to measure this stability constant, it was concluded[34] that the association of $Pb^{2+}$ with Ado is negligible. In the same study[34] also a constant for $Pb(Guo)^{2+}$ was measured: $\log K^{Pb}_{Pb(Guo)} = 0.48 \pm 0.14$ (20 °C; $I = 1$ M, $NaNO_3$). Though this value is considerably smaller than the present result (eq 9), the formation of the $Pb(Guo)^{2+}$ complex is confirmed; aside from possible difficulties due to the way the earlier experiments were performed,[34] the discrepancy between the constants probably stems from the use of a 10 times higher ionic strength (background electrolyte) in the earlier study.

In $M(Guo)^{2+}$ complexes the metal ions are coordinated to N7,[33] which is the most basic site, though possibly[20] also semichelates form involving a hydrogen bond between a $M^{2+}$-coordinated $H_2O$ molecule and the carbonyl oxygen at C6. For $M(Ado)^{2+}$ complexes it was previously shown that a N1 versus N7 dichotomy occurs,[31,32] N7 binding being favored for most metal ions, probably also for $Pb^{2+}$ (see also the considerations in section 3.7). Finally, it needs to be added that the stability of Pb(cytidine)$^{2+}$, where $Pb^{2+}$ coordinates via the N3/(C2)O site, with $\log K^{Pb}_{Pb(Cyd)} = 1.20 \pm 0.07$,[16] is very similar to the one of $Pb(Guo)^{2+}$ (see eq 9).

**3.3. Stability Constants of $Pb^{2+}$−Nucleotide Complexes.** The experimental data of the potentiometric pH titrations (see section 2.4) of the three $Pb^{2+}$/NMP systems, where NMP = AMP, IMP, or GMP, allow the determination of the stability constants defined by equilibria 10a and 11a:

$$Pb^{2+} + H(NMP)^- \rightleftharpoons Pb(H;NMP)^+ \quad (10a)$$

$$K^{Pb}_{Pb(H;NMP)} = [Pb(H;NMP)^+]/([Pb^{2+}][H(NMP)^-]) \quad (10b)$$

$$Pb^{2+} + NMP^{2-} \rightleftharpoons Pb(NMP) \quad (11a)$$

$$K^{Pb}_{Pb(NMP)} = [Pb(NMP)]/([Pb^{2+}][NMP^{2-}]) \quad (11b)$$

Overall, equilibria 4a, 5a, 10a, and 11a are sufficient to obtain excellent fitting of the titration data, provided the evaluation is not carried into the pH range where hydrolysis of $Pb(aq)^{2+}$ occurs, which was evident from the titrations without ligand.

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(33) Song, B.; Zhao, J.; Griesser, R.; Meiser, C.; Sigel, H.; Lippert, B. *Chem. Eur. J.* **1999**, *5*, 2374−2387.
(34) Fiskin, A. M.; Beer, M. *Biochemistry* **1965**, *4*, 1289−1294.

**Table 2.** Logarithms of the Stability Constants of the $Pb(H;NMP)^+$ (Eq 10) and Pb(NMP) Complexes (Eq 11), Together with the Negative Logarithms of the Acidity Constants for the $Pb(H;NMP)^+$ Species (Eqs 12 and 13), as Determined by Potentiometric pH Titrations in Aqueous Solution at 25 °C and $I = 0.1$ M ($NaNO_3$)[a]

| $NMP^{2-}$ | $\log K^{Pb}_{Pb(H;NMP)}$ | $\log K^{Pb}_{Pb(NMP)}$ | $pK^{H}_{Pb(H;NMP)}$ |
|---|---|---|---|
| $AMP^{2-}$ | $1.08 \pm 0.04$ | $2.92 \pm 0.08$ | $4.37 \pm 0.09$ |
| $IMP^{2-}$ | $1.30 \pm 0.15$[b] | $3.06 \pm 0.05$ | $4.46 \pm 0.16$ |
| $GMP^{2-}$ | $1.52 \pm 0.10$ | $3.23 \pm 0.08$ | $4.54 \pm 0.13$ |

[a] See footnote *b* in Table 1; the error limits of the derived data, in the present case for $pK^{H}_{Pb(H;NMP)}$, were calculated according to the error propagation after Gauss. [b] This value is an estimation which is mainly based on the lower basicity of N7 in the hypoxanthine moiety compared to that in the guanine residue (cf. Table 1).

The pH range where the N1-deprotonated $Pb(IMP-H)^-$ or $Pb(GMP-H)^-$ species might be formed is not reached. Of course, equilibria 10a and 11a are also connected via equilibrium 12a and the corresponding acidity constant (eq 12b) may be calculated with eq 13:

$$Pb(H;NMP)^+ \rightleftharpoons Pb(NMP) + H^+ \quad (12a)$$

$$K^{H}_{Pb(H;NMP)} = [Pb(NMP)][H^+]/[Pb(H;NMP)^+] \quad (12b)$$

$$pK^{H}_{Pb(H;NMP)} = pK^{H}_{H(NMP)} + \log K^{Pb}_{Pb(H;NMP)} - \log K^{Pb}_{Pb(NMP)} \quad (13)$$

The results are listed in Table 2. None of these values has been determined before.[13−15] Application of the constants of Tables 1 and 2 allows calculation of the formation degree of the various species as a function of pH. Two representative examples are shown in Figure 2; the concentrations used are close to some employed in the experiments.

Since the acidity constants of the $H(NMP)^-$ species are nearly identical (Table 1, column 3), an important conclusion follows immediately from the stability constants listed in column 3 of Table 2 for the Pb(NMP) complexes: There is a stability increase in the series, Pb(AMP) < Pb(IMP) < Pb(GMP), and this is only possible if the phosphate-coordinated $Pb^{2+}$ interacts to some extent also with N7 of the nucleobases; hence, equilibrium 1 exists. The increased stability of Pb(GMP), compared to that of Pb(AMP), is also evident from Figure 2. A quantitative evaluation of this observation is given in section 3.8.

**3.4. Structural Considerations on the Monoprotonated $Pb(H;NMP)^+$ Complexes. The Proton is at the Phosphate Group!** Potentiometric pH titrations allow determination of the stability constants of $Pb(H;NMP)^+$ complexes, but in order to locate the binding sites of the proton and the metal ion in these species, further information is needed. At first one best considers the proton because binding of a metal ion to a protonated ligand commonly leads to an acidification of the ligand-bound proton.[33,35] Indeed, the acidity constants of the $Pb(H;NMP)^+$ complexes given in column 4 of Table 2 ($pK^{H}_{Pb(H;NMP)} \simeq 4.45$) are on average 1.8 p*K* units smaller than the values listed in column 3 of Table 1 for the $H(NMP)^-$ species ($pK^{H}_{H(NMP)} \simeq 6.23$), but the acidity constants of the $Pb(H;NMP)^+$ complexes are also between about 0.6 and 3.2 p*K* units *larger* than the $pK^{H}_{H_2(NMP)}$ values (see column 2 in Table 1); hence, the proton must be located at the phosphate group of the NMPs in the $Pb(H;NMP)^+$ complexes.

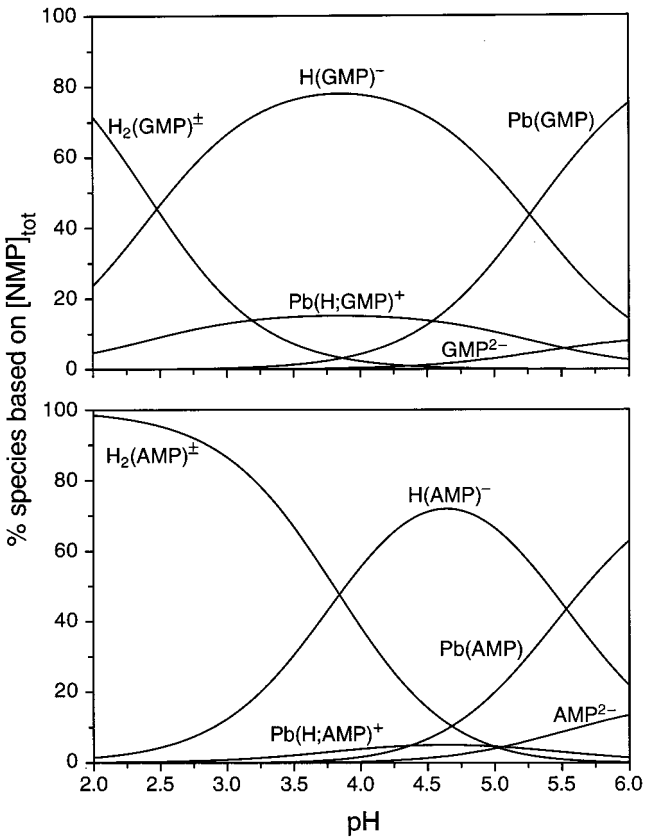


**Figure 2.** Effect of pH on the concentration of species present in the $Pb^{2+}$ systems with GMP (top) and AMP (bottom); the results are given as percentages of the total NMP concentration present. They were computed with the determined acidity (Table 1) and stability constants (Table 2) by using $[NMP]_{tot} = 0.0006$ M and $[Pb^{2+}]_{tot} = 0.006$ M (concentrations close to the experimental conditions; see section 2.4). In the pH range above 5 hydroxo complex formation occurs (see section 2.4), and this was ignored in the above plots.

However, where is the metal ion? Tentatively one might argue that if the proton is at the phosphate group then it appears likely that $Pb^{2+}$ is at the nucleobase residue. In fact, the increasing stability of the $Pb(H;NMP)^+$ complexes in the order $Pb(H;AMP)^+ < Pb(H;IMP)^+ < Pb(H;GMP)^+$ supports this suggestion because the binding tendency of $Pb^{2+}$ toward the $-P(O)_2(OH)^-$ residue should be identical for all three nucleotides, since this residue has the same basicity (Table 1, column 3). These tentative reasonings are largely confirmed by the following evaluation.

**3.5. Considerations on the Location of $Pb^{2+}$ in the $Pb(H;NMP)^+$ Complexes.** For the location of $Pb^{2+}$, in principle two possibilities exist: (i) the metal ion is at the phosphate group like the proton, symbolized by $(NMP\cdot Pb\cdot H)^+$; and (ii) it is at the nucleobase, symbolized by $(Pb\cdot NMP\cdot H)^+$. Hence, eq 10b may be rewritten in the form of eq 14:

$$K^{Pb}_{Pb(H;NMP)} = \frac{[(Pb\cdot NMP\cdot H)^+] + [(NMP\cdot Pb\cdot H)^+]}{[Pb^{2+}][H(NMP)^-]} \quad (14a)$$

$$= k^{Pb}_{Pb\cdot NMP\cdot H} + k^{Pb}_{NMP\cdot Pb\cdot H} \quad (14b)$$

Estimations for the micro stability constant $k^{Pb}_{Pb\cdot NMP\cdot H}$ may be made by using the known stability constants of the $Pb(Ns)^{2+}$ complexes (section 3.2).

For the GMP system, the stability constant $\log K^{Pb}_{Pb(Guo)} = 1.25 \pm 0.17$ (see eq 9) of $Pb(Guo)^{2+}$ needs to be corrected (i)

(35) Sigel, H.; Lippert, B. *Pure Appl. Chem.* **1998**, *70*, 845−854.

for the different basicities of the N7 site in $H(GMP)^-$ and Guo and (ii) for the charge effect that the $-P(O)_2(OH)^-$ group exerts on the $Pb^{2+}$ bound at N7 of the guanine residue in $(Pb{\cdot}GMP{\cdot}H)^+$. This estimation[36] results in $\log k^{Pb}_{Pb\cdot GMP\cdot H} = 1.76 \pm 0.23$ and this value is evidently identical within the error limits with the measured value, $\log K^{Pb}_{Pb(H;GMP)} = 1.52 \pm 0.10$, meaning that the stability of the $Pb(H;GMP)^+$ species is determined by the stability of the $(Pb{\cdot}GMP{\cdot}H)^+$ isomer (cf. eq 14) which carries $Pb^{2+}$ at N7 and the proton at the phosphate group and that the formation of the $(GMP{\cdot}Pb{\cdot}H)^+$ isomer with both $Pb^{2+}$ and $H^+$ at the phosphate group is negligible.

The same procedure may be applied for the AMP system by using $\log K^{Pb}_{Pb(Ado)} = 0.4 \pm 0.3$ (eq 8). This gives[37] $\log k^{Pb}_{Pb\cdot AMP\cdot H} = 0.90 \pm 0.35$ for the stability constant of the $(Pb{\cdot}AMP{\cdot}H)^+$ isomer. This micro stability constant is again within its error limits identical with the measured value $\log K^{Pb}_{Pb(H;AMP)} = 1.08 \pm 0.04$. Hence, it seems that also in this case the $(Pb{\cdot}AMP{\cdot}H)^+$ isomer with $Pb^{2+}$ at the adenine residue and $H^+$ at the phosphate group dominates.

However, at this point one may also ask, How stable is the $(NMP{\cdot}Pb{\cdot}H)^+$ isomer? Evidently, the stability of this isomer should only depend on the affinity of the $-P(O)_2(OH)^-$ group for $Pb^{2+}$, and therefore it should be the same for the AMP, IMP, and GMP systems. Unfortunately, no such value is available in the literature.[13−15] The stability of the $Pb(H_2PO_4)^+$ complex, $\log K^{Pb}_{Pb(H_2PO_4)} = 1.5 \pm 0.5$,[38] appears to be too large to be used here because comparisons of the stabilities of $M(HPO_4)$ complexes with those of the $M(CH_3OPO_3)$ species show[39] that the latter complexes are somewhat less stable. Based on the stability constants of diphosphate monoesters we estimate[40,41] $\log k^{Pb}_{NMP\cdot Pb\cdot H} = 0.7 \pm 0.4$ for the stability of the $(NMP{\cdot}Pb{\cdot}H)^+$ isomer which carries $Pb^{2+}$ and $H^+$ at the phosphate group. Comparison of this value with $\log k^{Pb}_{Pb\cdot AMP\cdot H} = 0.9 \pm 0.35$ reveals that both microconstants are of the same order; in fact, application of eq 14b gives $\log K^{Pb}_{Pb(H;AMP)} = \log [10^{(0.9\pm0.35)} + 10^{(0.7\ \pm\ 0.4)}] = 1.1\ (\pm 0.3)$. Evidently, this calculated value also agrees with the measured one, $\log K^{Pb}_{Pb(H;AMP)} = 1.08 \pm 0.04$ (Table 2). Application of the two estimated micro stability constants[37,40] allows calculation of the ratio $R$ for the two isomers:

$$R = \frac{[(Pb{\cdot}AMP{\cdot}H)^+]}{[(AMP{\cdot}Pb{\cdot}H)^+]} = \frac{k^{Pb}_{Pb\cdot AMP\cdot H}}{k^{Pb}_{AMP\cdot Pb\cdot H}} \quad (15a)$$

$$= \frac{10^{(0.9\pm0.35)}}{10^{(0.7\pm0.4)}} = \frac{1.6}{1} \simeq \frac{60}{40} \quad (15b)$$

Of course, the result of eq 15 must be considered as a rough estimate, but it indicates that most probably both isomers, $(Pb{\cdot}AMP{\cdot}H)^+$ and $(AMP{\cdot}Pb{\cdot}H)^+$, occur in aqueous solution with a possible dominance of the isomer with $Pb^{2+}$ at the adenine residue and the proton at the phosphate group. On the other hand, the above reasonings also indicate that the affinity of the adenine residue and that of a $-P(O)_2(OH)^-$ group for $Pb^{2+}$ are of comparable size.

Application of eq 14b to the GMP system gives $\log K^{Pb}_{Pb(H;GMP)} = \log [10^{(1.76\pm0.23)} + 10^{(0.7\pm0.4)}] = 1.8\ (\pm 0.3)$ which is still within its error limits identical with the measured one, $\log K^{Pb}_{Pb(H;GMP)} = 1.52 \pm 0.10$ (Table 2). Calculation of the ratio $R = [(Pb{\cdot}GMP{\cdot}H)^+]/[(GMP{\cdot}Pb{\cdot}H)^+] = 10^{(1.76\pm0.23)}/10^{(0.7\pm0.4)} = 11.5/1 = 92/8$ confirms the above conclusion that the isomer $(Pb{\cdot}GMP{\cdot}H)^+$, with $Pb^{2+}$ at N7 and $H^+$ at the phosphate group, strongly dominates with a formation degree of over 90%.

An analogous evaluation is not possible for $Pb(H;IMP)^+$ because the stability of the $Pb(Ino)^{2+}$ complex is unknown, but based on the results obtained for $Pb(H;AMP)^+$ and especially for the more closely related $Pb(H;GMP)^+$ system, it is safe to suggest that in this system also the $(Pb{\cdot}IMP{\cdot}H)^+$ isomer with $Pb^{2+}$ at N7 and $H^+$ at the phosphate group dominates.

To conclude, the various evaluations in this section show that $Pb^{2+}$ clearly has an affinity for the nucleobase residues considered here (Table 2); this affinity decreases in the order guanine > hypoxanthine > adenine in agreement with the results described in section 3.2.

**3.6. Evaluation of the Stability of the Pb(NMP) Complexes.** We have already noted in section 3.3 the increase in stability in the series Pb(AMP) < Pb(IMP) < Pb(GMP). Indeed, any macrochelate formation as described by equilibrium 1 must be reflected in an enhanced complex stability.[20,22,42]

Therefore, the stability of the Pb(NMP) complexes is now evaluated by making use of the previously established[16] straight-line correlation for a $\log K^{Pb}_{Pb(R-PO_3)}$ versus $pK^H_{H(R-PO_3)}$ plot, where $R-PO_3^{2-}$ represents phosphate monoester or phosphonate ligands[16,43] in which the residue R is unable to interact with the metal ion (eq 16):[16]

$$\log K^{Pb}_{Pb(R-PO_3)} = (m)pK^H_{H(R-PO_3)} + b \quad (16a)$$

$$= (0.493 \pm 0.033)pK^H_{H(R-PO_3)} - (0.122 \pm 0.213) \quad (16b)$$

The error limits of log stability constants calculated with given $pK^H_{H(R-PO_3)}$ values and eq 16 are $\pm 0.08$ log unit ($3\sigma$) in the $pK_a$ range 5−8.[16] This means that with a known $pK_a$ value for the deprotonation of a $-P(O)_2(OH)^-$ group an expected stability constant for a phosph(on)ate−$Pb^{2+}$ complex can be calculated.

(36) The stability constant of $Pb(Guo)^{2+}$, $\log K^{Pb}_{Pb(Guo)} = 1.25 \pm 0.17$ (eq 9), is corrected for the different basicities of N7 in $H(GMP)^-$ and Guo [i.e., $\Delta pK_a = pK^H_{H_2(GMP)} - pK^H_{H(Guo)} = (2.48 \pm 0.04) - (2.11 \pm 0.04) = 0.37 \pm 0.06$] by applying the estimated slope $m = 0.3$ for the regression line of the log $K$ versus $pK_a$ plot (this estimate is based on the slopes given in refs 31 and 32) and this leads to the "corrected" value $(1.25 \pm 0.17) + (0.11 \pm 0.06/\text{estimated error}) = 1.36 \pm 0.18$. This value needs to be further corrected for the charge effect which the $-P(O)_2(OH)^-$ group exerts on $Pb^{2+}$ at N7 [the effect of the same group on $(N7)H^+$ is taken care of via $\Delta pK_a$]; this effect corresponds to $0.40 \pm 0.15$ log unit, as is known[28] from various other cases where the distances between the positive and negative charges are of a comparable size. Hence, one obtains $\log k^{Pb}_{Pb\cdot GMP\cdot H} = (1.36 \pm 0.18) + (0.40 \pm 0.15) = 1.76 \pm 0.23$.

(37) Application of the procedure described in footnote 36 gives the correction term $[(3.84 \pm 0.02) - (3.61 \pm 0.03) = 0.23 \pm 0.04$ and multiplied by $m = 0.3] = 0.07 \pm 0.04$ for the different basicities of N1 in $AMP^{2-}$ and Ado; this together with the charge effect of $0.40 \pm 0.15$ leads to the micro stability constant $\log k^{Pb}_{Pb\cdot AMP\cdot H} = (0.4 \pm 0.3) + (0.07 \pm 0.04) + (0.40 \pm 0.15) = 0.9 \pm 0.35$.

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(39) Saha, A.; Saha, N.; Ji, L.-n.; Zhao, J.; Gregáň, F.; Sajadi, S. A. A.; Song, B.; Sigel, H. *J. Biol. Inorg. Chem.* **1996**, *1*, 231−238.

(40) Protonation of the $\beta$-phosphate group in $M(UDP)^-$ complexes (where $M^{2+} = Cu^{2+}$, $Zn^{2+}$, $Cd^{2+}$) destabilizes these complexes by about 2.1 log units.[41] Since in these cases $M^{2+}$ coordinates to the α- and $\beta$-phosphate groups, the effect of the proton is expected to be somewhat larger in the $(NMP{\cdot}Pb{\cdot}H)^+$ complexes, where only a single phosphate group is available. We estimate therefore $\log k^{Pb}_{NMP\cdot Pb\cdot H} = 2.9-2.2 = 0.7 \pm 0.4$ (estimated error/the value 2.9 is from column 3 in Table 2).

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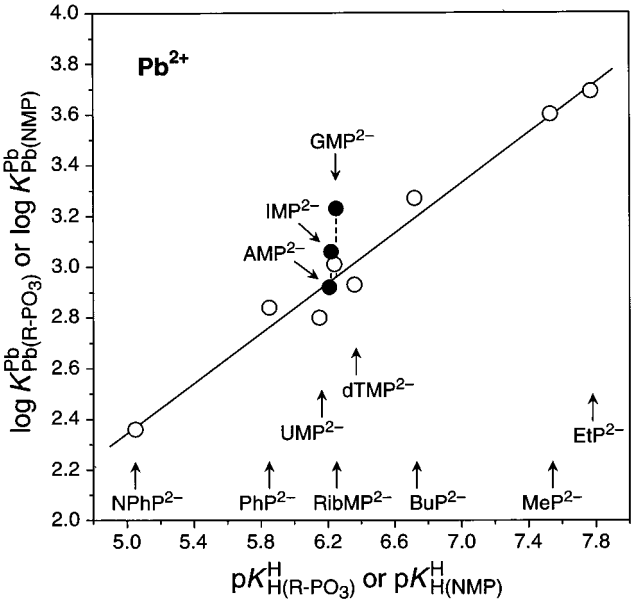


**Figure 3.** Evidence for an enhanced stability of the $Pb^{2+}$ 1:1 complexes formed with $IMP^{2-}$ and $GMP^{2-}$, and for the lack of such an enhanced stability of the Pb(AMP) complex (●), based on the relationship between log $K^{Pb}_{Pb(R-PO_3)}$ and $pK^{H}_{H(R-PO_3)}$ for the 1:1 complexes of $Pb^{2+}$ with some simple phosphate monoester or phosphonate ligands ($R-PO_3^{2-}$) (○): 4-nitrophenyl phosphate ($NPhP^{2-}$), phenyl phosphate ($PhP^{2-}$), uridine 5′-monophosphate ($UMP^{2-}$), D-ribose 5-monophosphate ($RibMP^{2-}$), thymidine [= 1-(2′-deoxy-$\beta$-D-ribofuranosyl)thymine] 5′-monophosphate ($dTMP^{2-}$), *n*-butyl phosphate ($BuP^{2-}$), methanephosphonate ($MeP^{2-}$), and ethanephosphonate ($EtP^{2-}$) (from left to right). The least-squares line (eq 16) is drawn through the corresponding eight data sets (○), which are taken from ref 16. The data points due to the equilibrium constants for the $Pb^{2+}$/AMP, /IMP, and /GMP systems (●) are based on the data given in columns 3 of Tables 1 and 2. The vertical broken lines emphasize the stability differences of the Pb(NMP) (●) complexes to the reference line; these differences are equal to log $\Delta_{Pb/NMP}$ (eq 17), the values of which are listed in column 4 of Table 3. All the plotted equilibrium constant values refer to aqueous solutions at 25 °C and $I = 0.1$ M ($NaNO_3$).

The plot of log $K^{Pb}_{Pb(R-PO_3)}$ versus $pK^{H}_{H(R-PO_3)}$ according to eq 16 is shown in Figure 3 for the 1:1 complexes of $Pb^{2+}$ with eight simple ligands allowing only a phosph(on)ate−$Pb^{2+}$ coordination. The solid points referring to the $Pb^{2+}$ complexes of $AMP^{2-}$, $IMP^{2-}$, and $GMP^{2-}$ prove an increased stability for the IMP and GMP systems. This observation can be evaluated quantitatively by calculating with the straight-line eq 16 and the $pK^{H}_{H(NMP)}$ values the expected (calcd) stabilities for the Pb(NMP) complexes having solely a phosphate−$Pb^{2+}$ coordination. The corresponding results are listed in column 3 of Table 3.

Comparison of the calculated (calcd) stability constants for the Pb(NMP) complexes with the measured (exptl) ones, i.e., according to eq 17a, leads to the stability differences given in

$$\log \Delta_{Pb/NMP} = \log K^{Pb}_{Pb(NMP)_{exptl}} - \log K^{Pb}_{Pb(NMP)_{calcd}} \quad (17a)$$

$$= \log K^{Pb}_{Pb(NMP)} - \log K^{Pb}_{Pb(NMP)_{op}} \quad (17b)$$

the fourth column of Table 3. The expression $K^{Pb}_{Pb(NMP)_{exptl}}$ (eq 17a) corresponds of course to $K^{Pb}_{Pb(NMP)}$ as used in Table 2 and $K^{Pb}_{Pb(NMP)_{calcd}}$ and $K^{Pb}_{Pb(NMP)_{op}}$ are synonymous (eq 17b) because the calculated value equals the stability constant of the "open" isomer, $Pb(NMP)_{op}$, of equilibrium 1 in which only a $-PO_3^{2-}$/$Pb^{2+}$ interaction occurs (see also section 3.8).

**Table 3.** Stability Constant Comparison for the Pb(NMP) Complexes between the Measured Stability Constants (exptl; Table 2, Column 3) and the Calculated Stability Constants (calcd) Based on the Basicity of the Phosphate Group in $AMP^{2-}$, $IMP^{2-}$, and $GMP^{2-}$ ($pK^{H}_{H(NMP)}$; Table 1, Column 3) and the Baseline Equation Established Previously,[16] (see Eq 16 and Figure 3), Together with the Stability Differences log $\Delta_{Pb/NMP}$ as Defined by Eq 17 (Aqueous Solution; 25 °C; $I = 0.1$ M, $NaNO_3$)[a]

| $NMP^{2-}$ | log $K^{Pb}_{Pb(NMP)exptl}$ | log $K^{Pb}_{Pb(NMP)calcd}$ | log $\Delta_{Pb/NMP}$ |
|---|---|---|---|
| $AMP^{2-}$ | 2.92 ± 0.08 | 2.94 ± 0.08 | −0.02 ± 0.11 |
| $IMP^{2-}$ | 3.06 ± 0.05 | 2.94 ± 0.08 | 0.12 ± 0.09 |
| $GMP^{2-}$ | 3.23 ± 0.08 | 2.96 ± 0.08 | 0.27 ± 0.11 |

[a] See footnote *a* in Table 2.

**3.7. Macrochelate Formation and Properties of the N7 Sites.** The vertical broken lines seen in Figure 3 correspond to the log $\Delta_{Pb/NMP}$ values (eq 17) given in column 4 of Table 3, and these prove for the Pb(IMP) and Pb(GMP) complexes an increased stability whereas the one of the Pb(AMP) complex is determined within the error limits by the basicity of the phosphate group. Previously it has been proven for several metal ions, including $Zn^{2+}$ and $Cd^{2+}$, by using tubercidine 5′-monophosphate ($TuMP^{2-}$ = 7-deaza-$AMP^{2-}$), that in the M(AMP) complexes macrochelate formation, which is responsible for the increased stability, occurs with N7.[22] Consequently, one expects that the extent of macrochelate formation depends on the basicity of N7, and indeed, this has also been proven for several series of M(AMP), M(IMP), and M(GMP) complexes.[20]

In the present situation the basicity of N7 is best described in a *relative* sense by the $pK^{H}_{H(Ns)}$ values given for $H(Ino)^+$ and $H(Guo)^+$ in Table 1, since the effect of the $-P(O)_2(OH)^-$ residue on N7 basicity is the same for all three NMPs considered here. However, for adenosine the situation is more complicated because the initial protonation of the purine ring occurs at N1 and *not* at N7; hence, a micro acidity constant quantifying the N7 basicity has to be determined in an indirect way and over the years several attempts have been made.[31,32,44,45] The micro acidity constant needed now is a *relative* one, i.e., one that takes the steric inhibition[46] exercized by the $(C6)NH_2$ group on metal ion coordination at the N7 site into account, i.e., $pK^{H}_{H(N7/Ado,NH_2)} = -0.2 \pm 0.3$.[31,47,48] In inosine and guanosine no competition between the proton affinity of N7 and another site exists; i.e., the measured macroconstants are identical with the corresponding microconstants, $pK^{H}_{H(Ns)} = pk^{H}_{H(N7/Ns)}$ (see Table 1).

Since the log $\Delta$ values according to eq 17 reflect the extent of macrochelate formation, they are plotted in Figure 4 as a function of the micro acidity constants, $pk^{H}_{H(N7/Ns)}$, discussed above; the result for the three Pb(NMP) complexes is a straight line. In addition, the log $\Delta_{M/NMP}$ values for the Zn(NMP) and Cd(NMP) complexes[21a] are plotted in the same figure to demonstrate that $Pb^{2+}$ is not a special case but behaves in its $NMP^{2-}$ complexes just as other metal ions with an affinity for N7. Only the affinity of $Pb^{2+}$ toward N7 is somewhat less pronounced (see Figure 4) than that of $Zn^{2+}$ or $Cd^{2+}$.[20,21] The straight lines seen in Figure 4 do not exclude the possibility

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(47) See also ref 21a, section 4.4 on p 167.
(48) The simple micro acidity constant for protonation at the N7 site of adenosine (i.e., without the consideration of the steric effect of the $(C6)NH_2$ group on metal ion binding at N7) has been estimated recently by Martin: $pk^{H}_{H(N7/Ado)} = 1.3$.[32]

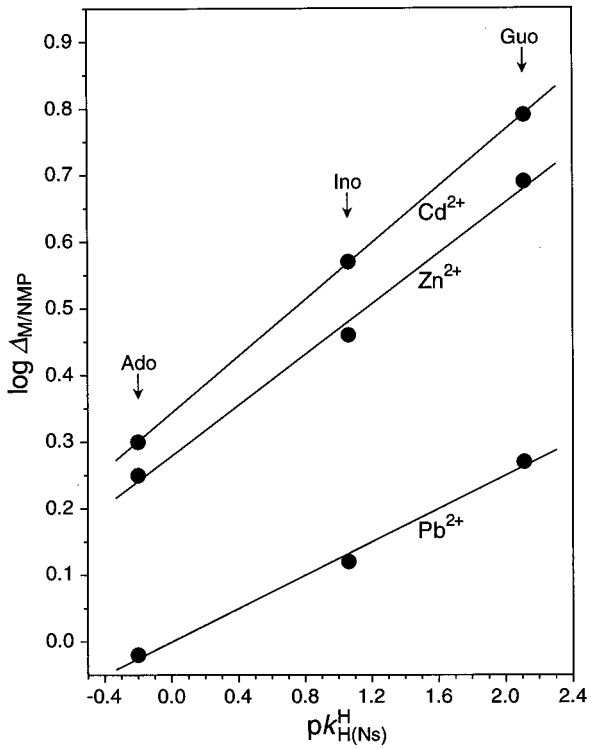


**Figure 4.** Relationship between log $\Delta_{Pb/NMP}$ (eq 17) for the $Pb^{2+}$ 1:1 complexes of $AMP^{2-}$, $IMP^{2-}$, and $GMP^{2-}$ (●) and $pk^{H}_{H(N7/Ns)}$ (see text in section 3.7) of the corresponding nucleosides (Ns), adenosine (Ado), inosine (Ino), and guanosine (Guo). The values for log $\Delta_{Pb/NMP}$ are from column 4 of Table 3 and those for $pk^{H}_{H(N7/Ns)}$ are from ref 31, ref 47 (section 3.7), and Table 1. For comparison, the same relationships are shown for the Zn(NMP) and Cd(NMP) complexes; the corresponding log $\Delta_{M/NMP}$ values are taken from tables 6 and 9 of ref 21a.

that to some extent a semichelate[20] is formed involving via H-bond formation the (C6)-carbonyl site and a metal ion coordinated water molecule in the case of the M(IMP) and M(GMP) complexes.

**3.8. Extent of Macrochelate Formation for the Pb(NMP) Complexes.** At this point the question arises about the formation degree of the two isomeric complexes seen in equilibrium 1. The position of this concentration-independent equilibrium between an "open" isomer, $Pb(NMP)_{op}$, and a "closed" or macrochelated species involving the N7 site, $Pb(NMP)_{cl}$, is defined by the intramolecular and, hence, dimensionless equilibrium constant $K_I$:

$$K_I = [Pb(NMP)_{cl}]/[Pb(NMP)_{op}] \qquad (18)$$

The connection between the values for log $\Delta_{Pb/NMP}$ (=log $\Delta$ in eq 19), which correspond to the vertical distances indicated by broken lines in Figure 3, and $K_I$ is given by eq 19 (for details see refs 20−22 and 42)

$$K_I = \frac{K^{Pb}_{Pb(NMP)}}{K^{Pb}_{Pb(NMP)_{op}}} - 1 \qquad (19a)$$

$$= 10^{\log \Delta} - 1 \qquad (19b)$$

and the percentage of the closed isomer occurring in equilibrium 1 follows from eq 20:

$$\% Pb(NMP)_{cl} = 100K_I/(1 + K_I) \qquad (20)$$

**Table 4.** Extent of Macrochelate Formation According to Equilibrium 1 for Pb(NMP) Complexes as Calculated from the Stability-Constant Differences, log $\Delta_{Pb/NMP}$ (Eq 17; Table 3, Column 4): Given Are the Intramolecular and Dimensionless Equilibrium Constant $K_I$ (Eqs 18 and 19) and Percentage (Eq 20) of the Closed Isomer, $Pb(NMP)_{cl}$, in Aqueous Solution at 25 °C and $I$ = 0.1 M ($NaNO_3$)[a]

| system | log $\Delta_{Pb/NMP}$ | $K_I$ | % $Pb(NMP)_{cl}$ |
|---|---|---|---|
| Pb(AMP) | −0.02 ± 0.11 | 0 (<0.23) | 0 (<19) |
| Pb(IMP) | 0.12 ± 0.09 | 0.32 ± 0.27 | 24 ± 16 |
| Pb(GMP) | 0.27 ± 0.11 | 0.86 ± 0.47 | 46 ± 14 |

[a] See footnote *a* in Table 2.

The corresponding results are listed in columns 3 and 4 of Table 4.

The reliability of any calculation for $K_I$ (eq 19) depends on the accuracy of the difference log $\Delta_{Pb/NMP}$, which becomes the more important the more similar the two constants in eq 17 are. Therefore, only well-defined error limits allow a quantitative evaluation of the extent of the possibly formed macrochelates.

For this reason the result for the Pb(AMP) complex may appear at first sight somewhat ambiguous: on one hand the formation degree of the $Pb(AMP)_{cl}$ macrochelate is zero within the error limits, while on the other hand one has to conclude that if such an isomer should exist, its formation degree is below 19% (see Table 4, column 4). For the Pb(IMP) and Pb(GMP) systems the macrochelate is a significant species with a formation degree of about 25−45%, respectively.

## 4. Conclusions

The results presented establish that $Pb^{2+}$ is able to form macrochelates with purine nucleotides, i.e., that a phosphate-coordinated $Pb^{2+}$ may also interact with N7 of the purine ring, especially in the case of a guanine or a hypoxanthine residue (sections 3.6−3.8). This result is in accord with the stability constants measured for $Pb(Ns)^{2+}$ complexes (section 3.2). However, the present stability data also allow quantification, at least approximately, of the affinity of $Pb^{2+}$ for nucleobase residues in nucleic acids; the order of the decreasing affinity is guanine (log $K^{Pb}$ = 1.6) ≃ cytosine (1.5) > hypoxanthine (1.2) > adenine (0.8) > uracil ≃ thymine.

The log affinity constants given in parentheses in the above series are based on the measured stability constants of the $Pb(Ns)^{2+}$ (section 3.2) and $Pb(H;NMP)^{+}$ (Table 2) complexes as well as on the estimated stabilities for the $(Pb{\cdot}NMP{\cdot}H)^{+}$ species (section 3.5). These constants reflect the affinities for $Pb^{2+}$ of the N7[(C6)O], N3/(C2)O, and N7/(N1) sites of the guanine, cytosine, and adenine residues, respectively, in single-stranded nucleic acids. The affinities of the adenine residue (log $K^{Pb}$ ≃ 0.8) and the phosphodiester linkage, $-O-P(O)_2^{-}-O-$ (∼0.7), are very similar, but the latter is overall more likely to coordinate with $Pb^{2+}$ because of its higher abundance. The (C2)O and (C4)O carbonyl sites of uracil and thymine are expected to interact only weakly with $Pb^{2+}$.

To summarize, the primary binding sites for $Pb^{2+}$ in a nucleic acid are the guanine and cytosine residues, followed by the $-O-P(O)_2^{-}-O-$ unit due to its abundance, whereas N7 and N1 of adenine and especially the carbonyl oxygens of uracil and thymine are expected to interact with $Pb^{2+}$ only if they are preoriented in a sterically favorable position by the initial or primary metal ion binding site.[49a] The latter also applies to the hydroxy groups of ribose residues: such −OH groups are known[49b,c] to be able to interact with metal ions, but only weakly.

With the above conclusions in mind it is interesting to note that in leadzyme, a ribozyme that requires $Pb^{2+}$, the cleavage site of the RNA is between a guanine and cytosine residue,[8a,10a,11a] though this does not necessarily mean that the metal ion is bound here. However, there is now much evidence[11a,12,50,51] including an X-ray structure[10b] that N7 [and possibly also (C6)O] of a guanine residue is essential for $Pb^{2+}$ binding and activity. Indeed, alterations at several of the guanine residues in leadzyme prove that these sites are crucial for the catalytic activity;[11a] apparently only three nucleobases, one cytosine and two guanines, are absolutely required for catalysis.[51] On the other hand, in the X-ray structure study,[10b] aside from $-O-P(O)_2^--O-/Pb^{2+}$ binding, also a N1(adenine)$-Pb^{2+}$ interaction is proposed, but this residue can be replaced by several other residues without severely impairing activity.[10a,50] In another leadzyme, which has two sites for $Pb^{2+}$ binding, also guanine residues are essential.[12] It has further been suggested that a lead-bound hydroxide ion in $Pb(OH)^+$ serves to activate the 2′-hydroxyl group of the cytidine residue.[10b,52] This is similar to the $M(OH)^+$-facilitated hydrolysis of nucleoside 5′-triphosphates where evidence exists[53] that an intramolecular nucleophilic attack of the coordinated hydroxide ion occurs (see also ref 54).

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