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Interactions of lead(II) with nucleotides and their constituents

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This article is dedicated to Professor Dr. A.B.P. Lever, the Founding Editor of Coordination Chemistry Reviews, on the occasion of his 65th birthday with the best wishes of the authors for all his future endeavors.

Contents

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Abstract

The coordination chemistry between the toxic lead(II) ion and nucleotides or nucleic acids is relatively poorly developed despite the known effects of Pb^{2+} on the structure and stability of nucleic acids. In this review information is summarized regarding the stability of Pb^{2+} 1:1 complexes formed in aqueous solution with simple phosphate monoester or phosphonate ligands ($R\text{-}PO_3^{2-}$). The plot of log $K^{Pb}_{Pb(R\text{-}PO_3)}$ (stability constants) versus $pK^{H}_{H(R\text{-}PO_3)}$ (acidity constants) results in a straight line on which also the data pairs for the Pb^{2+}-nucleotide complexes of UMP^{2-}, $dTMP^{2-}$, CMP^{2-} and AMP^{2-} ($= NMP^{2-}$) fit indicating that no significant nucleobase–Pb^{2+} interaction occurs in these Pb(NMP) species. This is different with the Pb(IMP) and Pb(GMP) complexes which are more stable than expected on the basis of the basicity of their phosphate group; in these instances the phosphate-coordinated Pb^{2+} forms a macrochelate by interacting with N7 of the purine residue giving thus rise to intramolecular equilibria. These observations are corroborated by results obtained for the stability of $Pb(nucleoside)^{2+}$ complexes. In the monoprotonated $Pb(H;NMP)^{+}$ complexes of CMP^{2-}, GMP^{2-}, IMP^{2-} and AMP^{2-} the proton is always at the phosphate group and Pb^{2+} mostly at the nucleobase residue. With regard to single-stranded nucleic acids it is concluded that for the affinity of Pb^{2+} toward the various constituents of nucleic acids the following order holds: guanine–N7(O6) $\gtrapprox$ cytosine–N3(O2) $\gtrapprox$ $R'OP(O)_2^-OR$ (phosphate-diester bridge) $\gtrapprox$ adenine > uracil ~ thymine. For the stability constants of the 1:1 complexes formed between Pb^{2+} and nucleoside 5′-diphosphates or 5′-triphosphates estimates are given. Stability studies with methyl thiophosphate and uridine 5′-*O*-thiomonophosphate show that the substitution of one of the terminal oxygens by a sulfur atom in the phosphate group of a phosphate monoester leads to a stability enhancement of about 2.4 log units compared with the original affinity of the phosphate group toward Pb^{2+}. This indicates that the insertion of an artificial thiophosphate group into a nucleic acid sequence makes this sulfur-containing group the preferred binding site for Pb^{2+}.

Keywords: Intramolecular isomeric equilibria; Lead(II) complexes of biological relevance; Nucleic acid–lead(II) interactions; Nucleoside–lead(II) complexes; Nucleotide–lead(II) complexes

1. Introduction

1.1. General considerations on the properties of lead(II) and its toxicity

Owing to its easy reduction from its ores upon heating, the metal lead has been known for 6000 years. Wealthy Romans suffered from lead toxicity because their food was cooked, their wine and acidic fruits stored, and their water piped in leaden vessels. What part such lead exposure played in the demise of Roman culture and empire has been debated [1].

Today food and water are the main sources of lead in the body, and children are more likely to absorb and retain lead [2,3]. The only oxidation state important in biological systems is Pb^{2+} [4]. It is not particularly carcinogenic, but quite toxic [2,5]. Acute toxicity is dealt with by infusion of Ca^{2+}–EDTA, with the intent of exchanging metal ions on the EDTA [2]. More than 95% of the body burden of lead

is stored inertly in bone [3,4]. Available Pb(II) affects the structure and function of the nervous system, renal system, and bone marrow, where it inhibits several enzymes involved in heme synthesis [2,4]. More than 95% of blood Pb(II) occurs in the red cells [2], where glutathione promises to be the strongest small-molecule Pb^{2+}-binder. Also in red cells, Pb^{2+} may induce a detoxifying metallothionein-like protein [6].

Interaction of Pb^{2+} with many components in the body severely restricts the free Pb^{2+} concentration in the blood or within cells to very low levels. For insoluble lead(II) hydrogen phosphate, $Pb(HOPO_3)$, the solubility product constant is reported as $10^{-11.4}$ [7]. We employ this value and the procedure developed for limited $Ca(HOPO_3)$ solubility [8], and we find for the extracellular fluid at pH 7.4 and 2 mM total inorganic phosphate an allowed free Pb^{2+} concentration of $2.5 \cdot 10^{-8}$ M; for the intracellular fluid at pH 6.6 and 10 mM phosphate we obtain $[Pb^{2+}]_{free} = 10^{-8}$ M. These very low permitted free Pb^{2+} concentrations in body fluids demonstrate that any significant quantities of total Pb(II) must be complexed in some way.

Pb^{2+} exhibits irregular coordination geometries (often with bridging ligands) and variable coordination numbers, 6 to 10 being common [9]. In a crystal structure of $Pb(H_2PO_4)_2$, phosphate oxygen-bridged Pb^{2+} is surrounded by a total of seven oxygen atoms from seven different $H_2PO_4^-$ groups, five at short distances and two farther away [10]. The variable and adaptive coordination sphere of Pb^{2+} presumably accounts for its ability to interact in biological systems at sites normally harboring smaller metal ions such as Ca^{2+} and Zn^{2+} [11–14].

Contrary to some expectations, Pb^{2+} is not an extraordinarily strong ligand binder, and favors oxygen as well a sulfur donors, vitiating application of the hard and soft concept [15]. We may relate Pb^{2+} binding in terms of the *Stability Ruler* that places ligand binding strengths on the scale of the usual stability order of dipositive metal ions: $Mg^{2+} < Mn^{2+} < Fe^{2+} < Co^{2+} < Ni^{2+} < Cu^{2+}$. With nitrogen donors Pb^{2+} binds at about the same strength as Fe^{2+} and more weakly than Cd^{2+} and Zn^{2+}, with amino acids Pb^{2+} moves up the ruler to the strength of Co^{2+} and binds at about the same strength as Cd^{2+} and Zn^{2+}, and with both oxygen and sulfur donors Pb^{2+} binds with about the same strength as Cu^{2+} and more strongly than Cd^{2+} and Zn^{2+} [11,14,16].

In the calmodulin family of proteins Pb^{2+} binds more strongly than the native Ca^{2+} to the calcium sites, composed solely of O donors. In oncomodulin and chick vitamin-D induced intestinal calcium-binding protein, Pb^{2+} binding occurs exclusively at the Ca^{2+} sites, despite the presence of a free sulfhydryl group on the proteins [17]. Calmodulin in which Pb^{2+} has replaced Ca^{2+} yields a protein that retains many of its native activities [18–20]. Such Pb^{2+} induced activities may be responsible for some of its toxicity. The chemical relationship between Pb^{2+} and Ca^{2+} is confirmed also by the already mentioned observation that more than 95% of the body burden of lead is stored in bones [3].

The rate of exchange of ligands into and out of the coordination sphere of Pb^{2+} is the fastest of almost any metal ion; there should be a rapidly attained equilibrium distribution of Pb^{2+} among ligand sites [11,12,14].

Aqueous Pb^{2+} undergoes loss of protons from bound water molecules with successive pK_a values of 7.8, 9.4, and 10.7 ($I = 0.16$ M; 25°C) [21]. Thus at low total lead(II) concentrations, both intracellular (pH 6.6) and extracellular (pH 7.4) body fluids contain mostly aquated Pb^{2+} with some aquated $Pb(OH)^{+}$. Assuming that a similar pK_a value of 7.8 prevails in Pb^{2+} complexes, an hydroxo complex may serve as a general base catalyst, a role that has been proposed several times. At the total concentrations of lead(II) prevailing in many experiments, soluble polynuclear complexes form: First to appear at about 0.010 mM total lead is $Pb_3(OH)_4^{2+}$ from pH 8 to 10. Greater total lead(II) concentrations yield polynuclear species across the entire pH scale [21].

Rarely cited, but with the proper geometry potentially more potent than general base catalysis, is the role of a metal ion bound hydroxide as a nucleophile [22]. Though a bound hydroxide is of greatly reduced basicity, it is of only modestly reduced nucleophilicity [23]. The nucleophilicity of the bound hydroxide is less than that of unbound hydroxide ion, but the former occurs in a much higher effective concentration when juxtaposed to a reactive site on a (macro)molecule. Thus in neutral solutions the nucleophilicity of a metal ion-bound hydroxide may be 10^7 times greater than that of unbound hydroxide [23]. Nucleophilic attack by a Pb^{2+}-bound OH^{-} would directly sever a phosphate bond without a cyclic intermediate and should be considered when a cyclic intermediate does not appear or if there is no lag phase in production of the final product [24,25]. Nucleophilic attack of metal ion- (including Pb^{2+}) bound OH^{-} has been suggested as a possible pathway in the metal ion catalyzed hydrolysis of uridine 2′,3′-cyclic monophosphate [26].

1.2. Interactions of lead(II) with nucleic acids and related compounds

Of eleven dipositive metal ions examined, Pb^{2+} is the most effective by a considerable margin in forming oligoadenylic acids from adenosine 5′-phosphorimidazolide, an activated derivative of adenylic acid [27,28].

Pb^{2+} is involved in both non-specific and specific cleavage of t-RNA molecules [29–31] and indeed, the Pb(II)-induced hydrolysis of RNA is much studied [32–36] and interactions with DNA are also considered [37–39]; e.g. lead(II) is especially effective in sequence-specific folding of DNA [37]. Furthermore, Pb^{2+} is by far the most effective metal ion catalyzing the depolymerization of RNA and homopolyribonucleotides [40]. Double stranded species resist hydrolysis. Increasing the concentrations of Pb(II) shifts the pH optimum to lower pH [40]. This result may be accounted for by the polynuclear complex formation mentioned above (Section 1.1) at the high concentrations of Pb(II) employed in these experiments, i.e. 0.125 and 1.25 mM.

Two independent crystal structure analyses reveal a specific cleavage between residues 17 and 18 in a loop region of yeast phenylalanine *t*-RNA induced by a nearby bound $Pb(OH)^{+}$ in the folded molecule [30,41–43]. Pb^{2+} is bound to N3 and (more weakly) O2 of cytosine(60), to an oxygen of phosphate(19) and to O4 of uracil(59). The bound hydroxide in the metal ion complex serves as a base for

removal of a 2′-ribose hydrogen, leaving the nucleophilic alcoholate oxygen to form a 2′,3′-cyclic phosphate and a free 5′-hydroxyl, cleaving the chain. That the role of Pb^{2+} in the cleavage is catalytic rather than merely stoichiometric has received emphasis [42,43]. This conclusion follows easily from the rapid equilibration of Pb^{2+} among ligand sites, as mentioned above under 'exchange' in Section 1.1, with tighter Pb^{2+} binding in the intact polymer. It is evident that Pb^{2+} performs two roles: it enforces the appropriate folding and provides the catalytic center.

In a relatively unique reaction, a small lead ribozyme, a so-called leadzyme, not only undergoes specific cleavage to yield a 2′,3′-cyclic phosphate, but the latter also undergoes hydrolysis to yield a 3′-phosphomonoester [44]. That the final product results from a second step through a cyclic phosphate intermediate and not a direct parallel reaction was established by an induction period in its rate of appearance. The cleavage reaction is highly specific for Pb^{2+}, and the pH dependence suggests that the reactive complex has again bound $Pb(OH)^{+}$ acting as a base [45]. Hydrolysis of the 2′,3′-cyclic phosphate may proceed by direct nucleophilic attack of the Pb^{2+}-bound OH^{-}. A crystal structure determination reveals Pb^{2+} binding to N7 and O6 of a guanine and to O4 of a uracil [46].

The universe of leadzymes, i.e. ribozymes that require Pb^{2+}, has been greatly expanded by in vitro selection of RNA molecules that undergo autolytic cleavage by Pb^{2+} [47]. A similar method was used to prepare a single stranded DNA enzyme that rapidly catalyzes the Pb(II)-dependent cleavage of an RNA–phosphoester bond [48].

With results of the kind indicated above in mind, we summarize below the Pb^{2+}-binding properties of phosphate groups and nucleobase residues as well as of nucleotides, i.e. of the constituents of nucleic acids.

2. Lead(II) complexes of phosphates

That Pb^{2+} easily forms solids with phosphates, e.g. $Pb(HPO_4)$ or $Pb_3(PO_4)_2$, is well known [7,49]. Much less information exists about the stability of Pb^{2+} complexes formed with phosphates and derivatives in aqueous solution [50–52]. Therefore, we shall at first consider the stabilities of the Pb^{2+} complexes formed with the phosphate monoester or phosphonate ligands seen in Fig. 1 [53].

2.1. Phosphate monoesters and phosphonates as ligands

The first proton from twofold protonated phosph(on)ate ligands, $H_2(R\text{-}PO_3)$, is released with $pK_a \lesssim 2.1$ [54,55] and thus is not of relevance for the physiological pH range. This is different for the release of the second proton which occurs, e.g. for ribose 5-monophosphate with a pK_a value of 6.24 [53,56]. It is also evident that monoprotonated *p*-nitrophenyl phosphate is more acidic [56] than its phenyl phosphate counterpart (Fig. 1) due to the electron withdrawing properties of the NO_2 substituent. Therefore, it was of interest to establish the relation between complex stability and phosphate group basicity; this reasoning has led to the

determination of the constants for the following two equilibria via potentiometric pH titrations:

$$H(R\text{-}PO_3)^- \rightleftharpoons H^+ + R\text{-}PO_3^{2-} \quad (1a)$$

$$K^{H}_{H(R\text{-}PO_3)} = [H^+][R\text{-}PO_3^{2-}]/[H(R\text{-}PO_3)^-] \quad (1b)$$

$$Pb^{2+} + R\text{-}PO_3^{2-} \rightleftharpoons Pb(R\text{-}PO_3) \quad (2a)$$

$$K^{Pb}_{Pb(R\text{-}PO_3)} = [Pb(R\text{-}PO_3)]/([Pb^{2+}][R\text{-}PO_3^{2-}]) \quad (2b)$$

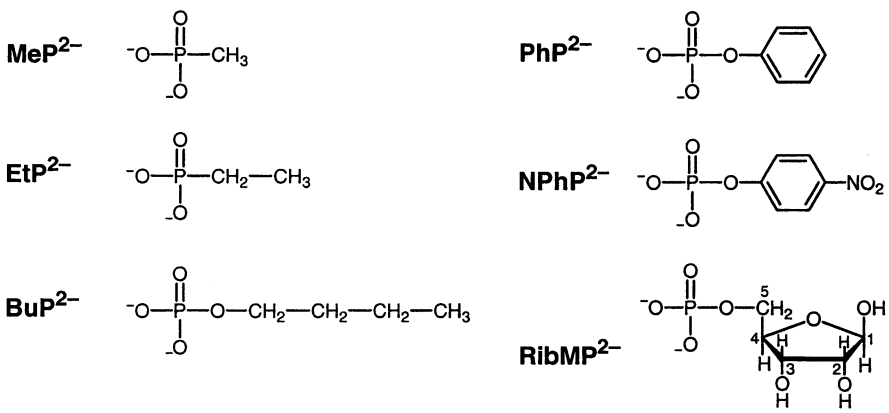


Fig. 1. Chemical structures of the simple phosphonates and phosphate monoesters ($= R\text{-}PO_3^{2-}$) methylphosphonate (MeP^{2-}), ethylphosphonate (EtP^{2-}), *n*-butyl phosphate (BuP^{2-}), phenyl phosphate (PhP^{2-}), 4-nitrophenyl phosphate ($NPhP^{2-}$), and D-ribose 5-monophosphate ($RibMP^{2-}$).

Table 1
Negative logarithms of the acidity constants of some $H(R\text{-}PO_3)^-$ or $H(NMP)^-$ species (Eq. (1)) and logarithms of the stability constants (Eq. (2)) of their corresponding $Pb(R\text{-}PO_3)$ or Pb(NMP) complexes (from [53]) [a,b,c]

No. [c]	$R\text{-}PO_3^{2-}$	$pK^{H}_{H(R\text{-}PO_3)}$	Log $K^{Pb}_{Pb(R\text{-}PO_3)}$
1	$NPhP^{2-}$	5.05 ± 0.01	2.36 ± 0.07
2	PhP^{2-}	5.85 ± 0.01	2.84 ± 0.04
3	$RibMP^{2-}$	6.24 ± 0.01	3.01 ± 0.05
4	BuP^{2-}	6.72 ± 0.02	3.27 ± 0.06
5	MeP^{2-}	7.53 ± 0.01	3.60 ± 0.02
6	EtP^{2-}	7.77 ± 0.01	3.69 ± 0.05
7	UMP^{2-}	6.15 ± 0.01	2.80 ± 0.04
8	$dTMP^{2-}$	6.36 ± 0.01	2.93 ± 0.03

[a] The data refer to aqueous solutions at 25°C and $I = 0.1$ M ($NaNO_3$). All equilibrium constants were measured by potentiometric pH titrations [53]; the acidity constants are so-called practical or mixed constants [53,57].

[b] The error limits given are three times the standard error of the mean value or the sum of the probable systematic errors, whichever is larger.

[c] For entries 7 and 8 holds $R\text{-}PO_3^{2-} = NMP^{2-}$.

The corresponding results [53] are summarized in Table 1 [57] and in Fig. 2 the values of log $K^{Pb}_{Pb(R\text{-}PO_3)}$ are plotted versus those of $pK^{H}_{H(R\text{-}PO_3)}$ [58–63].

From Fig. 2 it is evident that overall the coordinating properties of Pb^{2+} towards phosph(on)ate ligands correspond to those of other divalent metal ions like Ca^{2+} or Zn^{2+}. It follows further, as indicated already in Section 1.1, that the Pb(R-PO_3) complexes are more stable than those of Zn^{2+} or Ca^{2+}. The metabolic interdependencies [12] between these three metal ions are thus no surprise; at least as far as phosphate ligands are concerned, Pb^{2+} is clearly able to replace Ca^{2+} as well as Zn^{2+}.

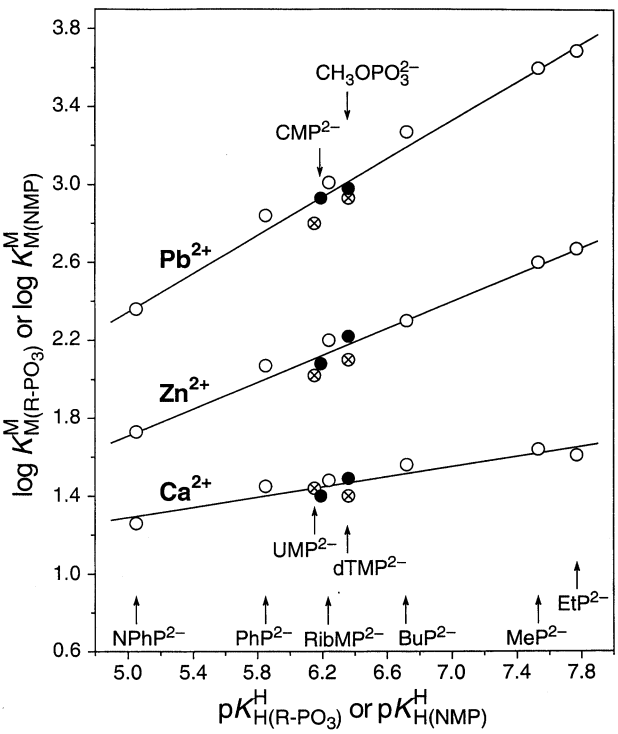


Fig. 2. Relationship between log $K^{M}_{M(R\text{-}PO_3)}$ or log $K^{M}_{M(NMP)}$ and $pK^{H}_{H(R\text{-}PO_3)}$ or $pK^{H}_{H(NMP)}$ for the 1:1 complexes of Pb^{2+} and for comparison also for those of Ca^{2+} and Zn^{2+} of the following R-PO_3^{2-} (○) and NMP^{2-} (⊗) ligands: 4-nitrophenyl phosphate ($NPhP^{2-}$), 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-}), methylphosphonate (MeP^{2-}), and ethylphosphonate (EtP^{2-}) (from left to right). The least-squares lines are drawn through the corresponding eight data sets given in Table 1 for the Pb^{2+} systems (see also Eq. (3)); for the Ca^{2+} and Zn^{2+} systems they are taken for the phosphate monoesters from Ref. [56] and for the phosphonates from Ref. [58]; the equations for these latter two reference lines are listed in Refs. [58–62]. The data points (●) for the Pb(CH_3OPO_3)/$CH_3OP(O)_2(OH)^-$ (Section 2 [54,63]) and Pb(CMP)/H(CMP)$^-$ systems (Section 4.1 [53]) are inserted for comparison. All plotted equilibrium constant values refer to aqueous solutions at 25°C and $I = 0.1$ M ($NaNO_3$).

A further interesting observation which follows from Fig. 2 is that the data points of the Pb^{2+}-phosph(on)ate systems for the first 6 entries of Table 1 fall on a straight line. However, on this same line fall within the error limits also the data points for the Pb^{2+} complexes of the two nucleoside 5′-monophosphates (NMP^{2-}), uridine 5′-monophosphate (UMP^{2-}) and thymidine 5′-monophosphate ($dTMP^{2-}$); i.e. these two NMPs behave like simple phosphate monoesters (see also Section 4.1). Consequently, the least-squares line was calculated [53] by employing all eight data pairs listed in Table 1:

$$\log K^{Pb}_{Pb(R\text{-}PO_3)} = m \cdot pK^{H}_{H(R\text{-}PO_3)} + b \quad (3a)$$

$$= (0.493 \pm 0.033) \cdot pK^{H}_{H(R\text{-}PO_3)} - (0.122 \pm 0.213) \quad (3b)$$

The application of Eq. (3) to any known $pK^{H}_{H(R\text{-}PO_3)}$ value of a $H(R\text{-}PO_3)^-$ species allows calculation of the stability constant of the corresponding $Pb(R\text{-}PO_3)$ complex; the error limit (3σ) of such a calculated value equals 0.08 log unit in the pK_a range (approximately) 5–8 (for details see [53]).

For example, for the deprotonation of $H_2PO_4^-$ $pK^{H}_{H_2PO_4} = 6.75 \pm 0.01$ was determined [54]; considering that in this case the micro acidity constant[3] $pk_{a/2} = 7.05$ needs to be applied to Eq. (3), one obtains $\log K = 3.35 \pm 0.08$ as stability constant[4]. However, the experimental results for ten metal ion complexes (alkaline earth ions and 3d transition ions) formed with HPO_4^{2-} show [54] that the $M(HOPO_3)$ complexes are on average by 0.08 ± 0.02 log unit more stable than expected on the basis of the basicity of HPO_4^{2-}; this slight stability increase has been attributed to a favored solvation of the $M(HOPO_3)$ complexes [54]. Indeed, it was very recently concluded that water binds very avidly to the ionic species derived from H_3PO_4 [64]. Assuming that the mentioned slight stability increase also applies to the $Pb(HOPO_3)$ complex the calculated stability constant amounts then to $\log K^{Pb}_{Pb(HPO_4)} = (3.35 \pm 0.08) + (0.08 \pm 0.02) = 3.43 \pm 0.08$. This calculated value is close to one measured under similar conditions, i.e. at 25°C and $I = 0.1$ M ($NaClO_4$): 3.27 ± 0.14 [65].

2.2. Stabilities of some Pb^{2+} complexes involving various phosphate esters

Considering the phosphate diester unit, $R'OP(O)_2^-OR$, which occurs as a singly negatively charged bridge in the backbone of nucleic acids, it is somewhat unfortunate that for the complex $Pb(H_2PO_4)^+$, [$= Pb(HOP(O)_2OH)^+$], only limited information exists; from solubility measurements $\log K^{Pb}_{Pb(H_2PO_4)} = 1.5 \pm 0.5$ was derived [7] (25°C; $I = 0$ M [49,66]). However, this value appears to be too large [67]; possibly because it was extrapolated to an ionic strength of zero. Recently, based on stability data of diphosphate monoesters, a value for the interaction between Pb^{2+} and a monoprotonated phosphate monoester, $ROP(O)_2^-OH$, was estimated:

[3] In this microconstant the statistical factor for the possible release of two protons is taken into account; see Eq. (6) of [54].

[4] In an earlier calculation only the macro acidity constant was considered [53].

$\log K^{Pb}_{Pb(ROP(O)_2OH)} = 0.7 \pm 0.4$ (25°C; $I = 0.1$ M, $NaNO_3$) [67]. We believe that this value describes the affinity of a phosphate diester unit toward Pb^{2+} relatively well [67]. Even though lead(IV) appears not to be of biological relevance (Section 1.1), the stability constant for the interaction between $(CH_3)_3Pb^+$ and HPO_4^{2-} may also be given: $\log K^{(CH_3)_3Pb}_{(CH_3)_3Pb(HPO_4)} = 1.88$ (25°C; $I = 0.3$ M) [68].

In contrast to the above discussed HPO_4^{2-} ligand the simplest orthophosphate monoester, $CH_3OPO_3^{2-}$, is a 'well-behaved' ligand [54]; this is evident from the data pair $pK^H_{CH_3OP(O)_2OH} = 6.36 \pm 0.01$ [54] and $\log K^{Pb}_{Pb(CH_3OPO_3)} = 2.98 \pm 0.11$ [63], which fits within the error limits on the straight-reference line as seen in Fig. 2. Indeed, the difference $\log \Delta$ between the experimental and the calculated values, by use of Eq. (3), amounts only to $(2.98 \pm 0.11) - (3.01 \pm 0.08) = -0.03 \pm 0.14$. It may be emphasized further that in agreement with the *Stability Ruler* discussed in Section 1.1, the stability of the $Cu(CH_3OPO_3)$ complex $\log K^{Cu}_{Cu(CH_3OPO_3)} = 2.94 \pm 0.03$ [54], is within the error limits identical with the value given above for $Pb(CH_3OPO_3)$.

In the above context it is interesting to compare the terms of the straight-line equation for Cu^{2+} complexes [58] given in Eq. (4) with those in Eq. (3) valid for the

$$\log K^{Cu}_{Cu(R\text{-}PO_3)} = (0.465 \pm 0.025) \cdot pK^H_{H(R\text{-}PO_3)} - (0.015 \pm 0.164) \quad (4)$$

Pb^{2+} complexes. Both straight-line equations are based on the same eight ligands (see Fig. 2) and hold for the pK_a range of about 5–8. It is evident that the slopes m and the intercepts b of the two equations are, within the error limits, identical. The validity of the *Stability Ruler* and the conclusion that Pb^{2+} has about the same affinity toward O donor sites as Cu^{2+} is further confirmed by the calculated stability constants based on Eqs. (3) and (4) and three different representative $pK^H_{H(R\text{-}PO_3)}$ values which cover the mentioned pK_a range:

$$pK^H_{H(R\text{-}PO_3)} = 5.00: \log K^{Pb}_{Pb(R\text{-}PO_3)} = 2.34 \pm 0.08 \text{ and} \quad (5a)$$

$$\log K^{Cu}_{Cu(R\text{-}PO_3)} = 2.31 \pm 0.06 \quad (5b)$$

$$pK^H_{H(R\text{-}PO_3)} = 6.50: \log K^{Pb}_{Pb(R\text{-}PO_3)} = 3.08 \pm 0.08 \text{ and} \quad (6a)$$

$$\log K^{Cu}_{Cu(R\text{-}PO_3)} = 3.01 \pm 0.06 \quad (6b)$$

$$pK^H_{H(R\text{-}PO_3)} = 8.00: \log K^{Pb}_{Pb(R\text{-}PO_3)} = 3.82 \pm 0.08 \text{ and} \quad (7a)$$

$$\log K^{Cu}_{Cu(R\text{-}PO_3)} = 3.71 \pm 0.06 \quad (7b)$$

Since the acidity constants of many monoprotonated nucleoside 5′-monophosphates are close to $pK^H_{H(NMP)} = 6.2$ (see also Fig. 2) [59,61,69] the calculation is also carried out for this pK_a value:

$$pK^H_{H(R\text{-}PO_3)} = 6.20: \log K^{Pb}_{Pb(R\text{-}PO_3)} = 2.93 \pm 0.08 \text{ and} \quad (8a)$$

$$\log K^{Cu}_{Cu(R\text{-}PO_3)} = 2.87 \pm 0.06 \quad (8b)$$

The above results give the necessary confidence to derive stability constants for the Pb^{2+} complexes of diphosphate ($R\text{-}DP^{3-}$) and triphosphate ($R\text{-}TP^{4-}$) monoesters. The knowledge of such values is desirable since no stability constants appear to be available for Pb^{2+} complexes of nucleoside di- and triphosphates

[50–52,70]. From the straight-line relation given in Ref. [71] (valid for the pK_a range 6.2–6.8) and represented in Eq. (9)

$$\log K^{Cu}_{Cu(R\text{-}DP)} = (1.283 \pm 0.115)\cdot pK^{H}_{H(R\text{-}DP)} - (2.939 \pm 0.738) \quad (9)$$

one calculates for $pK_a = 6.4$, an acidity constant valid for many diphosphate monosters [71], the following stability constant of the corresponding $Cu(R\text{-}DP)^-$ complex:

$$pK^{H}_{H(R\text{-}DP)} = 6.40: \log K^{Cu}_{Cu(R\text{-}DP)} = 5.27 \pm 0.04 \quad (10)$$

Since there are no indications that the Jahn–Teller distorted coordination sphere of Cu^{2+} [72] suffers any disadvantage in its binding to a diphosphate unit, compared to divalent metal ions with an octahedral coordination sphere [71], we conclude that for the stability of the $Pb(R\text{-}DP)^-$ complex the following value holds (error limit estimated):

$$pK^{H}_{H(R\text{-}DP)} = 6.40: \log K^{Pb}_{Pb(R\text{-}DP)} = 5.3 \pm 0.15 \quad (11)$$

For the representative acidity constant, $pK_a = 6.50 \pm 0.05$, of monoprotonated triphosphate monoesters [73,74] the stability of the $Cu(R\text{-}TP)^{2-}$ complex is known (see Table 7 in [71] or Table 3 in [75]):

$$pK^{H}_{H(R\text{-}TP)} = 6.50: \log K^{Cu}_{Cu(R\text{-}TP)} = 5.86 \pm 0.03 \quad (12)$$

However, since a triphosphate monoester is a potentially tridentate ligand, its binding to Cu^{2+} is hampered compared with metal ions having a coordination number larger than four [71]; this is also quite evident from the following differences (taken from Table 7 in [71]):

$$\log K^{Cu}_{Cu(R\text{-}TP)} - \log K^{Cu}_{Cu(R\text{-}DP)} = (5.86 \pm 0.03) - (5.27 \pm 0.04) = 0.59 \pm 0.05 \quad (13a)$$

$$\log K^{Co}_{Co(R\text{-}TP)} - \log K^{Co}_{Co(R\text{-}DP)} = (4.76 \pm 0.03) - (3.72 \pm 0.05) = 1.04 \pm 0.06 \quad (13b)$$

$$\log K^{Ni}_{Ni(R\text{-}TP)} - \log K^{Ni}_{Ni(R\text{-}DP)} = (4.50 \pm 0.03) - (3.54 \pm 0.06) = 0.96 \pm 0.07 \quad (13c)$$

$$\log K^{Zn}_{Zn(R\text{-}TP)} - \log K^{Zn}_{Zn(R\text{-}DP)} = (5.02 \pm 0.02) - (4.12 \pm 0.03) = 0.90 \pm 0.04 \quad (13d)$$

Hence, instead of using the result of Eq. (12) we believe that a more correct estimate for the stability constant of the $Pb(R\text{-}TP)^{2-}$ complex is obtained by adding 1.0 (± 0.1) log unit (= average of Eqs. (13b), (13c) and (13d)) to the result of Eq. (11); this then leads to the stability constant given in Eq. (14) (error limit estimated):

$$pK^{H}_{H(R\text{-}TP)} = 6.50: \log K^{Pb}_{Pb(R\text{-}TP)} = 6.3 \pm 0.25 \quad (14)$$

It is comforting to observe that the measured stability constant, $\log K^{Pb}_{Pb(HP_3O_{10})} = 6.32$ (30°C; $I = 1$ M, $NaClO_4$) [76,77], for the Pb^{2+} complex formed with hydrogen triphosphate, i.e. for $Pb(HP_3O_{10})^{2-}$, is very close to the above estimate for the $Pb(R\text{-}TP)^{2-}$ complex.

3. Nucleobase–lead(II) interactions

A nucleotide consists of three main subunits, the nucleobase residue, the sugar part and the phosphate group(s). The metal ion-binding properties of the last group(s) were discussed in Section 2 and the interactions between divalent metal ions and sugar residues are very weak [69]. For example, structural studies showed [78] that simple carbohydrates can bind Ca^{2+} only if they can provide three or more hydroxy groups in a geometrical arrangement fitting the coordination sphere of the metal ion [79]; the same may be assumed for the binding of Pb^{2+}. Similarly, hydroxy-group binding, e.g. to Cu^{2+}, occurs only under special conditions and the initial coordination to a neighboring high affinity site [62,80]; based on the *Stability Ruler* (Section 1.1) a similar weak interaction (if at all) is expected for Pb^{2+}.

The structures of the nucleobase residues of the most common nucleosides (Ns) are shown in Fig. 3. There is not much information known about the binding of Pb^{2+} to these ligands [50–52]; the available stability constants according to Eq. (15)

$$Pb^{2+} + Ns \rightleftharpoons Pb(Ns)^{2+} \tag{15a}$$

$$K^{Pb}_{Pb(Ns)} = [Pb(Ns)^{2+}]/([Pb^{2+}][Ns]) \tag{15b}$$

are summarized in Table 2 together with the corresponding data for some other metal ions [81–87].

Divalent metal ions bind to the neutral nucleosides via N7 in the case of guanosine and inosine [84,87], N1 and N7 of adenosine [81], and N3 of cytidine [82]; there is no indication for an interaction between neutral uridine or thymidine and Cu^{2+} or Pb^{2+} in aqueous solution [83]. Hence, according to the *Stability Ruler* (Section 1.1) one expects that Pb^{2+} binds to N donors with about the same strength as Fe^{2+} and more weakly than Cd^{2+} or Zn^{2+}, and of course, also than

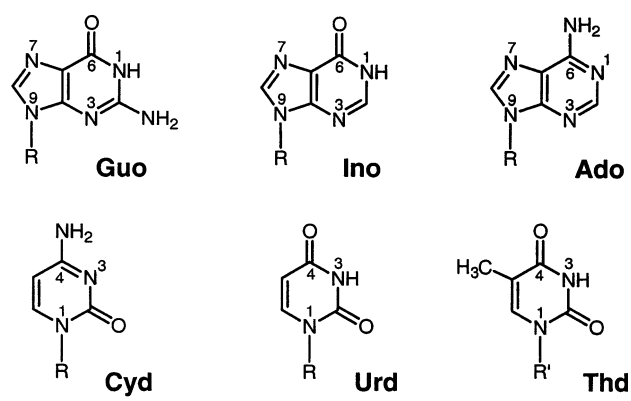


Fig. 3. Chemical structures of the nucleobase residues of the nucleosides (Ns), guanosine (Guo), inosine (Ino), adenosine (Ado), cytidine (Cyd) and uridine (Urd) where R = D-ribose, as well as of thymidine (Thd) where R′ = 2′-deoxy-D-ribose.

Table 2
Negative logarithms of the acidity constants of some $H(Ns)^+$ species and logarithms of the stability constants (Eq (15)) of their corresponding $Pb(Ns)^+$ complexes (25°C; $I = 0.1$ M, $NaNO_3$) together with the stabilities of related $M(Ns)^+$ complexes (close to 25°C and close to $I = 0.1$ M) [a,b]. The logarithms of the micro stability constants given in column 7 refer to the $(Pb{\cdot}NMP{\cdot}H)^+$ isomer in which the proton is at the phosphate group and Pb^{2+} at the nucleobase residue of the considered nucleoside 5′-monophosphate (NMP^{2-}) (25°C; $I = 0.1$ M, $NaNO_3$) [c]

Ns	$pK^{H}_{H(Ns)}$ [d]	Log $K^{Pb}_{Pb(Ns)}$	Log $K^{Cu}_{Cu(Ns)}$	Log $K^{Cd}_{Cd(Ns)}$	Log $K^{Zn}_{Zn(Ns)}$	Log $k^{Pb}_{Pb\cdot NMP\cdot H}$ [c]
Guo	2.11 ± 0.04 [e]	1.25 ± 0.17 [e]	2.15 ± 0.04 [h]	1.17 ± 0.06 [k]	0.80 ± 0.06 [k]	1.76 ± 0.23 [e]
Ino	1.06 ± 0.06 [e]	0.9 ± 0.25 [g]		0.86 ± 0.09 [k]	0.31 ± 0.06 [k]	1.41 ± 0.30 [c]
Ado	3.61 ± 0.03 [e]	0.4 ± 0.3 [e]	0.96 ± 0.06 [i]		0.2 ± 0.3 [i]	0.90 ± 0.35 [e]
Cyd	4.15 ± 0.01 [f]	1.20 ± 0.07 [f]	1.56 ± 0.06 [j]	0.91 ± 0.07 [j]	0.20 ± 0.11 [j]	1.65 ± 0.17 [f]

[a] For the error limits see footnote 'b' of Table 1.

[b] The binding constants should not show a pronounced ionic-strength dependence because the nucleosides are considered as ligands in their neutral form.

[c] These values were calculated [53,67] by using the constants listed in the third column and by taking into account the different basicities of the nucleobase residues in the Ns and $H(NMP)^-$ species, as well as the charge effect of the $-PO_3H^-$ group. Application of the route described in footnote (36) of Ref. [67] and use of the estimated value, $\log K^{Pb}_{Pb(Ino)} = 0.9 \pm 0.25$ (see above), and of the micro acidity constant, $pk^{IMP\cdot H}_{H\cdot IMP\cdot H} = 1.43 \pm 0.08$ (see Fig. 2 in [59]), gives $\log k^{Pb}_{Pb\cdot IMP\cdot H} = 1.41 \pm 0.30$.

[d] The proton is located at N7 in $H(Guo)^+$ and $H(Ino)^+$ [59,81], at N1 in $H(Ado)^+$ [59,81], and at N3 in $H(Cyd)^+$ [81,82].

[e] 25°C; $I = 0.1$ M, $NaNO_3$. From [67].

[f] 25°C; $I = 0.1$ M, $NaNO_3$. From [53].

[g] Estimate; see text in Section 3.

[h] 20°C; $I = 1$ M, $NaNO_3$. From [83]. For the Cu(2′-deoxyguanosine)$^{2+}$ complex $\log K^{Cu}_{Cu(dGuo)} = 2.12 \pm 0.14$ was determined (25°C; $I = 0.1$ M, $NaNO_3$) [84].

[i] Result of a re-calculation described in Section 5.2 (ii) of [85] based on the data published in [86] (25°C; $I = 1$ M, $NaClO_4$).

[j] 25°C; $I = 0.5$ M, $NaNO_3$. From [82].

[k] In D_2O at 27°C and $I = 0.1$–4 M ($NaNO_3$); determined by ^{1}H-NMR shift measurements [87].

Cu^{2+}. Indeed, all the stability constants of the $Pb(Ns)^{2+}$ complexes given in Table 2 are smaller than those for $Cu(Ns)^{2+}$ and, taking into account the large error limits the value for $Pb(Ado)^{2+}$ is about in the expected order. However, the stability constants of the $Pb(Guo)^{2+}$ and $Pb(Cyd)^{2+}$ complexes are clearly too large compared with those of the corresponding $Zn(Ns)^{2+}$ and $Cd(Ns)^{2+}$ complexes. This is a clear indication that in $Pb(Guo)^{2+}$ the (C6)O and in $Pb(Cyd)^{2+}$ the (C2)O groups participate in Pb^{2+} binding; this conclusion agrees with previous suggestions [53,67]. This participation of the carbonyl oxygens occurs probably via so-called indirect or semi-chelates involving a hydrogen bond between a M^{2+}-coordinated water molecule and the carbonyl O (for details regarding such structures see [59,82] for Guo and Cyd, respectively). The similarity of the stability constants of the $Pb(Ns)^{2+}$ and $Cd(Ns)^{2+}$ complexes allows to estimate the stability of the $Pb(Ino)^{2+}$ complex, the stability of which cannot easily be measured: $\log K^{Pb}_{Pb(Ino)} = 0.9 \pm 0.25$ (see column three of Table 2).

The isolated carbonyl oxygens of uridine and thymidine (Fig. 3) are unlikely to interact in aqueous solution with Pb^{2+}; the situation is very similar to the one discussed above for hydroxy groups. However, in the alkaline pH range upon deprotonation of the (N3)H sites, $Pb(Urd-H)^{+}$ and $Pb(Thd-H)^{+}$ complexes are known to form [83].

The micro stability constants listed in column seven of Table 2 will be discussed in Section 6; for the present it needs to be pointed out only that these values quantify the affinity of the nucleobase residues of phosphate-monoprotonated nucleoside 5'-monophosphate complexes, i.e. the stability of $(Pb{\cdot}NMP{\cdot}H)^{+}$ species. This situation is similar to that in nucleic acids where a singly negatively charged phosphate-diester unit is close to a nucleobase residue. Clearly, for the complexes with the composition $Pb(H;NMP)^{+}$ a further isomer is possible; i.e. one in which Pb^{2+} is next to the proton also phosphate-bound, NMP·Pb·H. Hence, the macro stability constant of the $Pb(H;NMP)^{+}$ complexes is defined by Eq. (16):

$$K^{Pb}_{Pb(H;NMP)} = \frac{[Pb(H;NMP)^{+}]}{[Pb^{2+}][H(NMP)^{-}]} \tag{16a}$$

$$= \frac{[(Pb{\cdot}NMP{\cdot}H)^{+}] + [(NMP{\cdot}Pb{\cdot}H)^{+}]}{[Pb^{2+}][H(NMP)^{-}]} \tag{16b}$$

$$= k^{Pb}_{Pb{\cdot}NMP{\cdot}H} + k^{Pb}_{NMP{\cdot}Pb{\cdot}H} \tag{16c}$$

Application of the values listed for $(Pb{\cdot}NMP{\cdot}H)^{+}$ in column seven of Table 2 and that given in Section 2.2 for the $Pb(ROP(O)_2OH)^{+}$ species, i.e. for the $(NMP{\cdot}Pb{\cdot}H)^{+}$ isomer, to Eq. (16c) leads to the calculated stability constants provided in Eqs. (17)–(20):

$$K^{Pb}_{Pb(H;GMP)} = 10^{(1.76\pm0.23)} + 10^{(0.7\pm0.4)} \tag{17a}$$

$$\log K^{Pb}_{Pb(H;GMP)} = 1.8 \pm 0.3 \text{ [67]} \tag{17b}$$

$$K^{Pb}_{Pb(H;IMP)} = 10^{(1.41\pm0.30)} + 10^{(0.7\pm0.4)} \tag{18a}$$

$$\log K^{Pb}_{Pb(H;IMP)} = 1.5 \pm 0.3 \tag{18b}$$

$$K^{Pb}_{Pb(H;AMP)} = 10^{(0.9\pm0.35)} + 10^{(0.7\pm0.4)} \tag{19a}$$

$$\log K^{Pb}_{Pb(H;AMP)} = 1.1 \pm 0.3 \text{ [67]} \tag{19b}$$

$$K^{Pb}_{Pb(H;CMP)} = 10^{(1.65\pm0.17)} + 10^{(0.7\pm0.4)} \tag{20a}$$

$$\log K^{Pb}_{Pb(H;CMP)} = 1.7 \pm 0.2 \tag{20b}$$

The results calculated above for the stabilities of the $Pb(H;GMP)^{+}$, $Pb(H;IMP)^{+}$, $Pb(H;AMP)^{+}$, and $Pb(H;CMP)^{+}$ complexes are in excellent agreement with the experimentally determined constants, $\log K^{Pb}_{Pb(H;GMP)} = 1.52 \pm 0.10$ ([67] and Table 4, vide infra), $\log K^{Pb}_{Pb(H;IMP)} = 1.30 \pm 0.15$ [67], $\log K^{Pb}_{Pb(H;AMP)} = 1.08 \pm 0.04$ [67], and $\log K^{Pb}_{Pb(H;CMP)} = 1.55 \pm 0.09$ ([53] and Table 3, vide infra), respectively, thus proving the internal consistency of all the equilibrium constants.

4. Stability and structure of lead(II)–nucleoside 5′-monophosphate complexes in aqueous solution

A comparison of the stability constants accumulated in Tables 1 and 2 immediately reveals that the affinity of Pb^{2+} toward phosphate monoesters is by factors of 10–100 or even more higher than its affinity toward the nucleobases. Hence, it is evident that the stability-determining binding site for the complexes formed with nucleoside 5′-monophosphates (NMP^{2-}) is the phosphate group and the question which arises is: do the nucleobase residues participate in metal ion binding in the Pb(NMP) complexes or not? This question will be dealt with below by considering first the pyrimidine–NMPs.

4.1. The stability of the Pb^{2+} complexes of pyrimidine–NMPs is solely determined by the basicity of the phosphate residue!

The structures of the three common pyrimidine–nucleoside 5′-monophosphates are shown in Fig. 4 [88,89]. For the Pb(UMP) and Pb(dTMP) complexes we have already seen in Section 2.1 that only equilibria (1) and (2) are of relevance. In the case of $H(CMP)^{-}$ a further protonation at the N3 site is possible and therefore the following four equilibria, written for NMPs in general, need to be considered; relations to equations considered in the preceding parts of this review are indicated:

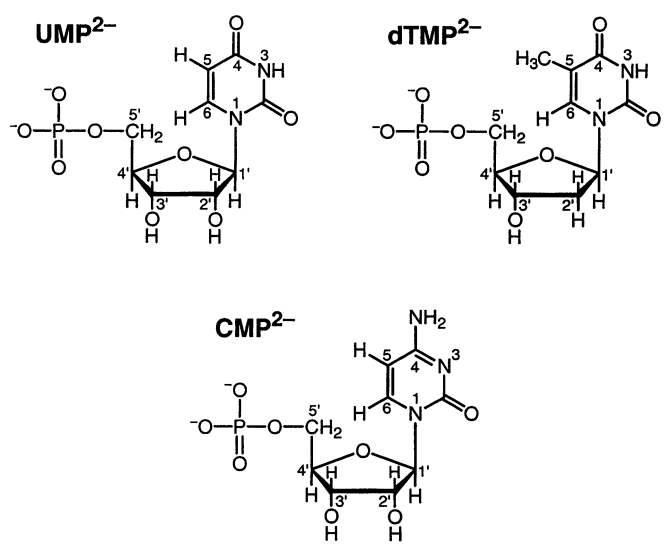


Fig. 4. Chemical structures of the pyrimidine–nucleoside 5′-monophosphates (NMPs), uridine 5′-monophosphate (UMP^{2-}), thymidine [= 1-(2′-deoxy-β-D-ribofuranosyl)thymine] 5′-monophosphate ($dTMP^{2-}$), and cytidine 5′-monophosphate (CMP^{2-}). The NMPs are shown in their predominant *anti* conformation [88,89].

Table 3
Negative logarithms of the acidity constants of the $H(NMP)^-$ species (Eq. (22)) and comparison of the logarithms of the measured (exp.) stability constants of the Pb(NMP) complexes (Eq. (24)) with those calculated (calc.) (Eq. (3)) based on the basicity of the phosphate group of the pyrimidine–nucleoside 5′-monophosphates (25°C; $I = 0.1$ M, $NaNO_3$) [a,b]

NMP^{2-}	$pK^H_{H(NMP)}$	Log $K^{Pb}_{Pb(NMP)}$	
		Exp.	Calc.
UMP^{2-}	6.15 ± 0.01	2.80 ± 0.04	2.91 ± 0.08
$dTMP^{2-}$	6.36 ± 0.01	2.93 ± 0.03	3.01 ± 0.08
CMP^{2-}	6.19 ± 0.02	2.93 ± 0.04	2.93 ± 0.08

[a] For the error limits see footnote 'b' of Table 1. The error limits of all derived data (3σ) were calculated according to the error propagation after Gauss.

[b] All values were determined by potentiometric pH titrations; they are from [53]; in addition it holds $pK^H_{H_2(CMP)} = 4.33 \pm 0.04$ (Eq. (21)) and $\log K^{Pb}_{Pb(H;CMP)} = 1.55 \pm 0.09$ (Eq. (23)).

$$H_2(NMP)^{\pm} \rightleftharpoons H^+ + H(NMP)^- \quad (21a)$$

$$K^H_{H_2(NMP)} = [H^+][H(NMP)^-]/[H_2(NMP)^{\pm}] \quad (21b)$$

$$H(NMP)^- \rightleftharpoons H^+ + NMP^{2-} \quad (1a) \triangleq (22a)$$

$$K^H_{H(NMP)} = [H^+][NMP^{2-}]/[H(NMP)^-] \quad (1b) \triangleq (22b)$$

$$Pb^{2+} + H(NMP)^- \rightleftharpoons Pb(H;NMP)^+ \quad (23a)$$

$$K^{Pb}_{Pb(H;NMP)} = [Pb(H;NMP)^+]/([Pb^{2+}][H(NMP)^-]) \quad (16) \triangleq (23b)$$

$$Pb^{2+} + NMP^{2-} \rightleftharpoons Pb(NMP) \quad (2a) \triangleq (24a)$$

$$K^{Pb}_{Pb(NMP)} = [Pb(NMP)]/([Pb^{2+}][NMP^{2-}]) \quad (2b) \triangleq (24b)$$

The equilibrium constants for the three mentioned pyrimidine–NMPs are summarized in Table 3.

Application of the acidity constants given in column 2 of Table 3 to the straight-line equation (3) leads to the calculated stability constants listed in the fourth column of Table 3; these constants reflect the expected stability based on the basicity of the $-PO_3^{2-}$ group of the NMPs. It is evident that the measured (exp.) and calculated (calc.) values are identical within their error limits; hence, there is no indication for the participation of any of the three nucleobase residues in Pb^{2+} binding, since such an interaction would have to be reflected in an increased stability of the complexes [90]. This result is no surprise as far as the Pb(UMP) and Pb(dTMP) complexes are concerned (see also Section 2.1). However, regarding the Pb(CMP) complex the mentioned result is at first sight a surprise, since the affinity of Pb^{2+} toward cytidine is quite pronounced (Table 2); yet, the fact that the data pair $pK^H_{H(CMP)}/\log K^{Pb}_{Pb(CMP)}$ fits exactly on the reference line (see Fig. 2) agrees with the results given in Table 3.

Indeed, the absence of an enhanced stability of the Pb(CMP) complex is in line with previous experience made with other M(CMP) complexes [56,91] and for related systems as well [61,69,71,74,92]. This observation is easily understood if one recalls that CMP^{2-} exists in aqueous solution predominantly in the *anti* conformation (Fig. 4) in which N3 of the cytosine residue points away from a metal ion coordinated at the phosphate group and therefore the latter binding site is solely responsible for the stability of the M(CMP) complexes.

Of course, the formation of the $Pb(H;CMP)^{+}$ species according to Eq. (23) means that this complex can also lose a proton as expressed in equilibrium (25a) in a general way. The acidity constant of the monoprotonated complex is defined by Eq. (25b) and values for it may be calculated with Eq. (26):

$$Pb(H;NMP)^{+} \rightleftharpoons Pb(NMP) + H^{+} \tag{25a}$$

$$K^{H}_{Pb(H;NMP)} = [Pb(NMP)][H^{+}]/[Pb(H;NMP)^{+}] \tag{25b}$$

$$pK^{H}_{Pb(H;NMP)} = pK^{H}_{H(NMP)} + \log K^{Pb}_{Pb(H;NMP)} - \log K^{Pb}_{Pb(NMP)} \tag{26}$$

The corresponding acidity constant, $pK^{H}_{Pb(H;CMP)} = 4.81 \pm 0.10$, [53] shows, in comparison with the acidity constants of $H_2(CMP)^{\pm}$ (see Table 3), that the proton must be located at the phosphate chain and indeed, it was previously concluded that Pb^{2+} is largely bound to the N3/[(C2)O] site [53]. This agrees with the result expressed below in Eq. (27), where the notations defined in Section 3 are also used:

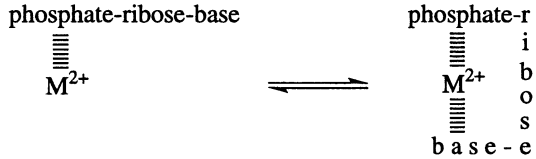
$$R = \frac{[(Pb{\cdot}CMP{\cdot}H)^{+}]}{[(CMP{\cdot}Pb{\cdot}H)^{+}]} = \frac{k^{Pb}_{Pb{\cdot}CMP{\cdot}H}}{k^{Pb}_{CMP{\cdot}Pb{\cdot}H}} \tag{27a}$$

$$= \frac{10^{(1.65 \pm 0.17)}}{10^{(0.7 \pm 0.4)}} = \frac{8.9}{1} \simeq \frac{90}{10} \tag{27b}$$

The microconstants employed in Eq. (27) and taken from Sections 2.2 and 3 indicate that about 90% of the protonated $Pb(H;CMP)^{+}$ species exist in the form of the $(Pb{\cdot}CMP{\cdot}H)^{+}$ isomer.

4.2. In Pb^{2+} complexes of purine–NMPs the nucleobase-N7 site may participate in metal ion binding!

The three most common purine–nucleoside 5′-monophosphates are shown in Fig. 5 [88,93,94]. After the discussion in Section 4.1 regarding the M(CMP) complexes and viewing the structures of Fig. 5 it is evident that this time a metal ion coordinated to the phosphate group may reach N7, which in fact is able to interact with metal ions as discussed in Section 3. Consequently, for the Pb^{2+} complexes of the purine–NMPs the occurrence of the intramolecular equilibrium (28) has to be expected:



(28)

Since the NMPs of Fig. 5 can also bind a proton at the nucleobase residue (see Section 3) for the reactions between Pb^{2+} and the $H_2(NMP)^{\pm}$ species Eqs. (21)–(24) are of relevance. The corresponding equilibrium constants are listed in Table 4.

From the two pK_a values of $H_2(NMP)^{\pm}$ and those of the $Pb(H;NMP)^{+}$ species (Table 4) follows that the proton in the latter complexes is located at the phosphate

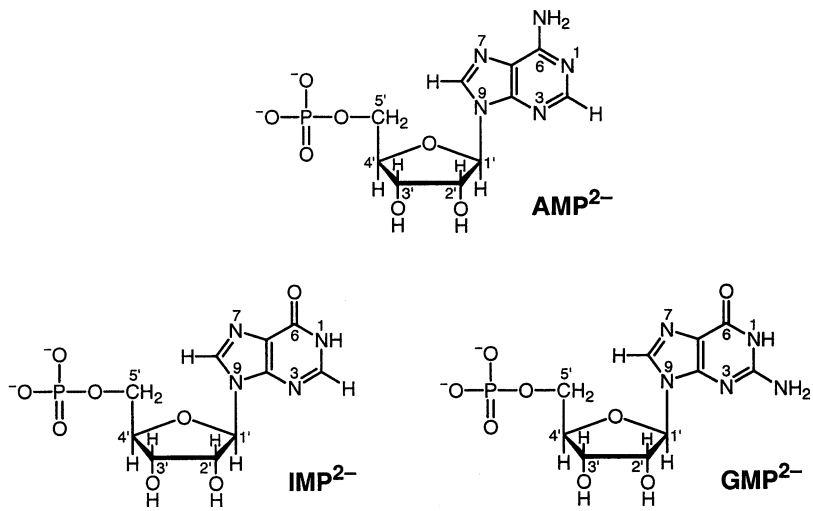


Fig. 5. Chemical structures of the purine-nucleoside 5′-monophosphates (NMPs), adenosine 5′-monophosphate (AMP^{2-}), inosine 5′-monophosphate (IMP^{2-}), and guanosine 5′-monophosphate (GMP^{2-}). The NMPs are shown in their predominant *anti* conformation [88,93,94].

Table 4
Negative logarithms of the acidity constants of twofold protonated purine-nucleoside 5′-monophosphates, $H_2(NMP)^{\pm}$ (Eqs. (21) and (22)), and logarithms of the stability constants of the $Pb(H;NMP)^{+}$ (Eq. (23)) and Pb(NMP) complexes (Eq. (24)), together with the negative logarithms of the acidity constants for the $Pb(H;NMP)^{+}$ species (Eqs. (25) and (26)) as determined by potentiometric pH titrations in aqueous solution at 25°C and $I = 0.1$ M ($NaNO_3$) [a]

NMP^{2-}	$pK^{H}_{H_2(NMP)}$ [b]	$pK^{H}_{H(NMP)}$	Log $K^{Pb}_{Pb(H;NMP)}$	Log $K^{Pb}_{Pb(NMP)}$	$pK^{H}_{Pb(H;NMP)}$
AMP^{2-}	3.84 ± 0.02	6.21 ± 0.01	1.08 ± 0.04	2.92 ± 0.08	4.37 ± 0.09
IMP^{2-}	1.30 ± 0.10	6.22 ± 0.01	1.30 ± 0.15	3.06 ± 0.05	4.46 ± 0.16
GMP^{2-}	2.48 ± 0.04	6.25 ± 0.02	1.52 ± 0.10	3.23 ± 0.08	4.54 ± 0.13

[a] The data are from [67]. For the error limits see footnote 'a' of Table 3.
[b] For the location of the proton at the nucleobase residue see footnote 'd' of Table 2.

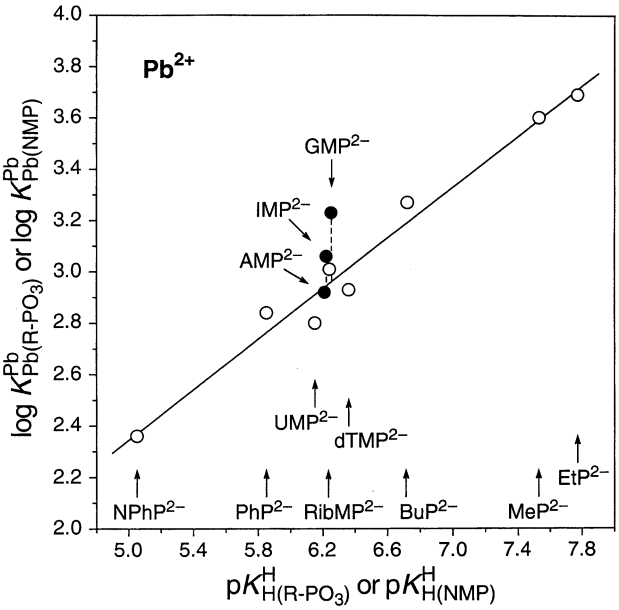


Fig. 6. Evidence for an enhanced stability of the Pb^{2+} 1:1 complexes formed with IMP^{2-} and GMP^{2-}, and for the lack of such an enhanced stability of the Pb(AMP) complex (●), based on the relationship between $\log K^{Pb}_{Pb(R\text{-}PO_3)}$ and $pK^{H}_{H(R\text{-}PO_3)}$ for the 1:1 complexes of Pb^{2+} with the eight simple phosphate monoester or phosphonate ligands ($R\text{-}PO_3^{2-}$) (○) named in the legend for Fig. 2. The least-squares line (Eq. (3)) is drawn through the corresponding eight data sets (Table 1). 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 and 5 of Table 4. 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. (29)), the values of which are listed in column 4 of Table 5. All the plotted equilibrium constant values refer to aqueous solutions at 25°C and $I = 0.1$ M ($NaNO_3$). Reproduced by permission of the American Chemical Society from Ref. [67].

group. Based on evaluations analogous to those indicated in Eq. (27) it was concluded [67] that the nucleobase-coordinated isomers also dominate now: $(Pb{\cdot}GMP{\cdot}H)^+$ (ca. 90% [67]), $(Pb{\cdot}IMP{\cdot}H)^+$ (ca. 80%)[5], and $(Pb{\cdot}AMP{\cdot}H)^+$ (about 60% [67]).

The data pairs $\log K^{Pb}_{Pb(NMP)}/pK^{H}_{H(NMP)}$ from Table 4, which are shown in Fig. 6, together with the reference line for a sole phosphate coordination, reveal a clear increased complex stability for the Pb(GMP) and Pb(IMP) species. This observation means that equilibrium (28) operates and that macrochelates are formed by an interaction of the phosphate-coordinated Pb^{2+} with N7 of the purine residue [67].

A quantitative evaluation of this situation is possible [59,90,95]: In the third column of Table 5 the calculated stability constants are listed. The difference

[5] Calculated in analogy to Eq. (27) with the microconstants given in Section 2.2 and Section 3 and Table 2: $R = \dfrac{[(Pb{\cdot}IMP{\cdot}H)^+]}{[(IMP{\cdot}Pb{\cdot}H)^+]} = \dfrac{k^{Pb}_{Pb\cdot IMP\cdot H}}{k^{Pb}_{IMP\cdot Pb\cdot H}} = \dfrac{10^{(1.41\pm0.30)}}{10^{(0.7\pm0.4)}} = \dfrac{5.13}{1} \simeq \dfrac{80}{20}$

Table 5
Comparison of the measured stability constants, $K^{Pb}_{Pb(NMP)}$ (Eq. (24)) [a], of the Pb(NMP) complexes with the calculated stability constants, $K^{Pb}_{Pb(NMP)op}$ [b], for the isomers with a sole Pb^{2+}–phosphate coordination, and extent of the intramolecular chelate formation according to equilibrium (28) for the Pb(NMP) species as defined by K_I (Eqs. (30) and (31)) and % $Pb(NMP)_{cl}$ (Eq. (32)) for aqueous solutions at 25°C and $I = 0.1$ M ($NaNO_3$) [a]

NMP^{2-}	Log $K^{Pb}_{Pb(NMP)}$	Log $K^{Pb}_{Pb(NMP)op}$ [b]	Log $\Delta_{Pb(NMP)}$ [c]	K_I	% $Pb(NMP)_{cl}$
AMP^{2-}	2.92 ± 0.08	2.94 ± 0.08	-0.02 ± 0.11	0 (<0.23)	0 (<19)
IMP^{2-}	3.06 ± 0.05	2.94 ± 0.08	0.12 ± 0.09	0.32 ± 0.27	24 ± 16
GMP^{2-}	3.23 ± 0.08	2.96 ± 0.08	0.27 ± 0.11	0.86 ± 0.47	46 ± 14

[a] The data are from [67]. For the error limits see footnote 'a' of Table 3.
[b] Calculated with the acidity constants $pK^{H}_{H(NMP)}$ given in Table 4 and the straight-line equation (3).
[c] See Eq. (29).

between the experimental (exp.) and the calculated (calc.) values according to Eq. (29)

$$\log \Delta_{Pb(NMP)} = \log K^{Pb}_{Pb(NMP)exp} - \log K^{Pb}_{Pb(NMP)calc} \quad (29a)$$

$$= \log \Delta = \log K^{Pb}_{Pb(NMP)} - \log K^{Pb}_{Pb(NMP)op} \quad (29b)$$

are given in column 4. The equivalence of the various terms in Eq. (29) is evident. The values for $\log K^{Pb}_{Pb(NMP)op}$ represent the stability of the open isomers in equilibrium (28), $Pb(NMP)_{op}$. If we define the closed or macrochelated isomer as $Pb(NMP)_{cl}$, the position of equilibrium (28) can be defined by the dimensionless constant K_I of Eq. (30):

$$K_I = [Pb(NMP)_{cl}]/[Pb(NMP)_{op}] \quad (30)$$

The interrelation between K_I and the stability difference $\log \Delta_{Pb(NMP)}$ is given by Eq. (31):

$$K_I = \frac{K^{Pb}_{Pb(NMP)}}{K^{Pb}_{Pb(NMP)op}} - 1 \quad (31a)$$

$$= 10^{\log \Delta} - 1 \quad (31b)$$

Knowledge of K_I allows then the calculation of the percentage of the closed isomer in equilibrium (28) according to Eq. (32):

$$\%\ Pb(NMP)_{cl} = 100 \cdot K_I/(1 + K_I) \quad (32)$$

The corresponding results are summarized in columns 4–6 of Table 5.

It is interesting to note that the formation degree of the macrochelated species decreases from remarkable 45% for $Pb(GMP)_{cl}$ via about 25% for $Pb(IMP)_{cl}$ to zero within the error limits for $Pb(AMP)_{cl}$ (Table 5). This result reflects nicely the decreasing affinity of the involved nucleobase residues as described in Section 3 (see also Table 2).

5. Lead(II) complexes of thiophosphates

The phosphorothioate group (PS^{2-}), in which a phosphate oxygen is replaced by a sulfur atom, is employed, e.g. in antisense oligonucleotides [96] and in studies of ribozymes [36,97]. Therefore, so-called nucleoside phosphorothioates [98] or thionucleotides [99] and their interaction with protons [100,101] and metal ions [99,102–105] are receiving increasing attention.

However, so far only very few equilibrium data [99,104,105] are available; in fact, the only Pb^{2+} complexes studied are those of uridine 5′-*O*-thiomonophosphate ($UMPS^{2-}$) and of the simple thio analogue of methyl phosphate, i.e. of methyl thiophosphate ($MePS^{2-}$) (see Fig. 7) [105]. The corresponding equilibrium constants are summarized in Table 6 together with those for their parent compounds, UMP^{2-} and $CH_3OPO_3^{2-}$. The relevant data pairs of Table 6 are inserted in Fig. 8 together with the reference line (Eq. (3)) representing the simple Pb^{2+}-phosphate interaction.

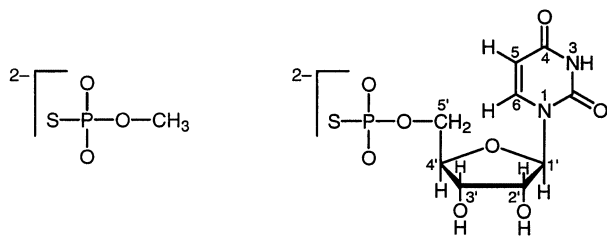


Fig. 7. Chemical structure of methyl thiophosphate ($MePS^{2-}$) and uridine 5′-*O*-thiomonophosphate ($UMPS^{2-}$).

Table 6
Negative logarithms of the acidity constants of the HL^- species (Eqs. (1) or (22)) of thiophosphate or phosphate monoesters (L^{2-}) and comparison of the logarithms of the measured (exp.) stability constants of the corresponding PbL complexes (Eqs. (2) or (24)) with those calculated (calc.) for a pure and unaltered Pb^{2+}–phosphate residue coordination (Eq. (3)) based on the basicity of the (thio)phosphate group of the various ligands, L^{2-}, in aqueous solution at 25°C and $I = 0.1$ M ($NaNO_3$) [a]

L^{2-}	pK_{HL}^{H}	Log K_{PbL}^{Pb}		Log Δ_{PbL} [b]
		Exp.	Calc.	
$CH_3OP(S)(O)_2^{2-}$ ($MePS^{2-}$)	4.96 ± 0.02 [105]	4.78 ± 0.07 [105]	2.32 ± 0.08	2.46 ± 0.11
$UMPS^{2-}$	4.78 ± 0.02 [105]	4.63 ± 0.05 [105]	2.23 ± 0.08	2.40 ± 0.09
$CH_3OPO_3^{2-}$	6.36 ± 0.01 [54]	2.98 ± 0.11 [63]	3.01 ± 0.08	-0.03 ± 0.14
UMP^{2-}	6.15 ± 0.01 [56]	2.80 ± 0.04 [53]	2.91 ± 0.08	-0.11 ± 0.09

[a] For the error limits see footnote 'a' of Table 3.
[b] Defined in analogy to Eq. (29).

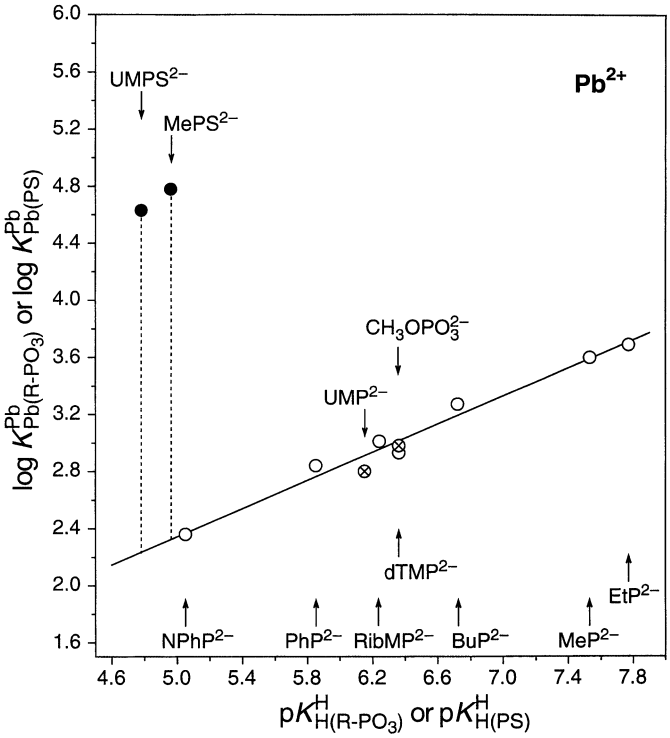


Fig. 8. Proof of an enhanced stability of the Pb^{2+} 1:1 complexes formed with $MePS^{2-}$ and $UMPS^{2-}$ (PS^{2-}) (●), and for the lack of such an enhanced stability with the parent ligands $CH_3OPO_3^{2-}$ and UMP^{2-} (⊗), based on the relationship between log $K^{Pb}_{Pb(R\text{-}PO_3)}$ and $pK^{H}_{H(R\text{-}PO_3)}$ for the 1:1 complexes of Pb^{2+} with the eight simple phosphate monoester or phosphonate ligands ($R\text{-}PO_3^{2-}$) (○) named in the legend for Fig. 2. The least-squares line (Eq. (3)) is drawn through the corresponding eight data sets (Table 1). The data points due to the equilibrium constants for the Pb^{2+}/MePS and UMPS (●) as well as their parent systems (⊗) are based on the data given in columns 2 and 3 of Table 6. The vertical broken lines emphasize the stability differences of the Pb(PS) complexes (●) to the reference line; these differences are equal to log Δ_{PbL} (Eq. (29) and Table 6, column 5). All the plotted equilibrium constant values refer to aqueous solutions at 25°C and $I = 0.1$ M ($NaNO_3$).

From Fig. 8 it is evident that whereas the data pairs for the UMP^{2-} and $CH_3OPO_3^{2-}$ systems fit on the reference line, the values for their thio derivatives are far above this line. In fact, the stability increases, log $\Delta_{Pb(PS)}$, as defined by Eq. (29) are with about 2.4 log units very large (Table 6, column 5). Interestingly, based on solubility and other data reflecting a metal ion-thio interaction, a stability increase of 2.35 ± 0.29 log units had been predicted [104] for Pb(PS) complexes; this prediction is in excellent agreement with the results of Table 6.

It may be mentioned that the corresponding stability increases for Zn(PS) and Cd(PS) complexes amount to 0.66 ± 0.21 and 2.37 ± 0.15 log units, respectively [104]. Hence, the affinities of Cd^{2+} and Pb^{2+} toward a thiophosphate group are quite alike, whereas the one of Zn^{2+} is lower. In fact, it has been calculated [104]

that in a Zn(PS) system Zn^{2+} binds to about 80% via sulfur leaving about 20% for oxygen coordination, whereas in the Cd(PS) species the Cd^{2+}-sulfur interaction is close to 100%; the latter applies evidently also to the Pb(PS) species.

The results of Table 6 which give on average a stability increase of $\log \Delta_{Pb(PS)} = 2.43 \pm 0.09$ for the two Pb(PS) complexes, if in the phosphate residue one of the terminal oxygens is replaced by a sulfur atom, allow now also to calculate the stability constant, $\log K^{Pb}_{Pb(PS)}$, for any Pb(PS) complex, provided the corresponding acidity constant, $pK^{H}_{H(PS)}$, is known. With the aid of Eq. (3) and $pK^{H}_{H(PS)}$ the stability constant, $\log K^{Pb}_{Pb(R\text{-}PO_3)}$, for the corresponding phosphate complex is calculated and to this value $\log \Delta_{Pb(PS)} = 2.43 \pm 0.09$ needs to be added to give the final stability constant, $\log K^{Pb}_{Pb(PS)}$, for the Pb(PS) complex considered. For example, for the stability of the Pb(AMPS) complex one calculates $\log K^{Pb}_{Pb(AMPS)} = (2.20 \pm 0.08) + (2.43 \pm 0.09) \simeq 4.65 \pm 0.15$ with $pk^{AMPS}_{AMPS\cdot H} = 4.71 \pm 0.04$ [101] and Eq. (3) by considering the mentioned $\log \Delta_{Pb(PS)}$ value, assuming that like in the parent Pb(AMP) complex (see Section 4.2 and Table 5) no significant Pb^{2+}/N7 interaction occurs.

6. Conclusions

In Section 4.1 it is shown that in the Pb^{2+} complexes of the pyrimidine–NMPs the nucleobase residues are not involved in metal ion binding; the same conclusion was recently reached for the corresponding $M(NDP)^{-}$ complexes of various metal ions [71] and it also holds for the Pb(AMP) complex as seen in Section 4.2. These observations plus the result given in Eq. (11) of Section 2.2 regarding Pb^{2+} complexes of diphosphate monoesters allow to conclude that the stability constants for the $Pb(NDP)^{-}$ complexes of UDP^{3-}, $dTDP^{3-}$, CDP^{3-}, and ADP^{3-} all equal $\log K^{Pb}_{Pb(NDP)} = 5.3 \pm 0.15$; for the acidity constants of these four monoprotonated nucleotides it holds $pK^{H}_{H(NDP)} = 6.40 \pm 0.05$ [71,106].

Based on the analogous reasoning one obtains with the aid of Eq. (14) estimates for the stabilities of the $Pb(NTP)^{2-}$ complexes, where $NTP^{4-} = UTP^{4-}$, $dTTP^{4-}$, CTP^{4-}, or ATP^{4-}, i.e. $\log K^{Pb}_{Pb(NTP)} = 6.3 \pm 0.25$; for the acidity constants of the corresponding $H(NTP)^{3-}$ species it holds $pK^{H}_{H(NTP)} = 6.50 \pm 0.05$ [73,74,107]. Furthermore, in a recent study devoted to the stabilities of the $M(ITP)^{2-}$ and $M(GTP)^{2-}$ complexes of alkaline earth ions and 3d transition metal ions [75] it was concluded by taking into account also previous results of M(IMP) and M(GMP) complexes [61] that for a given metal ion the extents of macrochelate formation according to equilibrium (28), i.e. the stability increases as expressed via $\log \Delta_{M(NTP)}$ or $\log \Delta_{M(NMP)}$ (cf. Eq. (29)) are very similar. Hence, by applying the $\log \Delta_{Pb(NMP)}$ values of Table 5 (column 4) we may conclude that the stability constants for the $Pb(ITP)^{2-}$ and $Pb(GTP)^{2-}$ complexes are $\log K^{Pb}_{Pb(ITP)} = (6.3 \pm 0.25) + (0.12 \pm 0.09) = 6.42 \pm 0.27$ and $\log K^{Pb}_{Pb(GTP)} = (6.3 \pm 0.25) + (0.27 \pm 0.11) = 6.57 \pm 0.27$, respectively. For $M(IDP)^{-}$ and $M(GDP)^{-}$ complexes not yet enough stability data are available [108] to justify the analogous conclusion, though as a first approximation the given procedure may also be applied if an estimation of the stability of the $Pb(IDP)^{-}$ or $Pb(GDP)^{-}$ complexes is needed. The estimations presented above are

important since so far no experimentally determined stability constants for the mentioned $Pb(NDP)^-$ and $Pb(NTP)^{2-}$ complexes are available [50–52,70].

Another point of general interest is the varying affinity of the various nucleobase residues toward Pb^{2+} (Section 3) which gives rise to selectivity regarding the coordination of Pb^{2+} to single-stranded nucleic acids. Assuming that the four main nucleobases of RNA (adenine, guanine, cytosine, uracil) or of DNA (adenine, guanine, cytosine, thymine) are present in about the same ratios then the anionic phosphate bridge occurs in a fourfold excess compared to each individual nucleobase residue. If this statistical factor is taken into account, one obtains, based on the estimated stability constant, $\log K^{Pb}_{Pb(R'OP(O)_2OH)} = (0.7 \pm 0.4)$ (Section 2.2), for the phosphate diester unit in a nucleic acid the micro stability constant $\log k^{Pb}_{Pb(R'OP(O)_2OR)} = (0.7 \pm 0.4) + 0.6 = 1.3 \pm 0.4$. Hence, by considering further the values given in column 7 of Table 2 the following affinity order evolves (the micro stability constants are given in parentheses): guanine–N7(O6) $(1.76 \pm 0.23) \gtrapprox$ cytosine–N3(O2) $(1.65 \pm 0.17) \gtrapprox R'OP(O)_2^-OR$ $(1.3 \pm 0.4) \gtrapprox$ adenine $(0.90 \pm 0.35) >$ uracil $\sim$ thymine.

For the carbonyl oxygens of the uracil and thymine residues no stability constants are known but their affinity toward Pb^{2+} in aqueous solution is certainly low. On the other hand it is necessary to point out that if a certain nucleic acid sequence or fold provides a suitably fitting coordination site for Pb^{2+}, e.g. via guanine–N7, cytosine–N3, and/or phosphate diester units, then close by and properly orientated carbonyl groups are also expected to be able to participate in binding as is known from other types of ligands [55,62]. However, such carbonyl groups cannot be expected to act as primary binding sites for Pb^{2+}. This conclusion is in accord with the RNA examples of Pb^{2+} binding [30,42,46] discussed in Section 1.2. The preferential binding of metal ions to N7 of guanines in oligonucleotides is also known for Pt^{2+}, Zn^{2+}, or Mn^{2+} [109].

In the case that in a nucleic acid sequence an artificial thiophosphate group is inserted by replacing an O by an S atom then it has to be expected that this is the site to which Pb^{2+} is coordinating, since the stability increase by exchanging an O by an S site amounts to about 2.4 log units (Section 5). An exception of this 'rule' can only be expected if the single-stranded nucleic acid exists in a special fold into which the coordination sphere of Pb^{2+} exactly fits; then one could imagine that the thiophosphate group is ignored by Pb^{2+}. For a protein, oncomodulin, such an exception is known (Section 1.1); Pb^{2+} binds exclusively at the Ca^{2+} sites despite the presence of a sulfhydryl group [17].

7. Abbreviations and definitions

The abbreviations of those ligands discussed in detail in this review are given, together with their structures, in Figs. 1, 3–5 and 7.

ADP^{3-}	adenosine 5′-diphosphate
$AMPS^{2-}$	adenosine 5′-*O*-thiomonophosphate

ATP^{4-}	adenosine 5′-triphosphate
CDP^{3-}	cytidine 5′-diphosphate
CTP^{4-}	cytidine 5′-triphosphate
$dTDP^{3-}$	thymidine 5′-diphosphate
$dTTP^{4-}$	thymidine 5′-triphosphate
$EDTA^{4-}$	1,2-diaminoethane-*N*,*N*,*N*′,*N*′-tetraacetate
GDP^{3-}	guanosine 5′-diphosphate
GTP^{4-}	guanosine 5′-triphosphate
I	ionic strength
IDP^{3-}	inosine 5′-diphosphate
ITP^{4-}	inosine 5′-triphosphate
L^{2-}	general ligand with a twofold negative charge
M^{2+}	general divalent metal ion
NDP^{3-}	nucleoside 5′-diphosphate
NMP^{2-}	nucleoside 5′-monophosphate
Ns	nucleoside
NTP^{4-}	nucleoside 5′-triphosphate
pK_a	negative logarithm of an acidity constant
PS^{2-}	phosphorothioate (group)
$R\text{-}DP^{3-}$	general diphosphate monoester
$R\text{-}PO_3^{2-}$	simple phosphate monoester or phosphonate ligand with R representing a non-interacting residue (see Fig. 1)
$R\text{-}TP^{4-}$	general triphosphate monoester
UDP^{3-}	uridine 5′-diphosphate
UTP^{4-}	uridine 5′-triphosphate

In formulas like $Pb(H;NMP)^{+}$ the H^{+} and the 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, e.g. Section 4.1).

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.

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