Inorg. Chem. **1988**, *27*, 1447–1453

Contribution from the Institute of Inorganic Chemistry,
University of Basel, Spitalstrasse 51, CH-4056 Basel, Switzerland

Metal Ion Coordinating Properties of Pyrimidine-Nucleoside 5′-Monophosphates (CMP, UMP, TMP) and of Simple Phosphate Monoesters, Including D-Ribose 5′-Monophosphate. Establishment of Relations between Complex Stability and Phosphate Basicity

Salah S. Massoud[1] and Helmut Sigel*

Received September 8, 1987

The stability constants of the 1:1 complexes formed between Mg^{2+}, Ca^{2+}, Sr^{2+}, Ba^{2+}, Mn^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, or Cd^{2+} and the pyrimidine-nucleoside 5′-monophosphates CMP^{2-}, UMP^{2-}, and TMP^{2-} were determined by potentiometric pH titration in aqueous solution (I = 0.1 M ($NaNO_3$), 25 °C). For comparison, the same values were measured for the corresponding complexes with the simple phosphate monoesters ($R\text{-}MP^{2-}$) 4-nitrophenyl phosphate ($NPhP^{2-}$), phenyl phosphate (PhP^{2-}), and *n*-butyl phosphate (BuP^{2-}); D-ribose 5′-monophosphate ($RibMP^{2-}$) was also included. By construction of log $K^{M}_{M(R\text{-}MP)}$ versus $pK^{H}_{H(R\text{-}MP)}$ plots for the complexes with $NPhP^{2-}$, PhP^{2-}, BuP^{2-}, and $RibMP^{2-}$ and comparison of the resulting straight lines with literature data for $M(HPO_4)$ and $M(CH_3OPO_3)$ complexes, it is shown that these preliminary base lines are free from any steric effects. The data for the UMP^{2-} and TMP^{2-} systems also fit these plots; this confirms the expectation that in the M(UMP) and M(TMP) complexes the metal ion is only phosphate-coordinated. Hence, final base-line plots were constructed by incorporating also the data for these two nucleotides; the corresponding straight-line equations together with the pK_a value of a given phosphate group allow us to predict the stability of the corresponding M(R-MP) complex. In fact, in this way the experimentally determined stability constants for the M(CMP) complexes are evaluated and it is concluded that the pyridine-like N(3) of the base residue does not participate in complex formation; i.e., the stability of the M(CMP) complexes is solely determined by the coordination tendency of the phosphate group. The possible binding modes of a substitution-labile divalent metal ion to a phosphate group are discussed: it is tentatively concluded by considering the slopes of the base lines that in aqueous solution four-membered chelate rings are hardly formed with a phosphate group, the dominating binding modes being monodentate phosphate oxygen coordination and possibly a six-membered "chelate" ring involving a coordinated water molecule and a hydrogen bond; outer-sphere complexation may also play a role.

Many natural coenzymes are esters of orthophosphoric acid,[2] and usually the related enzymic systems are metal ion dependent.[3] Therefore, we are studying by potentiometric pH titrations in a comprehensive effort the stability of nucleoside monophosphate (NMP^{2-})[4] complexes formed with Mg^{2+}, Ca^{2+}, Sr^{2+}, Ba^{2+}, Mn^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, and Cd^{2+}. In addition we are aiming to deduce from the measured stability data the structure of these complexes in solution.

Any kind of base back-binding or macrochelate formation in M(NMP) complexes will reveal itself by an enhanced complex stability.[5] Hence, well-defined error limits for all stability constants to be compared are important to achieve a quantitative evaluation of the extent of a possibly formed macrochelate. As the $pK^{H}_{H(NMP)}$ values for the deprotonation of the phosphate group of monoprotonated nucleoside monophosphates differ,[6–9] it is necessary to determine the metal ion affinity of different phosphate groups in dependence on their basicity; only thereafter can the expected stability constant for a pure phosphate coordination be calculated for a given acidity constant. Therefore, the stability constants for the complexes formed between the already mentioned metal ions and the following simple phosphate monoesters ($R\text{-}MP^{2-}$) carrying a noncoordinating organic residue were measured: phenyl phosphate (PhP^{2-}), 4-nitrophenyl phosphate ($NPhP^{2-}$), and *n*-butyl phosphate (BuP^{2-}). D-Ribose 5′-monophosphate ($RibMP^{2-}$) was also included in the study to see whether the ribose moiety has any influence on complex stability; this is not the case.

The equilibrium constants measured in aqueous solution for these mentioned systems allow us to establish correlations between complex stability and phosphate ligand basicity. Some results available in the literature for hydrogen phosphate (HPO_4^{2-})[10] and methyl phosphate ($CH_3OPO_3^{2-}$)[11] fit into these correlations. Furthermore, knowing that for most pyrimidine-nucleoside 5′-triphosphates complex stability is governed by the affinity of the phosphate chain for metal ions,[12,13] we expected the corresponding properties for the pyrimidine NMPs shown in Figure 1; indeed, the experiments confirm this, providing an even broader basis for the correlations between complex stability and phosphate basicity. These correlations may in future studies be used to evaluate the extent of base back-binding in all kinds of M(NMP) complexes, e.g. in those formed with adenosine 5′-monophosphate and related derivatives.[14]

So far practically no stability constants for complexes of simple phosphate monoesters, including $RibMP^{2-}$, are available.[6–8,15] There are apparently no data[6–8] for TMP^{2-} complexes and only very few reliable constants for CMP^{2-} (see also section 6 of the Results and Discussion)[6–8,15] and UMP^{2-} complexes.[6–8,16]

Experimental Section

Materials. The disodium salts of cytidine 5′-monophosphate, uridine 5′-monophosphate, thymidine 5′-monophosphate, and D-ribose 5′-monophosphate were purchased from Sigma Chemical Co., St. Louis, MO, and

---

(1) Work done during a leave from Alexandria University, Alexandria, Egypt.

(2) Westheimer, F. H. *Science (Washington, D.C.)* **1987**, *235*, 1173–1178.

(3) (a) Marzilli, L. G. *Prog. Inorg. Chem.* **1977**, *23*, 255–378. (b) Wu, F. Y.-H.; Wu, C.-W. *Met. Ions Biol. Syst.* **1983**, *15*, 157–192. (c) Kalbitzer, H. R. *Met. Ions Biol. Syst.* **1987**, *22*, 81–103.

(4) Abbreviations: BuP^{2-}, *n*-butyl phosphate; CMP^{2-} and CTP^{4-}, cytidine 5′-monophosphate and 5′-triphosphate; M^{2+}, bivalent metal ion; $NPhP^{2-}$, 4-nitrophenyl phosphate; NMP^{2-}, nucleoside 5′-monophosphate; PhP^{2-}, phenyl phosphate; $RibMP^{2-}$, D-ribose 5′-monophosphate; $R\text{-}MP^{2-}$, phosphate monoester (R may be any organic residue, e.g. phenyl or nucleosidyl); TMP^{2-} and TTP^{4-}, thymidine 5′-monophosphate and 5′-triphosphate; UMP^{2-} and UTP^{4-}, uridine 5′-monophosphate and 5′-triphosphate.

(5) Martin, R. B.; Sigel, H. *Comments Inorg. Chem.* **1988**, 285–314.

(6) (a) Sillén, L. G.; Martell, A. E. *Spec. Publ.—Chem. Soc.* **1964**, *No. 17*. (b) Sillén, L. G.; Martell, A. E. *Spec. Publ.—Chem. Soc.* **1971**, *No. 25* (Supplement 1).

(7) Perrin, D. D. *Stability Constants of Metal-Ion Complexes*; IUPAC Chemical Data Series; Pergamon: Oxford, England, 1979; Part B (Organic Ligands).

(8) (a) Smith, R. M.; Martell, A. E. *Critical Stability Constants*; Plenum: New York, London, 1975; Vol. 2. (b) Smith, R. M.; Martell, A. E. *Critical Stability Constants*; Plenum: New York, London, 1982; Vol. 5.

(9) Tribolet, R.; Sigel, H. *Eur. J. Biochem.* **1987**, *163*, 353–363.

(10) Sigel, H.; Becker, K.; McCormick, D. B. *Biochim. Biophys. Acta* **1967**, *148*, 655–664.

(11) (a) Brintzinger, H. *Helv. Chim. Acta* **1965**, *48*, 47–54. (b) Brintzinger, H.; Hammes, G. G. *Inorg. Chem.* **1966**, *5*, 1286–1287.

(12) Scheller, K. H.; Hofstetter, F.; Mitchell, P. R.; Prijs, B.; Sigel, H. *J. Am. Chem. Soc.* **1981**, *103*, 247–260.

(13) (a) Sigel, H.; Tribolet, R.; Malini-Balakrishnan, R.; Martin, R. B. *Inorg. Chem.* **1987**, *26*, 2149–2157. (b) Sigel, H. *Eur. J. Biochem.* **1987**, *165*, 65–72.

(14) Massoud, S. S.; Tribolet, R.; Sigel, H., submitted for publication.

(15) Frey, C. M.; Stuehr, J. E. *J. Am. Chem. Soc.* **1972**, *94*, 8898–8904.

(16) Sigel, H.; Scheller, K. H. *Eur. J. Biochem.* **1984**, *138*, 291–299.

0020-1669/88/1327-1447$01.50/0

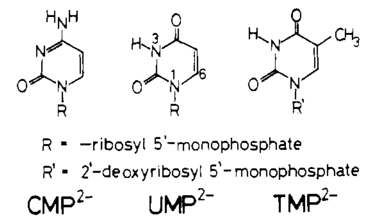

Figure 1. Structures of the pyrimidine-nucleoside 5'-monophosphates (NMP^{2-}) considered in this study.

Table I. Negative Logarithms of the Acidity Constants (Eq 1–3) of the Protonated Phosphate Ligands Considered in This Study As Determined by Potentiometric pH Titrations in Water at 25 °C and I = 0.1 M ($NaNO_3$)[a]

ligand[4]	$pK^H_{H_2(R\text{-}MP)}$	$pK^H_{H(R\text{-}MP)}$	pK^H_{NMP}
HPO_4^{2-}	1.86[b]	6.70 ± 0.02[c]	
$CH_3OPO_3^{2-}$		6.2[d]	
$NPhP^{2-}$		5.05 ± 0.01	
PhP^{2-}		5.85 ± 0.01	
BuP^{2-}		6.72 ± 0.02	
$RibMP^{2-}$		6.24 ± 0.01	
CMP^{2-}	4.33 ± 0.04[e]	6.19 ± 0.02	
UMP^{2-}	0.7 ± 0.3	6.15 ± 0.01	9.45 ± 0.02
TMP^{2-}		6.36 ± 0.01	9.90 ± 0.03

[a] The errors given are 3 times the standard error of the mean value or the sum of the probable systematic errors, whichever is larger. [b] This value for H_3PO_4, i.e. $pK^H_{H_3PO_4}$, is from ref 20 (I = 0.5 M (KCl), 22 °C); in this work is also given $pK^H_{H_2PO_4}$ = 6.69 in good agreement with the other value listed above. [c] This value for $H_2PO_4^-$, i.e. $pK^H_{H_2PO_4}$, is from ref 10 (I = 0.1 ($NaClO_4$), 25 °C). [d] From ref 11b (I = 0.1 (NaCl), 25 °C). [e] This value corresponds to the release of the proton from N(3) of the protonated pyrimidine ring residue (see also section 2).[21]

those of phenyl phosphate and 4-nitrophenyl phosphate from Fluka AG, Buchs, Switzerland. *n*-Butyl dihydrogen phosphate (an approximate 1:1 mixture of the mono- and diester) was from Merck-Schuchardt, Hohenbrunn (München), FRG. The aqueous stock solutions of the R-MPs were freshly prepared daily, and the pH was adjusted to about 8.5; the exact concentration was newly determined each time (see below).

The nitrate salts of Na^+, Mg^{2+}, Ca^{2+}, Sr^{2+}, Ba^{2+}, Mn^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, and Cd^{2+}, the disodium salt of EDTA, HNO_3, and NaOH (Titrisol) (all p.A.) were obtained from Merck AG, Darmstadt, FRG.

The titer of the NaOH used for the titrations was determined with potassium hydrogen phthalate (Merck AG); the exact concentrations of the R-MP solutions used in the titrations with metal ions (titrated in the presence of an excess of HNO_3; see below) were measured by titrations with NaOH. The concentrations of the stock solutions of the divalent metal ions were determined with EDTA.

Potentiometric pH Titrations. These were carried out with a Metrohm E536 potentiograph and an EA 121 macro glass electrode. The buffers (pH 4.64, 7.00, and 9.00) used for calibration were also from Metrohm AG, Herisau, Switzerland. The direct pH meter readings were used in the calculations of the acidity constants.

To obtain reliable equilibrium constants from a representative set of data (obtained in several instances independently by two different people) for all systems, at least eight independent pairs of titration curves were recorded for the acidity constants $K^H_{H_2(R\text{-}MP)}$, $K^H_{H(R\text{-}MP)}$, and $K^H_{(NMP)}$ and the results averaged. Similarly, for the determination of the stability constants $K^M_{M(R\text{-}MP)}$, always at least four independent pairs of titrations were made.

Determination of the Acidity Constants. $K^H_{H(R\text{-}MP)}$ of $H(R\text{-}MP)^-$ was determined by titrating 50 mL of aqueous 0.54 mM HNO_3 and $NaNO_3$ (I = 0.1; 25 °C) in the presence and absence of 0.3 mM $R\text{-}MP^{2-}$ under N_2 with 1 mL of 0.03 M NaOH and by using the differences in NaOH consumption between two such titrations for the calculations. For the determination of $K^H_{H_2(CMP)}$ and $K^H_{H(CMP)}$ of $H_2(CMP)^{\pm}$, where one proton is at the base moiety and the other at the phosphate group, the concentration of HNO_3 was doubled (=1.08 mM) and 2 mL of 0.03 M NaOH was used in the titrations. $K^H_{H_2(CMP)}$ and $K^H_{H(CMP)}$ were calculated with a Hewlett-Packard 9825A calculator (connected to a 7470A plotter) by a curve-fit procedure using a Newton-Gauss nonlinear-least-squares program within the pH range determined by the lowest point of neutralization reached by the experimental conditions (about 20% neutralization for the equilibrium $H_2(CMP)^{\pm}/H(CMP)^-$) and about 97% neutralization (for the equilibrium $H(CMP)^-/CMP^{2-}$). In those cases where only the constant $K^H_{H(R\text{-}MP)}$ is applicable, the calculation was carried out between 3% and 97% neutralization.

UMP^{2-} and TMP^{2-} can release a further proton from the H–N(3) unit of their base moieties (cf. Figure 1): this K^H_{NMP} value was determined under the conditions given above for $H(R\text{-}MP)^-$ by employing 2 mL of 0.03 M NaOH and evaluating the pH range 4.8–9.8 in the described way by taking into account also $K^H_{H(NMP)}$.

For an estimation of the acidity constant $K^H_{H_2(R\text{-}MP)}$ of $H_2(R\text{-}MP)$, i.e. for the release of the first proton from the diprotonated phosphate group, $H_2(UMP)$ was selected. A 15-mL sample of aqueous 31.7 mM HNO_3 and $NaNO_3$ (I = 0.1; 25 °C) was titrated with 2.5 mL of 0.2 M NaOH in the presence and absence of 6 mM UMP^{2-} under N_2. The experimental data were evaluated in the pH range 1.7–6.7 for $K^H_{H_2(UMP)}$ and $K^H_{H(UMP)}$ in the way described above. Evidently only an estimate can be obtained for $K^H_{H_2(UMP)}$ as the formation degree of $H_2(UMP)$ hardly reaches 10%.

Determination of the Stability Constants. The conditions for the determination of the stability constants $K^M_{M(R\text{-}MP)}$ of the binary M(R-MP) complexes (I = 0.1; 25 °C) were the same as for the acidity constants $K^H_{H(R\text{-}MP)}$ (see the preceding section), except $NaNO_3$ was partly or fully replaced by $M(NO_3)_2$. With Mg^{2+}, Ca^{2+}, Sr^{2+}, and Ba^{2+} [$M(NO_3)_2$] was 0.0333 M (i.e., R-MP:M^{2+} = 1:111); with Mg^{2+} and Ca^{2+} [$M(NO_3)_2$] was also 0.0267 M (R-MP:M^{2+} = 1:89). For Mn^{2+}, Co^{2+}, Ni^{2+}, Zn^{2+}, and Cd^{2+} [$M(NO_3)_2$] was 0.0167 M (R-MP:M^{2+} = 1:56); for Co^{2+} and Ni^{2+} [$M(NO_3)_2$] was also 0.0133 M (1:44), and for Mn^{2+}, Zn^{2+}, and Cd^{2+} [$M(NO_3)_2$] was also 0.0083 M (1:28). For Cu^{2+} [$M(NO_3)_2$] was 3.33 and 1.67 mM, i.e. the R-MP to Cu^{2+} ratios were 1:11 and 1:5.6. The stability constants $K^M_{M(R\text{-}MP)}$ were computed for each pair of titrations by taking into account the species H^+, $H_2(R\text{-}MP)$ where appropriate, $H(R\text{-}MP)^-$, $R\text{-}MP^{2-}$, M^{2+}, and $M(R\text{-}MP)$.[17] Throughout, the data were collected (every 0.1 pH unit) from about 5% complex formation to a neutralization degree of about 85% or to the beginning of the hydrolysis of $M(aq)^{2+}$, which was evident from the titrations without R-MP. The values calculated individually for log $K^M_{M(R\text{-}MP)}$ showed no dependence on pH or on the excess amount of M^{2+}.

One further experimental point warrants mentioning: The commercially available *n*-butyl phosphate, BuP^{2-}, contains about the same amount of the diester, $(Bu)_2P^-$. However, this "impurity" is without consequence, as for diesters $pK_a \simeq 1$;[18] i.e., the diester does not participate in any acid/base equilibrium in the pH range 4.8–8.4, which is important for the equilibrium $H(BuP)^-/BuP^{2-}$. Moreover, the metal ion affinity of $(Bu)_2P^-$, estimated from known constants,[19] is lower by a factor of about 0.06 (or smaller), compared with that for BuP^{2-}; i.e., even in the case of the Cu^{2+} system, where the M^{2+}:$R\text{-}MP^{2-}$ ratio is lowest, no influence of the diester can be expected, and this is confirmed by the results. Of course, the exact concentration of BuP^{2-} in the stock solutions was always determined.

Results and Discussion

1. Acidity Constants of Several Protonated Phosphate Monoesters, Including UMP and TMP.

Simple phosphate monoesters ($R\text{-}MP^{2-}$),[4] such as phenyl phosphate (PhP^{2-}), are dibasic species that may carry two protons at their phosphate group, and therefore the following two protonation equilibria must be considered:

$$H_2(R\text{-}MP) \rightleftharpoons H(R\text{-}MP)^- + H^+ \tag{1a}$$

$$K^H_{H_2(R\text{-}MP)} = [H(R\text{-}MP)^-][H^+]/[H_2(R\text{-}MP)] \tag{1b}$$

$$H(R\text{-}MP)^- \rightleftharpoons R\text{-}MP^{2-} + H^+ \tag{2a}$$

$$K^H_{H(R\text{-}MP)} = [R\text{-}MP^{2-}][H^+]/[H(R\text{-}MP)^-] \tag{2b}$$

In the case of the nucleoside 5'-monophosphates (NMP^{2-}) UMP^{2-} and TMP^{2-}, the proton from the H–N(3) unit of the neutral pyrimidine ring residue (see Figure 1) may also be released in the upper pH range, leading to the additional equilibrium 3. The

$$NMP^{2-} \rightleftharpoons (NMP\text{-}H)^{3-} + H^+ \tag{3a}$$

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

corresponding acidity constants, determined for this study by pH titrations, are listed in Table I, together with the pK_a values for H_3PO_4 and $H(CH_3OPO_3)^-$.[10,11b,20] The equilibria for $H_3(CMP)^+$

---

(17) Griesser, R.; Sigel, H. *Inorg. Chem.* **1970**, *9*, 1238–1243.

(18) Mahler, H. R.; Cordes, E. H. *Biological Chemistry*; Harper and Row: New York, Evanston, London, 1966; p 134.

(19) Diebler, H. *J. Mol. Catal.* **1984**, *23*, 209–217.

(20) Peacock, C. J.; Nickless, G. *Z. Naturforsch., A: Astrophys., Phys., Phys. Chem.* **1969**, *24A*, 245–247.

Table II. Logarithms of the Stability Constants of Simple M(R-MP) Complexes (Eq 4) As Determined by Potentiometric pH Titrations in Water at 25 °C and $I = 0.1$ M ($NaNO_3$)[a]

	$\log K^{M}_{M(R\text{-}MP)}$					
M^{2+}	M(NPhP)	M(PhP)	M(BuP)	M(RibMP)	$M(HPO_4)$[b]	$M(CH_3OPO_3)$[c]
Mg^{2+}	1.29 ± 0.03	1.53 ± 0.02	1.69 ± 0.02	1.58 ± 0.02		1.57
Ca^{2+}	1.26 ± 0.04	1.45 ± 0.01	1.56 ± 0.01	1.48 ± 0.01		1.49
Sr^{2+}	1.12 ± 0.02	1.26 ± 0.02	1.30 ± 0.02	1.25 ± 0.02		
Ba^{2+}	1.06 ± 0.02	1.19 ± 0.02	1.22 ± 0.02	1.17 ± 0.03		
Mn^{2+}	1.87 ± 0.04	2.12 ± 0.03	2.34 ± 0.01	2.20 ± 0.02		2.19
Co^{2+}	1.65 ± 0.05	1.94 ± 0.02	2.08 ± 0.03	2.00 ± 0.01	2.18	2.00
Ni^{2+}	1.59 ± 0.05	1.91 ± 0.03	2.08 ± 0.04	2.00 ± 0.01	2.08	1.91
Cu^{2+}	2.33 ± 0.04	2.77 ± 0.01	3.12 ± 0.06	2.96 ± 0.02	3.2	
Zn^{2+}	1.73 ± 0.03	2.07 ± 0.02	2.30 ± 0.02	2.20 ± 0.02	2.4	2.16
Cd^{2+}	2.05 ± 0.01	2.38 ± 0.02	2.61 ± 0.02	2.49 ± 0.02		

[a] The errors given are 3 times the standard error of the mean value or the sum of the probable systematic errors, whichever is larger. [b] From ref 10 ($I = 0.1$ M ($NaClO_4$), 25 °C). [c] From ref 11a ($I = 0.1$ M, 20 °C).

will be considered in section 2.[21]

The release of the first proton from monoesterified derivatives of phosphoric acid, i.e. from the $H_2(R\text{-}MP)$ species, occurs at a very low pH: usually $pK_a \approx 1$ (eq 1).[22] As an example, we measured the corresponding value for $H_2(UMP)$; the result $pK^{H}_{H_2(UMP)} = 0.7 \pm 0.3$ carries a large error because the formation degree of $H_2(UMP)$ hardly reaches 10% in the experiments (see Experimental Section). However, the given value is in excellent agreement with the following estimation: assuming the charge effect in the adenine nucleotides $H_3(ATP)^-$ and $H_3(AMP)^+$ is the same as in the uracil nucleotides $H_2(UTP)^{2-}$ and $H_2(UMP)^0$, then it holds that $pK^{H}_{H_3(ATP)} - pK^{H}_{H_3(AMP)} = (1.6 \pm 0.2)$[ref 23] $- (0.4 \pm 0.2)$[ref 9] $= 1.2 \pm 0.3 = pK^{H}_{H_2(UTP)} - pK^{H}_{H_2(UMP)}$; hence, $pK^{H}_{H_2(UMP)} = pK^{H}_{H_2(UTP)} - (1.2 \pm 0.3) = (2.0 \pm 0.1)$[ref 24] $- (1.2 \pm 0.3) = 0.8 \pm 0.3$. This result for $H_2(UMP)$ demonstrates that the first proton from the phosphoric acid residue in $H_2(R\text{-}MP)$ species is completely released at pH ≥ 3 and does therefore not affect the equilibrium $H(R\text{-}MP)^-/R\text{-}MP^{2-}$ and the complex formation between M^{2+} and $R\text{-}MP^{2-}$; the last two reactions occur only at pH >3.

The acidity constants determined for the $H(R\text{-}MP)^-$ species (Table I) are in the expected order.[22] The decreasing acidity within the series[4] $H(NPhP)^- > H(PhP)^- > H(CH_3OPO_3)^- > H(BuP)^- \approx H_2PO_4^-$ can be rationalized. The phenyl and especially the *p*-nitrophenyl groups have stronger electron-withdrawing properties than the methyl group. The higher acidity of $H(CH_3OPO_3)^-$ compared with that of $H_2PO_4^-$ probably reflects solvation differences: the methyl group is expected to disturb solvation somewhat, and therefore the release of the proton from $H(CH_3OPO_3)^-$ should be easier than from the better screened $H_2PO_4^-$. The lower acidity of $H(BuP)^-$, compared to that of $H(CH_3OPO_3)^-$, may be explained by the relatively large *n*-butyl residue leading to a reduced effective dielectric constant in its vicinity,[25] thus inhibiting the formation of a further charge, i.e. the release of the proton.[26] Correspondingly, one may explain the lower acidity of $H(TMP)^-$ compared with that of $H(UMP)^-$ (Table I), a trend paralleled by $H(TTP)^{3-}$ and $H(UTP)^{3-}$;[13a] the loss of a hydroxy group in the 2′-deoxyribosyl residue makes the thymidine nucleotides somewhat more "hydrophobic". The near-identity of the acidities of $H(CH_3OPO_3)^-$ and $H(RibMP)^-$ is probably a reflection of the pronounced hydrophilicity of the sugar ring allowing a solvation of the phosphate group similar to that in methyl phosphate.

The deprotonation of the H–N(3) unit in UMP^{2-} and TMP^{2-} occurs in the pH range above 8.5 (Table I). This is expected, as is the order of the acidity for $UMP^{2-} > TMP^{2-}$ (eq 3).[27] It is important to note with regard to the formation of M(NMP) complexes (see section 4) that this proton (eq 3) is not released in the pH range where complex formation between M^{2+} and NMP^{2-} occurs; therefore, eq 3 does not need to be considered in the corresponding evaluations.

2. Acidity Constants of $H_3(CMP)^+$. CMP is a special case compared with the other phosphate monoesters considered so far because N(3) of the pyrimidine ring residue (Figure 1) may be protonated.[21] Together with the two basic sites at the phosphate group this allows binding of three protons in all, forming $H_3(CMP)^+$. The first proton is released from the phosphoric acid residue, the second from the $^{+}H\text{–}N(3)$ unit, and the third from the still monoprotonated phosphate group. The corresponding acidity constants are $K^{H}_{H_3(CMP)} = [H_2(CMP)^{\pm}][H^+]/[H_3(CMP)^+]$, $K^{H}_{H_2(CMP)} = [H(CMP)^-][H^+]/[H_2(CMP)^{\pm}]$, and $K^{H}_{H(CMP)} = [CMP^{2-}][H^+]/[H(CMP)^-]$.

The value for $pK^{H}_{H_3(CMP)}$ was not determined, but it must obviously be lower than that for $H_2(UMP)$. An estimate for $pK^{H}_{H_3(CMP)}$ may be made by assuming that the charge effect in the pair $H_2(UMP)/H_3(AMP)^+$ corresponds to that for $H_2(UMP)/H_3(CMP)^+$: $pK^{H}_{H_2(UMP)} - pK^{H}_{H_3(AMP)} = (0.7 \pm 0.3)$[Table I] $- (0.4 \pm 0.2)$[ref 9] $= 0.3 \pm 0.4 = pK^{H}_{H_2(UMP)} - pK^{H}_{H_3(CMP)}$; hence, $pK^{H}_{H_3(CMP)} = pK^{H}_{H_2(UMP)} - (0.3 \pm 0.4) = (0.7 \pm 0.3) - (0.3 \pm 0.4) = 0.4 \pm 0.5$. This estimate is certainly correct within the given error limits, and therefore the corresponding equilibrium does not interfere with any acid/base reaction at pH >2.5.

The release of the proton from the $^{+}H\text{–}N(3)$ unit in $H_2(CMP)^{\pm}$ occurs with $pK^{H}_{H_2(CMP)} = 4.33$ (Table I) in the expected pH range;[13a,21] the same is true for $pK^{H}_{H(CMP)} = 6.19$ of $H(CMP)^-$ (Table I). The overlap between these two equilibria is small, as $\Delta pK_a = 1.86$, but one might still argue that in correlations between complex stability and ligand acidity a microconstant (see e.g. ref 28) for $H(CMP)^-$ should be employed that is corrected for any influence of the protonated base residue. Such a value is not easy to assess, but $pK^{H}_{H(UMP)} = 6.15$ from $H(UMP)^-$ may be considered a good estimate, and it confirms also that the mutual influence of the two equilibria is small: $pK^{H}_{H(UMP)}$ and $pK^{H}_{H(CMP)}$ hardly differ in a significant way (see Table I). However, in the evaluations of section 7 (Table VII) both acidity constants are used to demonstrate that this does not influence the conclusions regarding the structures of the M(CMP) complexes in solution.

3. Stability of Simple Phosphate Monoester Complexes. The experimental data of the potentiometric pH titrations may be completely described by considering equilibria 2 and 4 (see also section 1). The constants calculated individually show no de-

$$M^{2+} + R\text{-}MP^{2-} \rightleftharpoons M(R\text{-}MP) \tag{4a}$$

$$K^{M}_{M(R\text{-}MP)} = [M(R\text{-}MP)]/[M^{2+}][R\text{-}MP^{2-}] \tag{4b}$$

---

[21] (a) Martin, R. B.; Mariam, Y. H. *Met. Ions Biol. Syst.* **1979**, *8*, 57–124. (b) Martin, R. B. *Acc. Chem. Res.* **1985**, *18*, 32–38.

[22] In ref 18, p 131.

[23] Tribolet, R.; Sigel, H. *Eur. J. Biochem.* **1988**, *170*, 617–626.

[24] Tribolet, R.; Malini-Balakrishnan, R.; Sigel, H. *J. Chem. Soc., Dalton Trans.* **1985**, 2291–2303.

[25] Sigel, H.; Malini-Balakrishnan, R.; Häring, U. K. *J. Am. Chem. Soc.* **1985**, *107*, 5137–5148.

[26] Sigel, H.; Martin, R. B.; Tribolet, R.; Häring, U. K.; Malini-Balakrishnan, R. *Eur. J. Biochem.* **1985**, *152*, 187–193.

[27] (a) Sigel, H. *Eur. J. Biochem.* **1968**, *3*, 530–537. (b) Sigel, H. *J. Am. Chem. Soc.* **1975**, *97*, 3209–3214.

[28] Martin, R. B. *Met. Ions Biol. Syst.* **1979**, *9*, 1–39.

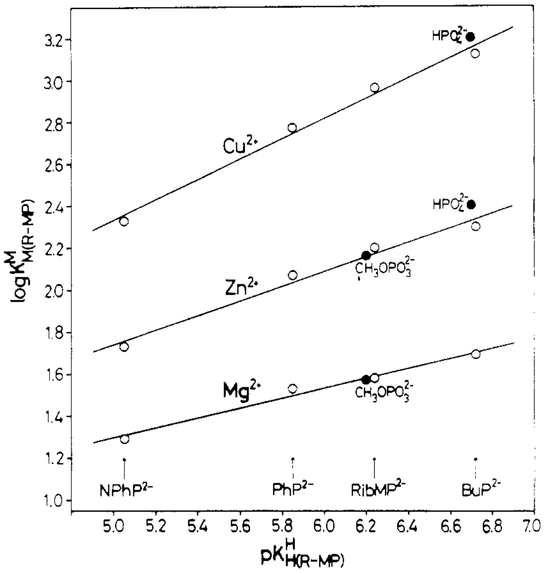

Figure 2. Relationship between $\log K^{M}_{M(R\text{-}MP)}$ and $pK^{H}_{H(R\text{-}MP)}$ for the Mg^{2+}, Zn^{2+}, and Cu^{2+} 1:1 complexes of 4-nitrophenyl phosphate ($NPhP^{2-}$), phenyl phosphate (PhP^{2-}), D-ribose 5′-monophosphate ($RibMP^{2-}$), and *n*-butyl phosphate (BuP^{2-}) (from left to right, ○). The least-squares lines are drawn through these data; the corresponding equations are given in Table III. The points due to the complexes formed with methyl phosphate ($CH_3OPO_3^{2-}$) and hydrogen phosphate (HPO_4^{2-}) (●) are inserted for comparison. The plotted equilibrium constant values are from Tables I and II.

Table III. Slopes (*m*) and Intercepts (*b*) for the Straight-Base-Line Plots of $\log K^{M}_{M(R\text{-}MP)}$ versus $pK^{H}_{H(R\text{-}MP)}$ As Calculated by the Least-Squares Procedure from the Data of Tables I and II for NPhP,[4] PhP, BuP, and RibMP (I = 0.1 M ($NaNO_3$), 25 °C)[a]

M^{2+}	*m*	*b*
Mg^{2+}	0.237 ± 0.023	0.110 ± 0.140
Ca^{2+}	0.177 ± 0.022	0.380 ± 0.134
Sr^{2+}	0.104 ± 0.027	0.612 ± 0.159
Ba^{2+}	0.091 ± 0.027	0.617 ± 0.163
Mn^{2+}	0.279 ± 0.013	0.471 ± 0.080
Co^{2+}	0.259 ± 0.040	0.372 ± 0.242
Ni^{2+}	0.298 ± 0.043	0.117 ± 0.259
Cu^{2+}	0.481 ± 0.037	−0.072 ± 0.221
Zn^{2+}	0.348 ± 0.038	0.000 ± 0.225
Cd^{2+}	0.338 ± 0.031	0.366 ± 0.185

[a] Straight-line equation: $y = mx + b$. The errors given with *m* and *b* correspond to 1 standard deviation (1σ).

Table IV. Logarithms of the Stability Constants of M(UMP) and M(TMP) Complexes (Eq 4) As Determined by Potentiometric pH Titrations in Water at 25 °C and I = 0.1 M ($NaNO_3$)[a] and for Comparison the Calculated Stability Constants Based on the $pK^{H}_{H(NMP)}$ Values (Eq 2) of Table I and the Base-Line Equations of Table III

	$\log K^{M}_{M(UMP)}$		$\log K^{M}_{M(TMP)}$	
M^{2+}	M^{2+}	calcd	exptl[a]	calcd
Mg^{2+}	1.56 ± 0.02	1.57	1.55 ± 0.02	1.62
Ca^{2+}	1.44 ± 0.05	1.47	1.40 ± 0.06	1.51
Sr^{2+}	1.25 ± 0.04	1.25	1.19 ± 0.06	1.27
Ba^{2+}	1.13 ± 0.06	1.18	1.11 ± 0.04	1.20
Mn^{2+}	2.11 ± 0.02	2.19	2.11 ± 0.05	2.25
Co^{2+}	1.87 ± 0.05	1.96	1.89 ± 0.05	2.02
Ni^{2+}	1.97 ± 0.05	1.95	1.92 ± 0.06	2.01
Cu^{2+}	2.77 ± 0.06	2.89	2.87 ± 0.05	2.99
Zn^{2+}	2.02 ± 0.07	2.14	2.10 ± 0.06	2.21
Cd^{2+}	2.38 ± 0.04	2.44	2.42 ± 0.03	2.52

[a] The errors given are 3 times the standard error of the mean value or the sum of the probable systematic errors, whichever is larger.

pendence on pH or the excess metal ion concentration, if the evaluation of the experimental data is not carried into the pH range where hydroxo complex formation occurs (cf. Experimental Section). The determined stability constants are listed in Table II together with some data from the literature[10,11a] for $M(HPO_4)$ and $M(CH_3OPO_3)$ complexes.

These stability constants show the usual trends: Complex stability of the alkaline-earth ions decreases with increasing ionic radii. For the divalent 3d metal ions the long-standing experience[10,29] is confirmed that the stabilities of metal ion/phosphate complexes often do not strictly follow the Irving–Williams sequence. The observed stability order for all phosphate ligands of Table II is $Ba^{2+} < Sr^{2+} < Ca^{2+} < Mg^{2+} < Ni^{2+} \lesssim Co^{2+} < Mn^{2+} < Cu^{2+} > Zn^{2+} < Cd^{2+}$.

However, more important is the following question: Is there a correlation between complex stability and phosphate group basicity? In other words, is there a linear relationship between $\log K^{M}_{M(R\text{-}MP)}$ and $pK^{H}_{H(R\text{-}MP)}$, as is known for other series[5] of structurally related ligands? Indeed, this is the case as is evident from Figure 2, where the corresponding data for the Mg^{2+}, Zn^{2+}, and Cu^{2+} complexes are plotted. It is clear that for a given metal ion the complexes with 4-nitrophenyl phosphate ($NPhP^{2-}$), phenyl phosphate (PhP^{2-}), and *n*-butyl phosphate (BuP^{2-}) fit within experimental error on a straight line; furthermore, the value for D-ribose 5′-monophosphate ($RibMP^{2-}$) fits also on this line, indicating that $RibMP^{2-}$ behaves like a simple phosphate monoester with a noncoordinating organic residue.

On the basis of this tentative result we carried out a careful check by calculating the least-squares lines of $\log K^{M}_{M(R\text{-}MP)}$ versus $pK^{H}_{H(R\text{-}MP)}$ for the series of the 10 metal ion complexes of Table II by using the acidity constants of Table I: the slopes and intercepts are listed in Table III; the error limits given show that the data for the four considered complexes fit well on a straight line.

A control of this correlation between complex stability and phosphate group basicity is possible for several metal ion systems by using the earlier equilibrium data for hydrogen phosphate (HPO_4^{2-})[10] and methyl phosphate ($CH_3OPO_3^{2-}$),[11] the most simple phosphate ligands to be thought of for a check. The four data points in Figure 2 show that the values for the HPO_4^{2-} and $CH_3OPO_3^{2-}$ systems fit excellently on the base lines despite the slightly different experimental conditions used in their determination (Tables I and II). A more careful judgement is possible from the *differences* between the experimentally determined values for $\log K^{M}_{M(R\text{-}MP)}$ (Table II) and the values calculated with $pK^{H}_{H(R\text{-}MP)}$ and the straight-line equations of Table III. It is satisfying that all differences[30] are within ±0.07 log unit, confirming that the correlation among all these equilibrium constants is excellent and that our base lines are free from any steric effects. It should especially be pointed out that this also proves that the D-ribose 5′-monophosphate systems have the properties of simple monophosphate esters in aqueous solution; in fact, the agreement between the values for M(RibMP) and $M(CH_3OPO_3)$ is striking (see Table II).

4. Stability and Structure of the M(UMP) and M(TMP) Complexes. The self-association of pyrimidine nucleotides is low,[12,31] and under the present experimental conditions (0.3 mM) no self-stacking occurs. Indeed, the experimental data are again satisfied by considering equilibria 2 and 4. The determined stability constants are listed in Table IV. The constants for the UMP^{2-} complexes with Mg^{2+}, Mn^{2+}, Cu^{2+}, and Zn^{2+} have been determined before;[16] the agreement between the two sets of data is good.

When $pK^{H}_{H(UMP)}$ and $pK^{H}_{H(TMP)}$ and the base-line equations of Table III are used, stability constants can be calculated that correspond to a pure phosphate coordination as determined by the basicity of the phosphate group (cf. also Figure 2). These constants are also listed in Table IV (error range about ±0.1 log unit).

---

[29] Sigel, H.; McCormick, D. B. *Acc. Chem. Res.* **1970**, *3*, 201–208.

[30] For $M(HPO_4)$ the differences are 0.07 (Co^{2+}), −0.03 (Ni^{2+}), 0.05 (Cu^{2+}), and 0.07 (Zn^{2+}), and for $M(CH_3OPO_3)$ they are −0.01 (Mg^{2+}), 0.01 (Ca^{2+}), −0.01 (Mn^{2+}), 0.02 (Co^{2+}), −0.05 (Ni^{2+}), and 0.00 (Zn^{2+}).

[31] Scheller, K. H.; Sigel, H. *J. Am. Chem. Soc.* **1983**, *105*, 5891–5900.

Table V. Correlations between M^{2+}–Phosphate Complex Stability and Phosphate Group Basicity: Slopes (m) and Intercepts (b) for the Straight-Base-Line Plots of log $K^{M}_{M(R\text{-}MP)}$ versus $pK^{H}_{H(R\text{-}MP)}$ As Calculated by the Least-Squares Procedure from the Experimental Equilibrium Constants Given in Tables I, II, and IV for NPhP,[4] PhP, BuP, RibMP, UMP, and TMP (I = 0.1 M ($NaNO_3$), 25 °C)[a]

M^{2+}	m	b	R^{b}
Mg^{2+}	0.224 ± 0.027	0.174 ± 0.167	0.971
Ca^{2+}	0.156 ± 0.039	0.487 ± 0.239	0.893
Sr^{2+}	0.089 ± 0.034	0.691 ± 0.206	0.794
Ba^{2+}	0.073 ± 0.036	0.706 ± 0.217	0.713
Mn^{2+}	0.250 ± 0.048	0.607 ± 0.293	0.933
Co^{2+}	0.230 ± 0.057	0.510 ± 0.345	0.897
Ni^{2+}	0.282 ± 0.045	0.201 ± 0.275	0.952
Cu^{2+}	0.453 ± 0.056	0.055 ± 0.340	0.971
Zn^{2+}	0.321 ± 0.057	0.125 ± 0.345	0.943
Cd^{2+}	0.317 ± 0.042	0.467 ± 0.253	0.967

[a] Straight-line equation: $y = mx + b$; x may represent the pK_a value of any phosphate monoester. The errors given with m and b correspond to 1 standard deviation (1σ). [b] Correlation coefficient.

Comparison of the experimental and calculated stability constants reveals an excellent agreement (Table IV). There is especially no hint of an increased complex stability compared with the stability expected from the phosphate group basicity; this means that the pyrimidine-base residues of UMP^{2-} and TMP^{2-} are not participating in complex formation. This result agrees with the situation in $M(UTP)^{2-}$ and $M(TTP)^{2-}$ complexes.[12,13] A metal ion–base interaction in this type of complexes is expected only when the H–N(3) units are deprotonated.[12,27] Hence, we conclude that the stability of the M(UMP) and M(TMP) complexes is solely determined by the basicity of the corresponding phosphate groups; i.e., these systems are further examples for simple monophosphate ester coordination.

These conclusions agree with crystal structure studies:[32] $[Co_2(H_2O)_4(UMP)_2]_n$ is a polymeric chain with Co^{2+} bound to phosphate oxygens and water molecules;[33] Mn^{2+} forms a similar compound.[33] Likewise, in $[Cu(UMP)(dpa)(H_2O)]_2 \cdot 5H_2O$ (dpa = di-2-pyridylamine) coordination of UMP^{2-} takes place exclusively through phosphate oxygens;[34] the same is true[35] in the polymeric complex $[Cd(UMP)(dpa)(H_2O)_2]_n$.

5. Correlations between Complex Stability and Ligand Basicity: Construction of Base-Line Plots of log $K^{M}_{M(R\text{-}MP)}$ versus $pK^{H}_{H(R\text{-}MP)}$. The acidity constants of many nucleoside monophosphates[6-9] are in the pH range 6–6.5, and this is also true for the $pK^{H}_{H(NMP)}$ values of $H(UMP)^-$ and $H(TMP)^-$, which are 6.15 and 6.36, respectively. Therefore, the UMP and TMP systems, which are also pure phosphate coordinators (section 4), are ideal for being incorporated into the final base-line construction: The equilibrium constants for the systems containing $NPhP^{2-}$, PhP^{2-}, BuP^{2-}, $RibMP^{2-}$, UMP^{2-}, and TMP^{2-} furnish six data points which cover the pH range 5–7 for each straight-line plot of log $K^{M}_{M(R\text{-}MP)}$ (Tables II and IV) versus $pK^{H}_{H(R\text{-}MP)}$ (Table I); the results of the corresponding least-squares calculations are summarized in Table V for the 10 metal ions considered. Comparison of these results with the preliminary calculations given in Table III shows that for all 10 systems the error limits for the slopes (m) and intercepts (b) of the least-squares lines overlap well.

Table VI lists the deviations from the least-squares line for each complex with the six phosphate ligands mentioned. The points for the TMP systems are farthest below the least-squares lines, and those for the PhP systems give the most positive deviations; however, all deviations are within ±0.09 log unit. To provide a reliable error limit for any stability constant calculated with the equations of Table V and a given pK_a value, for each metal ion the standard deviation of the six data points from the least-squares line was calculated; the corresponding values are given in Table VI in the column at the far right.

Users of the results described in this section are recommended to apply the equations of Table V for phosphate ligands in the pK_a range 5–7 and to consider as error limits of the calculated stability constant log $K^{M}_{M(R\text{-}MP)}$ 2 or 3 times the standard deviation given in Table VI for the corresponding metal ion system. An application of this procedure is given in section 7 for the evaluation of the structure of the M(CMP) complexes in solution.

6. Stability of the M(CMP) Complexes. $H_2(CMP)^{\pm}$ carries a proton not only at the phosphate group but also at N(3) of the pyrimidine residue (section 2). Therefore, one might expect not only that complexes according to equilibrium 5 are formed but

$$M^{2+} + CMP^{2-} \rightleftharpoons M(CMP) \tag{5a}$$

$$K^{M}_{M(CMP)} = [M(CMP)]/[M^{2+}][CMP^{2-}] \tag{5b}$$

also that monoprotonated $M(H\cdot CMP)^+$ complexes occur. However, in the pH range ≥4.7, which is defined by the ≥5% formation degree of M(CMP) (see Experimental Section), the experimental data of the potentiometric pH titrations may be completely described by taking into account equilibria 2, 3, and 5. This means, under the present experimental conditions, no evidence is obtained for the formation of $M(H\cdot CMP)^+$ complexes, and this observation agrees with an earlier study,[15] in which the Mg^{2+} and Ni^{2+} systems of CMP were investigated.

The stability constants determined now for the M(CMP) complexes are listed in Table VII. The values determined earlier[15] for the Mg^{2+} (1.75) and Ni^{2+} (1.90) complexes (I = 0.1 M (KNO_3)) are in fair agreement; in fact, the difference of 0.2 log unit in the case of Mg(CMP) may be a temperature effect, as the earlier study was done at 15 °C. However, the differences between the present results and those of two other recent studies (I = 0.1 M (KNO_3))[36,37] done at 35 °C are by far too large to be explained by a temperature effect. Furthermore, already the pK_a values for $H_2(CMP)^{\pm}$ differ considerably between the two studies despite the identity of the experimental conditions; similarly, the Co^{2+} and Cu^{2+} complexes are 0.7 log unit *more* stable in the first study[36] and the Ni^{2+} and Zn^{2+} complexes 0.7 log unit *less* stable, compared with the results in the second study.[37] This means that the agreement between the two studies[36,37] is poor and the corresponding log $K^{M}_{M(CMP)}$ values are between 0.2 and 1 log unit too large for the Co^{2+}, Ni^{2+}, Cu^{2+}, and Zn^{2+} complexes even if the value closest to our results (Table VII) is selected; even worse, the stability constants[37] for Ca(CMP) and Mg(CMP) are respectively 1 and 1.3 log units higher than our values. Though the reasons for these discrepancies are unclear, one has to conclude that the equilibrium constants given in these two recent studies[36,37] are not reliable. In contrast, the present results are not only in accord with the earlier values[15] for Mg(CMP) and Ni(CMP) but more important, as discussed in section 7, the stabilities of the M(CMP) complexes given in Table VII also fit into the general picture expected for pyrimidine-nucleoside 5′-monophosphates.

7. Evaluation of the Structures of M(CMP) Complexes in Solution. CMP^{2-} differs from UMP^{2-} and TMP^{2-} insofar as it offers an obvious binding site at the base moiety (Figure 1), i.e. the pyrimidine-like N(3), which is potentially available for coordination at pH >4.5. Indeed, spectrophotometric measurements[38] with Ni^{2+} and ^1H NMR shift experiments[39] with Cd^{2+} revealed that cytidine complexes form; in addition, the stability of $Cu(Cyd)^{2+}$ is known.[40] Therefore, to evaluate the situation with CMP^{2-} the stability of the M(CMP) complexes must be carefully compared with the stability of complexes capable of only a phosphate coordination. Such a pure phosphate coordination is defined by the base-line equations of Table V (see section 5), and the complex stability corresponding to the basicity of the phosphate group of CMP^{2-} may therefore be calculated. This was

---

(32) Gellert, R. W.; Bau, R. *Met. Ions Biol. Syst.* **1979**, *8*, 1–55.

(33) Cartwright, B. A.; Goodgame, D. M. L.; Jeeves, I.; Skapski, A. C. *Biochim. Biophys. Acta* **1977**, *477*, 195–198.

(34) Fischer, B. E.; Bau, R. *Inorg. Chem.* **1978**, *17*, 27–34.

(35) Aoki, K. *J. Chem. Soc., Chem. Commun.* **1979**, 589–591.

(36) Krishnamoorthy, C. R.; Sunil, S.; Ramalingam, K. *Polyhedron* **1985**, *4*, 1451–1456.

(37) Reddy, P. R.; Reddy, B. M. *Polyhedron* **1986**, *5*, 1947–1952.

(38) Mariam, Y. H.; Martin, R. B. *Inorg. Chim. Acta* **1979**, *35*, 23–28.

(39) See footnote 87 in ref 12.

(40) Kim, S. H.; Martin, R. B. *Inorg. Chim. Acta* **1984**, *91*, 19–24.

Table VI. Logarithmic Differences between the Experimental Stability Constants (log $K^{M}_{M(R\text{-}MP)}$; Tables II and IV) of the Complexes for $NPhP^{2-}$, PhP^{2-}, BuP^{2-}, $RibMP^{2-}$, UMP^{2-}, and TMP^{2-} and the Least-Squares Lines of log $K^{M}_{M(R\text{-}MP)}$ versus $pK^{H}_{H(R\text{-}MP)}$ As Determined by the Six Complex Systems Mentioned (Table V)[a]

M^{2+}	M(NPhP)	M(PhP)	M(BuP)	M(RibMP)	M(UMP)	M(TMP)	SD
Mg^{2+}	−0.02	0.05	0.01	0.01	0.01	−0.05	0.014
Ca^{2+}	−0.01	0.05	0.02	0.02	−0.01	−0.08	0.018
Sr^{2+}	−0.02	0.05	0.01	0.00	0.01	−0.07	0.016
Ba^{2+}	−0.01	0.06	0.02	0.01	−0.02	−0.06	0.017
Mn^{2+}	0.00	0.05	0.05	0.03	−0.03	−0.09	0.022
Co^{2+}	−0.02	0.08	0.02	0.05	−0.05	−0.08	0.024
Ni^{2+}	−0.04	0.06	−0.02	0.04	0.03	−0.07	0.021
Cu^{2+}	−0.01	0.06	0.02	0.08	−0.07	−0.07	0.026
Zn^{2+}	−0.02	0.07	0.02	0.07	−0.08	−0.07	0.027
Cd^{2+}	−0.02	0.06	0.01	0.04	−0.04	−0.06	0.019

[a] The farthest column to the right gives the standard deviation (SD) resulting from the listed differences.

Table VII. Logarithms of the Stability Constants of M(CMP) Complexes (Eq 5) As Determined by Potentiometric pH Titrations in Water at 25 °C and I = 0.1 M ($NaNO_3$)[a] and the Calculated Stability Constants Based on the Basicity of the Phosphate Group and the Base-Line Equations of Table V

M^{2+}	log $K^{M}_{M(CMP)}$ exptl[a]	log $K^{M}_{M(CMP)}$ calcd[b]	log K_{exptl} − log K_{calcd}[c]
Mg^{2+}	1.54 ± 0.05	1.55 (1.56) ± 0.04	−0.01 ± 0.06
Ca^{2+}	1.40 ± 0.05	1.45 (1.45) ± 0.05	−0.05 ± 0.07
Sr^{2+}	1.17 ± 0.04	1.24 (1.24) ± 0.05	−0.07 ± 0.06
Ba^{2+}	1.11 ± 0.03	1.15 (1.16) ± 0.05	−0.04 ± 0.06
Mn^{2+}	2.10 ± 0.04	2.14 (2.15) ± 0.07	−0.04 ± 0.08
Co^{2+}	1.86 ± 0.05	1.92 (1.93) ± 0.07	−0.06 ± 0.09
Ni^{2+}	1.94 ± 0.06	1.94 (1.95) ± 0.06	0.00 ± 0.08
Cu^{2+}	2.84 ± 0.06	2.84 (2.86) ± 0.08	0.00 ± 0.10
Zn^{2+}	2.06 ± 0.05	2.10 (2.11) ± 0.08	−0.04 ± 0.09
Cd^{2+}	2.40 ± 0.08	2.42 (2.43) ± 0.06	−0.02 ± 0.10

[a] The errors given are 3 times the standard error of the mean value or the sum of the probable systematic errors, whichever is larger. [b] The first value is calculated with the microconstant pk = 6.15 (=$pK^{H}_{H(UMP)}$, i.e. with the acidity constant corrected for the influence of the protonated base residue (see section 2). The value in parentheses is based on $pK^{H}_{H(CMP)}$ = 6.19. The error limits correspond to 3 times the standard deviations listed in Table VI. [c] The error limits for these differences were calculated according to the error propagation by the method of Gauss.

done by using $pK^{H}_{H(CMP)}$ = 6.19 as well as the microconstant pk = 6.15 (see section 2): evidently, the choice of the acidity constant has no significant influence on the results (third column of Table VII).

The experimentally determined and the calculated stability constants for the M(CMP) complexes are identical within the error limits (Table VII); i.e., there is no indication for an increased stability of any of the 10 complexes considered. Hence, there is no evidence for metal ion–base back-binding or macrochelation in these M(CMP) species; their stability is solely determined by the metal ion affinity of the phosphate group.

$M(CTP)^{2-}$ complexes have the same property; only $Cu(CTP)^{2-}$ shows some macrochelate formation, i.e. about 30% is back-bound to N(3).[13a] The reason for these observations is that the pyrimidine nucleotides exist in solution predominantly in the anti conformation;[21a] i.e., N(3) is pointing away from the phosphate group, and therefore a simultaneous coordination of the metal ion to both the phosphate residue and N(3) is not possible without a conformation change of the nucleotide into the less favored syn conformation. The energy barrier between the syn and anti conformations (about 6 kJ/mol for CTP^{4-})[13a] is considerable: None of the complexes in Table VII overcome it. The structures proposed in ref 36 and 37 involving the phosphate group *and* N(3) must therefore be rejected.

The fact that in aqueous solution the metal ion is only phosphate-coordinated in M(CMP) complexes is important and is only seemingly in contradiction with suggestions based on solid-state IR measurements[41] and results from crystal structure studies.[32]

The overwhelming part of the binary M(CMP) complexes is polymeric in the solid state, e.g. $[M(CMP)(H_2O)]_n$ with M^{2+} = Cd,[42,43] Co,[42] Zn,[44] and Mn:[45] the nucleotide coordinates from both ends, i.e. through the phosphate group and the base moiety. This is also the case in the dimeric ternary complex $[Pt(en)(CMP)]_2$[46] (en = ethylenediamine). The base moiety is usually N(3) coordinated;[32] an exception is the mentioned Mn^{2+} complex with a (C(2))O coordination.[45]

A particularly common type of ligation[42,43,45] in the polymers is one in which the base is coordinated via N(3) to one metal ion and the phosphate group to three others;[32] hence, metal–phosphate linkages predominate and they are probably the main binding force holding the lattice together, and it is perhaps for this reason that unusual bonding features, like the (C(2))O–Mn bond,[45] are sometimes found as part of a polymeric structure.[32] Furthermore, the formation of polymers in the crystalline state is actually not surprising, since in solid M(CMP) complexes also the *anti* conformation prevails.[32,42–46] However, if these solids are dissolved, the polymeric structure breaks down and in diluted aqueous solution monomeric M(CMP) complexes with only a phosphate coordination are formed. This structural result is the consequence of the anti conformation of CMP^{2-} and the higher metal ion affinity of the phosphate group compared with that of N(3) of the cytidine residue; even for Cu^{2+} it holds: log $K^{Cu}_{Cu(R\text{-}MP)}$ = 2.8 (Table VII) > log $K^{Cu}_{Cu(Cyd)}$ = 1.6–2.0.[40,47] Indeed, in the mixed-ligand complex $[Cu(CMP)(dpa)(H_2O)]_2$, where the coordination sphere of Cu^{2+} is partially saturated by the amine, CMP^{2-} coordinates only via the phosphate oxygens.[35] Hence, the different structures of the M(CMP) complexes in the solid state and in solution can now be well-understood.

General Conclusions

The described results show that in all the M^{2+} complexes formed with UMP^{2-}, TMP^{2-}, or CMP^{2-} the metal ion is only phosphate coordinated in aqueous solution and no metal ion–base interaction occurs. For the M(UMP) and M(TMP) complexes this is rather evident right from the beginning, while for the M(CMP) species due to the presence of the pyridine-like N(3) (Figure 1) this conclusion (section 7) is only possible from a careful analysis of the correlation between complex stability and ligand basicity (section 5).

The base-line correlations between pure phosphate coordination and phosphate group basicity are so well established for Mg^{2+}, Ca^{2+}, Sr^{2+}, Ba^{2+}, Mn^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, and Cd^{2+} (Table V) that the magnitudes of positive deviations for phosphate ligands with potentially coordinating side chains may be used to determine the extent of side-chain interaction with the metal ion already coordinated to the phosphate group. For example, it is thus possible to evaluate the formation degree of the macrochelates

---

[41] Ogawa, M.; Sakaguchi, T. *Yakugaku Zasshi* **1972**, *92*, 1166–1169.

[42] Clark, G. R.; Orbell, J. D. *J. Chem. Soc., Chem. Commun.* **1975**, 697–698.

[43] Shiba, J. K.; Bau, R. *Inorg. Chem.* **1978**, *17*, 3484–3488.

[44] Aoki, K. *Biochim. Biophys. Acta* **1976**, *447*, 379–381.

[45] Aoki, K. *J. Chem. Soc., Chem. Commun.* **1976**, 748–479.

[46] Louie, S.; Bau, R. *J. Am. Chem. Soc.* **1977**, *99*, 3874–3876.

[47] Fiskin, A. M.; Beer, M. *Biochemistry* **1965**, *4*, 1289–1294.

Table VIII. Slopes of log K^{M}_{ML} versus pK^{H}_{HL} Plots for Several Divalent Metal Ion Systems and Their Complexes with the Following Ligands (L): Simple Carboxylates (R-COO$^-$),[5,49] Phosphate Monoesters (R-MP^{2-}) (Table V), Amino Acid Anions (AA$^-$),[50] and N-Substituted Iminodiacetates (R-Ida^{2-})[5,a]

M^{2+}	M(R-COO)$^+$	M(R-COO)$^+$	M(R-MP)	M(AA)$^+$	M(R-Ida)
Mn^{2+}		0.16 (2)	0.25 (5)		0.57
Co^{2+}			0.23 (6)	0.38 (2)	0.48
Ni^{2+}			0.28 (5)	0.35 (5)	0.60
Cu^{2+}	0.19 (3)	0.42 (1)	0.45 (6)	0.55 (6)	0.79
Zn^{2+}	0.05 (3)	0.26 (1)	0.32 (6)	0.47 (5)	0.54

[a] All data refer to aqueous solutions, except those of the third column, M(R-COO)$^+$, which hold for 50% aqueous dioxane.[5] The numbers in parentheses represent 1 standard deviation in the last slope digit; the standard deviation in the slopes for the M(R-Ida) complexes varies from ±0.03 to ±0.06.

formed in certain metal ion complexes of adenosine 5′-monophosphate and some of its derivatives.[14] Recently, this quantification procedure for intramolecular equilibria was outlined[5] and applied to describe the situation in complexes of N-substituted iminodiacetates. Clearly, application of this method to nucleotide complexes will further improve our understanding of nucleotide coordination chemistry.[13,48]

The factors that determine the slopes of the base lines mentioned are difficult to establish. A very tentative interpretation is based on the slopes listed in Table VIII for plots of log K^{M}_{ML} versus pK^{H}_{HL} involving divalent metal ions and, as ligands (L), simple carboxylates (R-COO$^-$), phosphate monoesters (R-MP^{2-}), amino acid anions (AA$^-$), and N-substituted iminodiacetates (R-Ida^{2-}).[5,49,50] Comparison of columns 2 and 3 for the M(R-COO)$^+$ complexes indicates that the polarity of the solvent has an influence on the slopes: in a solvent with a lower dielectric constant (50% aqueous dioxane) the slope for the M(R-COO)$^+$ complexes is larger than the one determined for the aqueous solution; this may be indicative of a stronger shielding effect by the water molecules on the R-COO$^-$–M^{2+} interaction compared with the R-COO$^-$–H$^+$ release. However, for the higher charged interaction R-MP^{2-}–M^{2+} this influence is considerably smaller, as is indicated by the slopes determined in water, 30% dioxane–70% water, and 50% dioxane–50% water for the Cu^{2+} systems with NPhP^{2-}, PhP^{2-}, and RibMP^{2-};[4] for these three solvents the slopes are identical[51] within experimental error as well as identical with the value given in Table VIII. Some influence of the kind of donor atoms, O or N, is also expected.[52]

The slopes may also be influenced by the denticity of the ligands. For all metal ions of Table VIII the slope of the base lines increases in the order M(R-COO)$^+$ < M(R-MP) < M(AA)$^+$ < M(R-Ida), the last two ligands being bidentate and tridentate, respectively. Carboxylate ligands are usually viewed as monodentate for the type of substitution-labile metal ions considered here. What is the denticity of the phosphate group? Mostly, phoshate groups are also considered as monodentate, and both views are supported by the fact that in the solid state carboxylate[53,54] and phosphate[32] groups only rarely show the formation of strained four-membered chelate rings; instead, they act with their oxygen atoms as bridging groups between different metal ions. The order of the slopes observed for the two isocharged series, M(R-COO)$^+$ < M(AA)$^+$ and M(R-MP) < M(R-Ida) (Table VIII), may therefore be taken tentatively as an indication against a bidentate coordination of the phosphate group in solution. The four-membered chelate ring (Figure 3A) corresponding to this binding mode has repeatedly been proposed. Our suggestion does not mean that such a 4-ring may not be formed as an intermediate during a reaction but that its concentration in solution is considered as low. Instead, the slopes of the base lines appear to be more in accordance with a monodentate coordination (Figure 3B) or possibly also with a six-membered "chelate" involving a coordinated water molecule and a hydrogen bond (Figure 3C). Hence, we are tentatively suggesting that structure A of Figure 3 is of little relevance in aqueous solution while structures B and C are the important binding modes of phosphate groups to substitution-labile divalent metal ions. In addition, it should be mentioned that there are indications[11,55] that in equilibrium also various amounts of outer-sphere species may exist: their concentration seems to be appreciable with Ca^{2+}, Mg^{2+}, Mn^{2+}, Co^{2+} (cf. ref 11a), and Ni^{2+} (cf. ref 11), but negligible with Cu^{2+} and Zn^{2+}.[11a]

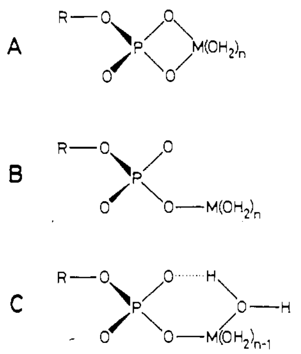

Figure 3. Metal ion binding modes in M(R-MP) complexes. Structure A is considered of little importance in aqueous solution, while structures B and C are proposed as the important binding modes (see text). In addition, there are indications[11,55] that in equilibrium also an outer-sphere binding mode occurs; i.e., the phosphate oxygens and the metal ion are separated by water molecules.

To conclude, when the available information is taken together, it must presently be assumed that M(R-MP) complexes in aqueous solution form intramolecular equilibria between several phosphate–metal ion binding modes; i.e., direct (Figure 3B, C) and indirect (outer-sphere) phosphate coordination probably occurs. Structure C of Figure 3 is obviously on the way from a sole inner-sphere coordination (Figure 3B) to a pure outer-sphere interaction. The ratios of these different phosphate–metal ion binding modes for M(R-MP) complexes are expected to depend on the metal ion considered (see above)[11a] but not on the nature of the noncoordinating residue R. These different ratios may possibly also be a reason why the Irving–Williams sequence is often not followed (see section 3) with phosphate complexes.

Acknowledgment. We thank Rita Baumbusch for technical assistance and the performance of some of the potentiometric pH titrations. A research grant from the Swiss National Science Foundation and a fellowship to S.S.M. from the Amt für Ausbildungsbeiträge des Kantons Basel-Stadt are also gratefully acknowledged.

Registry No. CMP, 63-37-6; UMP, 58-97-9; TMP, 365-07-1; RibMP, 4300-28-1.

---

(48) Sigel, H. *Chimia* **1987**, *41*, 11–26.
(49) Malini-Balakrishnan, R.; Scheller, K. H.; Häring, U. K.; Tribolet, R.; Sigel, H. *Inorg. Chem.* **1985**, *24*, 2067–2076.
(50) Martin, R. B. *Met. Ions Biol. Syst.* **1988**, *23*, 123–164.
(51) Liang, G.; Sigel, H., results to be submitted for publication.
(52) See Table 2 in: Martin, R. B. *Met. Ions Biol. Syst.* **1988**, *23*, 315–330.
(53) (a) Doedens, R. J. *Prog. Inorg. Chem.* **1976**, *21*, 209–231. (b) Cotton, F. A.; Wilkinson, G. *Advanced Inorganic Chemistry*; Wiley: New York, 1980; pp 817–818.
(54) Dubler, E.; Häring, U. K.; Scheller, K. H.; Baltzer, P.; Sigel, H. *Inorg. Chem.* **1984**, *23*, 3785–3792.
(55) Theophanides, T.; Tajmir-Riahi, H. A. *NATO ASI Ser., Ser. C* **1984**, *No. 139*, 137–152.