Acid−Base and Metal Ion-Coordinating Properties of Pyrimidine-Nucleoside 5′-Diphosphates (CDP, UDP, dTDP) and of Several Simple Diphosphate Monoesters. Establishment of Relations between Complex Stability and Diphosphate Basicity

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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′-diphosphates CDP^{3-}, UDP^{3-}, and $dTDP^{3-}$ (= NDP^{3-}) 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 diphosphate monoesters ($R\text{-}DP^{3-}$) phenyl diphosphate, methyl diphosphate, and *n*-butyl diphosphate. The acidity constants for $H_3(CDP)^{\pm}$, $H_2(UDP)^-$, $H_2(dTDP)^-$, and $H_2(R\text{-}DP)^-$ were measured also via potentiometric pH titration and various comparisons with related constants are made. By plotting log $K^{M}_{M(R\text{-}DP)}$ versus $pK^{H}_{H(R\text{-}DP)}$ for the complexes of all six diphosphates mentioned and by a careful evaluation of the deviation of the various data pairs from the straight-line correlations, the expectation is confirmed that in the $M(UDP)^-$ and $M(dTDP)^-$ complexes the metal ion is only diphosphate-coordinated. The straight-line equations, which result from the mentioned correlations, together with the pK_a value of a given monoprotonated diphosphate monoester allow now to predict the stability of the corresponding $M(R\text{-}DP)^-$ complexes. In this way, the experimentally determined stability constants for the $M(CDP)^-$ complexes are evaluated and it is concluded that the pyridine-like N3 of the cytosine residue does not participate in complex formation; i.e., the stability of the $M(CDP)^-$ complexes is also solely determined by the coordination tendency of the diphosphate residue. In all the monoprotonated M(H;NDP) and M(H;R-DP) complexes both, H^+ and M^{2+}, are bound at the diphosphate group. Only the Cu(H;CDP) complex exists in aqueous solution in the form of three different isomers: about 15% of the species have Cu^{2+} and H^+ at the diphosphate residue, in about 13% Cu^{2+} is bound at N3 and H^+ at the terminal β-phosphate group, and the dominating isomer with about 72% carries the proton at N3 and the metal ion at the diphosphate residue. Several general features of phosphate−metal ion coordination are discussed, and estimations for the stabilities of the Fe^{2+} complexes formed with mono-, di-, and triphosphate monoesters are provided.

1. Introduction

Nucleotides[2] are at the crossroads of many biological reactions,[3,4] in which they usually participate in the form of metal ion complexes.[5] Consequently, it is not surprising that a remarkable amount of thermodynamic data exists on the metal ion-binding properties of nucleotides in solution[6] and there is also significant information available on complexes in the solid state.[7,8] However, the available literature data[6−11] concern so far mostly complexes of nucleoside monophosphates and nucleoside triphosphates. The reasons for this, at least as far as solution studies are concerned, are most probably connected with the acid−base properties of the phosphate residues in the various nucleotides.

In a nucleoside triphosphate the terminal γ-phosphate group is relatively far away from the nucleobase moiety and thus, it is understandable that all the studied phosphate-monoprotonated nucleoside 5′-triphosphates[12,13] have pK_a values within a narrow range, i.e. $pK_a = 6.50 \pm 0.05$. Therefore, the stability constants of their complexes formed with a given metal ion can directly be compared and conclusions regarding their structures in solution can be drawn.[12−15] In this way it was possible, e.g., to determine the position of the following intramolecular equilibrium 1:

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(2) Abbreviations (see also Figure 1): ADP^{3-}, adenosine 5′-diphosphate; ATP^{4-}, adenosine 5′-triphosphate; $BuDP^{3-}$, *n*-butyl diphosphate; $BuMP^{2-}$, *n*-butyl monophosphate; CMP^{2-}, cytidine 5′-monophosphate; CTP^{4-}, cytidine 5′-triphosphate; Cyd, cytidine; dThd, thymidine; $dTMP^{2-}$, thymidine 5′-monophosphate; $dTTP^{4-}$, thymidine 5′-triphosphate; GDP^{3-}, guanosine 5′-diphosphate; I = ionic strength; M^{2+}, general divalent metal ion; $MeDP^{3-}$, methyl diphosphate; $MeMP^{2-}$, methyl monophosphate; N, nucleotide; NDP^{3-}, nucleoside 5′-diphosphate; NMP^{2-}, nucleoside 5′-monophosphate; NTP^{4-}, nucleoside 5′-triphosphate; $PhDP^{3-}$, phenyl diphosphate; $PhMP^{2-}$, phenyl monophosphate; $R\text{-}DP^{3-}$, general diphosphate monoester including the NDPs; $R\text{-}MP^{2-}$, general monophosphate monoester; $R\text{-}TP^{4-}$, general triphosphate monoester; UMP^{2-}, uridine 5′-monophosphate; Urd, uridine; UTP^{4-}, uridine 5′-triphosphate. Species given in the text without a charge either do not carry one or represent the species in general (i.e., independent from their protonation degree); which of the two versions applies is always clear from the context.

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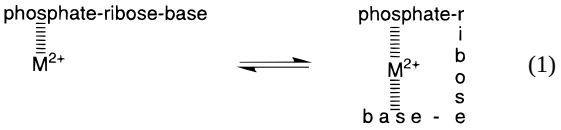

(1)

In the case of the purine-nucleoside 5′-triphosphates,[12−15] the macrochelated species indicated at the right hand side in equilibrium 1 is formed by an interaction of the phosphate-coordinated metal ion with N7 of the purine residue.

Since in the nucleoside monophosphates the nucleobase moiety is relatively close to the phosphate group, the release of the proton from the -$P(O)_2(OH)^-$ residue is affected giving thus rise to a wide range of pK_a values.[14−17] By including simple phosphate monoester ligands, like phenyl phosphate or *n*-butyl phosphate, log *K* versus pK_a straight-line plots could be established[18,19] for the alkaline earth and several divalent 3d metal ions in the pK_a range 5−7.5. The corresponding straight-line equations allow to calculate for a given pK_a value of a monoprotonated phosphate group the expected complex stability for a pure phosphate coordination.[20,21] As any further interaction has to be reflected by an increased complex stability,[22] in this way again the position of equilibrium 1 could be quantified, for example, for complexes of purine-nucleoside 5′-monophosphates.[14,15,23]

For nucleoside diphosphates intermediate properties are foreseen, i.e. an effect of the nucleobase on the acidity of the monoprotonated terminal β-phosphate group has to be expected, but it is also expected that this effect will be relatively small and thus possibly difficult to quantify. Knowing[24] that the pK_a value for the release of a proton from the terminal -$P(O)_2(OH)^-$ group of adenosine 5′-diphosphate (ADP^{3-}) is close to 6.4, we are aiming to establish the relation between complex stability and phosphate group basicity in the pK_a range of about 6−6.7 for the deprotonation of the -$P(O)_2^-$-O-$P(O)_2(OH)^-$ residue. To this end we are studying now the acid−base and metal ion-binding properties of methyl diphosphate, *n*-butyl diphosphate and phenyl diphosphate. From a very recent study of the Cu^{2+} complexes of cytidine 5′-diphosphate, uridine 5′-diphosphate and thymidine 5′-diphosphate we know[25] that the corresponding nucleobase moieties do not participate in complex formation. Consequently, we are using now the mentioned six diphosphate ligands, the structures[8,26] of which are shown in Figure 1

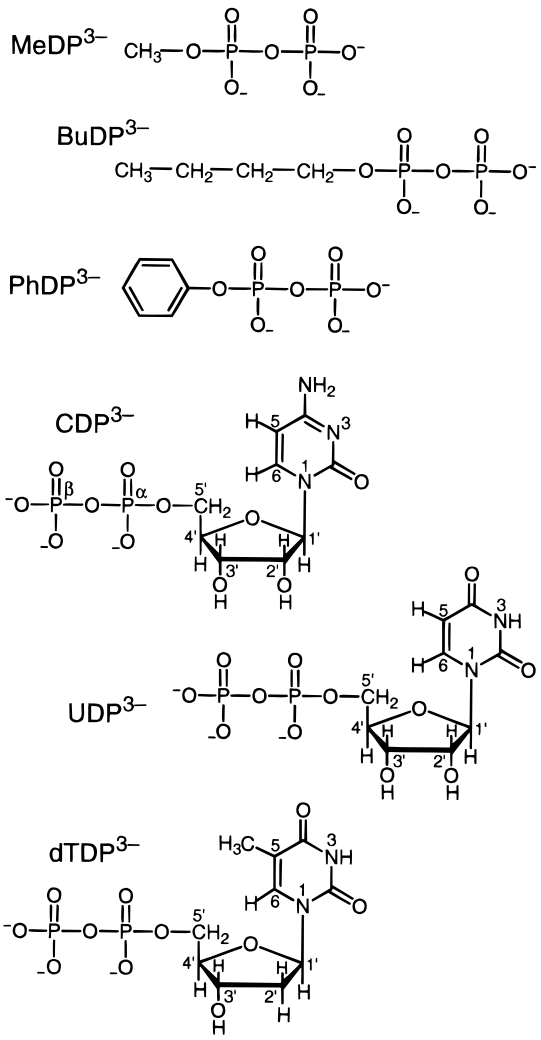

Figure 1. Chemical structures of the simple diphosphate monoesters, methyl diphosphate ($MeDP^{3-}$), *n*-butyl diphosphate ($BuDP^{3-}$), and phenyl diphosphate ($PhDP^{3-}$), as well as of the nucleoside 5′-diphosphates (NDPs), cytidine 5′-diphosphate (CDP^{3-}), uridine 5′-diphosphate (UDP^{3-}), and thymidine (= 1-(2′-deoxy-β-D-ribofuranosyl)thymine) 5′-diphosphate ($dTDP^{3-}$). The NDPs are shown in their predominant *anti* conformation.[8,26]

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together with the abbreviations employed in this study, to establish the mentioned correlation. The metal ions used in the present study are the alkaline earth ions and the divalent metal ions of the second half of the 3d series, including Zn^{2+} and Cd^{2+}. Indeed, the results presented now show that complex stability significantly depends on the basicity of the terminal phosphate group of diphosphate monoester ligands.

2. Experimental Section

2.1. Materials. The sodium salts of CDP (98%; 2.5 Na^+), UDP (99%; 3 Na^+), and dTDP (97%; 2 Na^+) were purchased from Sigma Chemical Co. (St. Louis, MO). The trisodium salts of methyl diphosphate, *n*-butyl diphosphate, and phenyl diphosphate were synthesized (by F.G.) according to published procedures.[27] The content of free, inorganic phosphate was determined in the six diphosphate monoesters via molybdate reagent;[28] it amounted to (mol% in parentheses): CDP (4.2), UDP (3.5), dTDP (1.7), MeDP (5.1), BuDP (5.4), and PhDP (6.1). The aqueous stock solutions of the six R-DPs were freshly prepared daily and their exact concentrations were newly measured each time by titrations with NaOH.

All other materials used in the experiments including the disodium salt of 1,2-diaminoethane-*N*,*N*,*N*′,*N*′-tetraacetic acid (Na_2H_2EDTA), potassium hydrogen phthalate, HNO_3, NaOH (Titrisol), and 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+} (all *pro analysi*) were from Merck AG (Darmstadt, Germany). All solutions were prepared with deionized, ultrapure (Milli-Q185 Plus; from Millipore S.A., 67120 Molsheim, France), and CO_2-free water.

The titer of the NaOH used for the titrations was established with potassium hydrogen phthalate. The exact concentrations of the stock solutions of the divalent metal ions were determined via their EDTA complexes.

2.2. Potentiometric pH Titrations. The pH titrations were carried out with a Metrohm E536 potentiograph equipped with an E655 dosimat and a 6.0202.100 (NB) or 6.0202.100 (JC) combined macro glass electrode. The buffer solutions [pH 4.00 or 4.64, 7.00, and 9.00; based on the NBS scale, now U.S. National Institute of Standards and Technology (NIST)] used for calibration were also from Metrohm AG (Herisau, Switzerland). The direct pH-meter readings were used to calculate the acidity constants; i.e., these constants are so-called practical, mixed or Brønsted constants.[29] Their negative logarithms given for aqueous solutions at $I = 0.1$ M ($NaNO_3$) and 25 °C may be converted into the corresponding concentration constants by subtracting 0.02 log unit[29] from the listed pK_a values. This conversion term contains both the junction potential of the glass electrode and the hydrogen ion activity.[29,30] No conversion is necessary for the stability constants of the metal ion complexes.

2.3. Determination of the Acidity Constants. The acidity constants $K^H_{H_2(CDP)}$ and $K^H_{H(CDP)}$ of $H_2(CDP)^-$ and $K^H_{H(R\text{-}DP)}$ of $H(MeDP)^{2-}$, $H(BuDP)^{2-}$, and $H(PhDP)^{2-}$ were determined by titrating 50 mL of aqueous 0.54 mM HNO_3 ($I = 0.1$ M, $NaNO_3$; 25 °C) in the presence and absence of 0.3 mM ligand under N_2 with 1 mL of 0.03 M NaOH. The acidity constants $K^H_{H(NDP)}$ and K^H_{NDP} of $H(UDP)^{2-}$ or $H(dTDP)^{2-}$ were measured under the same conditions but by using 2 mL of the 0.03 M NaOH in the titrations.

The calculations were carried out with IBM compatible computers with 80-486 or Pentium processors (connected with Epson Stylus 1000 printers and Hewlett-Packard 7475A plotters) by a curve-fitting procedure using a Newton−Gauss nonlinear least-squares program.

For the ligands MeDP, BuDP, and PhDP the pH range used in the calculations was defined by $pK^H_{H(R\text{-}DP)} \pm 1.2$ which corresponds to a neutralization degree between about 6 and 94% for the equilibrium $H(R\text{-}DP)^{2-}/R\text{-}DP^{3-}$; in this way the effect of the small amounts of inorganic phosphate present was further reduced. For $H_2(CDP)^-$ the pH range from 3.3 to 8.1 was employed corresponding to about 7% neutralization for the equilibrium $H_2(CDP)^-/H(CDP)^{2-}$ and about 98% neutralization for $H(CDP)^{2-}/CDP^{3-}$. For $H(UDP)^{2-}$ the pH range from 4.7 to 10.2 was taken, which corresponds to about 2% neutralization for the equilibrium $H(UDP)^{2-}/UDP^{3-}$ and about 84% neutralization for $UDP^{3-}/(UDP-H)^{4-}$; for $H(dTDP)^{2-}$ the pH range from 4.7 to 10.4 was used corresponding to about 2% neutralization for the equilibrium $H(dTDP)^{2-}/dTDP^{3-}$ and about 75% neutralization for the equilibrium $dTDP^{3-}/(dTDP-H)^{4-}$. In the case of the NDPs, the effect of the small amounts of inorganic phosphate present was largely eliminated by calculating the total ligand concentration via the NaOH consumption due to the acid−base reaction of the nucleobase residue.

Several experiments were also made by titrating 10 mL of aqueous 20.5 mM HNO_3 ($I = 0.1$ M, $NaNO_3$; 25 °C) in the presence of 3.0 or 4.3 mM ligand under N_2 with 2.1 mL of 0.1 M NaOH. In this way the pK_a values between about 1 to 1.6, i.e. $K^H_{H_3(CDP)}$ for $H_3(CDP)^{\pm}$ and $K^H_{H_2(R\text{-}DP)}$ for all the other $H_2(R\text{-}DP)^-$ species, could be obtained. The evaluation of the titration curves started with pH 1.9; this corresponds in the worst case, i.e. for $H_3(CDP)^{\pm}$, already to a neutralization degree of about 89% and consequently these results carry a relatively large error (see Section 3.1).

The final results are the averages of at least 4 independent pairs of titrations in the case of the pK_a values between 1 and 1.6. All the other acidity constants are the results of at least 20 independent pairs of titrations.

2.4. Determination of the Stability Constants. The stability constants $K^M_{M(H;R\text{-}DP)}$ and $K^M_{M(R\text{-}DP)}$, where $M^{2+} = Mg^{2+}$, Ca^{2+}, Sr^{2+}, Ba^{2+}, Mn^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, or Cd^{2+}, were determined for all the ligands under the same conditions as used for the acidity constants ($K^H_{H_2(CDP)}$ and) $K^H_{H(R\text{-}DP)}$, i.e. by titrating 50 mL of aqueous 0.54 mM HNO_3 in the presence and absence of 0.3 mM ligand under N_2 with 1 mL of 0.03 M NaOH, but $NaNO_3$ was partly replaced by $M(NO_3)_2$ ($I = 0.1$ M, $NaNO_3$; 25 °C). The M^{2+}/ligand ratios used in the experiments were 1:1 and 2:1 for all systems. In addition, in the Sr^{2+} and Ba^{2+} (and in some instances also in the Ca^{2+}) systems M^{2+}/ligand ratios of 5:1, 10:1, and 15:1 were also employed because of the low stability of the complexes. It should be emphasized that the results were independent of the excess of M^{2+} used. No constants could be determined for the Ba^{2+}/MeDP system due to the formation of a precipitate and there was not enough substance for measurements of MeDP with Zn^{2+} or Cu^{2+}.

The stability constants $K^M_{M(H;R\text{-}DP)}$ (see also below) and $K^M_{M(R\text{-}DP)}$ were calculated for each pair of titrations with the computers mentioned in Section 2.3 and a curve-fitting procedure by taking into account the species H^+, $H_2(R\text{-}DP)^-$, $H(R\text{-}DP)^{2-}$, $R\text{-}DP^{3-}$, M^{2+}, M(H;R-DP), and $M(R\text{-}DP)^-$.[31] The data were collected every 0.1 pH unit from the lowest pH which could be reached in the experiment (e.g., with Cu^{2+}) or from a formation degree of about 5% for $M(R\text{-}DP)^-$ to the beginning of the hydrolysis of $M(aq)^{2+}$ (e.g., with Cu^{2+} or Zn^{2+}), which was evident from the titrations without ligand, or to the beginning of the formation of $M(UDP-H)^{2-}$ or $M(dTDP-H)^{2-}$ complexes (e.g., with Ca^{2+}), or to a formation degree of about 85% for $M(R\text{-}DP)^-$.

The stability constants $K^M_{M(H;R\text{-}DP)}$ could only be determined for the M^{2+}/CDP systems but the formation degree of M(H;CDP) was small and therefore the error of the corresponding constants is large (vide infra, Section 3.3). For all other systems only estimates for the stabilities of M(H;R-DP) were possible; these are in part based on our previous experience with related ligands.[12,15] The formation degree of the M(H;-R-DP) species was usually ≤5% based on the total ligand concentration. The also estimated error limit for the estimated log $K^M_{M(H;R\text{-}DP)}$ values is ±0.3 log unit. Since the size of the stability constant $K^M_{M(H;R\text{-}DP)}$ affects in the calculations somewhat the result obtained for $K^M_{M(R\text{-}DP)}$ the following information, which is representative for all systems, is given for some M^{2+}/UDP systems. After the symbol of the metal ion first the estimate for the log stability constant, log $K^M_{M(H;UDP)}$, with its estimated error is given, the next number gives the effect in log units

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on log $K^{M}_{M(UDP)}$ if the *lower* limit of log $K^{M}_{M(H;UDP)}$ is used in the calculations, and the final number gives the same effect if the value resulting from the *upper* error limit is employed: Mg^{2+} (log $K^{Mg}_{Mg(H;UDP)} = 1.6 \pm 0.3/-0.01/+0.02$), Co^{2+} (log $K^{Co}_{Co(H;UDP)} = (2.0 \pm 0.3)/-0.01/+0.03$), Cu^{2+} (log $K^{Cu}_{Cu(H;UDP)} = (2.4 \pm 0.3)/-0.02/+0.03$), and Cd^{2+} (log $K^{Cd}_{Cd(H;UDP)} = (2.5 \pm 0.3)/-0.03/+0.05$); in the last case the effect is greatest. Overall, it is evident that the effect of $K^{M}_{M(H;R-DP)}$ on $K^{M}_{M(R-DP)}$ is relatively small, and, more importantly, an error in $K^{M}_{M(H;R-DP)}$ would affect *all* stability constants $K^{M}_{M(R-DP)}$ for a given metal ion to the same extent and would therefore give rise only to a small parallel shift of the straight-reference lines calculated in Section 3.6.

The final stability constants given in the tables are the results from the averages of at least 7 independent pairs of titrations carried out for each of the systems studied.

3. Results and Discussion

All potentiometric pH titrations ($I = 0.1$ M, $NaNO_3$; 25 °C), the results of which are summarized below, were carried out at ligand concentrations of 0.3 mM. Under these conditions self-stacking of the NDPs is negligible;[32] i.e., the results presented refer definitely to the monomeric species.

3.1. Acidity Constants of the Protonated Ligands. The deprotonated nucleotide CDP^{3-} can accept in total four protons to give the acid $H_4(CDP)^{+}$. First one of the two primary protons of the diphosphate residue is released; its pK_a is very low (<1). The next proton is the second primary proton from the diphosphate group and its acidity was measured (eq 1); next, deprotonation of the $(N3)H^{+}$ site (see Figure 1) occurs (eq 2; where $H_2(CDP)^{-} = H_2(R\text{-}DP)^{-}$) which is followed by the release of the secondary proton from the terminal β-phosphate group (eq 3).[18]

$$H_3(CDP)^{\pm} \rightleftharpoons H_2(CDP)^{-} + H^{+} \quad (1a)$$

$$K^{H}_{H_3(CDP)} = [H_2(CDP)^{-}][H^{+}]/[H_3(CDP)^{\pm}] \quad (1b)$$

$$H_2(R\text{-}DP)^{-} \rightleftharpoons H(R\text{-}DP)^{2-} + H^{+} \quad (2a)$$

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

$$H(R\text{-}DP)^{2-} \rightleftharpoons R\text{-}DP^{3-} + H^{+} \quad (3a)$$

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

The simple twofold-protonated diphosphate monoesters, $H_2(R\text{-}DP)^{-}$, as well as $H_2(UDP)^{-}$ and $H_2(dTDP)^{-}$ (see Figure 1) carry one primary proton either at the α- or the β-phosphate group (here overlapping equilibria must be assumed) and one secondary proton at the terminal β-phosphate; hence, these deprotonation reactions can also be described by equilibria 2a and 3a. However, UDP^{3-} and $dTDP^{3-}$ can release one more proton from the neutral (N3)H site (Figure 1) of their nucleobase residue; hence, in addition equilibrium 4a needs to be considered here.

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

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

For reasons of clarity it is emphasized again that the species $H_2(R\text{-}DP)^{-}$ in the case of CDP carries a proton at N3 and at the terminal β-phosphate group, whereas in all the other twofold protonated diphosphate ligands considered here, the two protons in $H_2(R\text{-}DP)^{-}$ reside at the diphosphate moiety.

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Table 1. Negative Logarithms of the Acidity Constants (Eqs 1−4) of the Protonated Diphosphate Ligands Considered in This Study and as Determined by Potentiometric pH Titrations in Aqueous Solutions at 25 °C and $I = 0.1$ M ($NaNO_3$)[a]

	pK_a for the sites			
acid	$-P_2(O)_4(OH)_2^{-}$	$(N3)H^{+}$	$-P(O)_2^{-}$-O-$P(O)_2(OH)^{-}$	(N3)H
$H_2(PhDP)^{-}$	1.32 ± 0.18		6.32 ± 0.02[c]	
$H_2(MeDP)^{-}$	1.62 ± 0.09		6.37 ± 0.02	
$H_2(UDP)^{-}$	1.26 ± 0.20		6.38 ± 0.02	9.47 ± 0.02
$H_3(CDP)^{\pm}$	1.0 ± 0.2	4.45 ± 0.02	6.39 ± 0.02	
$H_2(dTDP)^{-}$	1.3[b]		6.44 ± 0.01	9.93 ± 0.02
$H_2(BuDP)^{-}$	1.34 ± 0.16		6.65 ± 0.02[c]	

[a] So-called practical (or mixed) constants[29] are listed; see also Section 2.2. 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. [b] This value is an estimate based on the result measured for UDP. [c] See ref 33.

The measured acidity constants are summarized in Table 1. Previously, only the acidity constants of $H_2(CDP)^{-}$ and $H(CDP)^{2-}$ as well as of $H(UDP)^{2-}$ and UDP^{3-} had been determined;[6,9−11] those values,[10] i.e. p$K^{H}_{H_2(CDP)} = 4.45 \pm 0.05$, p$K^{H}_{H(CDP)} = 6.3 \pm 0.1$, p$K^{H}_{H(UDP)} = 6.4 \pm 0.1$, and p$K^{H}_{UDP} = 9.4$,[6] which have been measured under similar conditions as the present ones are in excellent agreement with our results. All the other constants[33] given in Table 1 have been determined for the first time; their order is as expected on the basis of previously obtained values for NMPs and NTPs.[13−15]

3.2. Comparison of Some Ligand Acid−Base Properties. There are many comparisons possible with the constants given in Table 1 and previously published data of mono- and triphosphate ligands, and a few follow here. The basicity enhancing effect of a second phosphate group on the release of the final primary proton, which is most probably distributed between the α- and β-phosphate groups in the case of the R-DPs, is evident from the data summarized in equations 5−7:

$$\Delta pK_{a/5} = pK^{H}_{H_2(MeDP)} - pK^{H}_{H_2(MeMP)} = (1.62 \pm 0.09) - (1.1 \pm 0.2/\text{from ref } 34) = 0.52 \pm 0.22 \quad (5)$$

$$\Delta pK_{a/6} = pK^{H}_{H_2(UDP)} - pK^{H}_{H_2(UMP)} = (1.26 \pm 0.20) - (0.7 \pm 0.3/\text{from ref } 18) = 0.56 \pm 0.36 \quad (6)$$

$$\Delta pK_{a/7} = pK^{H}_{H_3(CDP)} - pK^{H}_{H_3(CMP)} = (1.0 \pm 0.2) - (0.4 \pm 0.5/\text{from ref } 18) = 0.60 \pm 0.54 \quad (7)$$

Despite the large error limits (note, 3σ are given) it is evident that the effect of the second phosphate group is rather constant and independent from the residue R; the latter is especially clearly seen from eq 7 where the nucleobase residue of the two compared cytidine nucleotides carries a positive charge due to

(33) The acidity constants p$K^{H}_{H(PhDP)}$ and p$K^{H}_{H(BuDP)}$ as well as the stability constants log $K^{Cu}_{Cu(PhDP)}$ and log $K^{Cu}_{Cu(BuDP)}$ are the same as given very recently in the context of mixed ligand complex formation: Sajadi, S. A. A.; Song, B.; Gregáň, F.; Sigel, H. *Bull. Chem. Soc. Ethiop.* **1997**, *11*, 121−130.

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

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

NDP^{3-}	M^{2+}	log $K^{M}_{M(H;NDP)}$ [b]	log $K^{M}_{M(NDP)}$	p$K^{H}_{M(H;NDP)}$
CDP^{3-}	Mg^{2+}	1.6 ± 0.3	3.25 ± 0.03	4.74 ± 0.30
	Ca^{2+}	1.5 ± 0.3	2.87 ± 0.06	5.02 ± 0.31
	Sr^{2+}	1.2 ± 0.3	2.33 ± 0.04	5.26 ± 0.30
	Ba^{2+}	1.1 ± 0.3	2.27 ± 0.04	5.22 ± 0.30
	Mn^{2+}	2.30 ± 0.25	4.09 ± 0.04	4.60 ± 0.25
	Co^{2+}	2.1 ± 0.3	3.65 ± 0.06	4.84 ± 0.31
	Ni^{2+}	2.2 ± 0.3	3.45 ± 0.05	5.14 ± 0.30
	Cu^{2+}	3.23 ± 0.13	5.29 ± 0.08	4.33 ± 0.15
	Zn^{2+}	2.4 ± 0.3	4.10 ± 0.06	4.69 ± 0.31
	Cd^{2+}	2.50 ± 0.18	4.23 ± 0.03	4.66 ± 0.18
UDP^{3-}	Mg^{2+}	1.6	3.32 ± 0.05	4.65 ± 0.3
	Ca^{2+}	1.5	2.90 ± 0.05	5.0 ± 0.3
	Sr^{2+}	1.2	2.38 ± 0.05	5.2 ± 0.3
	Ba^{2+}	1.1	2.29 ± 0.04	5.2 ± 0.3
	Mn^{2+}	2.3	4.07 ± 0.05	4.6 ± 0.3
	Co^{2+}	2.0	3.68 ± 0.04	4.7 ± 0.3
	Ni^{2+}	2.2	3.50 ± 0.05	5.1 ± 0.3
	Cu^{2+}	2.4	5.21 ± 0.07	3.55 ± 0.3
	Zn^{2+}	2.3	4.07 ± 0.05	4.6 ± 0.3
	Cd^{2+}	2.5	4.22 ± 0.05	4.65 ± 0.3

[a] 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. The error limits of the derived data, in the present case for column 5, were calculated according to the error propagation after Gauss. [b] The values given for log $K^{M}_{M(H;UDP)}$ are estimates; their error limits are estimated as ±0.3 log unit. These same stability constants of the monoprotonated M(H;R-DP) complexes (eq 10) have also been used (as fixed values) in the calculations (Section 2.4) for the stability constants of all the other M(R-DP)$^-$ complexes (see Table 3 in Section 3.5).

the protonated N3 site (see Section 3.1). Similar results are obtained if diphosphates are compared with triphosphates.

$$\Delta pK_{a/8} = pK^{H}_{H_2(UTP)} - pK^{H}_{H_2(UDP)} = (2.0 \pm 0.1/\text{from ref 35}) - (1.26 \pm 0.20) = 0.74 \pm 0.22 \quad (8)$$

$$\Delta pK_{a/9} = pK^{H}_{H_3(CTP)} - pK^{H}_{H_3(CDP)} \simeq pK^{H}_{H_3(ATP)} - pK^{H}_{H_3(CDP)} = (1.7 \pm 0.1/\text{from refs 36 and 37}) - (1.0 \pm 0.2) = 0.7 \pm 0.2 \quad (9)$$

It is evident that the results of equations 5−9 provide confidence for extrapolating values needed for systems which have not yet been measured.

The effect of the residue R on the release of the secondary proton from the terminal β-phosphate group is relatively small in the H(R-DP)$^{2-}$ series (see Table 1). It spans only the narrow pK_a range of 6.3 − 6.65 by going from H(PhDP)$^{2-}$ to H(BuDP)$^{2-}$ whereas in the monophosphates the corresponding range[18] extends from $pK^{H}_{H(PhMP)} = 5.85 \pm 0.01$ to $pK^{H}_{H(BuMP)} = 6.72 \pm 0.02$.

The effects of the phosphate residues on the acid−base properties of the nucleobases are also interesting. The increasing influence of the negative charge of the phosphate residues (from 0 via −1 and −2 to −3) on the positively charged (N3)H$^+$ sites is evident for cytidine and its nucleotides: $pK^{H}_{H(Cyd)} = 4.14 \pm 0.02$ (cf. ref 38) < $pK^{H}_{H_2(CMP)} = 4.33 \pm 0.04$ (cf. ref 18) < $pK^{H}_{H_2(CDP)} = 4.45 \pm 0.02$ (Table 1) < $pK^{H}_{H_2(CTP)} = 4.55 \pm 0.03$ (cf. ref 12); the differences in this series are $0.19 \pm 0.04 > 0.12 \pm 0.04 \gtrsim 0.10 \pm 0.04$. The effect on the deprotonations of the neutral (N3)H sites in the uridine and thymidine residues is only pronounced from the nucleoside to the nucleoside 5′-monophosphate: $pK^{H}_{Urd} = 9.19$ (cf. ref 14a) < $pK^{H}_{UMP} = 9.45 \pm 0.02$ (cf. ref 18) ≃ $pK^{H}_{UDP} = 9.47 \pm 0.02$ (Table 1) ≃ $pK^{H}_{UTP} = 9.5$ (ref 39)/9.6 (ref 39)/9.70 (cf. ref 40) and $pK^{H}_{dThd} = 9.69$ (cf. ref 14a) < $pK^{H}_{dTMP} = 9.90 \pm 0.03$ (cf. ref 18) ≃ $pK^{H}_{dTDP} = 9.93 \pm 0.02$ (Table 1) ≃ $pK^{H}_{dTTP} = 9.8$(ref 39)/9.89 ± 0.03(ref 40)/10.1 (cf. ref 39); the charges on the phosphate residues vary in these series from 0 via −2 and −3 to −4.

3.3. Stability Constants of M^{2+} Complexes of CDP and UDP. The experimental data of the potentiometric pH titrations of the two mentioned M^{2+}/NDP systems, where M^{2+} = Mg^{2+}, Ca^{2+}, Sr^{2+}, Ba^{2+}, Mn^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, or Cd^{2+}, are completely described by equilibria 2, 3, 10, and 11 (where for

$$M^{2+} + H(R\text{-}DP)^{2-} \rightleftharpoons M(H;R\text{-}DP) \quad (10a)$$

$$K^{M}_{M(H;R\text{-}DP)} = [M(H;R\text{-}DP)]/([M^{2+}][H(R\text{-}DP)^{2-}]) \quad (10b)$$

$$M^{2+} + R\text{-}DP^{3-} \rightleftharpoons M(R\text{-}DP)^{-} \quad (11a)$$

$$K^{M}_{M(R\text{-}DP)} = [M(R\text{-}DP)^{-}]/([M^{2+}][R\text{-}DP^{3-}]) \quad (11b)$$

the present R-DP = CDP or UDP), if the evaluation is not carried into the pH range where either formation of hydroxo complexes or of the N3-deprotonated M(UDP−H)$^{2-}$ species occurs (see Section 2.4). The acidity constant of the connected equilibrium 12 may be calculated with equation 13.

$$M(H;R\text{-}DP) \rightleftharpoons M(R\text{-}DP)^{-} + H^{+} \quad (12a)$$

$$K^{H}_{M(H;R\text{-}DP)} = [M(R\text{-}DP)^{-}][H^{+}]/[M(H;R\text{-}DP)] \quad (12b)$$

$$pK^{H}_{M(H;R\text{-}DP)} = pK^{H}_{H(R\text{-}DP)} + \log K^{M}_{M(H;R\text{-}DP)} - \log K^{M}_{M(R\text{-}DP)} \quad (13)$$

The results obtained for equilibria 10a, 11a, and 12a concerning the M^{2+} complexes of CDP and UDP are listed in columns 3, 4, and 5 of Table 2, respectively.

For UDP^{3-} only stability constants for the Mg^{2+} complexes have been published;[6,9−11] these previous results[41] are in fair agreement with the present ones, especially if one considers the different experimental conditions and methods employed. For the M^{2+}/CDP systems some more data are available[6,9−11] and a set of values for the complexes of Mn^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, and Zn^{2+} exists,[42] but they differ from the present ones

(35) Tribolet, R.; Malini-Balakrishnan, R.; Sigel, H. *J. Chem. Soc., Dalton Trans.* **1985**, 2291−2303.
(36) Tribolet, R.; Sigel, H. *Eur. J. Biochem.* **1988**, *170*, 617−626.
(37) In a first approximation one may assume that the effect of the positively charged (N1)H$^+$ site of the adenine residue on the phosphate groups corresponds to that of the (N3)H$^+$ site of the cytosine residue. This assumption is confirmed by the identity of the values measured for $pK^{H}_{H_3(CDP)}$ and $pK^{H}_{H_3(ADP)}$ (H. Sigel et al., results to be published).
(38) Kinjo, Y.; Ji, L.-n.; Corfù, N. A.; Sigel, H. *Inorg. Chem.* **1992**, *31*, 5588−5596.
(39) Sigel, H. *Eur. J. Biochem.* **1968**, *3*, 530−537.
(40) Sigel, H. *J. Am. Chem. Soc.* **1975**, *97*, 3209−3214.
(41) (a) Walaas, E. *Acta Chem. Scand.* **1958**, *12*, 528−536. (b) Sari, J. C.; Belaich, J. P. *J. Am. Chem. Soc.* **1973**, *95*, 7491−7496.
(42) Manorik, P. A.; Davidenko, N. K.; Aleksyuk, N. P.; Lopatina, E. I. *Russ. J. Inorg. Chem.* **1984**, *29*, 424−427; *Zh. Neorg. Khim.* **1984**, *29*, 735−740.

in part by ±0.3 log unit. This contrasts with the results published by another group[43] for the CDP complexes with Mg^{2+} and Ni^{2+}, which are within the error limits identical with the values listed in Table 2.

3.4. Considerations on the Monoprotonated M(H;NDP) Complexes. Isomeric Equilibria. In the M(H;UDP) complexes (Table 2) both, H^+ and M^{2+}, must be bound at the diphosphate residue because the nucleobase moiety has no binding site to offer as long as (N3)H is not deprotonated (see Figure 1). Hence, there is only a single complex species present which may be formulated as UDP·M·H.

This situation differs in the case of the M(H;CDP) complexes because the N3 site of cytidine is known to be able to coordinate metal ions[38] and of course, also protons (Table 1). However, comparison of the stability constants $K^{M}_{M(H;NDP)}$ for the M(H;CDP) and M(H;UDP) complexes in column 3 of Table 2 reveals that in all instances, except for the Cu^{2+} complexes, the stability constants are identical within their error limits. Consequently, one has to conclude that in all M(H;CDP) complexes, except the one with Cu^{2+}, the metal ions and protons are also overwhelmingly located at the diphosphate residue. This conclusion also agrees with the acidity constants, $K^{H}_{M(H;NDP)}$, which are identical within their error limits for the M(H;UDP) and M(H;CDP) complexes for a given metal ion, the single exception being again the Cu^{2+} complexes.

Evidently, for Cu(H;CDP) and Cu(H;UDP) the stability difference, $\log \Delta = \log K^{M}_{M(H;CDP)} - \log K^{M}_{M(H;UDP)} = (3.23 \pm 0.13) - (2.4 \pm 0.3) = 0.83 \pm 0.33$, is significant. This means, in this case isomeric equilibria need to be considered.[12] In principle, the following possible binding sites for H^+ and Cu^{2+} are available: The proton and the metal ion may both be at the diphosphate group to give the CDP·Cu·H isomer; however, the metal ion could also be at the diphosphate residue and the proton at N3 of the nucleobase, leading to H·CDP·Cu. The formation of this latter isomer appears immediately as appealing because the acidity constant $pK^{H}_{Cu(H;CDP)} = 4.33 \pm 0.15$ of Cu(H;CDP) is somewhat smaller than the acidity constant of $H_2(CDP)^-$, $pK^{H}_{H_2(CDP)} = 4.45 \pm 0.02$, and upon complex formation some acidification is expected. A further isomer one may think of, namely Cu·CDP·H, carries the metal ion at N3 of the nucleobase residue and the proton at the terminal β-phosphate group. Macrochelates, C·Cu·DP·H, do not need to be considered because there is no indication for macrochelate formation, even in the deprotonated $Cu(CDP)^-$ species (see Section 3.7).

Hence, by taking into account the three mentioned isomers of Cu(H;CDP), which have a certain likelihood to exist, eq 10b may be rewritten:

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

$$= \frac{[CDP\cdot Cu\cdot H]}{[Cu^{2+}][H(CDP)^{2-}]} + \frac{[H\cdot CDP\cdot Cu]}{[Cu^{2+}][H(CDP)^{2-}]} + \frac{[Cu\cdot CDP\cdot H]}{[Cu^{2+}][H(CDP)^{2-}]} \quad (14b)$$

$$= k^{Cu}_{CDP\cdot Cu\cdot H} + k^{Cu}_{H\cdot CDP\cdot Cu} + k^{Cu}_{Cu\cdot CDP\cdot H} \quad (14c)$$

The overall stability constant $K^{Cu}_{Cu(H;CDP)}$ is known (Table 2) and for the microconstant $k^{Cu}_{CDP\cdot Cu\cdot H}$ one may assume that it is equal to $K^{Cu}_{Cu(H;UDP)}$ because the Cu(H;UDP) complexes exist in the form UDP·Cu·H which corresponds to CDP·Cu·H; clearly, in both species the nucleobase residue does not play a role. Hence, we are left with two unknown microconstants in equation 14c, but for $k^{Cu}_{Cu\cdot CDP\cdot H}$, which represents the isomer with Cu^{2+} at N3 and H^+ at the β-phosphate group, a value may be estimated because the stability of the $Cu(cytidine)^{2+}$ complex is known:[38] $\log K^{Cu}_{Cu(Cyd)} = 1.56 \pm 0.06$. Of course, this value needs to be corrected[44] for (i) the different basicities of N3 in $H(CDP)^{2-}$ and cytidine, and (ii) the charge effect which the $-P(O)_2^-$-O-$P(O)_2(OH)^-$ group exerts on Cu^{2+} at the N3 site; this then gives $\log k^{Cu}_{Cu\cdot CDP\cdot H} = 2.35 \pm 0.21$.[44] Consequently, we can now calculate $k^{Cu}_{H\cdot CDP\cdot Cu}$ according to eq 15, which follows from eq 14c:

$$k^{Cu}_{H\cdot CDP\cdot Cu} = K^{Cu}_{Cu(H;CDP)} - k^{Cu}_{CDP\cdot Cu\cdot H} - k^{Cu}_{Cu\cdot CDP\cdot H} \quad (15a)$$

$$= 10^{3.23\pm0.13} - 10^{2.4\pm0.3} - 10^{2.35\pm0.21} \quad (15b)$$

$$= 10^{3.09\pm0.19} \quad (15c)$$

From eqs 14a and 14b follows then eq 16:

$$[Cu(H;CDP)] = [CDP\cdot Cu\cdot H] + [H\cdot CDP\cdot Cu] + [Cu\cdot CDP\cdot H] \quad (16a)$$

$$10^{3.23\pm0.13} = 10^{2.4\pm0.3} + 10^{3.09\pm0.19} + 10^{2.35\pm0.21} \quad (16b)$$

$$1 = 10^{-0.83\pm0.33} + 10^{-0.14\pm0.23} + 10^{-0.88\pm0.25} \quad (16c)$$

$$1 = (0.148 \pm 0.112) + (0.724 \pm 0.384) + (0.132 \pm 0.076) \quad (16d)$$

$$100\% = (15 \pm 11)\% + (72 \pm 14\%;\ \text{cf. ref 45}) + (13 \pm 8)\% \quad (16e)$$

Consequently, from the Cu(H;CDP) species present in solution about 15% exist as CDP·Cu·H with both, Cu^{2+} and H^+, at the diphosphate residue, and another approximately 13% are present as Cu·CDP·H, where Cu^{2+} is at N3 and the proton at the β-phosphate group; however, despite the relatively large error limits it is clear that the dominating isomer with about 72% is H·CDP·Cu, i.e. the species which carries the proton at N3 and the metal ion at the diphosphate residue.

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

(44) (a) This estimate is made in the following way: The stability constant of $Cu(cytidine)^{2+}$, $\log K^{Cu}_{Cu(Cyd)} = 1.56 \pm 0.06$ (cf. ref 38), is corrected for the different basicities of the N3 site in $H(CDP)^{2-}$ and cytidine [i.e., $\Delta pK_a = pK^{H}_{H_2(CDP)} - pK^{H}_{H(Cyd)} = (4.45 \pm 0.02) - (4.24 \pm 0.02;$ cf. ref 38) $= 0.21 \pm 0.03$] by applying the slope ($m = 0.42$) of the regression lines for $\log K$ versus pK_a plots[44b] for N3- or pyridine-type ligands. This gives the "corrected" value $(1.56 \pm 0.06) + (0.09 \pm 0.03) = 1.65 \pm 0.07$, which needs to be further corrected for the charge effect that the $-P(O)_2^-$-O-$P(O)_2(OH)^-$ group exerts on Cu^{2+} at the N3 site [the effect of the same group on $(N3)H^+$ is taken care of by ΔpK_a] and which we estimate as being 0.7 ± 0.2 log unit. This estimate is based on our experience with the effect of 2+/1− or 2−/1+ charges where the distances are of a comparable size and which amounts to 0.40 ± 0.15 log units.[44c] Hence, $\log k^{Cu}_{Cu\cdot CDP\cdot H} = (1.65 \pm 0.07) + (0.7 \pm 0.2) = 2.35 \pm 0.21$. (b) Sigel, H.; Corfù, N. A.; Ji, L.-n.; Martin, R. B. *Comments Inorg. Chem.* **1992**, *13*, 35−59. (c) Bastian, M.; Sigel, H. *J. Coord. Chem.* **1991**, *23*, 137−154.

(45) The error limit for this value can either be taken from the corresponding term in eq 16d (±38%) or it can be calculated from the error limits of the two other terms in eq 16e according to the error propagation after Gauss which gives the more reasonable value of ±14%.

Table 3. Logarithms of the Stability Constants of the M(R-DP)$^{-}$ Complexes (Eq 11), where R-DP^{3-} = PhDP^{3-}, MeDP^{3-}, UDP^{3-}, CDP^{3-}, dTDP^{3-}, or BuDP^{3-} (see Figure 1), As Determined by Potentiometric pH Titrations in Aqueous Solution at 25 °C and I = 0.1 M ($NaNO_3$)[a]

	log $K^{M}_{M(R\text{-}DP)}$ for R-DP^{3-}					
M^{2+}	PhDP^{3-}	MeDP^{3-}	UDP^{3-}	CDP^{3-}	dTDP^{3-}	BuDP^{3-}
Mg^{2+}	3.24 ± 0.04	3.29 ± 0.10	3.32 ± 0.05	3.25 ± 0.03	3.34 ± 0.06	3.41 ± 0.06
Ca^{2+}	2.90 ± 0.03	2.87 ± 0.08	2.90 ± 0.05	2.87 ± 0.06	2.94 ± 0.04	3.00 ± 0.05
Sr^{2+}	2.38 ± 0.03	2.33 ± 0.10	2.38 ± 0.05	2.33 ± 0.04	2.40 ± 0.05	2.42 ± 0.05
Ba^{2+}	2.31 ± 0.03	*b*	2.29 ± 0.04	2.27 ± 0.04	2.33 ± 0.03	2.38 ± 0.06
Mn^{2+}	4.08 ± 0.03	4.10 ± 0.10	4.07 ± 0.05	4.09 ± 0.04	4.18 ± 0.03	4.32 ± 0.04
Co^{2+}	3.68 ± 0.05	3.73 ± 0.11	3.68 ± 0.04	3.65 ± 0.06	3.77 ± 0.04	3.89 ± 0.06
Ni^{2+}	3.51 ± 0.07	3.59 ± 0.13	3.50 ± 0.05	3.45 ± 0.05	3.57 ± 0.05	3.73 ± 0.08
Cu^{2+}	5.17 ± 0.05[c]	*b*	5.21 ± 0.07	5.29 ± 0.08	5.34 ± 0.05	5.59 ± 0.04[c]
Zn^{2+}	4.06 ± 0.04	*b*	4.07 ± 0.05	4.10 ± 0.06	4.15 ± 0.04	4.40 ± 0.06
Cd^{2+}	4.21 ± 0.03	4.27 ± 0.09	4.22 ± 0.05	4.23 ± 0.03	4.30 ± 0.03	4.51 ± 0.06

[a] The data of columns 4 (UDP^{3-}) and 5 (CDP^{3-}) are taken from Table 2. See also footnotes *a* and *b* of Table 2. [b] See Section 2.4. [c] See ref 33.

3.5. Stability Constants of Further M(R-DP)$^{-}$ Complexes. The stabilities of the complexes formed with dTDP^{3-} and the three simple diphosphate monoesters seen in Figure 1 were also calculated by taking into account equilibria 2, 3, 10, and 11. Evidently, eq 2 is of no relevance (see the p$K^{H}_{H_2(R\text{-}DP)}$ values given in column 2 of Table 1) and for the consideration of eq 10 the values estimated for the M(H;UDP) complexes (Table 2) were also used.

The results are summarized in Table 3, where the data for the M(CDP)$^{-}$ and M(UDP)$^{-}$ complexes are given again to provide a coherent picture. The order of the ligands in Table 3 (from left to right) follows the order of the p$K^{H}_{H(R\text{-}DP)}$ values given in column 4 of Table 1. Indeed, there is a general trend for an increasing complex stability with increasing ligand basicity.

To the best of our knowledge there are no stability constants available in the literature[6,9−11] for any of the M(dTDP)$^{-}$ complexes and also not for the M^{2+} complexes of the diphosphate monoesters PhDP^{3-}, MeDP^{3-}, or BuDP^{3-}.

All the stability constants of Table 3 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[46] is confirmed that the stabilities of phosphate−metal ion complexes often do not strictly follow the Irving−Williams[47] sequence. The observed stability order for all the diphosphate ligands of Table 3, in accordance with that for phosphate monoesters[18] and pyrimidine-nucleoside 5′-triphosphates,[13] is $Ba^{2+} < Sr^{2+} < Ca^{2+} < Mg^{2+} < Ni^{2+} < Co^{2+} < Mn^{2+} < Cu^{2+} > Zn^{2+} < Cd^{2+}$.

However, the most important question is now, is there a correlation between complex stability and diphosphate group basicity? In other words, is there a linear relationship between log $K^{M}_{M(R\text{-}DP)}$ and p$K^{H}_{H(R\text{-}DP)}$, as it is known[22] for other series of structurally related ligands? Indeed, this is the case as can be seen in Figure 2, where the data pairs for the systems of several metal ions are plotted.

Figure 2 reveals a number of remarkable points which deserve emphasis: (i) It is evident that the residue R in the R-DP^{3-} ligands only has a slight effect on the basicity of the terminal β-phosphate group and therefore the six ligands of Figure 1 span only the narrow pK_a range of about 6.3 to 6.7. (ii) In fact, only the three data points for PhDP^{3-}, dTDP^{3-}, and BuDP^{3-} and their M^{2+} complexes are about equally spaced in the mentioned pK_a range. The data points for the complexes of MeDP^{3-}, UDP^{3-}, and CDP^{3-} cluster together at pK_a about 6.38 because the corresponding p$K^{H}_{H(R\text{-}DP)}$ values (see Table 1, column 4) are identical within the error limits. However, (iii) the accumulation of data points at pK_a 6.4 is only an apparent handicap because it means that at this pK_a the position of the straight lines is especially well defined and this is important

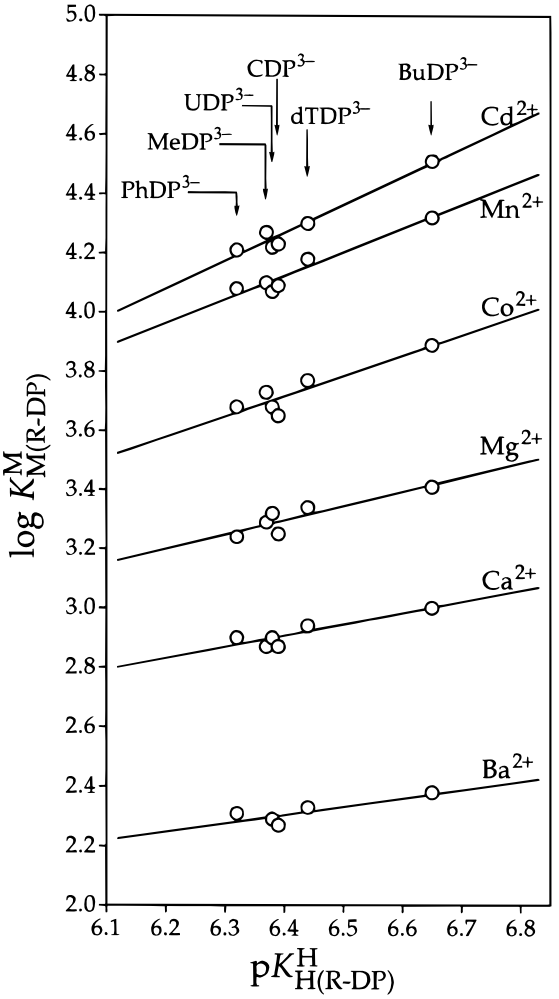

Figure 2. Relationship between log $K^{M}_{M(R\text{-}DP)}$ and p$K^{H}_{H(R\text{-}DP)}$ for the Ba^{2+}, Ca^{2+}, Mg^{2+}, Co^{2+}, Mn^{2+}, and Cd^{2+} 1:1 complexes of phenyl diphosphate (PhDP^{3-}), methyl diphosphate (MeDP^{3-}), uridine 5′-diphosphate (UDP^{3-}), cytidine 5′-diphosphate (CDP^{3-}), thymidine 5′-diphosphate (dTDP^{3-}), and *n*-butyl diphosphate (BuDP^{3-}) (from left to right). The least-squares lines are drawn through the indicated six (in the case of Ba^{2+}, five) data sets; the corresponding straight-line equations are given in Table 4. All the plotted equilibrium constant values refer to aqueous solutions at 25 °C and I = 0.1 M ($NaNO_3$).

(46) (a) Sigel, H.; McCormick, D. B. *Acc. Chem. Res.* **1970**, *3*, 201−208. (b) Sigel, H.; Becker, K.; McCormick, D. B. *Biochim. Biophys. Acta* **1967**, *148*, 655−664.

(47) (a) Irving, H.; Williams, R. J. P. *Nature* **1948**, *162*, 746−747. (b) Irving, H.; Williams, R. J. P. *J. Chem. Soc.* **1953**, 3192−3210.

with regard to comparisons to be made in the future for complexes of ADP^{3-}, GDP^{3-}, etc., because the $pK^{H}_{H(NDP)}$ values for these nucleotides are expected[24] to be close to 6.4. (iv) Most important, Figure 2 demonstrates that the data for the three NDPs fit for each metal ion on the same straight line as the data for the three diphosphate monoesters whose residues R are unable to interact with metal ions; i.e., the nucleobase residues do not participate in complex formation and consequently, these nucleotides behave like simple diphosphate monesters. This result is not surprising for UDP^{3-} and $dTDP^{3-}$ (see Figure 1), as their nucleobase residues offer no binding site for metal ions, but it may appear as surprising for CDP^{3-}; the situation for the latter will therefore be discussed in detail further below in Section 3.7.

3.6. Correlation between Complex Stability and Ligand Basicity: Construction of Straight-Line Plots of log $K^{M}_{M(R-DP)}$ versus $pK^{H}_{H(R-DP)}$. Since, as indicated above, UDP^{3-}, $dTDP^{3-}$, and CDP^{3-} act as pure phosphate coordinators, the data pairs for their complexes are being incorporated into the final straight-line construction. In doing so one could argue that all six diphosphate monoesters of Figure 1 form chelates with metal ions; i.e., the α- and the β-phosphate groups both are participating (possibly in part outersphere)[14a] in metal ion binding and that consequently log K versus pK_a plots should be constructed by employing the sum of the $pK^{H}_{H_2(R-DP)}$ and $pK^{H}_{H(R-DP)}$ values (Table 2). In principle, this request is sensible, however, the basicity of monoprotonated diphosphate residues is low and the values for $pK^{H}_{H_2(R-DP)}$ are thus not very well defined (large error limits), but fortunately, they are also all very similar. Therefore, in the construction of the plots only the very well defined acidity constants $pK^{H}_{H(R-DP)}$ are used as is seen also in Figure 2.

All together, the equilibrium constants for the systems containing $PhDP^{3-}$, $MeDP^{3-}$, UDP^{3-}, CDP^{3-}, $dTDP^{3-}$, and $BuDP^{3-}$ furnish six data points (in the case of Ba^{2+}, Cu^{2+}, Zn^{2+} only five) for each straight-line plot of log $K^{M}_{M(R-DP)}$ (Table 3) versus $pK^{H}_{H(R-DP)}$ (Table 1, column 4). The results of the corresponding least-squares calculations are summarized in Table 4 for the ten metal ions considered.

The slopes (m) of these straight lines are of a considerable size; they vary between about 0.2 (Sr^{2+}) to 0.7 (Ni^{2+}), except for Cd^{2+} ($m = 0.95$), Zn^{2+} (1.10), and Cu^{2+} (1.28) where the slopes are even larger. This contrasts with the results obtained previously[19] for M^{2+}/phosphate monoester systems where the slopes (m) vary between 0.087 (Sr^{2+}) and 0.465 (Cu^{2+}). We believe that these differences reflect a charge effect: In the latter systems the metal ion-to-phosphate charge ratio is 2+/2−, whereas in the diphosphate systems studied now the ratio is 2+/3−. This interpretation is confirmed by the Al^{3+}-monophosphate monoester systems[48] with a charge ratio of 3+/2− and an observed slope of 0.70.

Table 5 lists the deviations from the least-squares line for each individual complex with the six diphosphate ligands mentioned. The points for the UDP^{3-} and CDP^{3-} systems are farthest below the least-squares lines, and those for the $PhDP^{3-}$ and $dTDP^{3-}$ systems give the more positive deviations; however, *all* deviations are within ±0.08 log unit. To provide a reliable error limit for any stability constant calculated with the equations of Table 4 and a given pK_a value, for each of the ten metal ions studied, the standard deviation of the six data points from the relevant least-squares line was calculated; the corresponding values are given in Table 5 in the column at the far right under the heading "SD".

Users of the results described in this section are recommended to apply the equations of Table 4 for diphosphate ligands in the pK_a range 6.2−6.8 and to consider as error limits of the calculated stability constant log $K^{M}_{M(R-DP)}$ 2 or 3 times the standard deviation (SD) given in Table 5 for the corresponding metal ion system. An application of this procedure is given below in Section 3.7 for the evaluation of the structure of the $M(CDP)^{-}$ complexes in solution.

3.7. Structure of the $M(CDP)^{-}$ Complexes in Solution. On the one hand, we have already tentatively seen in Section 3.5 and Figure 2 that the data pairs for the M^{2+}/CDP^{3-} systems fit on their corresponding straight lines and this was also confirmed by the results given in Table 5 (column 5). On the other hand, CDP^{3-} differs from UDP^{3-} and $dTDP^{3-}$ in so far as it offers an obvious binding site at the nucleobase moiety (Figure 1), i.e., the pyridine-like N3, which is potentially available for coordination and which actually participates in the formation of the Cu·CDP·H isomer as discussed in Section 3.4. Further-

Table 4. Straight-Line Correlations for M^{2+}-Diphosphate Monoester Complex Stabilities and Diphosphate Monoester β-Group Basicities: Slopes (m) and Intercepts (b) for the Straight-Base-Line Plots of log $K^{M}_{M(R-DP)}$ (Eq 11) versus $pK^{H}_{H(R-DP)}$ (Eq 3) (Cf. Figure 2) As Calculated by the Least-Squares Procedure from the Experimental Equilibrium Constants Given in the Fourth Column of Table 1 (Eq 3) and in Table 3 (Eq 11) for $PhDP^{3-}$, $MeDP^{3-}$, UDP^{3-}, CDP^{3-}, $dTDP^{3-}$, and $BuDP^{3-}$ (Data Apply to Aqueous Solutions at 25 °C and $I = 0.1$ M ($NaNO_3$))[a]

M^{2+}	m	b	R^b
Mg^{2+}	0.485 ± 0.119	0.192 ± 0.764	0.90
Ca^{2+}	0.379 ± 0.097	0.481 ± 0.623	0.89
Sr^{2+}	0.202 ± 0.120	1.072 ± 0.772	0.64
Ba^{2+}	0.279 ± 0.104	0.518 ± 0.668	0.84
Mn^{2+}	0.800 ± 0.105	−0.998 ± 0.676	0.97
Co^{2+}	0.688 ± 0.152	−0.688 ± 0.974	0.92
Ni^{2+}	0.712 ± 0.223	−1.019 ± 1.430	0.85
Cu^{2+}	1.283 ± 0.115	−2.939 ± 0.738	0.99
Zn^{2+}	1.096 ± 0.097	−2.898 ± 0.621	0.99
Cd^{2+}	0.945 ± 0.104	−1.781 ± 0.668	0.98

[a] Straight-line equation: $y = mx + b$, where x represents the pK_a value of any monoprotonated diphosphate monoester and y the calculated stability constant (log K) of the corresponding $M(R-DP)^{-}$ complex; the errors given with m and b correspond to one standard deviation (1σ). It should be noted that the apparently large error limits of m and b are due to the small range of pK_a values covered by the experimental data. The third digit after the decimal point, however, is still needed because otherwise in Figure 2 the straight lines would no longer fit the data points in a balanced way. [b] Correlation coefficient.

Table 5. Logarithmic Differences between the Experimentally Determined Stability Constants (log $K^{M}_{M(R-DP)}$ of Table 3) of the M^{2+} complexes for $PhDP^{3-}$, $MeDP^{3-}$, UDP^{3-}, CDP^{3-}, $dTDP^{3-}$, and $BuDP^{3-}$ and the Least-Squares Lines of log $K^{M}_{M(R-DP)}$ versus $pK^{H}_{H(R-DP)}$ Plots (Table 4; Cf. also Figure 2) As Determined by the Mentioned Six Complex Systems[a]

M^{2+}	$PhDP^{3-}$	$MeDP^{3-}$	UDP^{3-}	CDP^{3-}	$dTDP^{3-}$	$BuDP^{3-}$	SD[a]
Mg^{2+}	−0.02	0.01	0.03	−0.04	0.02	−0.01	0.011
Ca^{2+}	0.02	−0.03	0.00	−0.03	0.02	0.00	0.009
Sr^{2+}	0.03	−0.03	0.02	−0.03	0.03	0.00	0.012
Ba^{2+}	0.03		−0.01	−0.03	0.02	0.01	0.011
Mn^{2+}	0.02	0.00	−0.04	−0.02	0.03	0.00	0.010
Co^{2+}	0.02	0.04	−0.02	−0.06	0.03	0.00	0.015
Ni^{2+}	0.03	0.07	−0.02	−0.08	0.00	0.01	0.021
Cu^{2+}	0.00		−0.04	0.03	0.02	0.00	0.012
Zn^{2+}	0.03		−0.02	−0.01	−0.01	0.01	0.009
Cd^{2+}	0.02	0.03	−0.03	−0.03	0.00	0.01	0.010

[a] SD is the standard deviation resulting from the listed differences.

(48) Atkári, K.; Kiss, T.; Bertani, R.; Martin, R. B. *Inorg. Chem.* **1996**, *35*, 7089−7094.

Table 6. Logarithms of the Stability Constants of $M(CDP)^-$ Complexes (Eq 11) As Determined by Potentiometric pH Titrations in Aqueous Solution at 25 °C and $I = 0.1$ M ($NaNO_3$)[a] and the Calculated Stability Constants Based on the Basicity of the Terminal β-Phosphate Group of CDP^{3-} and the Reference-Line Equations of Table 4

	$\log K^M_{M(CDP)}$		
M^{2+}	exptl[a]	calcd[b]	$\log K_{exptl} - \log K_{calcd}$[c]
Mg^{2+}	3.25 ± 0.03	3.29 ± 0.03	−0.04 ± 0.04
Ca^{2+}	2.87 ± 0.06	2.90 ± 0.03	−0.03 ± 0.07
Sr^{2+}	2.33 ± 0.04	2.36 ± 0.04	−0.03 ± 0.06
Ba^{2+}	2.27 ± 0.04	2.30 ± 0.03	−0.03 ± 0.05
Mn^{2+}	4.09 ± 0.04	4.11 ± 0.03	−0.02 ± 0.05
Co^{2+}	3.65 ± 0.06	3.71 ± 0.05	−0.06 ± 0.08
Ni^{2+}	3.45 ± 0.05	3.53 ± 0.06	−0.08 ± 0.08
Cu^{2+}	5.29 ± 0.08	5.26 ± 0.04	0.03 ± 0.09
Zn^{2+}	4.10 ± 0.06	4.11 ± 0.03	−0.01 ± 0.07
Cd^{2+}	4.23 ± 0.03	4.26 ± 0.03	−0.03 ± 0.04

[a] These values are from column 4 in Table 2; see also footnote *a* of Table 2. [b] Calculated with $pK_{H(CDP)} = 6.39$ and the straight-line equations given in Table 4. The error limits correspond to *three times* the standard deviations listed in Table 5 (column 8). [c] The error limit for these differences were calculated according to the error propagation after Gauss. The actual differences are identical, of course, with the values listed in column 5 of Table 5.

more, the formation of M(cytidine)$^{2+}$ complexes is well established,[38,49] and their stability constants have been determined.[38] Consequently, one could expect that a diphosphate-coordinated metal ion may interact to some extent also with N3 of the cytosine residue (Figure 1) giving thus rise to the formation of macrochelates (eq 1); a situation well known for complexes of purine-nucleotides.[12−16]

Clearly, any additional interaction, in the present case with N3, must be reflected by an increased complex stability;[22] therefore, the situation of CDP^{3-} in $M(CDP)^-$ 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 reference-line equations of Table 4 (Section 3.6), and the complex stability corresponding to the basicity of the β-phosphate group of CDP^{3-} may therefore be calculated with $pK^H_{H(CDP)} = 6.39$ (Table 1).

The situation regarding the $M(CDP)^-$ complexes is summarized in Table 6. The experimentally determined (column 2) and the calculated (column 3) stability constants are identical within the error limits (column 4); i.e., there is no indication for an increased stability of any of the ten complexes considered. Hence, there is no evidence for metal ion−nucleobase backbinding or macrochelation in these $M(CDP)^-$ species; their stability is solely determined by the metal ion affinity of the diphosphate group! This result then also justifies definitely the incorporation (Section 3.6) of the M^{2+}/CDP^{3-} data pairs into the calculations for the straight-line equations (Table 4).

N3 does not participate in metal ion binding in the $M(CDP)^-$ complexes because CDP^{3-} exists in solution largely in its anti conformation (see Figure 1) and therefore, a metal ion coordinated to the diphosphate residue can not reach N3 and the rotation barrier around the N1-C1′ bond, to bring CDP^{3-} into the syn conformation (about 6 kJ/mol for CTP^{4-}),[12] is evidently too large to be overcome by such an additional interaction. This result corroborates the previous[18] observations made with the M(CMP) complexes in which the nucleotide also behaves as a simple phosphate monoester ligand. Furthermore, also in all the $M(CTP)^{2-}$ complexes studied,[12,13] the metal ions only bind to the triphosphate chain, with the single exception of $Cu(CTP)^{2-}$ for which about 30% macrochelate formation was detected. These results with the cytidine phosphates demonstrate nicely how the structure of metal ion complexes formed in solution can be predetermined by the ligand conformation present in solution.

Table 7. Comparison of the Stability Constants of M^{2+} Complexes Formed with Mono- ($R\text{-}MP^{2-}$), Di- ($R\text{-}DP^{3-}$), and Triphosphate Monoesters ($R\text{-}TP^{4-}$) in Aqueous Solution at 25 °C and $I = 0.1$ M ($NaNO_3$)[a]

M^{2+}	$\log K^M_{M(R\text{-}MP)}$[b]	$\log K^M_{M(R\text{-}DP)}$[c]	$\log K^M_{M(R\text{-}TP)}$[d]
Mg^{2+}	1.56 ± 0.03	3.30 ± 0.03	4.24 ± 0.03
Ca^{2+}	1.45 ± 0.05	2.91 ± 0.03	3.90 ± 0.03
Sr^{2+}	1.24 ± 0.04	2.36 ± 0.04	3.30[e]
Ba^{2+}	1.16 ± 0.04	2.30 ± 0.03	3.20[e]
Mn^{2+}	2.16 ± 0.05	4.12 ± 0.03	4.93 ± 0.03
Co^{2+}	1.94 ± 0.06	3.72 ± 0.05	4.76 ± 0.03
Ni^{2+}	1.94 ± 0.05	3.54 ± 0.06	4.50 ± 0.03
Cu^{2+}	2.87 ± 0.06	5.27 ± 0.04	5.86 ± 0.03
Zn^{2+}	2.12 ± 0.06	4.12 ± 0.03	5.02 ± 0.02
Cd^{2+}	2.44 ± 0.05	4.27 ± 0.03	5.07 ± 0.03
pK_a	6.20	6.40	6.50

[a] See footnote *a* of Table 1. [b] Calculated with the pK_a value given in the bottom row of the table and the straight-line equations listed in Table 5 (and Table 6; error limits) of ref 19 or in Table 3 of ref 23. [c] Calculated with the pK_a value given in the bottom row of the table and the straight-line equations listed in Table 4; the error limits are three times the SD values given in Table 5. [d] Values taken from column 3 of Tables IV or 2 in refs 12 or 13, respectively (but above three times the standard errors of the mean values are given). [e] Estimated value based on the stability differences observed in the various series of complexes; the estimated error limit is ±0.1 log unit.

4. General Conclusions

The present study shows that the nucleotides CDP^{3-}, UDP^{3-}, and $dTDP^{3-}$ behave as simple diphosphate−monoester coordinators; their nucleobase residues have no effects on the stabilities of the complexes formed with the alkaline earth ions or the divalent metal ions of the second half of the 3d series, including Zn^{2+} and Cd^{2+}. The straight-line equations provided for the relation between complex stability and ligand basicity allow now for any diphosphate monoester ligand with a known pK_a value to calculate the stabilities of its M^{2+} complexes for a pure diphosphate binding. This means that now also the properties of complexes, like $M(ADP)^-$ or $M(GDP)^-$ can be evaluated and thus, it should be possible to quantify the extent of macrochelate formation (eq 1) in these species, if any.

However, most important is the fact that now for the first time the metal ion-properties of mono-, di-, and triphosphate monoesters can directly be compared in a quantitative way for a whole series of complexes. To this end we selected for the calculations summarized in Table 7 pK_a values which are representative for pyrimidine-nucleoside phosphates; i.e., $pK^H_{H(R\text{-}MP)} = 6.20$ for monophosphate monoesters,[18] $pK^H_{H(R\text{-}DP)} = 6.40$ for diphosphate monoesters (Table 1), and $pK^H_{H(R\text{-}TP)} = 6.50$ for triphosphate monoesters.[12] Application of the first mentioned pK_a value to the straight-line equations for M(R-MP) complexes[19,23] gives the results listed in column 2 of Table 7; column 3 gives the results based on the equations in Table 4 for the $M(R\text{-}DP)^-$ complexes, and column 4 provides the values for the $M(R\text{-}TP)^{2-}$ species.

To obtain a better overview, the results of Table 7 are plotted in an Irving-Williams sequence-type fashion in Figure 3. The figure confirms the previous observation that phosphate complexes do not strictly follow the Irving−Williams series, as

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

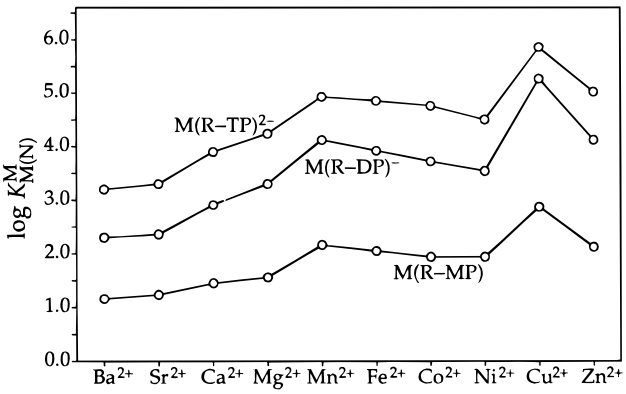

Figure 3. Irving−Williams sequence-type plot for the 1:1 complexes of Ba^{2+} through Zn^{2+} formed with mono- (R-MP^{2-}), di- (R-DP^{3-}), and triphosphate monoesters (R-TP^{4-}) (= N). The data are taken from Table 7; they represent also the stability constants of the M^{2+} complexes of pyrimidine-nucleoside 5′-mono-, di-, or triphosphate (except for Cu(CTP)$^{2-}$)[12] (25 °C; I = 0.1 M, $NaNO_3$). The values used for the Fe^{2+} complexes are the estimates given in the final paragraph of Section 4.

already discussed in Section 3.5. It also shows that addition of a further phosphate unit to R-MP^{2-}, giving R-DP^{3-}, increases the stability of the complexes by approximately 1.1 to 2.4 log units, the effect being especially pronounced for Cu^{2+}. The addition of one more phosphate unit, giving R-TP^{4-}, has a somewhat smaller effect, but the stability increase is still on the order of about 1 log unit throughout, only in the case of Cu^{2+} it reaches only 0.6 log units; the latter observation is certainly connected with the Jahn−Teller distorted octahedral coordination sphere of Cu^{2+} which allows strong coordination only in the equatorial but not at the apical positions of the coordination sphere.

The fact that the stability increase of the complexes varies significantly only from metal ion to metal ion by going from M(R-MP) to M(R-DP)$^{-}$, i.e. within the large span from 1.1 to 2.4 log units, whereas it is quite constant from M(R-DP)$^{-}$ to M(R-TP)$^{2-}$ (if the mentioned special case of Cu^{2+} is ignored), i.e. it stays within the narrow range of 0.8 to 1.0 log units, indicates in our view that outersphere species play a significant role in the M(R-MP) complexes,[18] but hardly in the corresponding di- and triphosphate species.

Finally, plots like those shown in Figure 3 (or equations like those listed in Table 4) also allow to estimate stability constants for the corresponding nucleotide complexes formed with Fe^{2+}. Values for Fe^{2+}−nucleotide complexes have hardly been measured[6,9−11] and a recent tabulation[6] of stability constants contains not a single "recommended" value for a Fe^{2+} complex of a nucleotide. The reason is that it is difficult to obtain Fe^{2+} solutions completely free of Fe^{3+} and especially to prevent oxidation of traces of Fe^{2+} (by traces of dioxygen from air) to Fe^{3+}, a reaction that is facilitated by phosphate coordination. In other words, there is a high danger for measuring artefacts. Interpolation of our data (Table 7 and Figure 3) for the Fe(R-MP), Fe(R-DP)$^{-}$, and Fe(R-TP)$^{2-}$ complexes gives the stability constants log $K^{Fe}_{Fe(R\text{-}MP)}$ = 2.05, log $K^{Fe}_{Fe(R\text{-}DP)}$ = 3.92, and log $K^{Fe}_{Fe(R\text{-}TP)}$ = 4.85, respectively, the estimated error limits being ±0.1 log unit.

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