

Stability and Structure of Metal Ion Complexes Formed in Solution with Acetyl Phosphate and Acetylphosphonate: Quantification of Isomeric Equilibria

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Abstract: The acidity constants of $\text{H}_2(\text{AcP})$ and $\text{H}_2(\text{AnP})$, where AcP^{2-} = acetyl phosphate and AnP^{2-} = acetylphosphonate, as well as 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 AcP^{2-} or AnP^{2-} were determined by potentiometric pH titrations in aqueous solution (25 °C; $I = 0.1 \text{ M}$, NaNO_3). On the basis of previously established $\log K_{\text{M}(\text{R}-\text{PO}_3)}^{\text{M}}$ versus $\text{p}K_{\text{H}(\text{R}-\text{PO}_3)}^{\text{H}}$ straight-line plots for phosph(on)ate ligands ($\text{R}-\text{PO}_3^{2-}$), which allow only a simple $-\text{PO}_3^{2-}$ coordination (Sigel, H.; et al. *Helv. Chim. Acta* **1992**, 75, 2634), it is concluded that the carbonyl oxygen atom of AcP^{2-} and AnP^{2-} participates in complex formation by giving rise to six-membered chelates for all of the mentioned systems except for $\text{Ba}(\text{AnP})$ and $\text{Sr}(\text{AnP})$, the stability of which corresponds to a simple $-\text{PO}_3^{2-}$ coordination. The formation degree of the chelates formed by the alkaline earth ions and AcP^{2-} is quite pronounced; it amounts, for example, for $\text{Mg}(\text{AcP})$ and $\text{Ca}(\text{AcP})$ to $41\% \pm 5\%$ and $48\% \pm 8\%$, respectively. The corresponding results for $\text{Zn}(\text{AcP})$ and $\text{Cu}(\text{AcP})$ are $59\% \pm 6\%$ and $76\% \pm 4\%$, respectively. The formation degree of the six-membered $\text{Cu}(\text{AnP})$ chelate increases in water containing 30% or 50% (v/v) 1,4-dioxane. This may also be surmised for the other divalent metal ions under conditions of lower solvent polarity and poorer solvating properties than water. Such conditions exist in active-site cavities of enzymes, and the expected effects are shortly discussed. The indicated measurements with AcP have only become possible after the stabilities of the corresponding $\text{M}(\text{HPO}_4)$ complexes had been determined (Saha, A.; et al. *J. Biol. Inorg. Chem.* **1996**, 1, 231) because AcP contains as an impurity always some phosphate; this phosphate content had to be quantified and its effect was carefully considered in the evaluations of the experimental data. Here is most probably also the main reason why previously published results regarding the stabilities of the $\text{Mg}(\text{AcP})$ and $\text{Ca}(\text{AcP})$ complexes vary widely; stability constants of the other $\text{M}(\text{AcP})$ complexes have not been determined previously.

1. Introduction

There is an intimate linking of the metabolism of Mg^{2+} , Ca^{2+} , and phosphorus (phosphate) in cells, involving in subtle ways also messengers that are controlled by zinc (hydrolysis) or copper enzymes (oxidation).¹ For example, sarcoplasmic reticulum Ca^{2+} -ATPase is activated in the catalytic cycle by Ca^{2+} and then the γ -phosphate group of ATP, which is bound to Mg^{2+} , is transferred to an Asp site² forming a phosphorylated enzyme intermediate;³ a reaction for which acetyl phosphate, a mixed anhydride of acetic acid and phosphoric acid (Figure 1, vide infra), can also act as a substrate.³ Similarly, a Ca^{2+} -ATPase

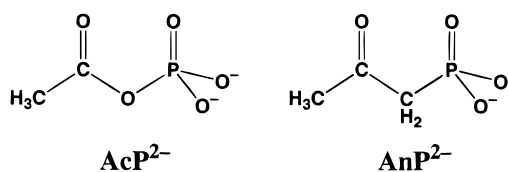


Figure 1. Structures of the ligands considered in this study: acetyl phosphate (AcP^{2-}) and acetylphosphonate (AnP^{2-}).

derived from vesicles of the dense tubular system of platelets can use either ATP or acetyl phosphate as a substrate in the

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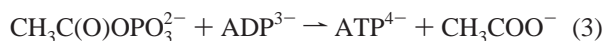
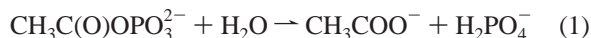
(1) Williams, R. J. P.; Fraústo da Silva, J. J. R. *The Natural Selection of the Chemical Elements*; Clarendon Press: Oxford, U.K., 1996; pp 513–516.

(2) Abbreviations: AcP^{2-} , acetyl phosphate; ADP^{3-} , adenosine 5'-diphosphate; AnP^{2-} , acetylphosphonate; Asp, aspartic acid; ATP^{4-} , adenosine 5'-triphosphate; Edta, 1,2-diaminoethane- N,N,N',N' -tetraacetic acid; I , ionic strength; M^{2+} , divalent metal ion; $\text{R}-\text{PO}_3^{2-}$, simple phosphate monoester or phosphonate ligand with R representing a noncoordinating residue (see also legend of Figure 2). In certain instances, where no confusion can occur, $\text{R}-\text{PO}_3^{2-}$ may also include AcP^{2-} and AnP^{2-} . Species which are 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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presence of Ca^{2+} .⁴ Indeed, a large number of enzymes employ acetyl phosphate as substrate,⁵ one of the most prominent reactions being the formation of $\text{Mg}(\text{ATP})^{2-}$ via $\text{Mg}(\text{ADP})^-$ by activating acetate with acetyl coenzyme A synthetase and synthesizing from acetyl coenzyme A with phosphotransacetylase acetyl phosphate which then, with an acetate kinase, provides ATP and acetate.^{6,7}

The last mentioned process involving acetyl phosphate produces upon hydrolysis according to reaction 1 a significant amount of free energy which is used in reaction 2 to form ATP. This means, for the overall reaction 3 holds⁸



$\Delta G_{(3)}^{\circ} = \Delta G_{(1)}^{\circ} + \Delta G_{(2)}^{\circ} = -43.1$ (kJ/mol; see ref 9) + 30.5 (kJ/mol; see ref 9) = -12.6 kJ/mol; hence, thermodynamically one may say that the hydrolysis of acetyl phosphate drives the phosphorylation of ADP to ATP.⁸ Indeed, also for commercial processes it was concluded that acetyl phosphate is the best phosphate donor to synthesize ATP.¹⁰

Divalent metal ions (M^{2+}) like Mg^{2+} and Ca^{2+} (refs 11–13) as well as Mn^{2+} , Co^{2+} , Ni^{2+} , and Zn^{2+} (ref 11) facilitate the hydrolysis of acetyl phosphate (AcP^{2-}),^{11–13} which is similar to observations made with ADP (ref 14) and ATP.^{14–16} Despite this effect only a few attempts have been made to determine the stability constants of $\text{M}(\text{AcP})$ complexes.^{17–19} In fact, data are only available for $\text{Mg}(\text{AcP})$ and $\text{Ca}(\text{AcP})$ and these differ

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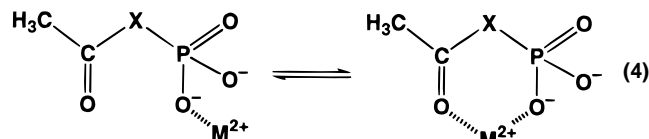
(17) *IUPAC Stability Constants Database*, Release 3, Version 3.02; compiled by Pettit, L. D.; Powell, H. K. J.: Academic Software, Timble, Otley, W. Yorks, U.K., 1998.

(18) *NIST Critically Selected Stability Constants of Metal Complexes*, Reference database 46, Version 5.0; data collected and selected by Smith, R. M.; Martell, A. E.; U.S. Department of Commerce, National Institute of Standards and Technology, Gaithersburg, MD, 1998.

widely; that is, the values^{11–13,20,21} $\log K_{\text{Mg}(\text{AcP})}^{\text{Mg}}$ vary between 0.76 (ref 11) and 2.03 (ref 20) and those^{11–13} for $\log K_{\text{Ca}(\text{AcP})}^{\text{Ca}}$ between 0.89 (ref 11) and 1.89 (ref 13). These differences are far too large to be attributable to different experimental conditions. However, considering that probes of the hydrolysis-sensitive acetyl phosphate practically always contain varying amounts of free phosphate as an impurity, these discrepancies do not appear very surprising in retrospect (see Experimental Section). Since we had recently measured the stability constants of M^{2+} complexes formed with hydrogen phosphate,²² we were now in the position to determine the stability constants of $\text{M}(\text{AcP})$ complexes and to take the formation degree and the stability of $\text{M}(\text{HPO}_4)$ species into account in the evaluation.

Considering the indicated difficulties with acetyl phosphate, we incorporated in our study also the hydrolysis-stable and structurally closely related acetylphosphonate (AnP^{2-}) (Figure 1). Of course, AnP^{2-} merits an investigation also on its own rights since, for example, it acts as a noncompetitive inhibitor of D-3-hydroxybutyrate dehydrogenase in the reduction of acetoacetate²³ as well as a competitive inhibitor of acetoacetate decarboxylase.²⁴ Its methyl ester was also employed as a substrate in various enzymatic and nonenzymatic reactions.²⁵ Furthermore, AnP^{2-} is a substrate for the P–C bond cleaving enzyme phosphonoacetaldehyde hydrolase; that is, it undergoes enzyme-catalyzed hydrolysis to hydrogen phosphate and acetone.²⁶ Again, almost no stability constants of $\text{M}(\text{AnP})$ complexes are available;^{17–19} only values for $\text{Mg}(\text{AnP})$ were published.²⁰

The primary metal ion binding site in AcP^{2-} and AnP^{2-} is certainly the PO_3^{2-} group (see Figure 1). However, since the carbonyl oxygen of the acetyl and acetyl residues is located such that six-membered chelates might possibly be formed, we had to take into account in our evaluations also the intramolecular equilibrium 4 where $\text{X} = \text{O}$ (AcP^{2-}) or CH_2 (AnP^{2-}):



Indeed, by employing recently determined,^{27,28} straight-line plots of $\log K_{\text{M}(\text{R}-\text{PO}_3)}^{\text{M}}$ versus $\text{p}K_{\text{H}(\text{R}-\text{PO}_3)}^{\text{H}}$, which are based on simple phosph(on)ate ligands, we are now able to show that with both

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ligands, depending on M^{2+} , varying amounts of the six-membered chelate are actually formed. For Cu(AnP) we could further show that reduction of the solvent polarity (by addition of 1,4-dioxane to the aqueous solution) facilitates chelate formation. This observation is important with regard to enzymes, since in their active site cavities a reduced solvent polarity exists.^{29,30}

2. Experimental Section

2.1. Materials. Two different lots of the lithium–potassium salt of acetyl phosphate (90%), one from Sigma Chemical Co., St. Louis, MO, and one from Aldrich Chemical Co., Milwaukee, WI, proved to be identical and were used as obtained (see also Section 2.2). Acetylphosphonic acid was synthesized (by F. G.) according to a published method.³¹ Methylphosphonic acid was purchased from Fluka Chemie AG, Buchs, Switzerland. Acetone, HClO₄ (70%), (NH₄)₆Mo₇O₂₄·4H₂O, and 1,4-dioxane (extra pure) were from Merck AG, Darmstadt, Germany. All of the other reagents were the same as those used recently,³² and the exact concentrations of the stock solutions of the divalent metal ions were determined as described.³²

The titer of the NaOH used for the titrations was established with potassium hydrogen phthalate. The aqueous stock solutions of acetyl phosphate and acetylphosphonate were freshly prepared daily, and the pH was adjusted to 8.0 and 8.8, respectively. The exact concentrations of both ligand solutions used in the titration experiments (titrated in the presence of an excess of HNO₃; see Section 2.5) were determined each time with the mentioned NaOH.

2.2. Stability of Acetyl Phosphate toward Dephosphorylation and Determination of Its Phosphate Content. The available acetyl phosphate (AcP) (Section 2.1) contained inorganic phosphate as an impurity. Its content was determined photometrically³³ with carefully standardized molybdate reagent using an adaptation^{33a} of the method developed by Hirata and Appleman.^{33b} In this procedure 1 mL of HClO₄ (70%) is applied for in total 20 mL solution containing all reactants. Since the hydrolysis of AcP is acid-catalyzed, AcP decomposes in this reagent mixture, and therefore it was added last to the other reagents; then the absorbance was measured immediately and continued for at least 10 min. From the increase in absorbance in dependence on time, it was possible to make a curve fit through the measured data to determine the initial free phosphate content of AcP by extrapolation to the initial time, $t = 0$.

According to this method the purchased AcP contained about 17% ± 1% (error estimated) free phosphate. This amount increased to about 28% after 8 h when a stock solution with pH 8.0 was stored at room temperature. Keeping the stock solutions at 0 °C prevented a further dephosphorylation during the time needed for the titration experiments (about 8 h).

The amount of free phosphate in AcP was independently determined in each potentiometric pH experiment by taking into account the acidity constant of H₂PO₄⁻ ($pK_{H_2PO_4}^H = 6.75$)²² in the curve-fitting procedure of the evaluation of the titration curves (see also Section 2.5). This method provided the amount of NaOH consumed by the H₂PO₄⁻ present in the solutions titrated. In this way 16.6% ± 1.1% (3σ) of free phosphate in AcP was determined, in excellent agreement with the percentage obtained by the method described above.

2.3. Potentiometric pH Titrations. These were carried out with a Metrohm E536 potentiograph equipped with an E535 or E665 dosimat and a 6.0202.100 (JC) combined macro glass electrode from Metrohm AG, Herisau, Switzerland. The buffer solutions (pH 4.00, 7.00, and

9.00; based on the NBS scale, now NIST) used for calibration were from the same company. The direct pH meter readings were used in the calculations of the acidity constants (Sections 2.4 and 2.5), which are so-called practical, mixed, or Brønsted constants.^{34a} Their negative logarithms given for aqueous solutions at $I = 0.1$ M and 25 °C may be converted into the corresponding concentration constants by subtracting 0.02 from the listed pK_a values;^{34a} this conversion term contains both the junction potential of the glass electrode and the hydrogen ion activity (γ).³⁴ No conversion is necessary for the stability constants of the M^{2+} complexes (Section 2.6).³⁵

All calculations were carried out with IBM-compatible desk computers with 80–486 or Pentium processors (connected with Epson Stylus 1000ESC/P 2 printers and a Hewlett-Packard Deskjet 1600C Color Smart printer or a Hewlett-Packard 7475A plotter) by a curve-fitting procedure using a Newton-Gauss nonlinear least-squares program.

2.4. Determination of the Acidity Constants of Methylphosphonic Acid and Acetylphosphonic Acid. The acidity constants $K_{H_3(MeP)}^H$ and $K_{H_2(MeP)}^H$ of methylphosphonic acid, H₂(MeP), were determined by titrating 25 mL of aqueous 0.01 M HNO₃ ($I = 0.1$ M, NaNO₃; 25 °C) in the presence and absence of 2.1 mM H₂MeP under N₂ with 2.7 mL of 0.1 M NaOH, and by means of the difference in NaOH consumption between the two titrations for the calculations.³⁵ For these the pH range 2.5–9.2 was used corresponding to a neutralization degree of about 72% at the beginning for the equilibrium H₂(MeP)/H(MeP)⁻ and finally of about 98% for H(MeP)⁻/MeP²⁻. Four independent pairs of titrations were carried out.

The acidity constants $K_{H_2(AnP)}^H$ and $K_{H(AnP)}^H$ of acetylphosphonic acid, H₂(AnP), were determined identically, but HNO₃ and H₂(AnP) were 7.2 and 1.3 mM, respectively, and 3.6 mL of 0.06 M NaOH was used for the titrations. Three independent pairs of titrations were made and the pH range 2.4–8.2 was used in the calculations; this corresponds to a neutralization degree of about 93% already at the beginning for the equilibrium H₂(AnP)/H(AnP)⁻ (hence, only an estimate was possible) and finally of about 98% for H(AnP)⁻/AnP²⁻.

2.5. Determination of the Acidity Constants of Monoprotonated Acetyl Phosphate and Acetylphosphonate. The acidity constants $K_{H(R-PO_3)}^H$ for H(AcP)⁻ and H(AnP)⁻ were determined by titrating 50 mL of aqueous 0.54 mM HNO₃ ($I = 0.1$ M, NaNO₃; 25 °C) in the presence and absence of 0.3 mM R–PO₃ under N₂ with 1 mL of 0.03 M NaOH and by using the differences in NaOH consumption between two such titrations for the calculations. For each pair of titrations the data were collected every 0.1 pH unit and evaluated by the mentioned curve-fitting procedure (Section 2.3) in the pH range of about 3.8–5.6 for acetyl phosphate, corresponding to about 8–85% neutralization of the equilibrium H(AcP)⁻/AcP²⁻, respectively, and between pH 4.8 and 8.2 for acetylphosphonate, corresponding to about 2–98% neutralization of the equilibrium H(AnP)⁻/AnP²⁻. This means that in the case of AcP the evaluation was carried out only up to a pH (= 5.60) where deprotonation of free H₂PO₄⁻ is still insignificant.

However, for AcP also a more sophisticated procedure which allowed an evaluation between pH 3.8 and 8.2 was used: The acidity constant $pK_{H(AcP)}^H$ was calculated in this case by taking into account the free phosphate (see Section 2.2), that is, by keeping the known²² acidity constant $pK_{H_2PO_4}^H = 6.75$ constant but allowing now variation for *three* unknowns, namely for the concentrations of H₂PO₄⁻/HPO₄²⁻ and H(AcP)⁻/AcP²⁻ as well as for $pK_{H(AcP)}^H$. It is comforting that the results obtained in both ways for $pK_{H(AcP)}^H$ are identical within the error limits.

The results given in Section 3.1 are the averages of 43 independent pairs of titrations for acetyl phosphate and of 32 independent pairs for acetylphosphonate.

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(35) It should be emphasized that the ionic product of water (K_w) and the mentioned conversion term do not enter into our calculation procedures because we evaluated the differences in NaOH consumption between solutions with and without ligand (see Sections 2.4–2.6).^{34a} The advantage of this procedure is (aside from not needing K_w and γ values) that impurities in the solvent or in the metal salts, as well as systematic errors, etc., cancel to a large part.

The acidity constants $pK_{\text{H(AcP)}}^{\text{H}}$ and $pK_{\text{H(AnP)}}^{\text{H}}$ were also measured in mixed solvents, where water was partially replaced by 1,4-dioxane (30% and 50% (v/v)). Seven pairs of independent titrations were carried out for each ligand in 30% dioxane within the pH range 3.8–6.4 for AcP corresponding to about 2–87% neutralization in the equilibrium $\text{H(AcP)}^-/\text{AcP}^{2-}$, and from pH 5.5 to 8.6 for AnP (2 to 96% neutralization of H(AnP)^-). In 50% dioxane six independent titration pairs were evaluated within the pH range 4.7–6.8 for AcP (4–85% neutralization of H(AcP)^-) and from pH 6.1 to 8.6 in the case of AnP (3–89% neutralization of H(AnP)^-). The values for $pK_{\text{H(AcP)}}^{\text{H}}$ of H(AcP)^- were this time only determined with the common procedure because $pK_{\text{H}_2\text{PO}_4}^{\text{H}}$ is not known for these mixed solvents.

2.6. Determination of the Stability Constants of the Complexes.

The conditions for the determination of the stability constants $K_{\text{M(R-PO}_3)}^{\text{M}}$ of the binary M(AcP) and M(AnP) complexes were the same as those given in Section 2.5 for the determination of the acidity constants; that is, 0.54 mM HNO_3 was titrated in the presence and absence of 0.3 mM R-PO_3 , but NaNO_3 was now partially or fully replaced by $\text{M(NO}_3)_2$. For Mg^{2+} , Ca^{2+} , Sr^{2+} , and Ba^{2+} with AcP, $[\text{M}^{2+}]$ was 33.33 or 26.67 mM; that is, the $\text{R-PO}_3/\text{M}^{2+}$ ratios were 1:111 or 1:89, respectively. For AnP, $[\text{M}^{2+}]$ was 33.33 or 26.67 mM ($\text{R-PO}_3/\text{M}^{2+} \approx 1:111$ or $1:89$) with Mg^{2+} and Ca^{2+} , and 33.33 mM ($\text{R-PO}_3/\text{M}^{2+} \approx 1:111$) with Sr^{2+} and Ba^{2+} . With Mn^{2+} , Co^{2+} , Zn^{2+} and Cd^{2+} , $[\text{M}^{2+}]$ was 16.67 or 8.33 mM ($\text{R-PO}_3/\text{M}^{2+} \approx 1:56$ or $1:28$) and with Cu^{2+} it was 3.33 or 1.67 mM ($\text{R-PO}_3/\text{M}^{2+} \approx 1:11$ or $1:5.6$) for both ligands. $[\text{Ni}^{2+}]$ was 24.90, 16.67, or 13.20 mM ($\text{R-PO}_3/\text{M}^{2+} \approx 1:83$, $1:56$, or $1:44$) with AcP and 16.67 or 8.33 mM ($\text{R-PO}_3/\text{M}^{2+} \approx 1:56$ or $1:28$) with AnP. In addition, experiments were made for AnP with $[\text{Mg}^{2+}] = 16.67$ mM ($\text{R-PO}_3/\text{M}^{2+} \approx 1:56$), and for AcP with Mn^{2+} or Co^{2+} and $[\text{M}^{2+}] = 24.90$ mM ($\text{R-PO}_3/\text{M}^{2+} \approx 1:83$).

For the M^{2+} systems with AcP, eight independent titration pairs were carried out for Mg^{2+} , Ca^{2+} , Sr^{2+} , Ba^{2+} , Zn^{2+} , and Cu^{2+} , nine pairs for Cd^{2+} , 10 pairs for Mn^{2+} and Ni^{2+} , and eleven pairs for Co^{2+} . With AnP six independent pairs were made with Mg^{2+} , Mn^{2+} , Co^{2+} , Ni^{2+} , Zn^{2+} , and Cd^{2+} , seven pairs with Ca^{2+} , and eight pairs with Sr^{2+} , Ba^{2+} , and Cu^{2+} . The data were collected every 0.1 pH unit starting from the lowest pH which could be reached in an experiment or from a formation degree of about 5% for $\text{M(R-PO}_3)$ 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 a deprotonation degree of about 90% for $\text{H(R-PO}_3)^-$. To avoid the influence of the phosphate impurity (Section 2.2), we stopped the evaluation of the data of the M(AcP) systems before the onset of the formation of the $\text{M(HPO}_4)$ complexes, which is known from our previous work.²²

The experimental data for all M^{2+} systems, except the one with Cu^{2+} , were evaluated by determining with the curve-fitting procedure (Section 2.3) the so-called³⁶ “apparent” acidity constant, K'_a , for the deprotonation of $\text{H(R-PO}_3)^-$ which is valid only for the given conditions and in the presence of a large excess of M^{2+} , that is, where $[\text{M}^{2+}]_{\text{free}} \approx [\text{M}^{2+}]_{\text{tot}}$. With the value determined for K'_a and the acidity constant $K_{\text{H(R-PO}_3)}^{\text{H}}$ determined previously (Section 2.5), the stability constant $K_{\text{M(R-PO}_3)}^{\text{M}}$ can be calculated according to eq 5:³⁶

$$K_{\text{M(R-PO}_3)}^{\text{M}} = (K'_a - K_{\text{H(R-PO}_3)}^{\text{H}}) / (K_{\text{H(R-PO}_3)}^{\text{H}} [\text{M}]_{\text{tot}}) \quad (5)$$

For the M^{2+}/AcP systems a further calculation procedure was employed, which takes into account also the known²² stability constants ($\log K_{\text{M(HPO}_4)}^{\text{M}}$) of $\text{M(HPO}_4)$ complexes which may form due to the phosphate impurity present (Section 2.2). This means, from the known stability constants for the $\text{M(HPO}_4)$ complexes,²² that the apparent K'_a values can be calculated for the various individual experimental conditions and these values were then taken into account in the curve-fitting procedure (analogously as mentioned in Section 2.5 for the determination of $pK_{\text{H(AcP)}}^{\text{H}}$). The results obtained by this more sophisticated method agree excellently with those obtained by the common method described above, provided that in the latter case the collection

(36) (a) Sigel, R. K. O.; Song, B.; Sigel, H. *J. Am. Chem. Soc.* **1997**, *119*, 744–755. (b) Ji, L.-n.; Corfù, N. A.; Sigel, H. *J. Chem. Soc., Dalton Trans.* **1991**, 1367–1375. (c) Sigel, H. *Chimia* **1967**, *21*, 489–500.

Table 1. Negative Logarithms of the Acidity Constants (Equations 6 and 7) in Aqueous Solution for $\text{H}_2(\text{AcP})$ and $\text{H}_2(\text{AnP})$ as Well as for Some Related Species at 25 °C and $I = 0.1$ M (NaNO_3) as Determined by Potentiometric pH Titrations^a

$\text{H}_2(\text{R-PO}_3)$	$pK_{\text{H}_2(\text{R-PO}_3)}^{\text{H}}$	$pK_{\text{H}(\text{R-PO}_3)}^{\text{H}}$	ΔpK_a^b
$\text{H}_2(\text{AcP})$	-0.4 ± 0.3^c	4.84 ± 0.02	
$\text{H}_2(\text{AnP})$	1.3 ± 0.2	6.49 ± 0.02	5.2 ± 0.2
$\text{CH}_3\text{OP(O)(OH)}_2$	1.1 ± 0.2^d	6.36 ± 0.01^d	5.3 ± 0.2
$\text{CH}_3\text{P(O)(OH)}_2$	2.10 ± 0.03	7.51 ± 0.01^e	5.41 ± 0.03

^a So-called practical (or mixed) constants³⁴ are listed; see Section 2.3. The errors given are *three times* the standard error of the mean value or the sum of the probable systematic errors, whichever is larger.

^b $\Delta pK_a = pK_{\text{H}(\text{R-PO}_3)}^{\text{H}} - pK_{\text{H}_2(\text{R-PO}_3)}^{\text{H}}$; see also footnote 42 regarding the estimate for $pK_{\text{H}_2(\text{AcP})}^{\text{H}}$.

^c Estimate; see footnote 42. ^d From ref 22. ^e This value from the present study is in excellent agreement with an earlier result: $pK_{\text{CH}_3\text{P(O)(OH)}_2}^{\text{H}} = 7.53 \pm 0.01^{27b}$

of the experimental data is stopped before the onset of the formation of the $\text{M(HPO}_4)$ species.

Since the excess of Cu^{2+} was not so large (see above), the stability constants of $\text{Cu(R-PO}_3)$ were calculated considering the species H^+ , $\text{H(R-PO}_3)^-$, R-PO_3^{2-} , M^{2+} , and $\text{M(R-PO}_3)^{37}$

The stability constant of the Cu(AnP) complex was also measured in water containing 30% or 50% (v/v) 1,4-dioxane. Six independent pairs of titrations were carried out each in the two mixed aqueous solvents under the conditions given in Section 2.5 for the acidity constant of H(AnP)^- . The Cu^{2+} concentrations were 1.67 and 0.83 mM, that is, $\text{AnP}/\text{Cu}^{2+} \approx 1:5.6$ and $1:2.8$. The evaluation of the experimental data was done as indicated in the preceding paragraph.

Finally it may be noted that the evaluations for all stability constants determined in this study showed no dependence on the excess amount of M^{2+} employed in the various experiments.

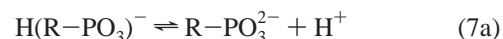
2.7. Semiempirical Molecular Orbital Calculations. The electronic structure and ESP partial atomic charges³⁸ of AcP^{2-} and AnP^{2-} were calculated using PM3 (see ref 39) and AM1 (see ref 40) Hamiltonians with the MOPAC 6 program. The geometries of the molecules were constructed from those of ADP^{3-} in its complex with herpes simplex virus type 1 thymidine kinase.⁴¹

3. Results and Discussion

3.1. Acidity Constants of Acetyl Phosphoric Acid and Acetonylphosphonic Acid. Simple phosphate and phosphonate species (R-PO_3^{2-}) including AcP^{2-} and AnP^{2-} are dibasic and may thus accept two protons at their $-\text{PO}_3^{2-}$ groups. Hence, for the release of these protons the following two equilibria need to be considered:



$$K_{\text{H}_2(\text{R-PO}_3)}^{\text{H}} = [\text{H(R-PO}_3)^-][\text{H}^+]/[\text{H}_2(\text{R-PO}_3)] \quad (6b)$$



$$K_{\text{H}(\text{R-PO}_3)}^{\text{H}} = [\text{R-PO}_3^{2-}][\text{H}^+]/[\text{H(R-PO}_3)^-] \quad (7b)$$

The corresponding results of the potentiometric pH titrations are listed in Table 1, where also the values for methyl phosphoric acid, $\text{CH}_3\text{OP(O)(OH)}_2$,²² and for methylphosphonic acid, $\text{CH}_3\text{P-}$

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

(38) Besler, B. H.; Merz, K. M., Jr.; Kollman, P. A. *J. Comput. Chem.* **1990**, *11*, 431–439.

(39) Stewart, J. J. P. *J. Comput. Chem.* **1989**, *10*, 209–220, 221–264.

(40) Dewar, M. J. S.; Zoebisch, E. G.; Healy, E. F.; Stewart, J. J. P. *J. Am. Chem. Soc.* **1985**, *107*, 3902–3909.

(41) (a) Wild, K.; Bohner, T.; Folkers, G.; Schulz, G. E. *Protein Sci.* **1997**, *6*, 2097–2106. (b) Entry 1VTK in the Protein Data Bank: Bernstein, F. C.; Koetzle, T. F.; Williams, G. J. B.; Meyer, E. F., Jr.; Rodgers, J. R.; Kennard, O.; Shimanouchi, T.; Tasumi, M. *J. Mol. Biol.* **1977**, *112*, 535–542.

Table 2. Logarithms of the Stability Constants of M(AcP) and M(AnP) Complexes (Equation 8) as Determined by Potentiometric pH Titrations (exptl)^a in Water at 25 °C and *I* = 0.1 M (NaNO₃), Together with the Corresponding Calculated Stability Constants for a Pure and Unaltered Metal Ion–Phosphate or –Phosphonate Coordination (calcd),^{a,b} and the Resulting Stability Differences log Δ_{M(R–PO₃)} (Equation 13)^{a,c}

M ²⁺	log K _{M(AcP)} ^M		log Δ _{M(AcP)} ^c	log K _{M(AnP)} ^M		log Δ _{M(AnP)} ^c
	exptl ^a	calcd ^b		exptl ^a	calcd ^b	
Mg ²⁺	1.51 ± 0.02	1.28 ± 0.03	0.23 ± 0.04	1.73 ± 0.02	1.62 ± 0.03	0.11 ± 0.04
Ca ²⁺	1.55 ± 0.04	1.27 ± 0.05	0.28 ± 0.06	1.55 ± 0.01	1.49 ± 0.05	0.06 ± 0.05
Sr ²⁺	1.47 ± 0.02	1.13 ± 0.04	0.34 ± 0.04	1.31 ± 0.05	1.26 ± 0.04	0.05 ± 0.06
Ba ²⁺	1.53 ± 0.03	1.04 ± 0.04	0.49 ± 0.05	1.23 ± 0.04	1.19 ± 0.04	0.04 ± 0.06
Mn ²⁺	1.95 ± 0.03	1.83 ± 0.05	0.12 ± 0.06	2.36 ± 0.02	2.23 ± 0.05	0.13 ± 0.05
Co ²⁺	1.83 ± 0.05	1.63 ± 0.06	0.20 ± 0.08	2.19 ± 0.02	2.00 ± 0.06	0.19 ± 0.06
Ni ²⁺	1.75 ± 0.04	1.61 ± 0.05	0.14 ± 0.06	2.14 ± 0.02	2.01 ± 0.05	0.13 ± 0.05
Cu ²⁺	2.86 ± 0.05	2.24 ± 0.06	0.62 ± 0.08	3.36 ± 0.03	3.00 ± 0.06	0.36 ± 0.07
Zn ²⁺	2.04 ± 0.03	1.65 ± 0.06	0.39 ± 0.07	2.47 ± 0.04	2.22 ± 0.06	0.25 ± 0.07
Cd ²⁺	2.18 ± 0.04	1.99 ± 0.05	0.19 ± 0.06	2.71 ± 0.03	2.53 ± 0.05	0.18 ± 0.06

^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 above case of log Δ_{M(R–PO₃)}, were calculated according to the error propagation after Gauss. ^b These values are based on straight-line equations,^{27,28} which quantify the relationship between complex stability and –PO₃^{2–}-group basicity (see also Figure 2); the latter is defined by the pK_{H(R–PO₃)}^H values of H(AcP)[–] and H(AnP)[–] (Table 1). ^c Log Δ_{M(R–PO₃)} = log K_{exptl} – log K_{calcd}; note, this difference corresponds also to that defined by eq 13.

(O)(OH)₂, determined now for comparison, are given. Due to the significant acid-catalyzed hydrolysis of AcP (see Section 2.2), we did not attempt to measure a value for pK_{H₂(AcP)}^H (eq 6) but preferred to estimate it via the application of various differences which follow from the constants in Table 1, resulting in pK_{H₂(AcP)}^H = –0.4 ± 0.3.⁴² The values available in the literature for pK_{H(AcP)}^H = 4.8 (see ref 20) and pK_{H(AnP)}^H = 6.3 (see refs 20, 24) are in fair agreement with the present results considering the differences in the experimental conditions (30 °C; *I* = 0.3 M).²⁰

The variations between the acidity constants in Table 1 may easily be rationalized: For example, monophosphate esters are expected to be more acidic than their related phosphonates due to the electron-withdrawing properties of the additional oxygen atom at the phosphorus. Indeed, the lower acidity constants of CH₃OP(O)(OH)₂, compared with those of CH₃P(O)(OH)₂ or the ones of H₂(AcP), compared with those of H₂(AnP), are in agreement with this expectation. Similarly, that an acetyl group has a stronger acidifying effect than a methoxy group is also expected and confirmed by the results listed in Table 1 for H₂(AcP) and methyl phosphoric acid.

With regard to the results discussed below one may note that the first proton from H₂(AcP) or H₂(AnP) is completely released at pH > 3.5; hence, the corresponding buffer regions (eq 6) do not overlap with the equilibria due to complex formation between M²⁺ and AcP^{2–} or AnP^{2–}.

3.2. Stability Constants of M(AcP) and M(AnP) Complexes. The experiments with AcP or AnP and the alkaline earth

(42) The estimate of the value for pK_{H₂(AcP)}^H was made in the following ways: (i) The differences pK_{CH₃P(O)(OH)₂}^H – pK_{CH₃OP(O)(OH)₂}^H = (7.51 ± 0.01) – (6.36 ± 0.01) = 1.15 ± 0.01 and pK_{CH₃OP(O)(OH)₂}^H – pK_{CH₃P(O)(OH)₂}^H = (2.10 ± 0.03) – (1.1 ± 0.2) = 1.0 ± 0.2 are identical within their error limits, indicating that the effects of substituents at the P atom, in the present case of the CH₃– versus the CH₃O– group, are similar for both acidity constants of the –P(O)(OH)₂ residue. Hence, the difference pK_{H(AnP)}^H – pK_{H(AcP)}^H = (6.49 ± 0.02) – (4.84 ± 0.02) = 1.65 ± 0.03 may be used to calculate a value for pK_{H₂(AcP)}^H, that is, pK_{H₂(AcP)}^H = pK_{H₂(AnP)}^H – (1.65 ± 0.03) = (1.3 ± 0.2) – (1.65 ± 0.03) = –0.35 ± 0.2. (ii) The other approach makes use of the differences, ΔpK_a = pK_{H(R–PO₃)}^H – pK_{H₂(R–PO₃)}^H, which are given in the fourth column of Table 1 and which are identical within their error limits for the three cases where both constants could be measured. Therefore, the corresponding average value, ΔpK_a = 5.3 ± 0.2, may be deduced from pK_{H(AcP)}^H to give pK_{H₂(AcP)}^H = (4.84 ± 0.02) – (5.3 ± 0.2) = –0.46 ± 0.2. Both estimates agree excellently within their error limits, and their average provides therefore the final value, that is, pK_{H₂(AcP)}^H = –0.4 ± 0.3, which is listed in Table 1.

ions, several divalent 3d ions, as well as Zn²⁺ or Cd²⁺ (M²⁺) may all be completely described by considering equilibria 7 and 8, as long as the evaluation is not carried into the pH range where hydroxo complexes form (see Section 2.6).



$$K_{M(R-PO_3)}^M = [M(R-PO_3)] / ([M^{2+}][R-PO_3^{2-}]) \quad (8b)$$

The stability constants determined in this study are listed in columns 2 and 5 of Table 2. So far practically none of the stabilities of the M(AcP) and M(AnP) complexes have been determined before.^{17–19} As already indicated, the few values available for Mg(AcP), Mg(AnP), and Ca(AcP) differ widely.⁴³ The value closest to one of this study is log K_{Ca(AcP)}^{Ca} = 1.70 (25 °C; *I* = 0.6 M)¹² being only 0.15 log units away from our result (log K_{Ca(AcP)}^{Ca} = 1.55); all of the other previous values differ by 0.25 log units or more. The reasons for these discrepancies are that previously inappropriate methods (like competition experiments) were applied and, most importantly, the phosphate impurity inevitably present in AcP preparations was apparently not considered.

The stability constants of the M(AnP) complexes (Table 2, column 5) show the usual trends, that is, that complex stability of the alkaline earth ions decreases with increasing ionic radii. For the divalent 3d metal ions the longstanding experience^{27b,28,44a} is confirmed that the stabilities of phosph(on)ate–metal ion complexes often do not strictly follow the Irving-Williams⁴⁴ sequence. The latter also applies for the stabilities of the M(AcP) complexes (Table 2, column 2).

However, the M(AcP) complexes of the alkaline earth ions behave differently: all of their values for log K_{M(AcP)}^M are close to 1.5 (Table 2, column 2). In fact, despite the lower basicity of AcP^{2–}, compared with that of AnP^{2–} (Table 1, column 3), Sr(AcP) and Ba(AcP) are more stable than the corresponding M(AnP) species. The stability of the Ca²⁺ complexes is the same with both ligands (see Table 2), and with Mg²⁺ and of all the other M²⁺ studied, the M(AnP) complexes are more stable, as is actually expected, because of the higher basicity of AnP^{2–}.

(43) For M(AcP) see Section 1. The published values²⁰ for log K_{Mg(AnP)}^{Mg} vary from 1.30 to 2.54.

(44) (a) Sigel, H.; McCormick, D. B. *Acc. Chem. Res.* **1970**, *3*, 201–208. (b) Irving, H.; Williams, R. J. P. *Nature* **1948**, *162*, 746–747. Irving, H.; Williams, R. J. P. *J. Chem. Soc.* **1953**, 3192–3210.

The "special properties" of the M(AcP) complexes with Ba²⁺, Sr²⁺, Ca²⁺, and Mg²⁺ will be discussed further in Section 3.4.

At this point it needs to be emphasized that the "increased" stabilities, which are especially pronounced for the Ba(AcP) and Sr(AcP) complexes (see Table 2), are real. They are not an artifact, for example, due to the phosphate impurity present in the AcP systems (cf. Section 2.2) because (i) this impurity was taken into account in the calculations for the stability constants as described in Section 2.6 and (ii), more revealing, the stability of the M(HPO₄) complexes is much lower for Ba²⁺ (log $K_{\text{Ba}(\text{HPO}_4)}^{\text{Ba}} = 1.36 \pm 0.02$)²² than for Mg²⁺ (log $K_{\text{Mg}(\text{HPO}_4)}^{\text{Mg}} = 1.83 \pm 0.03$)²²; in other words, the complexes of hydrogen phosphate²² show the common stability trend and can therefore not be responsible for the described observation.

3.3. Proof of an Increased Stability of M(AcP) and M(AnP) Complexes. The primary binding site for M²⁺ in M(AcP) and M(AnP) complexes is the phosph(on)ate group. The question which remains to be answered is the following: Does the carbonyl oxygen of the acetyl or acetonil residues also participate in M²⁺ binding and does the six-membered chelate indicated in equilibrium 4 form to some extent? Since any kind of chelate formation has to enhance complex stability,⁴⁵ an increased stability of M(AcP) or M(AnP) complexes, if compared with a pure phosph(on)ate coordination, has to be attributed to the participation of the carbonyl oxygen; that is, equilibrium 4 would then truly exist. The position of this concentration-independent equilibrium between an "open", M(R-PO₃)_{op}, and a "closed", M(R-PO₃)_{cl}, species is defined by the dimensionless constant K_1 :

$$K_1 = [\text{M}(\text{R}-\text{PO}_3)_{\text{cl}}]/[\text{M}(\text{R}-\text{PO}_3)_{\text{op}}] \quad (9)$$

It is evident that by taking into account the formation of the two isomeric complexes M(R-PO₃)_{op} and M(R-PO₃)_{cl} equilibrium 8a may be rewritten as given in eq 10. Correspondingly for eq 8b follows eq 11 and by taking into account also eq 9, eq 12 results,



$$K_{\text{M}(\text{R}-\text{PO}_3)}^{\text{M}} = \frac{[\text{M}(\text{R}-\text{PO}_3)]}{[\text{M}^{2+}][\text{R}-\text{PO}_3^{2-}]} = \frac{[\text{M}(\text{R}-\text{PO}_3)_{\text{op}}] + [\text{M}(\text{R}-\text{PO}_3)_{\text{cl}}]}{[\text{M}^{2+}][\text{R}-\text{PO}_3^{2-}]} \quad (11)$$

$$K_{\text{M}(\text{R}-\text{PO}_3)}^{\text{M}} = K_{\text{M}(\text{R}-\text{PO}_3)_{\text{op}}}^{\text{M}} + K_1 K_{\text{M}(\text{R}-\text{PO}_3)_{\text{op}}}^{\text{M}} \quad (12)$$

where $K_{\text{M}(\text{R}-\text{PO}_3)_{\text{op}}}^{\text{M}}$ quantifies the stability of the open isomer. If M(R-PO₃)_{cl} does not form, $K_1 = 0$, and hence, the (requested) increased stability disappears and $K_{\text{M}(\text{R}-\text{PO}_3)}^{\text{M}} = K_{\text{M}(\text{R}-\text{PO}_3)_{\text{op}}}^{\text{M}}$.

The values of $K_{\text{M}(\text{R}-\text{PO}_3)_{\text{op}}}^{\text{M}}$ needed for the above evaluation are not accessible by a simple direct experimental determination. However, this problem was recently resolved²⁷ by constructing log $K_{\text{M}(\text{R}-\text{PO}_3)}^{\text{M}}$ versus $\text{p}K_{\text{H}(\text{R}-\text{PO}_3)}^{\text{H}}$ plots for M(R-PO₃) complexes, where R-PO₃²⁻ are simple phosph(on)ate ligands; that is, their residue R cannot participate in complex formation. Plots of this type result in straight lines as expected;^{45a} the parameters of these reference lines (least-squares) are summarized in table 5 of ref 27b (cf. also table 3 in ref 27a). This achievement allows now the calculation of the stability constant for a pure phos-

ph(on)ate coordination with the known acidity constant of any monoprotonated phosph(on)ate group.

With these reference-line equations and $\text{p}K_{\text{H}(\text{R}-\text{PO}_3)}^{\text{H}}$ of H(AcP)⁻ and H(AnP)⁻ (Table 1), the logarithms of the stability constants, log $K_{\text{M}(\text{R}-\text{PO}_3)_{\text{op}}}^{\text{M}}$, for the open isomers M(AcP)_{op} and M(AnP)_{op} were calculated (Table 2, columns 3 and 6). Now the differences between the measured and the calculated stability constants can be formed according to eq 13,

$$\log \Delta = \log \Delta_{\text{M}(\text{R}-\text{PO}_3)} = \log K_{\text{M}(\text{R}-\text{PO}_3)}^{\text{M}} - \log K_{\text{M}(\text{R}-\text{PO}_3)_{\text{op}}}^{\text{M}} \quad (13)$$

(see columns 4 and 7 of Table 2). All of these stability differences, except for Ba(AnP) and Sr(AnP), which are zero within their error limits, are clearly positive; hence, an increased stability is observed for most of the M(AcP) and M(AnP) complexes meaning that the carbonyl oxygen participates in complex formation and equilibrium 4 exists.

The described result probably becomes more apparent from the four examples of log $K_{\text{M}(\text{R}-\text{PO}_3)}^{\text{M}}$ versus $\text{p}K_{\text{H}(\text{R}-\text{PO}_3)}^{\text{H}}$ plots in Figure 2:⁴⁶ The data pairs for the Ba²⁺, Mg²⁺, Zn²⁺, and Cu²⁺ complexes with AcP²⁻ and those for Mg²⁺, Zn²⁺, and Cu²⁺ with AnP²⁻ are clearly above the reference lines. Only the data pair for Ba(AnP) falls within the error limits on its line.

3.4. Extent of Chelate Formation in Aqueous Solution for M(AcP) and M(AnP) Complexes. With the results described above in mind, it is evident that values for the intramolecular equilibrium constant K_1 (eq 9) have to be the aim. Indeed, from eq 12 a further definition for K_1 (eq 14) follows in which the stability difference log Δ is defined by eq 13:

$$K_1 = \frac{K_{\text{M}(\text{R}-\text{PO}_3)}^{\text{M}}}{K_{\text{M}(\text{R}-\text{PO}_3)_{\text{op}}}^{\text{M}}} - 1 = 10^{\log \Delta} - 1 \quad (14)$$

The constant K_1 can now be obtained because $K_{\text{M}(\text{R}-\text{PO}_3)}^{\text{M}}$ is known (Table 2, columns 2 and 5) and $K_{\text{M}(\text{R}-\text{PO}_3)_{\text{op}}}^{\text{M}}$ was calculated as described (Section 3.3; Table 2, columns 3 and 6).

The vertical distances indicated by broken lines in Figure 2 are identical with the stability differences log $\Delta_{\text{M}(\text{R}-\text{PO}_3)}$ as defined in eq 13 and listed in columns 4 and 7 of Table 2. Clearly, the reliability of any calculation for K_1 (eq 14) depends on the accuracy of the difference log $\Delta_{\text{M}(\text{R}-\text{PO}_3)}$, which becomes more important the more similar the two constants in eq 13 are. Therefore, only well-defined error limits allow a quantitative evaluation of the extent of the possibly formed six-membered chelate. Finally, if K_1 is known, the percentage of the closed (chelated) species occurring in equilibrium 4 follows from eq 15:

$$\% \text{M}(\text{R}-\text{PO}_3)_{\text{cl}} = 100K_1/(1 + K_1) \quad (15)$$

Application of this procedure^{27,45} yields the results of Table 3. The values in column 4 show that for all the M(AcP) and M(AnP) species substantial percentages of chelates are formed; only for Ba(AnP) and Sr(AnP) they are zero within the error limits.

Comparisons of the results in Table 3 show that the formation degrees of chelates formed between Mn²⁺, Co²⁺, Ni²⁺, or Cd²⁺ and AcP²⁻ or AnP²⁻ are identical within the error limits for a given M²⁺. In contrast, the M(AcP) complexes of the alkaline earth ions and of Cu²⁺ or Zn²⁺ have larger chelate formation

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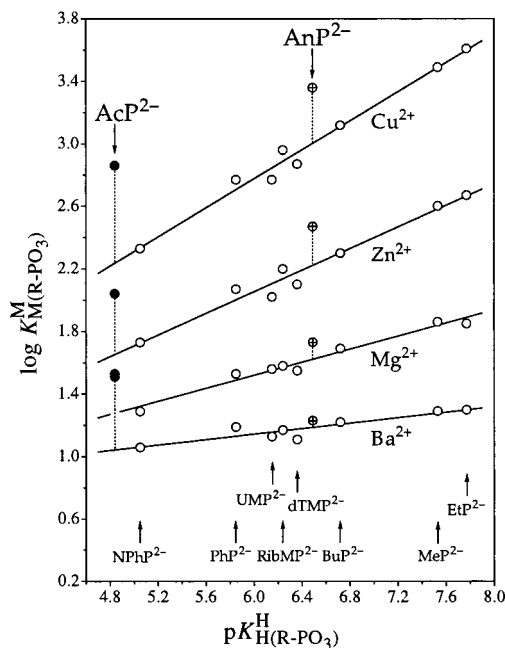


Figure 2. Evidence for an enhanced stability of the M(AcP) (●) complexes with Ba²⁺, Mg²⁺, Zn²⁺, and Cu²⁺ [the data points for the Mg²⁺ and Ba²⁺ complexes are nearly on top of each other; Ba(AcP) is slightly more stable than Mg(AcP)] and of several M(AnP) (○) complexes based on the relationship between $\log K_{M(R-PO_3)}^M$ and $pK_{H(R-PO_3)}^H$ for the 1:1 complexes of Ba²⁺, Mg²⁺, Zn²⁺, and Cu²⁺ with some simple phosphate monoesters or phosphonate ligands (R-PO₃²⁻): 4-nitrophenyl phosphate (NPhP²⁻), phenyl phosphate (PhP²⁻), uridine 5'-monophosphate (UMP²⁻), D-ribose 5-monophosphate (RibMP²⁻), thymidine 5'-monophosphate (dTMP²⁻), *n*-butyl phosphate (BuP²⁻), methylphosphonate (MeP²⁻), and ethylphosphonate (EtP²⁻) (from left to right) (○). The least-squares lines are drawn through the corresponding eight data sets, which are taken for the phosphate monoesters from ref 46 and for the phosphonates from ref 27b; the equations for these reference lines are listed in refs 27 and 28. The data points due to the equilibrium constants for the AcP and AnP systems are based on the data given in Tables 1 and 2. The vertical dotted lines emphasize the stability differences of the M(AcP) (●) and M(AnP) (○) complexes to the corresponding reference lines; these differences are equal to $\log \Delta_{M(R-PO_3)}$ (eq 13), the values of which are listed in columns 4 and 7 of Table 2. All of the plotted equilibrium constant values refer to aqueous solutions at 25 °C and *I* = 0.1 M (NaNO₃).

degrees. Maybe the reason is, especially in the case of Cu²⁺ and Zn²⁺ which usually do not have a regular octahedral coordination sphere,⁴⁷ the smaller angle around the “anhydride” O (assumed to be close to 104° as in H₂O) in AcP²⁻ compared with the C-(CH₂)-P angle (assumed to be close to 109° as in CH₄) in AnP²⁻. Theoretical calculations⁴⁸ also indicate that the CH₃C(O)-O bond, that is, the one to the “anhydride” O, has some double bond contribution, which is meaningful for a preorientation of the ligand toward complex formation.

Most intriguing, however, is the situation of the alkaline earth ion complexes. Those with AnP²⁻ show the usual properties (Section 3.2), whereas the complexes with AcP²⁻ show not only a relatively high formation degree of the chelates but also a surprising order for the stability increase $\log \Delta_{M(AcP)}$, that is, Mg²⁺ < Ca²⁺ < Sr²⁺ < Ba²⁺ (Table 3, column 2). This order parallels the ionic radii, and therefore it cannot be explained

Table 3. Increased Complex Stability, $\log \Delta_{M(R-PO_3)}$ (Equation 13), and Extent of Chelate Formation (Equation 4) in the M(AcP) (upper part) and M(AnP) Complexes (lower part) as Quantified by the Dimensionless Equilibrium Constant K_I (Equations 9 and 14) and the Percentage of M(R-PO₃)_{cl} (Equation 15) for Aqueous Solutions at 25 °C and *I* = 0.1 M (NaNO₃)^a

M ²⁺	$\log \Delta_{M(AcP)}^b$	K_I	% M(AcP) _{cl}
Mg ²⁺	0.23 ± 0.04	0.70 ± 0.14	41 ± 5
Ca ²⁺	0.28 ± 0.06	0.91 ± 0.28	48 ± 8
Sr ²⁺	0.34 ± 0.04	1.19 ± 0.23	54 ± 5
Ba ²⁺	0.49 ± 0.05	2.09 ± 0.36	68 ± 4
Mn ²⁺	0.12 ± 0.06	0.32 ± 0.18	24 ± 10
Co ²⁺	0.20 ± 0.08	0.58 ± 0.29	37 ± 11
Ni ²⁺	0.14 ± 0.06	0.38 ± 0.20	28 ± 11
Cu ²⁺	0.62 ± 0.08	3.17 ± 0.75	76 ± 4
Zn ²⁺	0.39 ± 0.07	1.45 ± 0.38	59 ± 6
Cd ²⁺	0.19 ± 0.06	0.55 ± 0.23	35 ± 10

M ²⁺	$\log \Delta_{M(AnP)}^b$	K_I	% M(AnP) _{cl}
Mg ²⁺	0.11 ± 0.04	0.29 ± 0.11	22 ± 6
Ca ²⁺	0.06 ± 0.05	0.15 ± 0.13	13 ± 10
Sr ²⁺	0.05 ± 0.06	0.12 ± 0.17	11 ± 13
Ba ²⁺	0.04 ± 0.06	0.10 ± 0.14	9 ± 12
Mn ²⁺	0.13 ± 0.05	0.35 ± 0.17	26 ± 9
Co ²⁺	0.19 ± 0.06	0.55 ± 0.23	35 ± 9
Ni ²⁺	0.13 ± 0.05	0.35 ± 0.17	26 ± 9
Cu ²⁺	0.36 ± 0.07	1.29 ± 0.35	56 ± 7
Zn ²⁺	0.25 ± 0.07	0.78 ± 0.30	44 ± 9
Cd ²⁺	0.18 ± 0.06	0.51 ± 0.20	34 ± 9

^a See footnote a of Table 2. ^b Values from columns 4 and 7 of Table 2.

by these; however, the hydrated radii⁴⁹ show the reverse order, that is, Ba²⁺ has the smallest and Mg²⁺ the largest one. Consequently, this indicates that complex formation occurs, at least to some extent, in an outersphere fashion. If this interpretation is correct, then hydrogen bonding is important and here AcP²⁻ is clearly to be favored over AnP²⁻ because the “anhydride” oxygen may participate in it whereas the methylene group is unable to do so. Into this same argument also fits that the methylene group will hinder solvation of the complex whereas the “anhydride” oxygen will favor it.

However, overall it is clear that in both complex series, M(AcP) and M(AnP), chelate formation plays a significant role and that this is even somewhat more pronounced in the M(AcP) species (Table 3, column 4).⁵⁰ In this context, recent ab initio molecular orbital calculations⁴⁸ carried out for AcP²⁻ at the STO-3G level in the gas phase are of interest. These calculations provided on average a net atomic charge of -0.697 for each of the three terminal oxygens of the -PO₃²⁻ group and a charge of -0.473 for the carbonyl oxygen of the acetyl residue. This relatively high charge density on the carbonyl oxygen makes this atom evidently quite suitable for an interaction with metal ions, and in fact, this is what the present results prove.

With the above conclusions in mind, we carried out some semiempirical calculations for AcP²⁻ and AnP²⁻ in the gas phase with the aim to see whether the charge density on the carbonyl oxygen in the two ligands (see Figure 1) differs significantly. The calculations based on the PM3 method of

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(49) *Gmelins Handbuch der Anorganischen Chemie*, 8. völlig neu bearbeitete Auflage: Verlag Chemie GmbH, Weinheim: (a) *Magnesium* (Syst.-Nr. 27), Teil A, 1952; p 172. (b) *Calcium* (Syst.-Nr. 28), Teil A, 1957; p 390. (c) *Strontium* (Syst.-Nr. 29), 1931; p 40. (d) *Barium* (Syst.-Nr. 30), 1932; p 39.

(50) It is worthwhile to note that the changes in energy involved in this kind of chelate formation are very small:^{45a} for example, $\Delta G^\circ = -0.57$ kJ/mol corresponds to $\log \Delta = 0.10$ (eqs 13, 14) and a formation degree of already 20% for the closed species (eq 4). When half of the complexes (i.e., 50%) exist as chelates, $\log \Delta = 0.30$ and $\Delta G^\circ = -1.71$ kJ/mol. For further details on these interrelations, ref 45a should be consulted.

Table 4. Negative Logarithms of the Acidity Constants (Equation 7)^a for H(AcP)⁻ and H(AnP)⁻ as Determined by Potentiometric pH Titrations in Water^b and in Water Containing 30% or 50% (v/v) 1,4-dioxane at 25 °C and *I* = 0.1 M (NaNO₃), Together with Some Properties^c of the Mentioned Solvents

H(R-PO ₃) ⁻	% (v/v) dioxane	mol fraction	ε ^c	pK _{H(R-PO₃)}} ^H
H(AcP) ⁻	0	0	78.5	4.84 ± 0.02 ^b
	30	0.083	52.7	5.56 ± 0.01
	50	0.175	35.2	6.03 ± 0.02
H(AnP) ⁻	0	0	78.5	6.49 ± 0.02 ^b
	30	0.083	52.7	7.23 ± 0.01
	50	0.175	35.2	7.67 ± 0.02

^a See footnote a of Table 1. No "corrections" were applied for the change in solvent from water to the dioxane-water mixtures, though correction factors have been published for such⁵² and related mixtures.⁵³ In this context it should be noted that the *difference* of pairs of titrations, that is, with and without R-PO₃, was evaluated (see Sections 2.3 and 2.4).³⁵ ^b Values from Table 1. ^c The dielectric constants (ε) for the dioxane-water mixtures are interpolated from the data given in ref 54.

Stewart³⁹ yielded ESP charges for the carbonyl O of -0.611 and -0.571 for AcP²⁻ and AnP²⁻, respectively; application of the AM1 technique of Dewar et al.⁴⁰ gave -0.604 and -0.550. Hence, the charge density on the carbonyl O in the two ligands is rather similar, the O of AcP²⁻ being only slightly favored, and consequently, the above conclusions regarding the importance of hydrogen bonding and solvation in several of the M(AcP) complexes are supported.

3.5. A Reduced Solvent Polarity Promotes Chelate Formation! Since charge-type interactions in the M(AcP) and M(AnP) complexes are clearly important, we also studied the effect of a decreasing solvent polarity on the stability of the Cu(AnP) complex. Cu(AcP) was not considered directly because the stability of the Cu(HPO₄) complex in the mixed solvents is not known, but these data would be needed for a careful evaluation (see Section 2.6).

Increasing amounts of 1,4-dioxane in an aqueous solution are expected to render solvation of metal ions more difficult, and hence, the participation of the carbonyl oxygen in complex formation should become promoted. Cu²⁺ was selected for these measurements because reference lines for log K_{Cu(R-PO₃)}}^{Cu} versus pK_{H(R-PO₃)}}^H plots for simple phosph(on)ate ligands with a noncoordinating group have previously been published⁵¹ for various water-dioxane mixtures.

The acidity constants (eq 7) determined for H(AcP)⁻ and H(AnP)⁻ using water and water containing 30% or 50% (v/v) 1,4-dioxane as solvent are listed in Table 4 together with some information on the solvents.⁵²⁻⁵⁴ AcP was included in these measurements, since any interference by phosphate can easily be detected; these constants are helpful to make estimations regarding the stability increases to be expected for Cu(AcP) in the various solvents (see below).

Table 4 shows that the effect of a decreasing solvent polarity on both H(AcP)⁻ and H(AnP)⁻ is quite dramatic; that is, the release of the proton from the -P(O)₂(OH)⁻ group is considerably

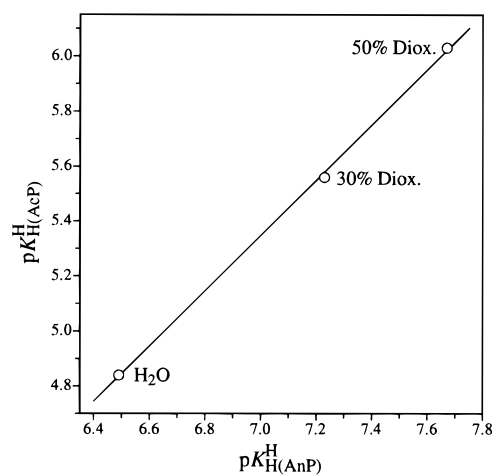


Figure 3. Plot of the negative logarithms of the acidity constants, pK_{H(R-PO₃)}}^H, of H(AcP)⁻ and H(AnP)⁻ versus each other. The points of all three data pairs fit exactly a straight line with a slope of one, which proves that the solvent effect (increasing amounts of 1,4-dioxane from the left to the right) is identical on both acids (see text in Section 3.5).

inhibited. In fact, this is expected: In a solvent of a lower polarity charge separation should become more difficult. It has to be emphasized that the solvent influence on the acidity constants of H(AcP)⁻ and H(AnP)⁻ is completely identical; that is, within the error limits the pK_{H(R-PO₃)}}^H values, if plotted toward each other, fit a straight line with a slope of one (Figure 3).

The stability of the Cu(AnP) complex has been determined in the same solvent mixtures. To be able to evaluate the solvent influence, one needs the solvent effect on a sole phosphonate-metal ion coordination. The corresponding reference lines for log K_{Cu(R-PO₃)}}^{Cu} versus pK_{H(R-PO₃)}}^H plots have previously been constructed^{51a} and their straight-line equations, which are based on the various simple Cu²⁺/R-PO₃ systems defined in the legend of Figure 4, are given below:^{51b,c}

$$\text{water: } \log K_{\text{Cu(R-PO}_3\text{)}}^{\text{Cu}} = (0.465 \pm 0.025)pK_{\text{H(R-PO}_3\text{)}}^{\text{H}} - (0.015 \pm 0.164) \quad (16)$$

$$30\% \text{ diox.: } \log K_{\text{Cu(R-PO}_3\text{)}}^{\text{Cu}} = (0.559 \pm 0.015)pK_{\text{H(R-PO}_3\text{)}}^{\text{H}} - (0.089 \pm 0.106) \quad (17)$$

$$50\% \text{ diox.: } \log K_{\text{Cu(R-PO}_3\text{)}}^{\text{Cu}} = (0.571 \pm 0.022)pK_{\text{H(R-PO}_3\text{)}}^{\text{H}} + (0.190 \pm 0.160) \quad (18)$$

The error limits of log stability constants calculated with given pK_{H(R-PO₃)}}^H values and eqs 16, 17, and 18 are ±0.06, ±0.03, and ±0.03 log units (3σ), respectively.⁵¹

The straight lines defined by eqs 16, 17, and 18 are shown in Figure 4 together with the results obtained for Cu²⁺/AnP in the various solvents. Cu(AnP) is in all instances more stable than expected for a sole phosphate coordination. In fact, from the vertical distances (broken lines) of the data points due to Cu(AnP) to the corresponding reference lines, it can be seen that its relative stability increases with decreasing solvent polarity. These qualitative impressions are quantified in Table 5, where column 2 provides the experimentally measured stability constants and column 3 provides the calculated ones based on eqs 16, 17, and 18 and the pK_{H(AnP)}}^H values of Table 4. The stability differences log Δ_{Cu(AnP)} clearly increase with increasing dioxane concentration, indicating that equilibrium 4 is progressively shifted toward its right-hand side.

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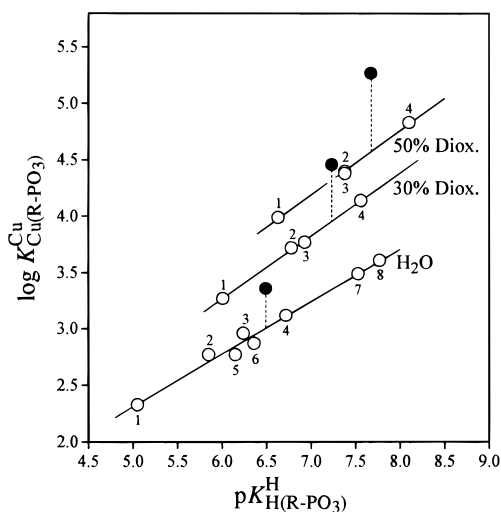


Figure 4. Evidence for an enhanced stability of the Cu(AnP) complex (●) in 1,4-dioxane–water mixtures as solvents based on the relationship between $\log K_{\text{CuCu(R-PO}_3\text{)}}^{\text{Cu}}$ and $\text{p}K_{\text{H(R-PO}_3\text{)}}^{\text{H}}$ for the Cu^{2+} 1:1 complexes of 4-nitrophenyl phosphate (1), phenyl phosphate (2), D-ribose 5-monophosphate (3), *n*-butyl phosphate (4), uridine 5'-monophosphate (5), thymidine 5'-monophosphate (6), methylphosphonate (7), and ethylphosphonate (8) in water and in water containing 30% or 50% (v/v) 1,4-dioxane. The least-squares lines are drawn in each case through the data sets shown (○); ^{27b,51a} these reference lines^{27,51} are defined in eqs 16–18. It needs to be emphasized here that the data pairs for Cu^{2+} /methylphosphonate or ethylphosphonate also fit within their error limits on the straight lines valid for the mixed solvents (eqs 17, 18),^{51b,c} thus proving that these reference lines also hold for phosphonates. The data pairs for the points due to the Cu^{2+} 1:1 complexes formed with AnP^{2-} (●) in the three mentioned solvents are taken from Tables 4 and 5. The vertical broken lines emphasize the stability differences to the corresponding reference lines; these differences are equal to $\log \Delta_{\text{Cu(AnP)}}$ (eq 13), the values of which are listed in column 4 of Table 5. All of the plotted equilibrium constants refer to 25 °C and $I = 0.1 \text{ M}$ (NaNO_3).

Table 5. Logarithms of the Stability Constants of the Cu(AnP) Complex (Equation 8) as Determined by Potentiometric pH Titrations (exptl)^a in Water^b and in Water Containing 30% or 50% (v/v) 1,4-Dioxane at 25 °C and $I = 0.1 \text{ M}$ (NaNO_3), Together with the Corresponding Calculated Stability Constants for a Sole Phosphonate Coordination of Cu^{2+} (Calcd)^{a,c}

% (v/v) dioxane	$\log K_{\text{Cu(AnP)}}^{\text{Cu}}$		$\log \Delta_{\text{Cu(AnP)}}^d$	K_1	% Cu(AnP) _{cl}
	exptl	calcd ^c			
0 ^b	3.36 ± 0.03	3.00 ± 0.06	0.36 ± 0.07	1.29 ± 0.35	56 ± 7
30	4.46 ± 0.03	3.95 ± 0.03	0.51 ± 0.04	2.24 ± 0.32	69 ± 3
50	5.27 ± 0.02	4.57 ± 0.03	0.70 ± 0.04	4.01 ± 0.42	80 ± 2

^a The observed stability increase (see also Figure 4) is quantified via $\log \Delta_{\text{Cu(AnP)}}$ (eq 13) (see footnote *d*) and the extent of chelate formation (eq 4) by the dimensionless equilibrium constant K_1 (Eqs 9 and 14) and the percentage of Cu(AnP)_{cl} (eq 15). See footnote *a* of Table 2. ^b The values for water as solvent are taken from Tables 2 and 3. ^c Calculated with the $\text{p}K_{\text{H(AnP)}}^{\text{H}}$ values of Table 4 and the straight-line eqs 17 and 18. ^d See footnote *c* of Table 2.

Of course, the values for $\log \Delta_{\text{Cu(AnP)}}$ of Table 5 (column 4) can now be used to calculate the dimensionless constant K_1 (eq 9) of equilibrium 4 according to eq 14 (column 5) and then with eq 15 also the percentage of the chelated isomer Cu(AnP)_{cl} (column 6). These results confirm the mentioned expectation that, with decreasing solvent polarity, the participation of the carbonyl oxygen in complex formation increases, and they are also in accord with previous results⁵⁵ regarding the coordinating properties of dihydroxyacetone phosphate.

(55) Liang, G.; Chen, D.; Bastian, M.; Sigel, H. *J. Am. Chem. Soc.* **1992**, *114*, 7780–7785.

The strictly equivalent effect of the solvent changes on the acidity constants of H(AcP)^- and H(AnP)^- (see Figure 3) allows the conclusion that in a first approximation this should also be true for the stability differences, $\log \Delta_{\text{M(R-PO}_3\text{)}}$, which quantify the participation of the carbonyl group in metal ion binding. Consequently, the experimental results obtained for Cu(AnP) (Table 5) allow one to calculate (estimate)⁵⁶ $\log \Delta_{\text{Cu(AcP)}} = 0.62$ (taken from Table 2), 0.88 (cf.⁵⁶), and 1.21 for the solvents water and water containing 30% and 50% 1,4-dioxane, respectively. From these values follows then the formation degree for Cu(AcP)_{cl} which amounts to 76% (Table 3), 87%, and 94%, respectively, in the mentioned solvents. Similar estimates would be possible for other M^{2+} systems; in any case, it can be concluded that the metal ion interaction with the carbonyl oxygen will be promoted in the mixed solvents for all metal ions considered.

4. Conclusions

The stability of the complexes formed between M^{2+} and AcP^{2-} or AnP^{2-} (Figure 1) is largely governed by the metal ion affinity of the phosph(on)ate group (Figure 2). However, the present study proves that the carbonyl O is also able to interact with metal ions, thus giving rise to the formation of six-membered chelates (eq 4), the formation degree of which can be promoted by a reduced solvent polarity as shown for Cu(AnP) in water containing 30% or 50% (v/v) 1,4-dioxane.

The observation that chelate formation is favored under conditions of a lower polarity with poorer solvating properties than those of water is meaningful for biological systems. Since the so-called “effective” or “equivalent solution” dielectric constants in proteins^{57,58} or in active-site cavities of enzymes²⁹ are reduced compared to the situation in bulk water, the probability for a carbonyl oxygen–metal ion interaction is favored exactly at those locations where AcP^{2-} (or AnP^{2-}) is employed as substrate. It may be pointed out that a decrease in the dielectric constant from about 80 to 35, as it occurs by going from water to water containing 50% dioxane (Table 4), is also reached in active site cavities of enzymes.²⁹

From the results summarized in Tables 4 and 5 it is evident that a shift of a substrate by a few Ångströms at the “surface” of a protein may drastically alter the acid–base properties of a phosphate group as well as the metal ion affinity of a carbonyl oxygen. It needs to be emphasized that weak interactions between metal ions and carbonyl oxygen atoms (or oxygens of hydroxy groups)⁵⁵ on one hand and nitrogen atoms on the other, as they occur for example in the formation of macrochelates with purine nucleotides where a phosphate-coordinated M^{2+} may also interact with N7,^{27a,28b} are affected very differently by a decreasing solvent polarity.⁵⁹

This is demonstrated in Figure 5, where the formation degree of the six-membered chelate of Cu(AnP) is plotted as a function of the percentage of 1,4-dioxane added to water and where the solvent effect⁵⁹ on the formation degree of the macrochela^{45b}

(56) Example for the estimate: $\log \Delta_{\text{Cu(AcP)}} / \log \Delta_{\text{Cu(AnP)}} = 0.62 / 0.36$ [for water; Table 2] = $x / 0.51$ [for 30% diox.; Table 5]; hence, $x = 0.88 = \log \Delta_{\text{Cu(AcP)}}$ for 30% dioxane.

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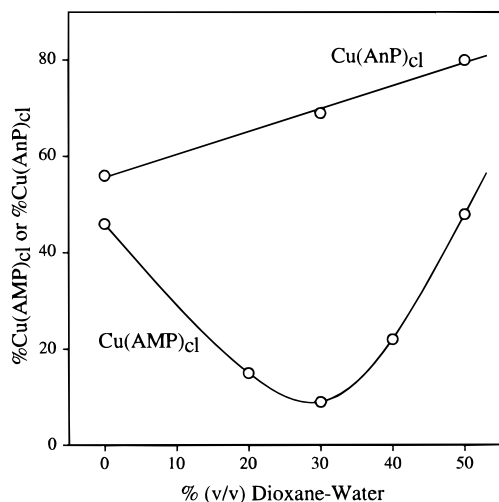


Figure 5. Formation degree of the chelated isomers in the Cu(AnP) and Cu(AMP) complex systems as a function of the percentage of 1,4-dioxane added to the aqueous reagent mixtures at 25 °C and $I = 0.1$ M (NaNO₃). Cu(AMP)_{cl} represents the macrochelate^{27a,28b,45b} involving the phosphate group and N7 of the adenine residue; the data are from ref 59. Cu(AnP)_{cl} corresponds to the six-membered chelate in equilibrium 4; the data are from Table 5 (see also text in Section 3.5).

formed by the phosphate-coordinated Cu²⁺ in its adenosine 5'-monophosphate (AMP²⁻) complex with N7 of the adenine residue is also shown. The formation degree of the macrochelate involving N7 passes through a minimum in its dependence on

the solvent composition,⁵⁹ whereas the formation degree of the six-membered chelate with the carbonyl O atom continuously increases (Table 5). These results provide a hint for the subtle ways in which nature may alter the structure of a substrate for a recognition reaction.⁵⁰

A final point to be emphasized with regard to AcP²⁻ is that metal ions such as Mg²⁺, Ca²⁺, or Zn²⁺, that is, those which generally occur in enzymatic systems involved in transphosphorylations, possess already a quite pronounced affinity toward the carbonyl O in aqueous solution (Table 3), and therefore, it is safe to assume that the effects described for Cu²⁺ apply also to these M²⁺. One may further mention here that the metal ion-promoted hydrolysis of alanyl ethyl phosphate, a mixed anhydride of alanine and phosphoric acid, possibly also involves bidentate metal ion binding,⁶⁰ thus indicating that the presented data are of relevance beyond AcP²⁻. In addition, the involvement of the carbonyl O in M²⁺ binding in mixed ligand (ternary) complexes⁶¹ corresponds to that described for binary complexes; this observation is important with regard to the formation of enzyme-metal ion-substrate complexes. It is thus evident that acetyl phosphate, despite its seemingly simple structure, is a ligand with many intriguing facets regarding its coordination chemistry.

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