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Intramolecular chelate formation involving the carbonyl oxygen of acetyl phosphate or acetonylphosphonate in mixed ligand copper(II) complexes containing also 2,2′-bipyridine or 1,10-phenanthroline. A decreased solvent polarity favours the metal ion–carbonyl oxygen recognition†

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The stability constants of the mixed ligand complexes formed by Cu^{2+}, 2,2′-bipyridine or 1,10-phenanthroline (= Arm) and acetyl phosphate (AcP^{2-}) or acetonylphosphonate (AnP^{2-}) were determined by potentiometric pH titrations in water and in water containing 30 or 50% (v/v) 1,4-dioxane (25 °C; $I = 0.1$ M, $NaNO_3$). Previous measurements with simple phosph(on)ate ligands, $R\text{-}PO_3^{2-}$ (R being a non-interacting residue), had established log $K^{Cu(Arm)}_{Cu(Arm)(R\text{-}PO_3)}$ *versus* $pK^{H}_{H(R\text{-}PO_3)}$ straight-line plots and these were used now to prove that the Cu(Arm)(AcP) and Cu(Arm)(AnP) complexes possess a higher stability than is expected for a sole phosph(on)ate–Cu^{2+} coordination. This increased stability is attributed to the formation of six-membered chelates involving the carbonyl oxygen present in AcP^{2-} and AnP^{2-}. The formation degree of the six-membered chelates in the Cu(AcP), Cu(Bpy)(AcP), and Cu(Phen)(AcP) systems is very close to 75% in all three cases. For the corresponding systems with AnP^{2-} it is shown that increasing amounts of 1,4-dioxane added to aqueous solutions favour the formation of the six-membered chelates in both the binary and the ternary complexes. It is concluded with regard to biological systems that such six-membered chelates will also be formed in mixed ligand complexes of other metal ions and that their formation degree will also be favoured by a reduced solvent polarity; both points are relevant for the situation in active-site cavities of enzymes.

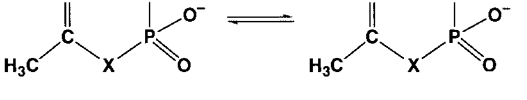
1. Introduction

Acetyl phosphate (AcP^{2-}),[2] which is derived from acetic acid and orthophosphoric acid, is one of the so-called "energy-rich" anhydrides. It plays a significant role in biology and participates in many regulatory systems;[3] *e.g.*, in the activation of the phosphate regulon,[4,5] in flagellar expression,[6] or in the anaerobic respiratory gene expression of *Escherichia coli*.[7] Acetyl phosphate is also a non-nucleotide substrate of the sarcoplasmic reticulum Ca^{2+}-ATPase, where it provides the energy for the active accumulation of Ca^{2+};[8] furthermore, it is an important factor in the regeneration of ATP.[4,9,10] Together with acetate kinase, acetyl phosphate forms the best enzyme/substrate pair for the commercial synthesis of ATP.[11]

In all the indicated roles and reactions of acetyl phosphate, enzymes are involved, and in general, enzymes participating in phosphate-transfer reactions also depend on the presence of metal ions.[3*c*,*d*,9,12] Hence, the metal ion-binding properties of AcP^{2-} are of interest and indeed, we measured recently[13] the stability constants of various M(AcP) complexes including those with the alkaline earth ions and the divalent ions of the second half of the 3d series *via* potentiometric pH titrations.[13] The stability determining binding site of acetyl phosphate is mostly the phosphate group, however, it became clear that the carbonyl oxygen atom of the acetyl residue may participate as well in complex formation, giving rise to six-membered chelates.[13]

Since acetyl phosphate is a rather reactive compound which easily undergoes hydrolysis in aqueous solution,[13,14] we included in our studies acetonylphosphonate (AnP^{2-}), which has a similar structure without being hydrolysis-sensitive. Of course, AnP also merits to be investigated in its own rights, since it is a biologically active compound. For example, it acts as a non-competitive inhibitor of D-3-hydroxybutyrate dehydrogenase[15] and it is a substrate for the P–C bond-cleaving enzyme phosphonoacetaldehyde hydrolase, forming hydrogen phosphate and acetone.[16]

For both compounds, *i.e.* AcP^{2-} and AnP^{2-}, we determined[13] for several metal ions the position of the following intramolecular equilibrium (1), where X = O or CH_2, respectively, in aqueous solution:



(1)

For example, the formation degree of the six-membered chelate amounts for Mg(AcP) and Cu(AcP) to 41 ± 5% and 76 ± 4%, respectively, and for Mg(AnP) and Cu(AnP) to 22 ± 6% and 56 ± 7%.

Since in an enzymatic reaction complex formation at the active site often involves several species,[12] the formation of higher order complexes is of interest. We studied as model systems the mixed ligand complexes consisting of Cu^{2+}, AcP^{2-}

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† This is part 64 of the series 'Ternary Complexes in Solution'; for parts 63 and 62 see ref. 1.

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or AnP^{2-}, plus the heteroaromatic amines (Arm) 2,2′-bipyridine (Bpy) or 1,10-phenanthroline (Phen) as further strongly coordinating ligands. Owing to the very high stability of the binary $Cu(Bpy)^{2+}$ and $Cu(Phen)^{2+}$ complexes,[17] only two positions for binding of AcP^{2-} or AnP^{2-} remain in the equatorial coordination sphere[18] of Cu^{2+} and the question then is: Does the carbonyl oxygen of acetyl phosphate or of acetonylphosphonate still participate in complex formation under such limiting conditions?

Considering that in active site cavities of enzymes[19] and metalloproteins[20] the so-called "effective" or "equivalent solution" dielectric constants (relative permittivities) are reduced compared with the situation in bulk water, *i.e.* the activity of water is decreased[21] due to the presence of aliphatic and aromatic amino acid side chains at the protein–water interface, experiments under conditions of a reduced solvent polarity were also carried out by adding 1,4-dioxane to the aqueous reagent mixtures, to simulate in this way to some degree the situation in active-site cavities.[22] In fact, the "equivalent solution" relative permittivity in active-site cavities of certain enzymes is estimated to be of the order of 35, a value[19] which corresponds to an aqueous solution containing 50% (v/v) of 1,4-dioxane.[23]

There are two main reasons for selecting Cu^{2+} as metal ion for the present study: (*i*) For the evaluation of the effect of the carbonyl oxygen on complex stability plots of log $K^{M}_{M(R\text{-}PO_3)}$ *versus* p$K^{H}_{H(R\text{-}PO_3)}$ for simple phosph(on)ate ligands, $R\text{-}PO_3^{2-}$, where R represents a non-interacting group, are needed and these are available for this metal ion not only for the solvent water[24,25] but also for water containing various amounts of 1,4-dioxane.[26,27] (*ii*) Acetyl phosphate is always contaminated with some phosphate[13] and this needs to be considered in the calculations for the stabilities of the complexes;[13] since we determined recently not only the stability constants of binary[28] $M(HPO_4)$ but also of the ternary $Cu(Bpy)(HPO_4)$ and $Cu(Phen)(HPO_4)$ complexes,[29] we were in the position to cope with this difficulty, at least for aqueous solutions and ternary Cu^{2+} complexes. The results presented now prove that equilibrium (1) also operates in the indicated mixed ligand complexes.

2. Experimental

The heteroaromatic amines (Arm) 2,2′-bipyridine and 1,10-phenanthroline monohydrate were obtained from Merck AG, Darmstadt, Germany. All the other reagents were identical with those used previously.[13]

The apparatus for the potentiometric pH titrations, the computers, the experimental and the calculation procedures used now are the same as given in ref. 13; the phosphate content of AcP was again carefully determined and taken into account in the evaluations (for details see Sections 2.2, 2.5 and 2.6 in ref. 13). The titration apparatus was calibrated with aqueous buffer solutions based on the NIST scale,[13] and for the titrations in all instances 1 mL of aqueous 0.03 M NaOH was used. The given acidity constants[13] are so-called practical, mixed or Brønsted constants;[30] no "corrections" were applied for the change in solvent from water to the dioxane–water mixtures, though correction factors have been published for such[31] and related mixtures.[32] In this context it should be noted that the *difference* of pairs of titrations, *i.e.* with and without $R\text{-}PO_3$, was evaluated;[13] due to this procedure the ionic product of water (K_w) does not enter into the calculations.[13] The stability constants presented are, as usual, concentration constants.

The stability constants of the ternary complexes in aqueous solution were determined exactly as described for the binary ones;[13] *i.e.*, AcP or AnP were 0.3 mM and ratios of $R\text{-}PO_3 : Cu^{2+} : Arm = 1 : 11 : 11$ and $1 : 5.6 : 5.6$ were employed. Similarly, for the experiments with water containing 30 or 50% (v/v) 1,4-dioxane, the AnP : (Cu^{2+}/Arm) ratios were 1 : 5.6 and 1 : 2.8. Under the experimental conditions the formation of the $Cu(Arm)^{2+}$ complexes is practically complete[17] (titrations of solutions with HNO_3 and HNO_3 plus Cu^{2+}/Arm were identical in the lower pH range) and therefore the evaluation of the titration data of the ternary systems could be done in the way described for the binary ones.[13] For each pair of titrations the data were evaluated every 0.1 pH unit in the pH range 3.6–5.0 for the aqueous systems with AcP; for the AnP systems the pH ranges were 3.8–5.4, 3.9–4.9 and 3.9–4.7 in water and in water containing 30 or 50% (v/v) 1,4-dioxane, respectively. The onset of the formation of hydroxo complexes was evident from the titrations without ligand.

The results listed for the equilibrium constants in the tables (*vide infra*) are the averages of the evaluations of at least six, usually eight, independent pairs of titration curves ($I = 0.1$ M, $NaNO_3$; 25 °C). The calculated stability constants of the ternary complexes showed neither a dependence on pH nor on the excess of Cu^{2+}/Arm employed.

3. Results and discussion

3.1. Stability of the ternary complexes formed between $Cu(Arm)^{2+}$ and AcP^{2-} or AnP^{2-}

The release of the first proton from twofold protonated phosph(on)ates, $H_2(R\text{-}PO_3)$, occurs at a rather low[13] pH and is therefore not of relevance in the present context.[13] The pertinent deprotonation equilibrium is the release of the second proton as expressed in equilibrium (2a):

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

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

The experimental data of the potentiometric pH titrations of the considered $Cu(Arm)^{2+}/R\text{-}PO_3$ systems are completely described by the mentioned eqn. (2) and the following complex formation (3), where M^{2+} represents Cu^{2+}, $Cu(Bpy)^{2+}$, or $Cu(Phen)^{2+}$, as long as the evaluation is not carried into the pH range where formation of hydroxo complexes occurs.

$$M^{2+} + R\text{-}PO_3^{2-} \rightleftharpoons M(R\text{-}PO_3) \qquad (3a)$$

$$K^{M}_{M(R\text{-}PO_3)} = [M(R\text{-}PO_3)]/([M^{2+}][R\text{-}PO_3^{2-}]) \qquad (3b)$$

Under the present experimental conditions the formation of $Cu(Arm)^{2+}$ is practically complete in the pH ranges needed for the evaluation of the ternary complexes; this was evident from the titrations in the absence of $R\text{-}PO_3$ and is also in agreement with the high stability[17] of the $Cu(Bpy)^{2+}$ and $Cu(Phen)^{2+}$ complexes.

The results regarding eqn. (2) and (3) for the AcP and AnP systems in water and in water containing 30 or 50% (v/v) 1,4-dioxane are listed in columns 3, 5, and 7 of Table 1, respectively. None of these stability constants has been determined before.[33–35] The stability constants of the binary Cu(AcP) and Cu(AnP) complexes are given for comparison (column 4).[13] The stability constants of the binary Cu(AcP) and ternary Cu(Arm)(AcP) complexes in the dioxane–water solvents were not determined because the constants for the corresponding HPO_4^{2-} complexes in these solvents are not known and hence, a reliable evaluation by taking into account the phosphate impurity always present in the AcP probes[13] would not have been possible. Therefore, we restricted ourselves regarding the mixed solvents to the complexes formed with AnP^{2-}. However, from these latter results it follows that the overall complex stability (columns 4, 5 and 7) increases dramatically with a decreasing solvent polarity regardless if the binary or ternary complexes are considered. Clearly, if one compares the solvent effect on the acidity constants of $H(AcP)^-$ and $H(AnP)^-$ (column 3), which is quite alike for both acids, it is evident that an analogous stability increase must also occur for the complexes with AcP^{2-}, if the solvent polarity decreases.

Table 1 Negative logarithms of the acidity constants [eqn. (2)] of $H(AcP)^-$ and $H(AnP)^-$, logarithms of the stability constants of the corresponding binary $Cu(R\text{-}PO_3)$ and ternary $Cu(Arm)(R\text{-}PO_3)$ complexes [eqn. (3)] as determined by potentiometric pH titrations in water and in water containing 30 or 50% (v/v) 1,4-dioxane at 25 °C and $I = 0.1$ M ($NaNO_3$). The resulting values for $\Delta \log K_{Cu/Arm/R\text{-}PO_3}$ are also given [eqn. (4), (5)][a,b]

$R\text{-}PO_3^{2-}$	% (v/v) Dioxane	$pK^H_{H(R\text{-}PO_3)}$	$\log K^{Cu}_{Cu(R\text{-}PO_3)}$	$\log K^{Cu(Bpy)}_{Cu(Bpy)(R\text{-}PO_3)}$	$\Delta \log K_{Cu/Bpy/R\text{-}PO_3}$	$\log K^{Cu(Phen)}_{Cu(Phen)(R\text{-}PO_3)}$	$\Delta \log K_{Cu/Phen/R\text{-}PO_3}$
AcP^{2-}	0	4.84 ± 0.02	2.86 ± 0.05	2.87 ± 0.08	0.01 ± 0.09	2.89 ± 0.05	0.03 ± 0.07
	30	5.56 ± 0.01					
	50	6.03 ± 0.02					
AnP^{2-}	0	6.49 ± 0.02	3.36 ± 0.03	3.40 ± 0.03	0.04 ± 0.04	3.38 ± 0.04	0.02 ± 0.05
	30	7.23 ± 0.01	4.46 ± 0.03	4.53 ± 0.02	0.07 ± 0.04	4.53 ± 0.03	0.07 ± 0.04
	50	7.67 ± 0.02	5.27 ± 0.02	5.33 ± 0.02	0.06 ± 0.03	5.32 ± 0.02	0.05 ± 0.03

[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 $\Delta \log K_{Cu/Arm/R\text{-}PO_3}$, were calculated according to the error propagation after Gauss. [b] The entries in columns 3 and 4 are from our previous work;[13] the constants for all the ternary systems have been measured now.

Table 2 Baseline correlations for Cu^{2+}–phosph(on)ate complex stabilities and phosph(on)ate group basicities in the solvents water and water containing 30 or 50% (v/v) 1,4-dioxane (25 °C; $I = 0.1$ M, $NaNO_3$),[a] together with some properties of the mentioned solvents. In the straight-line equations, $y = m \cdot x + b$, a value for x represents the acidity constant, $pK^H_{H(R\text{-}PO_3)}$, of a monoprotonated phosphate monoester or phosphonate ligand, $H(R\text{-}PO_3)^-$, and y the calculated stability constant, $\log K^{Cu}_{Cu(R\text{-}PO_3)}$ or $\log K^{Cu(Arm)}_{Cu(Arm)(R\text{-}PO_3)}$, of the corresponding binary $Cu(R\text{-}PO_3)$ or ternary $Cu(Arm)(R\text{-}PO_3)$ complex;[a] the errors given with the slopes (m) and the y-axis intercepts (b) correspond to one standard deviation (1σ). The listed $3 \times SD$ values (column at the right)[b] are considered as reasonable error limits for any stability constant calculation in the $pK^H_{H(R\text{-}PO_3)}$ range 5–8 for aqueous solution, 6–8.5 for 30%, and 6.5–9 for 50% (v/v) dioxane–water mixtures

No.	M^{2+}	% (v/v) Dioxane	Mol fraction	ε^c	m	b	$3 \times SD^b$
1	Cu^{2+}	0	0	78.5	0.465 ± 0.025	−0.015 ± 0.164	0.06
2	Cu^{2+}	30	0.083	52.7	0.559 ± 0.015	−0.089 ± 0.106	0.03
3	Cu^{2+}	50	0.175	35.2	0.571 ± 0.022	0.190 ± 0.160	0.03
4	$Cu(Bpy)^{2+}$	0	0	78.5	0.465	0.009	0.07
5	$Cu(Bpy)^{2+}$	30	0.083	52.7	0.559	−0.007	0.04
6	$Cu(Bpy)^{2+}$	50	0.175	35.2	0.571	0.334	0.07
7	$Cu(Phen)^{2+}$	0	0	78.5	0.465	0.018	0.06
8	$Cu(Phen)^{2+}$	30	0.083	52.7	0.559	0.015	0.04
9	$Cu(Phen)^{2+}$	50	0.175	35.2	0.571	0.382	0.08

[a] The straight-line parameters are taken from our earlier work, *i.e.* from Tables 2 and 3 of ref. 26(*a*) for the binary complexes (*cf.* also ref. 43) and from Table II of ref. 27 for the ternary ones. The slopes (m) and the intercepts (b) for the $\log K^M_{M(R\text{-}PO_3)}$ *versus* $pK^H_{H(R\text{-}PO_3)}$ plots are based on the equilibrium constants determined for simple phosph(on)ate ligands like 4-nitrophenyl phosphate, phenyl phosphate, *n*-butyl phosphate, D-ribose 5-monophosphate, methylphosphonate, ethylphosphonate, *etc.* [b] The *SD* values result from the standard deviation of the individual experimental points on which a straight-line is based.[24,26a,27] [c] The dielectric constants (ε) for the dioxane–water mixtures are interpolated from the data given in ref. 23.

One way to quantify the stability of mixed ligand complexes[36–38] toward their binary parent complexes is by considering equilibrium (4a):

$$Cu(Arm)^{2+} + Cu(R\text{-}PO_3) \rightleftharpoons Cu(Arm)(R\text{-}PO_3) + Cu^{2+} \quad (4a)$$

$$10^{\Delta \log K_{Cu/Arm/R\text{-}PO_3}} = \frac{[Cu(Arm)(R\text{-}PO_3)][Cu^{2+}]}{[Cu(Arm)^{2+}][Cu(R\text{-}PO_3)]} \quad (4b)$$

The corresponding equilibrium constant is calculated with eqn. (5):

$$\Delta \log K_{Cu/Arm/R\text{-}PO_3} = \log K^{Cu(Arm)}_{Cu(Arm)(R\text{-}PO_3)} - \log K^{Cu}_{Cu(R\text{-}PO_3)} \quad (5)$$

According to the general rule for complex stabilities, $K_1 > K_2$, one expects that equilibrium (4a) is on its left side with negative values for $\Delta \log K_{Cu/Arm/R\text{-}PO_3}$ [eqn. (5)], which is in agreement with statistical considerations: For the coordination of a monodentate ligand to the Jahn–Teller-distorted Cu^{2+}, at which two binding sites are already occupied, one estimates $\Delta \log K_{Cu/statist} \approx -0.5$ (*cf.* ref. 39) and for the coordination of a bidentate ligand $\Delta \log K_{Cu/statist} \approx -0.9$.[36]

The results listed in columns 6 and 8 of Table 1 for $\Delta \log K_{Cu/Bpy/R\text{-}PO_3}$ and $\Delta \log K_{Cu/Phen/R\text{-}PO_3}$ are close to zero or slightly positive, *i.e.* $10^{\Delta \log K} \gtrsim 1$; hence, the above equilibrium (4a) is about midway or slightly shifted to its right side in contrast to the expected behaviour according to the statistics. This observation is independent from the solvent employed. However, this increased stability of the Cu(Arm)(AcP) and Cu(Arm)(AnP) complexes corresponds to previous experience with mixed ligand complexes formed by a divalent transition metal ion of the second half of the 3d series, a heteroaromatic N base and an O donor ligand.[36,37,39,40] The synergism operating here is clearly important for biological systems[40] and has also been observed for the corresponding ternary complexes with the related ligands dihydroxyacetone phosphate and glycerol 1-phosphate.[41] Such an increased stability is also to be expected for mixed ligand coordination spheres in which the heteroaromatic amine is represented by imidazole residues which are often present in active-site cavities of enzymes.[40]

3.2. Evidence for the participation of the carbonyl oxygen in the formation of mixed ligand complexes

The results discussed in Section 3.1 proved an increased stability of the ternary Cu(Arm)(AcP) and Cu(Arm)(AnP) complexes, but so far they gave no hint for the participation of the carbonyl oxygen in metal ion binding.

Here one should recall that for families of structurally related ligands plots of $\log K^M_{ML}$ *versus* pK^H_{HL} should lead to straight lines.[42] Indeed, recently such plots have been constructed for binary[24–26] and ternary[27] complexes formed by various metal ions with phosph(on)ate ligands, $R\text{-}PO_3^{2-}$, where R represents a non-interacting residue. The pertinent straight-line equations of such plots, valid for water and water containing 30 or 50% (v/v) 1,4-dioxane are summarized[26,27,43] in Table 2 together with some properties of the mentioned solvents.[23] It is evident that any participation in complex formation of the carbonyl oxygen

Table 3 Logarithms of the stability constants of the binary Cu(R-PO$_3$) and ternary Cu(Arm)(R-PO$_3$) complexes [eqn. (3), (8a)] of AcP^{2-} and AnP^{2-} as determined by potentiometric pH titrations in water and in water containing 30 or 50% (v/v) 1,4-dioxane at 25 °C and $I = 0.1$ M (NaNO$_3$),[a] together with the corresponding calculated stability constants for a sole phosph(on)ate coordination of Cu^{2+} (M(R-PO$_3$)$_{op}$).[b] The observed stability increase (see also Fig. 1) is quantified *via* log $\Delta_{M(R\text{-}PO_3)}$ [eqn. (6)], and the extent of chelate formation according to equilibrium (1) by the dimensionless equilibrium constant K_I [eqn. (7), (9)] and the percentage of M(R-PO$_3$)$_{cl}$ [eqn. (10)][a]

No.	$M^{2+}/R\text{-}PO_3^{2-}$	% (v/v) Dioxane	$\log K^{M}_{M(R\text{-}PO_3)}$ [c]	$\log K^{M}_{M(R\text{-}PO_3)_{op}}$ [b]	$\log \Delta_{M(R\text{-}PO_3)}$	K_I	% M(R-PO$_3$)$_{cl}$
1	Cu^{2+}/AcP^{2-}	0	2.86 ± 0.05	2.24 ± 0.06	0.62 ± 0.08	3.17 ± 0.75	76 ± 4
2	Cu(Bpy)$^{2+}$/AcP^{2-}	0	2.87 ± 0.08	2.26 ± 0.07	0.61 ± 0.11	3.07 ± 1.00	75 ± 6
3	Cu(Phen)$^{2+}$/AcP^{2-}	0	2.89 ± 0.05	2.27 ± 0.06	0.62 ± 0.08	3.17 ± 0.75	76 ± 4
4	Cu^{2+}/AnP^{2-}	0	3.36 ± 0.03	3.00 ± 0.06	0.36 ± 0.07	1.29 ± 0.35	56 ± 7
5	Cu(Bpy)$^{2+}$/AnP^{2-}	0	3.40 ± 0.03	3.03 ± 0.07	0.37 ± 0.08	1.34 ± 0.41	57 ± 7
6	Cu(Phen)$^{2+}$/AnP^{2-}	0	3.38 ± 0.04	3.04 ± 0.06	0.34 ± 0.07	1.19 ± 0.36	54 ± 8
7	Cu^{2+}/AnP^{2-}	30	4.46 ± 0.03	3.95 ± 0.03	0.51 ± 0.04	2.24 ± 0.32	69 ± 3
8	Cu(Bpy)$^{2+}$/AnP^{2-}	30	4.53 ± 0.02	4.03 ± 0.04	0.50 ± 0.04	2.16 ± 0.33	68 ± 3
9	Cu(Phen)$^{2+}$/AnP^{2-}	30	4.53 ± 0.03	4.06 ± 0.04	0.47 ± 0.05	1.95 ± 0.34	66 ± 4
10	Cu^{2+}/AnP^{2-}	50	5.27 ± 0.02	4.57 ± 0.03	0.70 ± 0.04	4.01 ± 0.42	80 ± 2
11	Cu(Bpy)$^{2+}$/AnP^{2-}	50	5.33 ± 0.02	4.71 ± 0.07	0.62 ± 0.07	3.17 ± 0.70	76 ± 4
12	Cu(Phen)$^{2+}$/AnP^{2-}	50	5.32 ± 0.02	4.76 ± 0.08	0.56 ± 0.08	2.63 ± 0.69	72 ± 5

[a] See footnote *a* of Table 1. The values for Cu(AcP) in water and for Cu(AnP) in water as well as in water containing 30 or 50% 1,4-dioxane, *i.e.* entries 1, 4, 7 and 10, are from ref. 13. [b] Calculated with the acidity constants, p$K^{H}_{H(R\text{-}PO_3)}$, listed in Table 1 and the straight-line equations summarized in Table 2. [c] The values in this column are from Table 1.

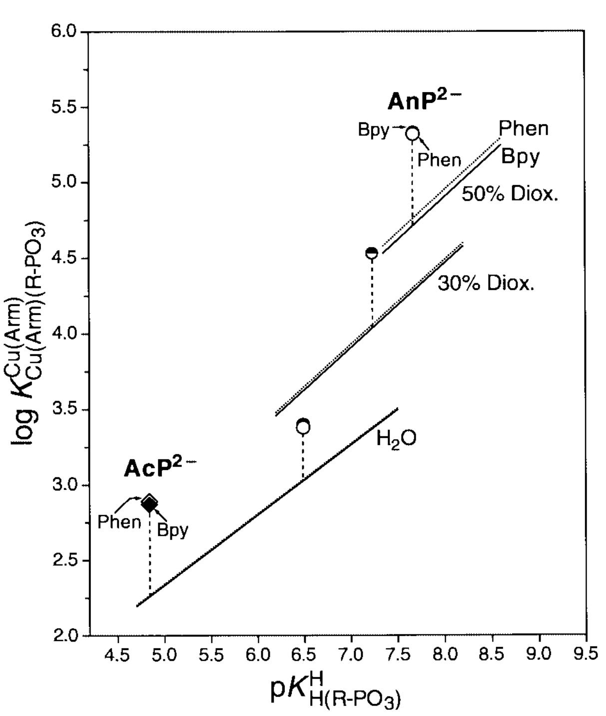

Fig. 1 Evidence for an enhanced stability of the ternary Cu(Bpy)(AcP) (◆) and Cu(Phen)(AcP) (◇) complexes in water as well as of the ternary Cu(Bpy)(AnP) (●) and Cu(Phen)(AnP) (○) complexes in water and in water containing 30 or 50% (v/v) 1,4-dioxane based on the relationship between log $K^{Cu(Arm)}_{Cu(Arm)(R\text{-}PO_3)}$ and p$K^{H}_{H(R\text{-}PO_3)}$ for R-PO$_3^{2-}$ derivatives with a group R unable to undergo any kind of interaction. These reference lines are drawn by using the parameters for the straight-line equations given in entries 4–9 of Table 2; the solid lines hold for Arm = Bpy and the dotted lines for Arm = Phen. The vertical broken lines emphasize the stability differences to the corresponding reference lines; these differences are equal to log $\Delta_{Cu(Arm)(AcP)}$ and log $\Delta_{Cu(Arm)(AnP)}$ [eqn. (6)] the values of which are listed in column 6 of Table 3. All the plotted equilibrium constants refer to 25 °C and $I = 0.1$ M (NaNO$_3$); the data pairs for Cu(Arm)(AcP)/H(AcP)$^-$ and Cu(Arm)(AnP)/H(AnP)$^-$ are taken from Table 1.

should lead to a deviation from these straight reference lines. In fact, it is well known[42,44] that any kind of chelate formation as indicated, *e.g.*, in equilibrium (1), must be reflected in an increased stability.

Therefore, we have used the equations of Table 2 to construct the straight lines of the log $K^{Cu(Arm)}_{Cu(Arm)(R\text{-}PO_3)}$ *versus* p$K^{H}_{H(R\text{-}PO_3)}$ plots seen in Fig. 1, where also the corresponding data pairs for the Cu(Bpy)$^{2+}$/AnP and Cu(Phen)$^{2+}$/AnP systems are inserted for the three different solvents as well as those for AcP referring to aqueous solution. The resulting eight points for the Cu(Arm)(AnP) and Cu(Arm)(AcP) complexes are considerably above the corresponding reference lines, thus proving an increased stability for these mixed ligand complexes. This stability increase must be attributed to the participation of the carbonyl oxygen in metal ion binding, since no other further site is available in AcP^{2-} and AnP^{2-}.

A quantitative evaluation of the situation reflected in Fig. 1 is possible by calculating with the p$K^{H}_{H(AcP)}$ and p$K^{H}_{H(AnP)}$ values given in column 3 of Table 1 and the straight-line equations summarized in Table 2 the expected stabilities for Cu(Arm)(R-PO$_3$) complexes having solely a phosph(on)ate–Cu^{2+} coordination. The corresponding results, designated as $K^{M}_{M(R\text{-}PO_3)_{op}}$ because they describe the stabilities of the open Cu(R-PO$_3$) and Cu(Arm)(R-PO$_3$) species in equilibrium (1), are listed in column 5 of Table 3. Comparison with the measured stability constants (column 4 of Table 3) according to eqn. (6),

$$\log \Delta_{M(R\text{-}PO_3)} = \log \Delta = \log K^{M}_{M(R\text{-}PO_3)} - \log K^{M}_{M(R\text{-}PO_3)_{op}} \quad (6)$$

where $M^{2+} = Cu^{2+}$, Cu(Bpy)$^{2+}$ or Cu(Phen)$^{2+}$, leads to the stability differences given in column 6 of Table 3, log $\Delta_{M(AcP)}$ or log $\Delta_{M(AnP)}$, which correspond to the vertical broken-line distances shown in Fig. 1.

Comparison of the values listed for log $\Delta_{M(R\text{-}PO_3)}$ in column 6 of Table 3 reveals several remarkable points: (*i*) The stability increase is, with approximately 0.3 to 0.7 log units, quite significant. (*ii*) All of the log $\Delta_{M(R\text{-}PO_3)}$ values are very similar for a given ligand and a given solvent; *i.e.* the stability increase is approximately the same for a binary and its corresponding ternary complexes. (*iii*) With decreasing solvent polarity the observed stability increase is further enhanced (see also Section 3.3) as is evident from the Cu(Arm)(AnP) complexes. (*iv*) In aqueous solution, where a comparison is possible, the stability enhancement is more pronounced for the AcP^{2-} complexes than for those with AnP^{2-} (see also Section 3.3).

3.3. Formation degree of the chelated isomer involving the carbonyl oxygen in the Cu(Arm)(AcP) and Cu(Arm)(AnP) species

The increased stability of the Cu(Arm)(AcP) and Cu(Arm)(AnP) complexes can only be attributed, as already indicated above, to the carbonyl oxygen because aside from the phosph(on)ate group, AcP^{2-} and AnP^{2-} [see eqn. (1)] do not contain any other binding site; *i.e.* equilibrium (1) must operate. The position of this concentration-independent equilibrium between the simple phosph(on)ate-bound species, which we designate as the "open" isomer, $M(R\text{-}PO_3)_{op}$, and the six-membered chelate involving the carbonyl oxygen, designated as the "closed" species, $M(R\text{-}PO_3)_{cl}$, is defined by the intramolecular and hence, dimensionless, equilibrium constant K_I:

$$K_I = [M(R\text{-}PO_3)_{cl}]/[M(R\text{-}PO_3)_{op}] \qquad (7)$$

Of course, the experimentally measured stability constant for $M(R\text{-}PO_3)$ is defined by eqn. (3), yet due to equilibrium (1) this expression may be rewritten as given in eqn. (8a) and further developed[42,44,45] to eqn. (8c) and (9):

$$K^{M}_{M(R\text{-}PO_3)} = \frac{[M(R\text{-}PO_3)]}{[M^{2+}][R\text{-}PO_3^{2-}]} = \frac{[M(R\text{-}PO_3)_{op}] + [M(R\text{-}PO_3)_{cl}]}{[M^{2+}][R\text{-}PO_3^{2-}]} \qquad (8a)$$

$$= K^{M}_{M(R\text{-}PO_3)_{op}} + K_I \cdot K^{M}_{M(R\text{-}PO_3)_{op}} \qquad (8b)$$

$$= K^{M}_{M(R\text{-}PO_3)_{op}}(1 + K_I) \qquad (8c)$$

$$K_I = \frac{K^{M}_{M(R\text{-}PO_3)}}{K^{M}_{M(R\text{-}PO_3)_{op}}} - 1 = 10^{\log \Delta} - 1 \qquad (9)$$

The stability constant of the open isomer, $K^{M}_{M(R\text{-}PO_3)_{op}}$, is not directly accessible by experiments, yet it may be calculated as described in Section 3.2; the corresponding values are listed in column 5 of Table 3. The stability difference as defined in eqn. (6) corresponds to the one needed for the calculation of K_I in eqn. (9). Knowledge of K_I [eqn. (7), (9)] allows one then to calculate according to eqn. (10) the percentage of the closed form, $M(R\text{-}PO_3)_{cl}$, occurring in equilibrium (1):

$$\%\ M(R\text{-}PO_3)_{cl} = 100 \cdot K_I/(1 + K_I) \qquad (10)$$

The use of eqn. (6), (9), and (10) leads to the results summarized in columns 6, 7, and 8 of Table 3, respectively. These results prove that the intramolecular equilibrium (1) operates not only in the binary Cu(AcP) and Cu(AnP) species (rows 1, 4, 7, 10 of Table 3),[13] but also in the ternary Cu(Arm)(AcP) and Cu(Arm)(AnP) complexes (rows 2, 3, 5, 6, 8, 9, 11, 12) and that both types of isomers are formed in appreciable amounts. It is interesting to see that the formation degrees of the closed species are relatively independent of the presence of a second ligand (*e.g.* rows 1-6 of Table 3); *i.e.*, the binary and ternary complexes behave quite alike in this respect.

As far as the solvent influence is concerned, it is evident from the complexes containing AnP^{2-} (Table 3, rows 4–12) that a decreasing solvent polarity favours the participation of the carbonyl oxygen in metal ion binding. This result appears as logical since 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. Comparison of rows 10–12 in Table 3 seems to indicate that the chelated isomer in water containing 50% 1,4-dioxane is slightly more stable in the binary Cu(AnP) complex than in the ternary Cu(Bpy)(AnP) and Cu(Phen)(AnP) species. Maybe this results from the solvation of the heteroaromatic amines, especially of 1,10-phenanthroline, by 1,4-dioxane, which may slightly screen the access of the carbonyl oxygen to the coordination sphere of Cu^{2+}.

Another interesting result is the higher formation degree of the closed isomers in the complexes containing AcP^{2-} compared with those formed with AnP^{2-} (rows 1–6 in Table 3). Since there is no difference in this respect between the binary and ternary complexes the difference must be due to properties of AcP^{2-} or AnP^{2-}, respectively. In fact, for the binary M(AcP) and M(AnP) complexes previously the same observation has been made.[13] It appears that AcP^{2-} is somewhat favoured possibly because of a smaller angle around the 'anhydride' O atom (assumed to be close to 104° as in water) compared with the one of the CH_2 bridge (assumed to be close to 109° as in CH_4) in AnP^{2-}, as well as by a possibly favoured solvation, *i.e.* hydrogen bonding in which the 'anhydride' oxygen may participate whereas the methylene group is unable to do so.

4. Conclusions

The present study proves that the carbonyl oxygen in AcP^{2-} and AnP^{2-} is able to participate in the formation of mixed ligand complexes and to do so also under a reduced solvent polarity; in fact, under the latter conditions the metal ion–carbonyl oxygen interaction is even favoured. Both observations are clearly meaningful with regard to biological systems: In an active site cavity a reduced water activity is expected and the coordination sphere of a potentially present metal ion will certainly be partially occupied by ligating groups other than H_2O.

There can be no doubt that the observations described here for Cu^{2+} are valid for other metal ions too, especially if one considers that many of these also have a significant affinity for the carbonyl oxygen atom. For example,[13] the formation degree of the chelated species of Ca(AcP) and Zn(AcP) amounts to 48 ± 8 and 59 ± 6%, respectively.

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Notes and references

1 (*a*) Part 63, E. M. Bianchi, S. A. A. Sajadi, B. Song and H. Sigel, *Inorg. Chim. Acta*, 2000, **300**, in the press; (*b*) Part 62, M. S. Lüth, L. E. Kapinos, B. Song, B. Lippert and H. Sigel, *J. Chem. Soc., Dalton Trans.*, 1999, 357.

2 *Abbreviations and definitions*: AcP^{2-}, acetyl phosphate; AnP^{2-}, acetonylphosphonate; Arm, heteroaromatic nitrogen base, *e.g.* Bpy or Phen; ATP^{4-}, adenosine 5′-triphosphate; Bpy, 2,2′-bipyridyl; *I*, ionic strength of a solution; L, general ligand; M^{2+}, general divalent metal ion also including in part $Cu(Arm)^{2+}$; Phen, 1,10-phenanthroline; $R\text{-}PO_3^{2-}$, simple phosphate monoester or phosphonate ligand with R representing a non-interacting residue. In certain instances, where no confusion can occur, $R\text{-}PO_3^{2-}$ may also include AcP^{2-} and AnP^{2-}. Species which are given in 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.

3 (*a*) T. L. Mayover, C. J. Halkides and R. C. Stewart, *Biochemistry*, 1999, **38**, 2259; (*b*) S. S. Da Re, D. Deville-Bonne, T. Tolstykh, M. Véron and J. B. Stock, *FEBS Lett.*, 1999, **457**, 323; (*c*) C. L. Sodré, H. M. Scofano and H. Barrabin, *Biochim. Biophys. Acta*, 1999, **1419**, 55; (*d*) T. Tsuda, S. Kaya, T. Yokoyama, Y. Hayashi and K. Taniguchi, *J. Biol. Chem.*, 1998, **273**, 24339.

4 S.-K. Kim, M. R. Wilmes-Riesenberg and B. L. Wanner, *Mol. Microbiol.*, 1996, **22**, 135.

5 K. Hiratsu, A. Nakata, H. Shinagawa and K. Makino, *Gene*, 1995, **161**, 7.

6 S. Shin and C. Park, *J. Bacteriol.*, 1995, **177**, 4696.

7 (*a*) A. J. Darwin and V. Stewart, *J. Mol. Biol.*, 1995, **251**, 15; (*b*) C. G. Head, A. Tardy and L. J. Kenney, *J. Mol. Biol.*, 1998, **281**, 857.

8 (*a*) D. B. McIntosh, D. G. Woolley, B. Vilsen and J. P. Andersen, *J. Biol. Chem.*, 1996, **271**, 25778; (*b*) S. Engelender, H. Wolosker and L. de Meis, *J. Biol. Chem.*, 1995, **270**, 21050; (*c*) K. Kimura, H. Suzuki, T. Daiho, K. Yamasaki and T. Kanazawa, *J. Biol. Chem.*, 1996, **271**, 28933.

9 A.-K. Bock, J. Glasemacher, R. Schmidt and P. Schönheit, *J. Bacteriol.*, 1999, **181**, 1861.

10 H. Ishikawa, M. Shiroshima, A. Widjaja, H. Nakajima and R. Tsurutani, *J. Chem. Eng. Jpn.*, 1995, **28**, 517.

11 L. A. Ryabova, L. M. Vinokurov, E. A. Shekhovtsova, Y. B. Alakhov and A. S. Spirin, *Anal. Biochem.*, 1995, **226**, 184.

12 J. J. R. Fraústo da Silva and R. J. P. Williams, *The Biological Chemistry of the Elements*: *The Inorganic Chemistry of Life*, Clarendon Press, Oxford, 1991, pp. 180–181.

13 H. Sigel, C. P. Da Costa, B. Song, P. Carloni and F. Gregáň, *J. Am. Chem. Soc.*, 1999, **121**, 6248.

14 (*a*) C. H. Oestreich and M. M. Jones, *Biochemistry*, 1966, **5**, 2926; (*b*) P. J. Briggs, D. P. N. Satchell and G. F. White, *J. Chem. Soc. B*, 1970, 1008; (*c*) J. P. Klinman and D. Samuel, *Biochemistry*, 1971, **10**, 2126.

15 R. Kluger, K. Nakaoka and W.-C. Tsui, *J. Am. Chem. Soc.*, 1978, **100**, 7388.

16 (*a*) D. B. Olsen, T. W. Hepburn, S.-l. Lee, B. M. Martin, P. S. Mariano and D. Dunaway-Mariano, *Arch. Biochem. Biophys.*, 1992, **296**, 144; (*b*) D. B. Olsen, T. W. Hepburn, M. Moos, P. S. Mariano and D. Dunaway-Mariano, *Biochemistry*, 1988, **27**, 2229.

17 (*a*) G. Anderegg, *Helv. Chim. Acta*, 1963, **46**, 2397; (*b*) H. Irving and D. H. Mellor, *J. Chem. Soc.*, 1962, 5222.

18 H. Sigel and R. B. Martin, *Chem. Rev.*, 1982, **82**, 385.

19 H. Sigel, R. B. Martin, R. Tribolet, U. K. Häring and R. Malini-Balakrishnan, *Eur. J. Biochem.*, 1985, **152**, 187. See also the footnote on p. 258 in: M. Bastian and H. Sigel, *Inorg. Chim. Acta*, 1990, **178**, 249.

20 (*a*) D. C. Rees, *J. Mol. Biol.*, 1980, **141**, 323; (*b*) G. R. Moore, *FEBS Lett.*, 1983, **161**, 171; (*c*) N. K. Rogers, G. R. Moore and M. J. E. Sternberg, *J. Mol. Biol.*, 1985, **182**, 613; (*d*) G. Iversen, Y. I. Kharkats and J. Ulstrup, *J. Mol. Phys.*, 1998, **94**, 297.

21 L. de Meis, *Biochim. Biophys. Acta*, 1989, **973**, 333.

22 H. Sigel, *Pure Appl. Chem.*, 1989, **61**, 923.

23 (*a*) G. Åkerlöf and O. A. Short, *J. Am. Chem. Soc.*, 1936, **58**, 1241; (*b*) F. E. Critchfield, J. A. Gibson, Jr. and J. L. Hall, *J. Am. Chem. Soc.*, 1953, **75**, 1991; (*c*) G. Åkerlöf and O. A. Short, *J. Am. Chem. Soc.*, 1953, **75**, 6357.

24 H. Sigel, D. Chen, N. A. Corfù, F. Gregáň, A. Holý and M. Strašák, *Helv. Chim. Acta*, 1992, **75**, 2634.

25 H. Sigel, S. S. Massoud and N. A. Corfù, *J. Am. Chem. Soc.*, 1994, **116**, 2958.

26 (*a*) M. C. F. Magalhães and H. Sigel, *J. Indian Chem. Soc.*, 1992, **69**, 437; (*b*) M. Bastian and H. Sigel, *Inorg. Chim. Acta*, 1991, **187**, 227.

27 M. Bastian, D. Chen, F. Gregáň, G. Liang and H. Sigel, *Z. Naturforsch., Teil B*, 1993, **48**, 1279.

28 A. Saha, N. Saha, L.-n. Ji, J. Zhao, F. Gregáň, S. A. A. Sajadi, B. Song and H. Sigel, *J. Biol. Inorg. Chem.*, 1996, **1**, 231.

29 J. Zhao, B. Song, N. Saha, A. Saha, F. Gregáň, M. Bastian and H. Sigel, *Inorg. Chim. Acta*, 1996, **250**, 185.

30 H. Sigel, A. D. Zuberbühler and O. Yamauchi, *Anal. Chim. Acta*, 1991, **255**, 63.

31 (*a*) Y. K. Agrawal, *Talanta*, 1973, **20**, 1354; (*b*) L. G. Van Uitert and W. C. Fernelius, *J. Am. Chem. Soc.*, 1954, **76**, 5887; (*c*) L. G. Van Uitert and C. G. Haas, *J. Am. Chem. Soc.*, 1953, **75**, 451.

32 R. G. Bates, *Determination of pH: Theory and Practice*, Wiley, New York, 1973, pp. 243–249.

33 *IUPAC Stability Constants Database*, Release 3, Version 3.02 (compiled by L. D. Pettit and H. K. J. Powell), Academic Software, Timble, UK, 1998.

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

35 *Joint Expert Speciation System* (*JESS*), Version 5.3b (joint venture by K. Murray and P. M. May), Division of Water Technology, CSIR, Pretoria, South Africa and School of Mathematical and Physical Sciences, Murdoch University, Murdoch, Western Australia, 1996.

36 H. Sigel, *Angew. Chem.*, 1975, **87**, 391; *Angew. Chem., Int. Ed. Engl.*, 1975, **14**, 394.

37 H. Sigel, in *Coordination Chemistry-20*, ed. D. Banerjea, IUPAC through Pergamon Press, Oxford, UK, New York, 1980, pp. 27–45.

38 (*a*) N. Saha and H. Sigel, *J. Am. Chem. Soc.*, 1982, **104**, 4100; (*b*) R. Tribolet, R. B. Martin and H. Sigel, *Inorg. Chem.*, 1987, **26**, 638.

39 R. Malini-Balakrishnan, K. H. Scheller, U. K. Häring, R. Tribolet and H. Sigel, *Inorg. Chem.*, 1985, **24**, 2067.

40 (*a*) H. Sigel, B. E. Fischer and B. Prijs, *J. Am. Chem. Soc.*, 1977, **99**, 4489; (*b*) H. Sigel, *Inorg. Chem.*, 1980, **19**, 1411.

41 (*a*) G. Liang, D. Chen, M. Bastian and H. Sigel, *J. Am. Chem. Soc.*, 1992, **114**, 7780; (*b*) H. Sigel and L. E. Kapinos, *Coord. Chem. Rev.*, 2000, **200**, in the press.

42 R. B. Martin and H. Sigel, *Comments Inorg. Chem.*, 1988, **6**, 285.

43 D. Chen, F. Gregáň, A. Holý and H. Sigel, *Inorg. Chem.*, 1993, **32**, 5377.

44 H. Sigel, S. S. Massoud and R. Tribolet, *J. Am. Chem. Soc.*, 1988, **110**, 6857.

45 H. Sigel and B. Song, in *Interactions of Metal Ions with Nucleotides, Nucleic Acids and Their Constituents*, vol. 32 of *Metal Ions in Biological Systems*, eds. A. Sigel and H. Sigel, Dekker, New York, 1996, pp. 135–205.

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