

Stability and structure of the Mg^{2+} , Ca^{2+} and Cu^{2+} complexes of orotidinate 5'-monophosphate (OMP^{3-}) in various aqueous 1,4-dioxane mixtures

Matthias Bastian and Helmut Sigel*

Institute of Inorganic Chemistry, University of Basel, Spitalstrasse 51, CH-4056 Basel (Switzerland)

(Received March 11, 1991; revised May 13, 1991)

Abstract

The stability constant of the 1:1 complexes formed between Mg^{2+} , Ca^{2+} or Cu^{2+} and orotidinate 5'-monophosphate (OMP^{3-}) were determined by potentiometric pH titrations in water containing 30 or 50% (vol./vol.) 1,4-dioxane ($I=0.1$ M, $NaNO_3$; 25 °C). In addition to the stability constants of these $M(OMP)^-$ complexes, for $Mg(OMP)^-$ and $Ca(OMP)^-$ the acidity constants for the release of the proton from the H(N-3) site were also calculated, i.e. the formation of the corresponding $M(OMP-H)^{2-}$ complexes was quantified. All the corresponding equilibrium constants for aqueous solution are known from a previous study (M. Bastian and H. Sigel, *J. Coord. Chem.*, 23 (1991) 137) and in the discussions these data are also taken into account. On the basis of recent measurements in aqueous dioxane mixtures with simple phosphate monoesters (M. C. F. Magalhães and H. Sigel, *J. Indian Chem. Soc.*, in press), evidence is provided that the somewhat increased stability of the $Cu(OMP)^-$ complex in the various solvents is mainly the result of a charge effect of the carboxylate group (in position 6 of OMP^{3-}) and *not* of a direct participation in complex formation, i.e. there are no indications for the formation of significant amounts of macrochelates involving the phosphate and the carboxylate groups. This result is in agreement with the dominating *syn* conformation of OMP^{3-} in which the 5'-phosphate and 6-carboxylate groups are pointing away from each other. However, for the Cu^{2+} complex of salicyl phosphate (SaP^{3-}) evidence is given by evaluating previously published equilibrium constants (R. W. Hay, A. K. Basak, M. P. Pujari and A. Perotti, *J. Chem. Soc., Dalton Trans.*, (1986) 2029) that a simultaneous coordination of a phosphate and a carboxylate group to the same metal ion is possible; it is estimated that the eight-membered chelate of $Cu(SaP)^-$ reaches a formation degree of about 65 (± 25)%.

Introduction

Orotidine and its derivatives are important intermediates in the metabolism of pyrimidines [1], which involves also metal ions [2]. For this reason we have endeavoured to study the metal ion-binding properties of orotidinate (Oro^-) [3] and also of orotidinate 5'-monophosphate (OMP^{3-}) [4]. It became evident that the carboxylate group of Oro^- is only weakly basic with a relatively low affinity for divalent metal ions like Cu^{2+} or Zn^{2+} ; yet overall, Oro^- behaves in a first approximation as a simple carboxylate ligand [3]. The stability of the complexes formed between divalent metal ions and OMP^{3-} in aqueous solution is governed mainly by the metal ion affinity of the phosphate group; the relatively small extra stability due to the presence of the

carboxylate group in position 6 of the pyrimidine ring may largely be attributed to a charge effect [4]. This means, it was concluded that direct participation of the carboxylate group in metal ion-binding in $M(OMP)^-$ complexes does hardly or not at all occur in aqueous solution**.

As one might think that the indicated carboxylate-metal ion interaction could be favored in solvents with a polarity lower than that of water we have now studied the acid base properties of OMP , as well as the stability of several $M(OMP)^-$ complexes

**Abbreviations: Ac^- , acetate; L, general ligand with undefined charge; M^{2+} , divalent metal ion; NMP, nucleoside 5'-monophosphate; $NPhP^{2-}$, 4-nitrophenyl phosphate; OMP^{3-} , orotidinate 5'-monophosphate; Oro^- , orotidinate; $RibMP^{2-}$, D-ribose 5'-monophosphate; $R-MP^{2-}$, phosphate monoester where R is an organic residue; SaP^{3-} , salicyl phosphate (= 2-carboxyphenyl phosphate); UMP^{2-} , uridine 5'-monophosphate; UTP^{4-} , uridine 5'-triphosphate.

*Author to whom correspondence should be addressed.

in various 1,4-dioxane–water mixtures. Furthermore, biological decarboxylation [1] of OMP^{3-} leads to UMP^{2-} , i.e. these two NMPs are closely related (Fig. 1), and therefore the acidity constants of $\text{H}_2(\text{UMP})$ were also measured in various dioxane–water mixtures to allow some additional comparisons.

OMP^{3-} exists in solution predominantly in the *syn* form [5–7] (Fig. 1), i.e. the carbonyl O-2 and N-3 are above the ribose ring [8], while the carboxylate group at position 6 is pointing away. This *syn* conformation is expected because the carboxylate group, which is also highly solvated due to its negative charge, is much larger than the carbonyl group at position 2. In contrast, for UMP^{2-} the *anti* conformation is dominating [7, 8] (Fig. 1); its population in aqueous solution has been determined as $64 (\pm 2)\%$ [7]. Unfortunately no quantitative values are available for OMP^{3-} , but for orotidinate a population of the *syn* form of $86 (\pm 2)\%$ was calculated [7]. Hence, one may conclude for OMP^{3-} that the *syn* conformer (Fig. 1) should occur to more than 86% due to the larger repulsion in the *anti* conformation between the negatively charged 5'-phosphate and 6-carboxylate groups. It is evident [4] that a simultaneous phosphate and carboxylate binding of metal ions in $\text{M}(\text{OMP})^-$ complexes is only possible in those species with the nucleotide in the *anti* conformation.

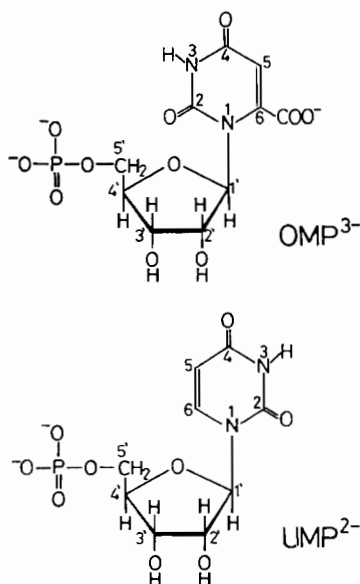


Fig. 1. Chemical structures of orotidinate 5'-monophosphate (OMP^{3-}) and uridine 5'-monophosphate (UMP^{2-}). OMP^{3-} is shown in its dominating *syn* conformation [6, 7] and UMP^{2-} in its dominating *anti* conformation [7, 8].

Experimental

Materials, apparatus and procedures

1,4-Dioxane (*reinst*) was from Merck AG, Darmstadt, F.R.G. The disodium salt of uridine 5'-monophosphoric acid and the trisodium salt of orotidine (=3- β -D-ribofuranosyl orotic acid) 5'-monophosphoric acid were from Sigma-Aldrich Co., St. Louis, MO, U.S.A. The latter nucleotide, all the other reagents [4], and the apparatus used in this study are identical with those used previously [3, 4].

The direct pH meter readings were used in the calculations of the acidity constants, i.e. they are so-called practical, 'mixed' or Brønsted constants; for details see refs. 3, 4, 9. No 'corrections' were applied for the change in solvent from water to the dioxane–water mixtures, though correction factors have been published [10, 11]. The stability constants are defined, as always, as concentration constants.

Conditions for the determination of the acidity constants

The acidity constants $K_{\text{H}_2(\text{OMP})}^{\text{H}}$, $K_{\text{H}(\text{OMP})}^{\text{H}}$ and $K_{\text{OMP}}^{\text{H}}$ of $\text{H}_2(\text{OMP})^-$ in water containing 30 or 50% (vol./vol.) dioxane were determined by titrating 50 ml of 0.0095 M HNO_3 and NaNO_3 ($I=0.1$ M; 25 °C) in the presence and absence of 1.8 mM OMP^{3-} under N_2 with 3 ml of 0.2 M NaOH and by using the difference in NaOH consumption between two such titrations for the calculations in the pH range of about 2.6 to 10.8. Three independent pairs of such titrations were carried out in the two solvent mixtures leading by average to the final result for $K_{\text{H}_2(\text{OMP})}^{\text{H}}$ in each solvent.

In another set of experiments (again) $K_{\text{H}(\text{OMP})}^{\text{H}}$ and $K_{\text{OMP}}^{\text{H}}$ of $\text{H}(\text{OMP})^{2-}$ were determined by titrating 50 ml of 0.54 mM HNO_3 and NaNO_3 ($I=0.1$ M; 25 °C) in the presence and absence of 0.3 mM OMP^{3-} with 1.5 ml of 0.03 M NaOH and by evaluating for water containing 30% (vol./vol.) dioxane the pH range of 5 to 10, and for 50% (vol./vol.) dioxane–water the pH range 5.7–10.5; the number of titration pairs in the two solvents were 6 and 7, respectively. Hence, the final results for $K_{\text{H}(\text{OMP})}^{\text{H}}$ and $K_{\text{OMP}}^{\text{H}}$ in water containing 30 and 50% (vol./vol.) dioxane are the averages of nine or ten pairs of titrations, respectively.

The acidity constants $K_{\text{H}_2(\text{UMP})}^{\text{H}}$, $K_{\text{H}(\text{UMP})}^{\text{H}}$ and $K_{\text{UMP}}^{\text{H}}$ of $\text{H}_2(\text{UMP})$ in water containing 30 or 50% (vol./vol.) dioxane were determined in six independent pairs of titrations as described above for $\text{H}_2(\text{OMP})^-$. In a further set of experiments $K_{\text{H}(\text{UMP})}^{\text{H}}$ and $K_{\text{UMP}}^{\text{H}}$ of $\text{H}(\text{UMP})^-$ were additionally determined in both solvent mixtures from two pairs of titrations (0.54 mM HNO_3 ; 0.3 mM UMP^{2-} ; see

above for $H(OMP)^{2-}$). Hence, the results for $K_{H_2(UMP)}^H$ are in both solvent mixtures the averages of six titration pairs, and the results for $K_{H(UMP)}^H$ and K_{UMP}^H of eight titration pairs.

Conditions for the determination of the stability constants

The stability constants $K_{M(OMP)}^M$ of the binary $M(OMP)^-$ complexes ($I=0.1$ M; 25°C) were determined in both solvent mixtures under the same conditions as the two acidity constants, $K_{H_3(OMP)}^H$ and $K_{H_2(OMP)}^H$, of $H(OMP)^{2-}$ (i.e. with 0.3 mM OMP^{3-}), except $NaNO_3$ was partly or fully replaced by $M(NO_3)_2$. With Mg^{2+} and Ca^{2+} $[M(NO_3)_2]$ was 0.0333 or 0.0267 M, i.e. $OMP:M^{2+}=1:111$ or $1:89$, respectively. For Cu^{2+} $[Cu(NO_3)_2]$ was 3.33 and 1.67 mM, i.e. the OMP to Cu^{2+} ratios were $1:11$ and $1:5.6$. For each system at least four independent pairs of titrations were made.

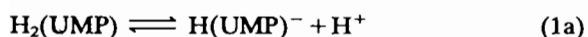
The constants $K_{M(OMP)}^M$ were computed from the pH range below the onset of the formation of $M(OMP-H)^{2-}$ or the hydrolysis of $Cu(aq)^{2+}$ as previously reported [4]. In the cases of Mg^{2+} and Ca^{2+} , where $M(aq)^{2+}$ does not hydrolyze before the onset of the formation of $M(OMP-H)^{2-}$, the experimental data were also analyzed with a curve-fitting procedure [4], and in this way the stability constant $K_{M(OMP)}^M$ plus the acidity constant $K_{M(OMP)}^H$ of the $M(OMP)^-$ species were determined. The agreement of the values obtained for $K_{M(OMP)}^M$ in the two evaluation procedures was excellent.

Results and discussion

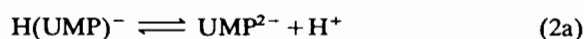
The conditions for the experiments were selected such that self-association of OMP (or of UMP [12]) is expected to be negligible [4]. The values given below for the equilibrium constants valid for aqueous solutions are from our previous studies [4] while all the results presented for the solvents water containing 30 or 50% (vol./vol.) 1,4-dioxane have only now been measured.

1. Acidity constants of $H_2(UMP)$ and $H_3(OMP)$

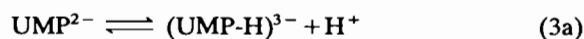
From the chemical structure shown in Fig. 1 for UMP^{2-} it is evident that this nucleotide may accept two protons at the phosphate group; in addition it is well known that the $H(N-3)$ site of the nucleic base moiety may release a further proton [13]. Hence, the following three deprotonation equilibria have to be considered:



$$K_{H_2(UMP)}^H = [H^+][H(UMP)^-]/[H_2(UMP)] \quad (1b)$$

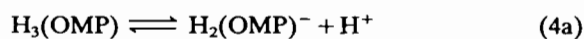


$$K_{H(UMP)}^H = [H^+][UMP^{2-}]/[H(UMP)^-] \quad (2b)$$

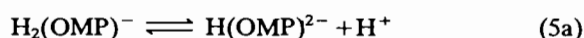


$$K_{UMP}^H = [H^+][(UMP-H)^{3-}]/[UMP^{2-}] \quad (3b)$$

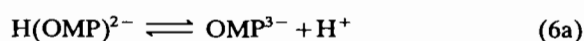
In the case of OMP an additional carboxylate group is present at position 6 of the pyrimidine ring (Fig. 1). Accordingly, the species $H_3(OMP)$ may be formed and therefore the following four equilibria have to be considered:



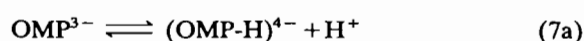
$$K_{H_3(OMP)}^H = [H^+][H_2(OMP)^-]/[H_3(OMP)] \quad (4b)$$



$$K_{H_2(OMP)}^H = [H^+][H(OMP)^{2-}]/[H_2(OMP)^-] \quad (5b)$$



$$K_{H(OMP)}^H = [H^+][OMP^{3-}]/[H(OMP)^{2-}] \quad (6b)$$



$$K_{OMP}^H = [H^+][(OMP-H)^{4-}]/[OMP^{3-}] \quad (7b)$$

It may be added that the ribose residue of nucleosides and nucleotides may also be ionized but this reaction even occurs in aqueous solution [14, 15] only at $pH > 12$ and is therefore not studied in the present context.

The results obtained via potentiometric pH titrations for the various acidity constants of the three-proton-donors H_2UMP and $H_2(OMP)^-$ for water and water containing 30 or 50% (vol./vol.) dioxane are listed in Table 1. It appears safe to assume that the acidities of $H_2(UMP)$ (eqn. (1)) and $H_3(OMP)$ (eqn. (4)) are very similar due to their near structural identity (see Fig. 1), i.e. $pK_{H_2(UMP)}^H \approx pK_{H_3(OMP)}^H$; hence, the first proton is released from the $-PO(OH)_2$ group in both NMPs. The release of the next proton in $H_2(OMP)^-$ (eqn. (5)) occurs from the carboxylic acid group in position 6; it is apparent from the values given in Table 1 that deprotonation of $H_3(OMP)$ and $H_2(OMP)^-$ occurs to some extent in overlapping pH ranges. The third proton from $H(OMP)^{2-}$ (eqn. (6)) and the second one from $H(UMP)^-$ (eqn. (2)) are from the $-PO(O^-)(OH)$ group. The slightly larger acidity in aqueous solution of the singlefold negatively charged $H(UMP)^-$ compared with the twofold negatively charged $H(OMP)^{2-}$ is expected [4], though this difference disappears with increasing dioxane content in the solvent mixture (Table 1). This disappearance evidently results from a smaller effect of dioxane on $K_{H(OMP)}^H$ compared with $K_{H_2(UMP)}^H$; probably a stronger solvation of $H(OMP)^{2-}$ by water molecules due to the presence of the 6-carboxylate group leads to a larger 'effective'

TABLE 1. Negative logarithms of the acidity constants for orotidine 5'-monophosphoric acid, $H_3(OMP)$ (eqns. (4)–(7)), and uridine 5'-monophosphoric acid, $H_2(UMP)$ (eqns. (1)–(3)), as determined by potentiometric pH titrations in water and in water containing 30 or 50% (vol./vol.) 1,4-dioxane at 25 °C and $I=0.1$ M ($NaNO_3$)^a

Acid	pK_a for the sites			
	$-PO(OH)_2$	$-COOH$	$-PO(O^-)(OH)$	$>N(3)H$
<i>In water</i>				
$H_3(OMP)$	~ 0.7 (eqn. (4))	1.46 ± 0.10 (eqn. (5))	6.40 ± 0.02 (eqn. (6))	9.35 ± 0.02 (eqn. (7))
$H_2(UMP)$	0.7 ± 0.3 (eqn. (1))		6.15 ± 0.01 (eqn. (2))	9.45 ± 0.02 (eqn. (3))
<i>In 30% dioxane</i>				
$H_3(OMP)$		2.18 ± 0.13	7.09 ± 0.02	9.85 ± 0.05
$H_2(UMP)$	1.64 ± 0.07		6.97 ± 0.04	10.10 ± 0.02
<i>In 50% dioxane</i>				
$H_3(OMP)$		2.80 ± 0.05	7.46 ± 0.04	10.17 ± 0.06
$H_2(UMP)$	2.27 ± 0.05		7.48 ± 0.05	10.51 ± 0.03

^aThe errors given are three times the standard error of the mean value or the sum of the probable systematic errors, whichever is larger. The acidity constants for $H_3(OMP)$ and $H_2(UMP)$ in water are from refs. 4 and 13, respectively.

dielectric constant in the close vicinity of OMP (compared with the bulk solvent and the situation for $H(UMP)^-$) and thus to a lower pK_a value (see also Section 4). The possibility of somewhat different solvation effects on the two NMPs is also confirmed by the varying differences between pK_{OMP}^H and pK_{UMP}^H . The fact that the H(N-3) site in OMP^{3-} (eqn. (7)) is deprotonated at a somewhat lower pH than the same site in UMP^{2-} (eqn. (3)), i.e. $pK_{OMP}^H < pK_{UMP}^H$, in all three solvents may at first sight appear as surprising yet this observation is also understood [4] and due to the electron-withdrawing properties of the carboxylate group.

Plots of the negative logarithms of the acidity constants for $H_3(OMP)$ in water containing 30 or 50% (vol./vol.) dioxane versus the corresponding values in water as solvent are shown in Fig. 2. It is interesting to observe that straight lines result from these plots. This allows several conclusions. (i) Addition of dioxane to an aqueous solution of $H_3(OMP)$ affects the various acidity constants of this species in a systematic way. (ii) Comparison of the slopes of the two straight lines suggests that the effect on small pK_a values is slightly larger than on higher pK_a values. (iii) The assumption that $pK_{H_2(UMP)}^H \approx pK_{H_3(OMP)}^H$ is confirmed, as the corresponding data points fit on these lines. The same type of plots may be constructed for $H_2(UMP)$; again straight lines result which allow the corresponding conclusions.

2. Stability and acidity constants of $M(OMP)^-$ complexes

The experimental data of the potentiometric pH titrations of $H(OMP)^{2-}$ in the presence of Mg^{2+} ,

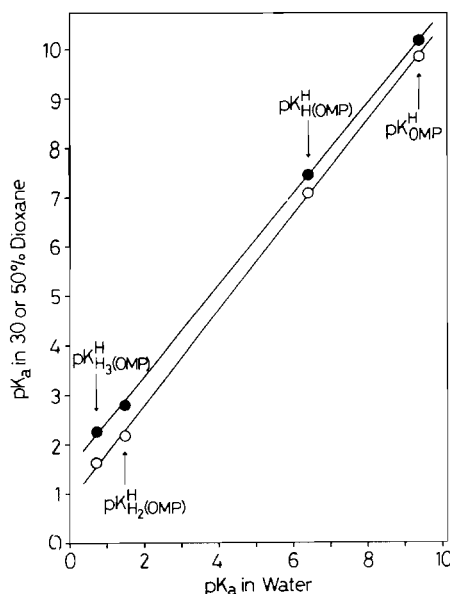


Fig. 2. Relationship between $pK_{H_3(OMP)}^H$, $pK_{H_2(OMP)}^H$, $pK_{H(OMP)}^H$ and $pK_{OMP}^H (=pK_a)$ in water containing 30 (○) or 50% (●) (vol./vol.) 1,4-dioxane and the corresponding pK_a values for $H_3(OMP)$ in water. The two times four data pairs are from Table 1 with the assumption that $pK_{H_2(UMP)}^H \approx pK_{H_3(OMP)}^H$. The least-squares regression line through the 4 data points for 30% dioxane (○) has the slope $m=0.961 \pm 0.015$ (1σ) and the intercept $b=y_0=0.885 \pm 0.084$, and the one for 50% dioxane has $m=0.922 \pm 0.012$ and $b=y_0=1.545 \pm 0.069$.

Ca^{2+} or Cu^{2+} may be completely described by considering equilibria (5) (which actually plays only a very minor role), (6) and (8), as long as the evaluation is not carried into the pH range, where formation of $M(OMP-H)^{2-}$ species or of hydroxo complexes

occurs (see 'Experimental' and ref. 4).



$$K_{\text{M}(\text{OMP})}^{\text{M}} = [\text{M}(\text{OMP})^{-}] / ([\text{M}^{2+}][\text{OMP}^{3-}]) \quad (8b)$$

In the Mg^{2+} and Ca^{2+} systems, where deprotonation of the H(N-3) site in $\text{M}(\text{OMP})^{-}$ takes place before the onset of the hydrolysis of $\text{M}(\text{aq})^{2+}$, the equilibrium constant for the following reaction could be determined as well.



$$K_{\text{M}(\text{OMP})}^{\text{H}} = [\text{H}^{+}][\text{M}(\text{OMP-H})^{2-}] / [\text{M}(\text{OMP})^{-}] \quad (9b)$$

The results obtained for the solvents water, and water containing 30 or 50% (vol./vol.) dioxane are given in Table 2. It is evident that increasing amounts of dioxane to an aqueous solution lower the polarity of the solvent; hence, the formation of uncharged or lower charged species should be favored while the creation of charged sites should be inhibited. Indeed, this is the case: the stability of the $\text{M}(\text{OMP})^{-}$ complexes (eqn. (8)) increases for all three metal ions with an increasing dioxane concentration, while the formation of the $\text{M}(\text{OMP-H})^{2-}$ complexes (eqn. (9)), which result from a deprotonation of the H(N-3) site (Fig. 1), is increasingly inhibited.

3. Structural considerations on the $\text{M}(\text{OMP})^{-}$ complexes

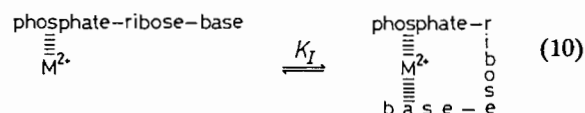
As indicated already in the 'Introduction', one of the questions one may ask regarding $\text{M}(\text{OMP})^{-}$

TABLE 2. Logarithms of the stability constants of the $\text{M}(\text{OMP})^{-}$ complexes (eqn. (8)) with Mg^{2+} , Ca^{2+} and Cu^{2+} , together with the negative logarithms of the acidity constants for the $\text{Mg}(\text{OMP})^{-}$ and $\text{Ca}(\text{OMP})^{-}$ complexes (eqn. (9)), as determined by potentiometric pH titrations in water and in water containing 30 or 50% (vol./vol.) 1,4-dioxane at 25 °C and $I=0.1$ M (NaNO_3)^a

M^{2+}	$\log K_{\text{M}(\text{OMP})}^{\text{M}}$	$\text{p}K_{\text{M}(\text{OMP})}^{\text{H}}$
<i>In water</i>		
Mg^{2+}	1.93 ± 0.02	8.89 ± 0.02
Ca^{2+}	1.76 ± 0.02	8.77 ± 0.02
Cu^{2+}	3.29 ± 0.05	
<i>In 30% dioxane</i>		
Mg^{2+}	2.57 ± 0.02	9.32 ± 0.11
Ca^{2+}	2.36 ± 0.02	9.15 ± 0.06
Cu^{2+}	4.32 ± 0.01	
<i>In 50% dioxane</i>		
Mg^{2+}	2.96 ± 0.02	9.54 ± 0.15
Ca^{2+}	2.73 ± 0.01	9.27 ± 0.15
Cu^{2+}	5.03 ± 0.02	

^aThe errors given are three times the standard error of the mean value or the sum of the probable systematic errors, whichever is larger. The equilibrium constants in water are from ref. 4.

complexes is: does the carboxylate group bind to the metal ion? As also indicated: such a metal-carboxylate binding in $\text{M}(\text{OMP})^{-}$ complexes is only possible in the *anti* conformation of the nucleotide (see Fig. 1). However, in any case, any kind of base backbinding or macrochelate formation has to enhance complex stability [16–18]. A possibly increased stability of $\text{M}(\text{OMP})^{-}$ complexes could therefore be attributed to the participation of the carboxylate group in complex formation, i.e. the position of the following intramolecular equilibrium has to be evaluated:



The position of this concentration-independent equilibrium (eqn. (10)) between an 'open' isomer, $\text{M}(\text{OMP})_{\text{op}}^{-}$, and a closed species, $\text{M}(\text{OMP})_{\text{cl}}^{-}$, is defined by the dimensionless equilibrium constant K_I :

$$K_I = [\text{M}(\text{OMP})_{\text{cl}}^{-}] / [\text{M}(\text{OMP})_{\text{op}}^{-}] \quad (11)$$

Values for K_I may be calculated with eqn. (12) from an experimentally accessible (overall) stability constant, $K_{\text{M}(\text{OMP})}^{\text{M}}$, provided the stability constant, $K_{\text{M}(\text{OMP})_{\text{op}}}^{\text{M}*}$, of the open isomer is also known.

$$K_I = \frac{K_{\text{M}(\text{OMP})}^{\text{M}}}{K_{\text{M}(\text{OMP})_{\text{op}}}^{\text{M}*}} - 1 = 10^{\log \Delta^*} - 1 \quad (12)$$

$$\log \Delta^* = \log K_{\text{M}(\text{OMP})}^{\text{M}} - \log K_{\text{M}(\text{OMP})_{\text{op}}}^{\text{M}*} \quad (13)$$

It is evident that obtaining the difference defined in eqn. (13) is the crucial part of any evaluation, and the reliability of any calculations for K_I (eqn. (12)) depends on the accuracy of this difference.

Recently, one aspect of this problem has been resolved by constructing $\log K_{\text{Cu}(\text{R-MP})}^{\text{Cu}}$ versus $\text{p}K_{\text{H}(\text{R-MP})}^{\text{H}}$ plots for $\text{Cu}(\text{R-MP})$ complexes where R-MP^{2-} is a simple phosphate monoester, i.e. the group R does not participate in complex formation. According to experience [17], plots of this type should result in straight lines for a series of structurally related ligands; this is indeed the case [13, 19], establishing thus a relationship between Cu^{2+} -phosphate coordination and phosphate group basicity. The equations for the indicated baseline correlations [13, 19] are listed in Table 3 [20]. These results allow us to calculate for any known acidity constant ($\text{p}K_{\text{H}(\text{R-MP})}^{\text{H}}$) of a monoprotonated phosphate residue, $\text{H}(\text{R-MP})^{-}$, the corresponding stability constant ($\log K_{\text{Cu}(\text{R-MP})}^{\text{Cu}}$) of the $\text{Cu}(\text{R-MP})$ complex. In other words, the stability of the 'open' species in equilibrium (10) can be calculated for *uncharged* $\text{Cu}(\text{NMP})$ complexes and thus K_I may be obtained via equations analogous to (12) and (13).

TABLE 3. Baseline correlations for Cu^{2+} -phosphate coordination and phosphate group basicity for the solvents water and water containing 30 or 50% (vol./vol.) 1,4-dioxane ($I=0.1$ M, NaNO_3 ; 25°C),^a together with some properties of the mentioned solvents.^b Straight-line equation: $y=mx+b$, where x represents the $\text{p}K_{\text{a}}$ value of any phosphate monoester, $\text{H}(\text{R-MP})^-$, and y the calculated $\log K_{\text{Cu}(\text{R-MP})}^{\text{Cu}}$ value of the corresponding $\text{Cu}(\text{R-MP})$ complex; the errors given with the slopes (m) and the y -axis intercepts (b) correspond to one standard deviation (1σ). The listed SD values^c (column at the right) times 2 or 3 are considered as reasonable error limits for any stability constant calculation in the $\text{p}K_{\text{a}}$ range 5–7 for aqueous solution, 6–7.5 for 30% and 6.5–8 for 50% (vol./vol.) dioxane–water mixtures^d

No. ^d	% Dioxane (vol./vol.)	Mol fraction	e^b	m	b	SD^c
1	0	0	78.5	0.453 ± 0.056	0.055 ± 0.340	0.026
2	30	0.083	52.7	0.559 ± 0.015	-0.089 ± 0.106	0.008
3	50	0.175	35.2	0.571 ± 0.022	0.190 ± 0.160	0.01

^aThe slopes (m) and intercepts (b) for the straight baselines from plots of $\log K_{\text{Cu}(\text{R-MP})}^{\text{Cu}}$ vs. $\text{p}K_{\text{H}(\text{R-MP})}^{\text{H}}$ are calculated from equilibrium constants determined earlier [13, 19] for the following simple phosphate monoesters: (i) for the aqueous solution (entry 1) with 4-nitrophenyl phosphate, phenyl phosphate, *n*-butyl phosphate, *D*-ribose 5'-monophosphate, uridine 5'-monophosphate and thymidine 5'-monophosphate [13]; (ii) for the dioxane–water mixtures (entries 2 and 3) with 4-nitrophenyl phosphate, phenyl phosphate, *n*-butyl phosphate and *D*-ribose 5'-monophosphate [19]. ^bThe dielectric constants for the dioxane–water mixtures are interpolated from the data given in ref. 20. ^cThe standard deviations (SD) result from the differences between the experimental and calculated values for the mentioned six (i/entry 1)^{*} and four (ii/entries 2 and 3)^{*} ligand systems [13, 19]. ^dThe data for m , b and SD for entry 1 are from Tables V and VI of ref. 13, and those for entries 2 and 3 from Tables 1 and 2 of ref. 19.

The problem in the present case is that the different charges of $\text{H}(\text{R-MP})^-$ and $\text{H}(\text{OMP})^{2-}$ prevent a simple evaluation. This is why $\log K_{\text{M}(\text{OMP})_{\text{op}}}^{\text{M}}$ and $\log \Delta^*$ (eqns. (12) and (13)) carry an asterisk (see also Sections 4 and 5); we define here for the present that the values with an asterisk are *corrected* for the pure charge effect (see also the section 'Some general conclusions'). To say it differently, the stability of the negatively charged $\text{Cu}(\text{OMP})_{\text{op}}^-$ has to be somewhat higher than that of the corresponding neutral $\text{Cu}(\text{NMP})_{\text{op}}$ species. This increased stability is then represented by straight lines parallel to those defined in Table 3 (or shown below in Fig. 3).

4. The increased stability of $\text{Cu}(\text{OMP})^-$ and some reasonings about the influence of a charge effect

For water and water containing 30 or 50% (vol./vol.) dioxane the plots of $\log K_{\text{Cu}(\text{R-MP})}^{\text{Cu}}$ versus $\text{p}K_{\text{H}(\text{R-MP})}^{\text{H}}$ for the results summarized in Table 3 are shown in Fig. 3, together with the data pairs for $\text{Cu}(\text{OMP})^-/\text{H}(\text{OMP})^{2-}$ (see Tables 1 and 2) which are given as full circles. (The data referring to the full triangle [21] are discussed in the final section 'Some general conclusions'). Evidently for all three solvents these data pairs do not fit on the reference lines; the points due to $\text{Cu}(\text{OMP})^-$ are above, indicating an increased complex stability, and this might be taken as a hint that the intramolecular equilibrium (10) is existing because participation of a further binding site should lead to an increased complex stability [16–18].

However, such a simple conclusion is not justified, as indicated already in Section 3: because the charges

of $\text{H}(\text{R-MP})^-$ and $\text{H}(\text{OMP})^{2-}$, and also of $\text{Cu}(\text{R-MP})$ and $\text{Cu}(\text{OMP})^-$, are different. For example, the negative charge of the carboxylate group in position 6 of the pyrimidine ring (Fig. 1) increases in aqueous solution the affinity for a proton of the phosphate group in OMP by 0.25 log unit, as it is evident from $\text{p}K_{\text{H}(\text{OMP})}^{\text{H}}=6.40$ and $\text{p}K_{\text{H}(\text{UMP})}^{\text{H}}=6.15$ (Table 1). Clearly, the effect of the negatively charged carboxylate group on the coordination of the dipositively charged Cu^{2+} is expected to be even somewhat larger than that on the binding of the singlefold positively charged proton.

A further difficulty is that the mentioned charge effect of 0.25 log unit for the proton diminishes in 30% dioxane and disappears in 50% dioxane (see Table 1). This is probably the result of a better solvation by dioxane of the neutral uracil moiety compared with the negatively charged orotate residue which attracts water (Section 1); the more intense dioxane solvation of the uracil residue would then lead to a lower effective dielectric constant in the neighborhood of the phosphate group of UMP^{2-} , thus enhancing its proton affinity compared to that of the somewhat less dioxane-solvated OMP^{3-} . In agreement with this interpretation is the decreasing acidity of the $\text{H}(\text{N-3})$ site in UMP^{2-} with increasing amounts of dioxane; i.e. the difference $\text{p}K_{\text{UMP}^{2-}}^{\text{H}}-\text{p}K_{\text{OMP}^{3-}}^{\text{H}}$ increases from water to 50% dioxane–water (Table 1). These comparisons demonstrate how difficult it is to make a reliable estimate about the charge effect.

To gather at least some tentative feeling about the size of the charge effect and the way it is influenced

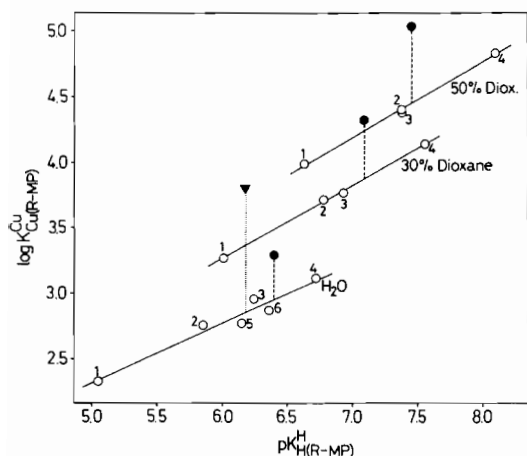


Fig. 3. Relationship between $\log K_{\text{Cu}^{2+}/\text{Cu(R-MP)}}^{\text{Cu}}$ and $\text{p}K_{\text{H}^{\text{H}}(\text{R-MP})}^{\text{H}}$ for the Cu^{2+} 1:1 complexes of 4-nitrophenyl phosphate (1), phenyl phosphate (2), D-ribose 5'-monophosphate (3), n-butyl phosphate (4), uridine 5'-monophosphate (5), and thymidine 5'-monophosphate (6) in water and in water containing 30 or 50% (vol./vol.) dioxane. The least-squares lines are drawn in each case through the corresponding six or four data sets [13, 19]; the equations for these reference lines are given in Table 3. All these plotted equilibrium constants refer to solutions at 25 °C and $I=0.1$ M (NaNO_3). The points due to the Cu^{2+} 1:1 complexes formed with OMP^{3-} (●) in the three mentioned solvents are inserted for comparison (see Section 4); the corresponding equilibrium constants are taken from Tables 1 and 2 ($I=0.1$ M, NaNO_3 ; 25 °C). For further comparison the data pair ($\log K_{\text{Cu}(\text{SaP})}^{\text{Cu}}=3.8$; $\text{p}K_{\text{H}(\text{SaP})}^{\text{H}}=6.18$) for the Cu^{2+} complex of salicyl phosphate (SaP^{3-} ; ▼) in aqueous solution ($I=0.1$ M, NaClO_4 ; 25 °C [21]) is inserted; see the final section 'Some general conclusions'. The vertical dotted and broken lines emphasize the stability differences to the corresponding reference lines.

by increasing amounts of dioxane we made the following reasonings and calculations. The average increase in stability of the $\text{M}(\text{OMP})^-$ complexes of Mg^{2+} , Ca^{2+} , Sr^{2+} , Ba^{2+} , Mn^{2+} , Co^{2+} , Ni^{2+} , Cu^{2+} , Zn^{2+} and Cd^{2+} in aqueous solution corresponds to 0.40 ± 0.06 log unit (=log Δ ; see also Section 5 and Table 5, *vide infra*) [4]; as in several examples where no direct interaction between the charged sites is possible (but the distances are comparable), the electrostatic effect amounts also to about 0.4 log unit, it is concluded [4], "that the somewhat increased stability of all the mentioned $\text{M}(\text{OMP})^-$ complexes is mainly the result of a charge effect of the carboxylate group (in position 6 of OMP^{3-}) and *not* of a direct participation in complex formation, i.e. there are no indications for the formation of significant amounts of macrochelates involving the phosphate and the carboxylate groups".

A further observation for aqueous solutions [4] is that the stability increase for $\text{Cu}(\text{OMP})^-$ amounts to 0.45 ± 0.09 log unit (i.e. it is identical within the

error limits with the average given in the preceding paragraph) and that this value is very close to the average of the electrostatic acidification effects of Mg^{2+} , Ca^{2+} , Sr^{2+} , Ba^{2+} and Mn^{2+} on $\text{H}(\text{N-3})$ in $\text{M}(\text{OMP})^-$ complexes, i.e. $\text{p}K_{\text{OMP}^-}^{\text{H}} - \text{p}K_{\text{M}(\text{OMP})}^{\text{H}} = 0.52 \pm 0.09$, and especially close to the one for $\text{Mg}(\text{OMP})^-$, i.e. $\text{p}K_{\text{OMP}^-}^{\text{H}} - \text{p}K_{\text{Mg}(\text{OMP})}^{\text{H}} = 0.46 \pm 0.03$ [4], (while the one for $\text{Ca}(\text{OMP})^{2-}$, i.e. $\text{p}K_{\text{OMP}^{2-}}^{\text{H}} - \text{p}K_{\text{Ca}(\text{OMP})^{2-}}^{\text{H}} = 0.58 \pm 0.03$, appears to be somewhat larger). Hence, we used the data of Tables 1 and 2 and calculated the differences $\Delta \text{p}K_a = \text{p}K_{\text{OMP}^-}^{\text{H}} - \text{p}K_{\text{M}(\text{OMP})}^{\text{H}}$ for the Mg^{2+} and Ca^{2+} complexes to obtain some information on the electrostatic effect in the mixed solvents (Table 4).

5. Does the carboxylate group directly coordinate to Cu^{2+} in $\text{Cu}(\text{OMP})^-$?

To answer this question at first the results of Table 4 need to be evaluated somewhat further with regard to the size of the expected electrostatic effect. The values of $\Delta \text{p}K_a$ given for the Mg^{2+} and Ca^{2+} systems indicate, despite the relatively large error limits, that the acidification of the $\text{H}(\text{N-3})$ site by Ca^{2+} increases stronger than the one by Mg^{2+} with increasing amounts of dioxane in the aqueous solvent mixture. As the charge density on Mg^{2+} is larger than on Ca^{2+} , because Mg^{2+} prefers coordination number 6 with an ionic radius of 0.72 Å and Ca^{2+} 8 with a radius of 1.12 Å [22], there has to be a special interaction as otherwise the reverse effect would be expected. Possibly a phosphate-coordinated Ca^{2+} , due to its larger size, is able to interact in addition to some extent also with the carbonyl oxygen in position 2, while the smaller Mg^{2+} cannot reach this carbonyl oxygen conveniently. Indeed, from crystal structure studies Ca^{2+} is well known [23] to undergo carbonyl interactions and in the preferred *syn* conformation of OMP its 2-carbonyl group is directed towards the 5'-phosphate residue (Fig. 1). Such a (weak) Ca^{2+} -carbonyl interaction close to the $\text{H}(\text{N-3})$ site would evidently facilitate the release of a proton from this site. In any case, it appears that indications about the size of the expected electrostatic effects operating in the three solvents in $\text{M}(\text{OMP})^-$ complexes between the phosphate-coordinated metal ion and the not coordinated carboxylate group can best be obtained from the $\Delta \text{p}K_a$ values of the Mg^{2+} systems in Table 4.

With this in mind one has to ask next: how large is the stability increase for the $\text{Cu}(\text{OMP})^-$ complexes in the several solvents? Is the stability increase larger than the expected charge effect? Consequently, the increased stability of the $\text{Cu}(\text{OMP})^-$ complex (Fig. 3) needs now to be quantified: OMP^{3-} and UMP^{2-} differ, aside from their preferred conformations, only

TABLE 4. Acidification of the H(N-3) site in OMP^{3-} (see Fig. 1) in several solvents ($I=0.1$ M, NaNO_3 ; 25 °C) by the formation of Mg^{2+} or Ca^{2+} complexes, as expressed by $\Delta pK_a = pK_{\text{OMP}}^{\text{H}} - pK_{\text{M(OMP)}}^{\text{H}}$

No. ^a	% Dioxane (vol./vol.)	$pK_{\text{OMP}}^{\text{H}}$	M^{2+}	$pK_{\text{M(OMP)}}^{\text{H}}$	ΔpK_a
1a	0	9.35 ± 0.02	Mg^{2+}	8.89 ± 0.02	0.46 ± 0.03
1b	0		Ca^{2+}	8.77 ± 0.02	0.58 ± 0.03
2a	30	9.85 ± 0.05	Mg^{2+}	9.32 ± 0.11	0.53 ± 0.12
2b	30		Ca^{2+}	9.15 ± 0.06	0.70 ± 0.08
3a	50	10.17 ± 0.06	Mg^{2+}	9.54 ± 0.15	0.63 ± 0.16
3b	50		Ca^{2+}	9.27 ± 0.15	0.90 ± 0.16

^aThe values for $pK_{\text{OMP}}^{\text{H}}$ and $pK_{\text{M(OMP)}}^{\text{H}}$ are from Tables 1 and 2, respectively; the error limits for the differences ΔpK_a were calculated according to the error propagation after Gauss.

by the presence of the 6-carboxylate group (Fig. 1). Hence, the acidity constant for the monoprotonated phosphate group in H(UMP)^- reflects certainly the properties of the same group in H(OMP)^{2-} in the absence of any carboxylate influence; in other words, the negative logarithm of this micro acidity constant, $pK_{\text{OMP-H}}^{\text{H}}$ (see, for example, ref. 18), is well represented by the $pK_{\text{H(UMP)}}^{\text{H}}$ values given in Table 1 for the several solvents. With this micro acidity constant and the reference-line equations of Table 3 for the $\log K_{\text{Cu(R-MP)}}^{\text{Cu}}$ versus $pK_{\text{H(R-MP)}}^{\text{H}}$ plots (see also Fig. 3), the logarithm of the stability constants for Cu(OMP)^- complexes without the influence of the carboxylate group may be calculated, and the following stability difference with the experimentally determined values may then be defined:

$$\log \Delta = \log K_{\text{Cu(OMP)}}^{\text{Cu}}_{\text{exp}} - \log K_{\text{Cu(OMP)}}^{\text{Cu}}_{\text{calc}} \quad (14)$$

The results of these calculations are presented in Table 5. An argument against this procedure could be that the experimentally determined values for $\log K_{\text{Cu(OMP)}}^{\text{Cu}}_{\text{exp}}$ in the three solvents are based on the

corresponding macro acidity constants, i.e. the $pK_{\text{H(OMP)}}^{\text{H}}$ values of Table 1. Therefore for completeness, the basicity adjusted 'experimental' values are given in parentheses with the second column of Table 5 and the connected values for $\log \Delta$, also in parentheses, with the fourth column at the right. It is evident that the interpretation will not be significantly influenced by the small changes in these data; in fact, the parenthesized values for $\log \Delta$ are even more in favor of the following conclusion.

For aqueous solutions of M(OMP)^- complexes it has been concluded earlier [4] (see also Section 4) that the stability enhancing effect of the carboxylate group amounts to about 0.4 log unit, which is in agreement with the ΔpK_a value of the $\text{Mg}^{2+}/\text{OMP}$ system (0.46 ± 0.03 ; Table 4) and both values are close to the $\log \Delta$ value given for Cu(OMP)^- ($0.45 \pm 0.09/0.34$) in Table 5; hence, this is evidence that the open species in equilibrium (10) dominates. More important, the values for $\log \Delta$ of the Cu^{2+} system (0.51 ± 0.03 (0.45); 0.57 ± 0.04 (0.58); Table 5) and those for ΔpK_a of the Mg^{2+} system (0.53 ± 0.12 ; 0.63 ± 0.16 ; Table 4) in the aqueous solvents con-

TABLE 5. Stability constant comparisons (eqn. (14)) for the Cu(OMP)^- complex formed in different solvents between the measured stability constants (exp.) from Table 2 and the calculated stability constants for a pure Cu^{2+} -phosphate coordination (calc.) based on the straight-line equations^a quantifying the relationship between complex stability and phosphate group basicity (see Fig. 3) and the micro acidity constant $k_{\text{OMP-H}}^{\text{OMP}}$,^b not affected by the negatively charged carboxylate group in position 6 of OMP^{3-} (see Fig. 1)^{c, d}

% Dioxane (vol./vol.)	$\log K_{\text{Cu(OMP)}}^{\text{Cu}}$		$\log \Delta = \log K_{\text{exp}} - \log K_{\text{calc}}$
	exp.	calc.	
0	3.29 ± 0.05 (3.18)	2.84 ± 0.08	0.45 ± 0.09 (0.34)
30	4.32 ± 0.01 (4.26)	3.81 ± 0.03	0.51 ± 0.03 (0.45)
50	5.03 ± 0.02 (5.04)	4.46 ± 0.03	0.57 ± 0.04 (0.58)

^aThe corresponding parameters are listed in Table 3. ^b $pK_{\text{OMP-H}}^{\text{OMP}} = pK_{\text{H(UMP)}}^{\text{H}}$ (see text in Section 5 and Table 1). ^cThe error limits (see also Tables 2 and 3) for the differences $\log \Delta$ were calculated according to the error propagation after Gauss. ^dThe data in parentheses are basicity corrected values (via the straight-line equations); see text in Section 5. The error limits of these values are the same as those given to their left.

taining 30 or 50% (vol./vol.) dioxane also correspond very well with each other. In other words, after correcting the stability of $\text{Cu}(\text{OMP})_{\text{op}}^-$ for the charge effect, $\log \Delta^*$ (eqn. (13)) approaches a value of zero. Therefore, these observations provide evidence that in the $\text{Cu}(\text{OMP})^-$ complex in all three solvents mainly an electrostatic charge effect of the carboxylate group is operating and that this group does *not* directly interact to a significant extent with the phosphate-coordinated metal ion.

6. Some further considerations about the solvent influence on $M(\text{OMP})^-$ systems

To see if there is a systematic proton-affinity/complex-stability relationship for OMP under the influence of a changing solvent polarity $\log K_{M(\text{R-MP})}^M$ versus $\text{p}K_{\text{H}(\text{R-MP})}^{\text{H}}$ plots were constructed for the OMP systems with Cu^{2+} , Mg^{2+} and Ca^{2+} with the data of Tables 1 and 2. These plots are shown in Fig. 4, together with the corresponding plots for the 4-nitrophenyl phosphate (NPhP^{2-}) and D-ribose 5'-monophosphate (RibMP^{2-}) systems with Cu^{2+}

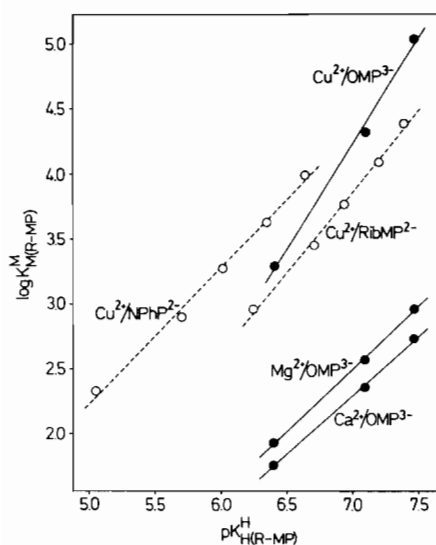


Fig. 4. Relationship between $\log K_{M(\text{R-MP})}^M$ and $\text{p}K_{\text{H}(\text{R-MP})}^{\text{H}}$ for the Mg^{2+} , Ca^{2+} and Cu^{2+} complexes of OMP^{3-} (●) (see Tables 1 and 2), as well as for the Cu^{2+} complexes of 4-nitrophenyl phosphate (NPhP^{2-}) and D-ribose 5'-monophosphate (RibMP^{2-}) (○) [24], as they result from the addition of increasing amounts of dioxane to the aqueous solutions of the components ($I=0.1$ M, NaNO_3 ; 25°C). The point at the left for each straight line refers to water and the point most to the right to 50% (vol./vol.) dioxane-water; for the $\text{M}^{2+}/\text{OMP}^{3-}$ systems the point in between refers to 30% (vol./vol.) dioxane-water mixtures, and for the $\text{Cu}^{2+}/\text{NPhP}^{2-}$ or RibMP^{2-} systems the three points in between hold for 20, 30 or 40% (vol./vol.) dioxane-water mixtures. The slopes (m) and intercepts (b) defining the least-squares regression lines for the five $\text{M}^{2+}/\text{R-MP}^{2-}$ systems shown above are listed in Table 6.

[24]. Straight lines are observed for all five systems; the results of the least-squares regression calculations are summarized in Table 6, together with some further related examples [24].

Comparison of the slopes (m) listed in Table 6 for the Cu^{2+} and Zn^{2+} /acetate systems (nos. 7 and 8) might indicate that different slopes are observed for different metal ions. To some extent this may be true and appears to be confirmed for the OMP systems with Mg^{2+} , Ca^{2+} and Cu^{2+} (nos. 1–3). In fact, for the latter three systems the slopes differ considerably (see also Fig. 4), and the increased slope observed for $\text{Cu}(\text{OMP})^-/\text{H}(\text{OMP})^{2-}$ might be taken as an indication that with a decreasing solvent polarity OMP^{3-} behaves increasingly as a tridentate ligand towards Cu^{2+} , while it does not do so with Mg^{2+} and Ca^{2+} .

However, this latter conclusion is most probably not justified, as the increased slope may have another origin. The slopes for such different Cu^{2+} systems as those with NPhP^{2-} , UTP^{4-} and Ac^- (nos. 5–7 of Table 6) are all close to 1 (average: $m=1.01$), while that for the $\text{Cu}^{2+}/\text{RibMP}^{2-}$ system is larger ($m=1.24$; no. 4). This latter observation was already previously recognized and attributed [24] to specific solvation properties of RibMP^{2-} : the hydroxo groups of the ribose ring probably form hydrogen bonds with water molecules of the solvent and this may lead to slightly higher water concentrations close to the phosphate group than is expressed by the percentage of the dioxane-water mixtures; it is clear, a slightly higher water concentration means a somewhat lower $\text{p}K_{\text{H}(\text{RibMP})}^{\text{H}}$ value and also, but less pronounced, $\log K_{\text{Cu}(\text{RibMP})}^{\text{Cu}}$ value (cf. Fig. 3); consequently, a somewhat increased slope is expected (see also refs. 19, 24).

Similar solvation effects may operate with OMP. Indeed, that the negatively charged carboxylate group in position 6 (Fig. 1) does have an effect in this respect was already discussed in Sections 1 and 4 in connection with the somewhat different effects of increasing dioxane concentrations on the acidity constants of $\text{H}(\text{OMP})^{2-}$ and $\text{H}(\text{UMP})^-$ (see also Table 1). Finally, though it would be appealing to attribute the relatively steep slope of the $\text{Cu}^{2+}/\text{OMP}^{3-}$ system in Fig. 4 (see also Table 6) to an increasing participation of the 6-carboxylate group in metal ion coordination with an increasing dioxane content in the solvent, such a conclusion is not justified.

Conclusions

The present study of M^{2+}/OMP systems in water containing 30 or 50% (vol./vol.) dioxane confirms to

TABLE 6. Slopes (m) and y -axis intercepts (b) for regression lines of plots of $\log K_{M(L)}^M$ versus $pK_{H(L)}^H$ for various ligands (L) and several of their complexes ($I=0.1$ M; 25°C). In the straight-line equation, $y=mx+b$, x represents the pK_a value of $H(L)$ and y the stability constant, $\log K_{M(L)}^M$, of the corresponding $M(L)$ complex; the errors given with m and b correspond to one standard deviation (1σ)^a

No. ^a	M(L)/H(L)	m	b	R^b
1	Mg(OMP) ⁻ /H(OMP) ²⁻	0.966 ± 0.032	-4.260 ± 0.225	0.9994
2	Ca(OMP) ⁻ /H(OMP) ²⁻	0.909 ± 0.033	-4.067 ± 0.232	0.9993
3	Cu(OMP) ⁻ /H(OMP) ²⁻	1.623 ± 0.109	-7.118 ± 0.759	0.9978
4	Cu(RibMP)/H(RibMP) ⁻	1.242 ± 0.047	-4.827 ± 0.325	0.9979
5	Cu(NPhP)/H(NPhP) ⁻	1.052 ± 0.046	-3.029 ± 0.272	0.9972
6	Cu(UTP) ²⁻ /H(UTP) ³⁻	0.930 ± 0.011	-0.201 ± 0.077	0.9999
7	Cu(Ac) ⁺ /H(Ac)	1.056 ± 0.010	-2.997 ± 0.053	0.9999
8	Zn(Ac) ⁺ /H(Ac)	0.860 ± 0.100	-2.869 ± 0.531	0.9933

^aThe least-squares regression lines for entries 1–3 were calculated with the data listed in Tables 1 and 2; see also Fig. 3. Entries 4–8 were taken from Table II of ref. 24. ^bCorrelation coefficient.

a large part the previous results for aqueous solutions [4]. The energy barrier between the *syn* and *anti* conformation of OMP³⁻ (Fig. 1) is evidently too large to be overcome in the Cu(OMP)⁻ complex and to allow a significant extent of direct coordination of the 6-carboxylate group. It should be recalled (see 'Introduction') that in the dominating *syn* conformation a simultaneous binding of a metal ion to the phosphate and carboxylate groups is *not* possible, and that the sterically suitable *anti* conformer occurs to less than 14%.

Furthermore, considering that the basicity of the carboxylate group of OMP is very low (Table 1) it is not surprising that also the metal ion affinity of this group is small, as was shown recently in studies with orotidine [3]. It is apparent that the stability gain by carboxylate coordination is not enough to significantly overcome the *syn-anti* energy barrier. It may be added that formation of 10% of a macrochelate in equilibrium (10) means only a stability increase (compared to the stability of the open form) of 0.05 log unit; a formation degree of 20% for the macrochelate corresponds to a stability enhancement of 0.1 log unit and this appears to be close to the limit that would possibly have been recognized (beyond the charge effect). Thus, we may conclude with some confidence for the Cu(OMP)⁻ complex that in all three solvents studied here, if at all, not more than 20% form a macrochelate involving simultaneous metal ion coordination to the 5'-phosphate group and the 6-carboxylate residue (Fig. 1).

However, it may also be emphasized that a simultaneous coordination of a phosphate and carboxylate group to a metal ion is possible, as may be demonstrated with salicyl phosphate (SaP³⁻). The negative logarithms of the acidity constants of H₂(SaP)⁻ in aqueous solution are $pK_{H_2(SaP)}^H =$

3.57 ± 0.02 (analogous to eqn. (5)) and $pK_{H(SaP)}^H = 6.18 \pm 0.01$ (analogous to eqn. (6)) [21]; the first value refers to the release of the proton from the carboxylic acid group and the second one to that of the monoprotonated phosphate residue. The stability of the Cu(SaP)⁻ complex is quantified by $\log K_{Cu(SaP)}^{Cu} = 3.8 \pm 0.1$ [21]. The corresponding data pair is inserted in Fig. 3 as the full triangle point. This point is rather far above the reference line indicating a significant stability increase for the Cu(SaP)⁻ complex. It should be noted that all the given equilibrium constants refer to 10°C and $I=0.1$ M (NaClO₄) [21]; however, to a first approximation it may be assumed (see, for example, ref. 25) that at 25°C the data point is simply somewhat shifted parallel to the reference line without a large effect on the distance from this line. Hence, these equilibrium constants may be directly evaluated.

Use of $pK_{H(SaP)}^H = 6.18$ and the straight line equation for aqueous solutions of Table 3 gives $\log K_{Cu(SaP)}^{Cu} = 2.85 \pm 0.08$, which is the stability constant of the 'open' Cu(SaP)⁻ complex (see equilibrium (10)) in which the charge effect of the carboxylate group has *not* been considered; hence, $\log \Delta = \log K_{Cu(SaP)}^{Cu}/\text{exp} - \log K_{Cu(SaP)}^{Cu}/\text{calc} = (3.8 \pm 0.1) - (2.85 \pm 0.08) = 0.95 \pm 0.13$ (analogous to eqn. (14)). If one assumes that the stability of the open Cu(SaP)⁻ species, in which the metal ion is only phosphate-coordinated, is enhanced by the presence of the negatively charged (but not coordinated) carboxylate group by about 0.5 log unit (see also the reasonings in Sections 4 and 5) one is left with a stability increase of 0.45 log unit to be attributed to chelate formation. As this evaluation contains several assumptions it seems appropriate to apply a large error range; we take ± 0.3 log unit; hence, one obtains for $\log \Delta^* = 0.45 \pm 0.3$ (analogous to eqn. (13); see also Section 3). Application of this value to the equation analogous to (12) results in $K_1 = 1.82$, from which

follows that the 'closed' isomer in equilibrium (10) occurs to 65 (± 25)%. This then is clear evidence that a significant percentage of the $\text{Cu}(\text{SaP})^-$ species contains Cu^{2+} that is coordinated simultaneously to the phosphate and to the carboxylate group in forming an eight-membered chelate.

Finally, it seems worthwhile to point out that even though macrochelated species of $\text{M}(\text{OMP})^-$ complexes occur evidently only in low amounts or even not at all (see Sections 5 and 6) this does not mean that in the biological context any carboxylate-metal ion interaction can be neglected. It could, for example, well be that in an enzymic process the 6-carboxylate group of OMP^{3-} is directed towards a metal ion such that coordination occurs and that in this way decarboxylation of OMP^{3-} to UMP^{2-} is facilitated.

Acknowledgement

The support of this work by a research grant from the Swiss National Science Foundation is gratefully acknowledged.

References

- 1 (a) J. D. Rawn, *Biochemistry*, Patterson, Burlington, NC, 1989; (b) P. Karlson, *Biochemie*, Thieme, Stuttgart, 1988.
- 2 (a) I. Lieberman, A. Kornberg and E. S. Simms, *J. Biol. Chem.*, 215 (1955) 403; (b) J. Victor, L. B. Greenberg and D. L. Sloan, *J. Biol. Chem.*, 254 (1979) 2647; (c) J. Victor, A. Leo-Mensah and D. L. Sloan, *Biochemistry*, 18 (1979) 3597.
- 3 M. Bastian and H. Sigel, *Inorg. Chim. Acta*, 178 (1990) 249.
- 4 M. Bastian and H. Sigel, *J. Coord. Chem.*, 23 (1991) 137.
- 5 F. E. Hruska, *Can. J. Chem.*, 49 (1971) 2111.
- 6 H. Follmann, R. Pfeil and H. Witzel, *Eur. J. Biochem.*, 77 (1977) 451.
- 7 D. B. Davies, P. Rajani and H. Sadikot, *J. Chem. Soc., Perkin Trans. II*, (1985) 279.
- 8 R. B. Martin and Y. H. Mariam, *Met. Ions Biol. Syst.*, 8 (1979) 57.
- 9 H. Sigel, O. Yamauchi and A. D. Zuberbühler, *Anal. Chim. Acta*, in press.
- 10 Y. K. Agrawal, *Talanta*, 20 (1973) 1354.
- 11 (a) L. G. Van Uitert and W. C. Fernelius, *J. Am. Chem. Soc.*, 76 (1954) 5887; (b) L. G. Van Uitert and C. G. Haas, *J. Am. Chem. Soc.*, 75 (1953) 451.
- 12 H. Sigel, *Biol. Trace Element Res.*, 21 (1989) 49.
- 13 S. S. Massoud and H. Sigel, *Inorg. Chem.*, 27 (1988) 1447.
- 14 (a) J. J. Christensen, J. H. Rytting and R. M. Izatt, *Biochemistry*, 9 (1970) 4907; (b) *J. Chem. Soc. B*, (1970) 1643.
- 15 Y. Kinjo, R. Tribolet, N. A. Corfù and H. Sigel, *Inorg. Chem.*, 28 (1989) 1480.
- 16 H. Sigel, *Eur. J. Biochem.*, 165 (1987) 65.
- 17 R. B. Martin and H. Sigel, *Comments Inorg. Chem.*, 6 (1988) 285.
- 18 H. Sigel, S. S. Massoud and R. Tribolet, *J. Am. Chem. Soc.*, 110 (1988) 6857.
- 19 M. C. F. Magalhães and H. Sigel, *J. Indian Chem. Soc.*, in press.
- 20 (a) G. Åkerlöf and O. A. Short, *J. Am. Chem. Soc.*, 58 (1936) 1241; (b) F. E. Critchfield, J. A. Gibson, Jr. and J. L. Hall, *J. Am. Chem. Soc.*, 75 (1953) 1991; (c) G. Åkerlöf and O. A. Short, *J. Am. Chem. Soc.*, 75 (1953) 6357.
- 21 R. W. Hay, A. K. Basak, M. P. Pujari and A. Perotti, *J. Chem. Soc., Dalton Trans.*, (1986) 2029.
- 22 R. B. Martin, *Met. Ions Biol. Syst.*, 17 (1984) 1.
- 23 H. Einspahr and C. E. Bugg, *Met. Ions Biol. Syst.*, 17 (1984) 51.
- 24 G. Liang, N. A. Corfù and H. Sigel, *Z. Naturforsch., Teil B*, 44 (1989) 538.
- 25 (a) A. E. Martell and R. M. Smith, *Critical Stability Constants*, Vol. 3, *Other Organic Ligands*, Plenum, New York, 1977, p. 24; (b) L. G. Sillén and A. E. Martell, *Stability Constants of Metal-Ion Complexes*, Suppl. 1, Special Publication No. 25, The Chemical Society, London, 1971, p. 518.