

Influence of Decreasing Solvent Polarity (Dioxane-Water Mixtures) on the Stability of Metal Ion Complexes Formed with Phosphate Monoesters

Guogang Liang*, Nicolas A. Corfù, and Helmut Sigel**

Institute of Inorganic Chemistry, University of Basel,
Spitalstraße 51, CH-4056 Basel, Switzerland

Z. Naturforsch. **44b**, 538–542 (1989); received November 28, 1988

Phosphate Monoester, Metal Ion Complexes, Solvent Influence, Stability Constants, Active-Site Cavities of Enzymes

The acidity constants of $\text{H}(\text{R}-\text{MP})^-$, where $\text{R}-\text{MP}^{2-} = 4\text{-nitrophenyl phosphate (NPhP}^{2-})$, phenyl phosphate (PhP^{2-}) and $D\text{-ribose } 5'\text{-monophosphate (RibMP}^{2-})$, and the stability constants of the binary $\text{Cu}(\text{R}-\text{MP})$ complexes were determined by potentiometric pH titrations in aqueous solution and in 20, 30, 40 and 50% (v/v) dioxane-water mixtures. The solvent influence on the corresponding equilibrium constants is compared with the same influence on previously studied systems containing uridine 5'-triphosphate, formate or acetate. The influence of the solvent composition on the various ligand (L) systems was evaluated by constructing $\log K_{\text{M(L)}}^{\text{M}}$ versus $\text{p}K_{\text{H(L)}}^{\text{H}}$ plots; in all cases straight lines are obtained with slopes close to 1. This indicates that in all these systems, despite the different negative charges of the involved ligands, the solvent effect on proton binding and on metal ion binding is approximately of the same size: A decreasing solvent polarity resulting from the addition of increasing amounts of organic solvent to the aqueous solutions favors the affinity of the negatively charged ligands for protons and metal ions as well. Information of this type is considered important because the 'effective' or 'equivalent solution' dielectric constants in active-site cavities of enzymes are reduced compared with the dielectric constant of bulk water; *i. e.*, in protein cavities also a decreased 'solvent polarity' is occurring and this is expected to affect the stability of metal ion-ligand bonds.

Introduction

In recent years it has been well established that the so-called 'effective' or 'equivalent solution' dielectric constants in proteins [1–3] or in the active-site cavities of metalloenzymes [4] are reduced compared to the situation in bulk water. It is now generally agreed that different types of water exist in cells [5].

Proteins reduce the effective dielectric constant, in other words the solvent polarity, at their surface or in clefts *via* hydrophobic regions; *i. e.*, aliphatic or aromatic side chains of amino acid residues at the protein-water interface are responsible for this effect [6]. Of course, the mentioned amino acid side-chain residues are also able to interact in a selective way [7, 8] with low-molecular-weight solutes; for example, the aromatic indole group of a tryptophanyl residue of creatine kinase interacts with the purine moiety of adenosine 5'-diphosphate [9], and in

adenylate kinase the aliphatic groups of *iso*-leucyl and valyl residues are close to the purine moiety of adenosine 5'-triphosphate [10].

Based on the described situation for proteins one is tempted to conclude that at the protein-water surface to some extent the properties of a 'mixed solvent' are created. Indeed, the equivalent solution dielectric constants at the Zn^{2+} -region in the active-site cavities of bovine carboxypeptidase A and carbonic anhydrase are in the order of 70 and 35, respectively [4]; these dielectric constants correspond to aqueous solutions which contain about 10% and 50% dioxane, respectively (*vide infra*). Hence, one may expect to simulate to some extent the solution properties of active-site cavities of proteins by employing aqueous organic solvent mixtures in experiments.

So far not much quantitative knowledge has been accumulated on the extent to which a decreasing dielectric constant or solvent polarity affects the stability of metal ion complexes [4, 7, 11]. Some information exists about the stability of amino acid complexes [12, 13] in mixed solvents and also on metal ion-carboxylate complexes [4, 14, 15]. No detailed and comprehensive information on phosphate com-

* Work done at the University of Basel during a leave from the Academy of Beijing Traditional Medicine, People's Republic of China.

** Reprint requests to Prof. Dr. Helmut Sigel.

plexes is yet available. Considering that many natural coenzymes are esters of orthophosphoric acid [16], and that usually the related enzymic systems are metal ion dependent [17] respective knowledge is clearly desirable [18,19]. We have therefore studied by potentiometric pH titrations the stabilities of the Cu^{2+} complexes formed with 4-nitrophenyl phosphate (NPhP^{2-})*, phenyl phosphate (PhP^{2-}), and *D*-ribose 5'-monophosphate (RibMP^{2-}) and quantified the influence on the complex stability of increasing amounts of dioxane added to aqueous solutions of the reactants: From the results it is evident that with decreasing solvent polarity the stability of the complexes is significantly increasing.

Experimental Section

Materials

All solutions were prepared with distilled and CO_2 -free water. 1,4-Dioxane (extra pure) was from Merck AG, Darmstadt, FRG. All the other reagents were the same as used recently [20].

Determination of equilibrium constants

The pH titrations were carried out with a Metrohm potentiograph E536, dosimat E535 and macro EA 121 glass electrodes. The buffers used for calibration (pH 4.64 and 7.00) were also from Metrohm AG, Herisau, Switzerland. The direct pH-meter readings were used in the calculations for the acidity constants; no "corrections" were applied for the change in solvent from water to the dioxane-water mixtures, though correction factors have been published for such [21] and related solvent mixtures [22]. The mixed solvents (v/v) were prepared by taking, *e. g.*, 25 ml aqueous solution and adding 25 ml dioxane.

The acidity constant, $K_{\text{H(R-MP)}}^{\text{H}}$, of the H(R-MP)^- species (where R-MP^{2-} = 4-nitrophenyl phosphate, phenyl phosphate or *D*-ribose 5'-monophosphate) and the stability constant, $K_{\text{Cu(R-MP)}}^{\text{Cu}}$, of the binary Cu(R-MP) complexes were determined exactly as described recently [20]; this includes the concentrations of the reactants, as well as the calculation procedures. However, a large part of the experiments

was also carried out by employing about twice the reagent concentrations used in [20]: *i. e.*, 50 ml of 0.9 mM HNO_3 and NaNO_3 ($I = 0.1 \text{ M}$; 25°C) in the presence and absence of 0.6 mM R-MP^{2-} were titrated under N_2 with 1 ml of 0.05 M NaOH ; the corresponding $K_{\text{H(R-MP)}}^{\text{H}}$ values were calculated from at least four independent pairs of titrations within the pH range determined by $\text{p}K_{\text{H(R-MP)}}^{\text{H}} \pm 1.7$ and by taking into account the species H^+ , H(R-MP)^- and R-MP^{2-} .

The conditions for the determination of the stability constant $K_{\text{Cu(R-MP)}}^{\text{Cu}}$ of the binary Cu(R-MP) complexes ($I = 0.1 \text{ M}$, NaNO_3 ; 25°C) were in a large part of the experiments the same as given above for the acidity constant (see also [20]), but NaNO_3 was partly replaced by 3.33 mM $\text{Cu(NO}_3)_2$, *i. e.* the R-MP to Cu^{2+} ratio was in these cases 1:5.56. The stability constant was computed by taking into account the species H^+ , H(R-MP)^- , R-MP^{2-} , Cu^{2+} , and Cu(R-MP) ; the data were collected from about 5% complex formation to the beginning of the hydrolysis of $\text{Cu}_{\text{aq}}^{2+}$, which was evident from the titrations without R-MP^{2-} . The individually calculated values for $\log K_{\text{Cu(R-MP)}}^{\text{Cu}}$ showed no dependence on pH or on the excess of Cu^{2+} . For all systems at least four independent pairs of titration curves were recorded and the results averaged.

Results and Discussion

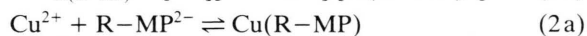
Simple phosphate monoesters (R-MP^{2-}), such as phenyl phosphate (PhP^{2-}), 4-nitrophenyl phosphate (NPhP^{2-}) or *D*-ribose 5'-monophosphate (RibMP^{2-}), are dibasic species that may carry two protons at their phosphate group. The release of the first proton from monoesterified derivatives of phosphoric acid, *i. e.* from the $\text{H}_2(\text{R-MP})$ species, occurs in water at a very low pH: usually $\text{p}K_{\text{a}} \approx 1$ [23]; for example, the first proton from diprotonated uridine 5'-monophosphate, $\text{H}_2(\text{UMP})$, is released with $\text{p}K_{\text{H}_2(\text{UMP})}^{\text{H}} = 0.7 \pm 0.3$ [20]. Hence, the first proton from the phosphoric acid residue in $\text{H}_2(\text{R-MP})$ species is completely ionized at $\text{pH} \geq 3$ and does therefore not affect the equilibrium $\text{H(R-MP)}^-/\text{R-MP}^{2-}$ and the complex formation between Cu^{2+} and R-MP^{2-} ; the last two reactions occur only at $\text{pH} > 3$.

Indeed, the experimental data of the potentiometric pH titrations carried out with the mentioned three R-MP systems may be completely described in all solvents employed in this study by considering the following two equilibria:

* *Abbreviations.* Aside from the three definitions given above the following abbreviations are used: Ac^- , acetate; AMP, adenosine monophosphate; L, general ligand with undefined charge; M^{2+} , divalent metal ion; R-MP^{2-} , phosphate monoester (R may be any organic residue, *e. g.* phenyl or nucleosidyl); UTP^{4-} , uridine 5'-triphosphate.



$$K_{\text{H(R-MP)}}^{\text{H}} = [\text{H}^+][\text{R-MP}^{2-}]/[\text{H(R-MP)}^-] \quad (1b)$$



$$K_{\text{Cu(R-MP)}}^{\text{Cu}} = [\text{Cu(R-MP)}]/([\text{Cu}^{2+}][\text{R-MP}^{2-}]) \quad (2b)$$

The determined acidity constants of the H(R-MP)^- species and the stability constants of the corresponding Cu(R-MP) complexes are listed in Table I, together with some details on the solvent mixtures [24] and also with data on some related systems taken from the literature and used for comparisons. These latter data refer to H(L) species and their M(L) complexes, where L = uridine 5'-triphosphate (UTP^{4-}) [25], formate (HCOO^-) [4] or acetate (Ac^-) [4]; the acidity constants, $K_{\text{H(L)}}^{\text{H}}$ and the stability constants, $K_{\text{M(L)}}^{\text{M}}$, are defined analogously to equations 1 and 2, respectively.

From the results given in Table I it is immediately evident that in all cases the negative logarithms of the acidity constants of the H(L) species and the logarithms of the stability constants of the M(L) complexes increase considerably with increasing amounts of dioxane present in the aqueous solvent mixtures. It is thus clear that a decreasing polarity resulting from the increasing amounts of organic solvent favors the affinity of negatively charged species for protons and metal ions as well; *i.e.*, the interaction between oppositely charged ions is favored in agreement with earlier observations [4, 11].

The interrelations between ligand basicity and complex stability, and the effect of the decreasing solvent polarity on these interrelations is more clearly seen from Fig.1, where the values for $\log K_{\text{M(L)}}^{\text{M}}$

Ligand/ M^{2+}	% (v/v) dioxane	mol fract.	ϵ^b	salt ^c	$\text{p}K_{\text{H(L)}}^{\text{H}}$	$\log K_{\text{M(L)}}^{\text{M}}$
$\text{NPhP}^{2-}/\text{Cu}^{2+}$	0	0	78.5	NaNO_3	5.05 ± 0.01	2.33 ± 0.04
	20	0.050	61.3	NaNO_3	5.70 ± 0.01	2.90 ± 0.01
	30	0.083	52.7	NaNO_3	6.01 ± 0.01	3.27 ± 0.01
	40	0.124	44.1	NaNO_3	6.34 ± 0.01	3.63 ± 0.01
	50	0.175	35.2	NaNO_3	6.63 ± 0.01	3.99 ± 0.02
$\text{PhP}^{2-}/\text{Cu}^{2+}$	0	0	78.5	NaNO_3	5.85 ± 0.01	2.77 ± 0.01
	20	0.050	61.3	NaNO_3	6.46 ± 0.01	3.35 ± 0.01
	30	0.083	52.7	NaNO_3	6.78 ± 0.01	3.72 ± 0.01
	40	0.124	44.1	NaNO_3	7.11 ± 0.01	4.12 ± 0.02
	50	0.175	35.2	NaNO_3	7.38 ± 0.01	4.40 ± 0.03
$\text{RibMP}^{2-}/\text{Cu}^{2+}$	0	0	78.5	NaNO_3	6.24 ± 0.01	2.96 ± 0.02
	20	0.050	61.3	NaNO_3	6.70 ± 0.01	3.45 ± 0.01
	30	0.083	52.7	NaNO_3	6.93 ± 0.01	3.77 ± 0.02
	40	0.124	44.1	NaNO_3	7.19 ± 0.01	4.09 ± 0.03
	50	0.175	35.2	NaNO_3	7.38 ± 0.01	4.38 ± 0.05
$\text{UTP}^{4-}/\text{Cu}^{2+}$	0	0	78.5	NaNO_3	6.46 ± 0.01	5.81 ± 0.06
	30	0.083	52.7	NaNO_3	6.84 ± 0.01	6.16 ± 0.05
	50	0.175	35.2	NaNO_3	6.92 ± 0.01	6.24 ± 0.03
$\text{HCOO}^-/\text{Cu}^{2+}$	0	0	78.5	KNO_3	3.59 ± 0.02	1.65 ± 0.09
	10	0.023	70.1	KNO_3	3.71 ± 0.01	1.79 ± 0.03
	30	0.083	52.7	K, NaNO_3	4.14 ± 0.02	2.20 ± 0.01
	50	0.175	35.2	NaClO_4	4.73 ± 0.02	2.79 ± 0.02
$\text{HCOO}^-/\text{Zn}^{2+}$	0	0	78.5	KNO_3	3.59 ± 0.02	1.07 ± 0.05
	30	0.083	52.7	K, NaNO_3	4.14 ± 0.02	1.24 ± 0.02
	50	0.175	35.2	NaClO_4	4.73 ± 0.02	1.96 ± 0.01
$\text{Ac}^-/\text{Cu}^{2+}$	0	0	78.5	KNO_3	4.58 ± 0.02	1.85 ± 0.05
	10	0.023	70.1	KNO_3	4.79 ± 0.01	2.05 ± 0.02
	30	0.083	52.7	K, NaNO_3	5.31 ± 0.01	2.61 ± 0.01
	50	0.175	35.2	NaClO_4	5.97 ± 0.01	3.31 ± 0.02
$\text{Ac}^-/\text{Zn}^{2+}$	0	0	78.5	KNO_3	4.58 ± 0.02	1.11 ± 0.02
	30	0.083	52.7	K, NaNO_3	5.31 ± 0.01	1.62 ± 0.01
	50	0.175	35.2	NaClO_4	5.97 ± 0.01	2.31 ± 0.01

Table I. Negative logarithms of the acidity constants of several H(L) species (analogous to eq. 1) and logarithms of the corresponding M(L) complexes (analogous to eq. 2) in dependence on the amount of dioxane added to water and on the resulting dielectric constant ($I = 0.1 \text{ M}$; 25°C)^a.

^a The errors given are 3 times the standard error of the mean value or the sum of the probable systematic errors, whichever is larger. Some of the equilibrium constants are taken from our earlier work: Values for NPhP^{2-} , PhP^{2-} and RibMP^{2-} in water from Tables I and II of [20]; UTP^{4-} from Table 4 of [25]; HCOO^- and Ac^- from Table I of [4]; ^b the dielectric constants for the dioxane-water mixtures are interpolated from the data given in [24]; ^c salt used to adjust the ionic strength to $I = 0.1 \text{ M}$.

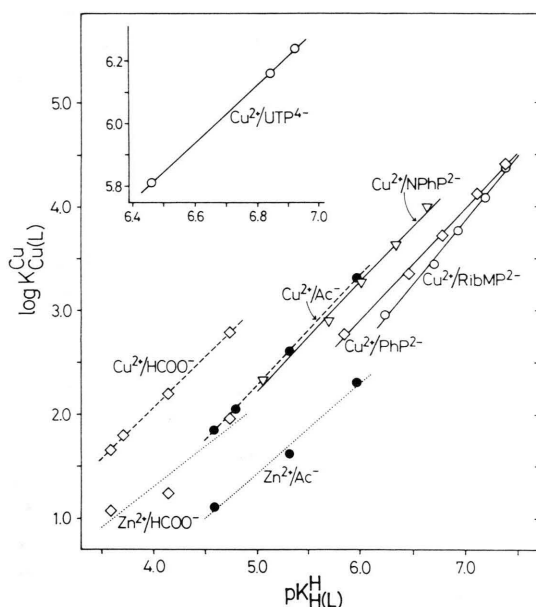


Fig. 1. Relationship between $\log K_{M(L)}^M$ (analogous to eq. 2) and $pK_{H(L)}^H$ (analogous to eq. 1) for the $M(L)$ complexes of Table I as they result from the addition of increasing amounts of dioxane to the aqueous solutions of the components ($I = 0.1$ M; 25°C). The point at the left for each straight line refers to water and the point most to the right to 50% (v/v) dioxane-water; the points in between cover various dioxane-water mixtures (see Table I). The slopes (m) and intercepts (b) defining the regression lines for the eight M^{2+}/L systems shown above are summarized in Table II; the corresponding calculations are based on the equilibrium constants listed in Table I.

versus $pK_{H(L)}^H$ are plotted. It is evident that for all eight systems listed in Table I straight lines are obtained; the slopes and intercepts characterizing these straight lines are summarized in Table II. These results allow interesting conclusions; some are given below:

(i) For all eight systems the slope of the straight lines is very close to one (Table II); this indicates that the solvent effect on proton binding and on metal ion binding is approximately of the same size.

(ii) This observation is surprising because the phosphate ligands, $R-MP^{2-}$, have a twofold negative charge, UTP^{4-} is fourfold negatively charged, and the two carboxylate ligands carry only a single charge, and still in all these cases a slope of about one is observed.

(iii) Correspondingly, the similarity of the results with the Cu^{2+} and Zn^{2+} systems indicates that the kind of metal ion has also only a small influence on the size of the slope (the slopes of the carboxylate pairs are within 2σ identical); this is despite the fact that the stabilities of the Cu^{2+} complexes are significantly larger than those of the Zn^{2+} complexes for a given carboxylate ligand.

The mentioned three points suggest that the results obtained for the polarity range covered between water and 50% (v/v) dioxane-water are of a general nature and apply probably in a first approximation also for other negatively charged ligands with O-donor atoms and their complexes with divalent metal ions. For the carboxylates indications [4] exist that this linearity between $\log K$ and pK_a holds even for solvent mixtures up to 90% (v/v) dioxane-water; moreover, for ethanol-water mixtures the corresponding results [4] have been obtained.

There is also a finer detail which warrants recognition and which also indicates that generalizations should be made only with great care. The properties of the $RibMP^{2-}$ system differ somewhat from those of the other ones: the slope of the regression line is clearly somewhat larger than one (Fig. 1 and Table II), and this has its origin mainly in a smaller effect of the organic solvent on $pK_{H(RibMP)}^H$ compared with

$M(L)/H(L)$	m	b	R^b
$Cu(NPhP)/H(NPhP)^-$	1.052 ± 0.046	-3.029 ± 0.272	0.9972
$Cu(PhP)/H(PhP)^-$	1.078 ± 0.032	-3.570 ± 0.213	0.9987
$Cu(RibMP)/H(RibMP)^-$	1.242 ± 0.047	-4.827 ± 0.325	0.9979
$Cu(UTP)^{2-}/H(UTP)^{3-}$	0.930 ± 0.011	-0.201 ± 0.077	0.9999
$Cu(HCOO)^+/HCOOH$	0.992 ± 0.012	-1.901 ± 0.050	0.9998
$Cu(Ac)^+/H(Ac)$	1.056 ± 0.010	-2.997 ± 0.053	0.9999
$Zn(HCOO)^+/HCOOH$	0.79 ± 0.26	-1.84 ± 1.10	0.948
$Zn(Ac)^+/H(Ac)$	0.860 ± 0.100	-2.869 ± 0.531	0.9933

^a In the straight-line equation, $y = m \cdot x + b$, x represents the pK_a value of $H(L)$ and y the corresponding $\log K_{M(L)}^M$ value; the errors given with m and b correspond to one standard deviation (1σ); ^b correlation coefficient.

Table II. Slopes (m) and intercepts (b) for the regression lines of the $\log K_{M(L)}^M$ versus $pK_{H(L)}^H$ plots shown in Fig. 1. The least-squares regression lines were calculated with the data listed in Table I ($I = 0.1$ M; 25°C)^a.

$\log K_{\text{Cu(RibMP)}}^{\text{Cu}}$; the largest difference for H(RibMP)^- is $\Delta\text{p}K_{\text{a}} = 1.14$, whereas for the H(NPhP)^- and H(PhP)^- systems the corresponding values are $\Delta\text{p}K_{\text{a}} = 1.58$ and 1.53 , respectively (Table I). Certainly, there are also differences in the solvent effects on the stability constants of the Cu(R-MP) complexes, but these are less pronounced. Tentatively, one may conclude that RibMP differs from the two other phosphate monoesters mainly by the absence of a hydrophobic residue; or to say it differently: the hydroxy groups of the ribose ring form probably hydrogen bonds with water molecules of the solvent and this may possibly lead to a slightly higher water concentration 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 $\text{p}K_{\text{H(RibMP)}}^{\text{H}}$ (and also, but less pronounced $\log K_{\text{Cu(RibMP)}}^{\text{Cu}}$) value.

It may be added that plots of $\log K_{\text{Cu(R-MP)}}^{\text{Cu}}$ versus $\text{p}K_{\text{H(R-MP)}}^{\text{H}}$ for the NPhP, PhP and RibMP systems and a given solvent lead also to straight lines; in these cases the slopes are close to 0.5 for all five solvents

studied (Table I). This is interesting because it means that in a given solvent with different ligands the effect of the residue R in R-MP^{2-} is larger on the basicity of the phosphate group than it is on the stability of the Cu^{2+} complex [26]. From experiments in aqueous solution it is also known [20] that for different metal ions different slopes for the $\log K_{\text{M(R-MP)}}^{\text{M}}$ versus $\text{p}K_{\text{H(R-MP)}}^{\text{H}}$ plots are observed.

For the Cu(AMP) complexes with $2'\text{-AMP}^{2-}$, $3'\text{-AMP}^{2-}$ [27] and $5'\text{-AMP}^{2-}$ [28] it is known that macrochelates involving metal ion binding to the phosphate group and the purine system are formed. It is our next aim to quantify the solvent influence on the extent of base-backbinding in these Cu(AMP) complexes [26], and if possible, to include into the study also the solvent effect on the intramolecular stack formation in $\text{Cu(1,10-phenanthroline)(AMP)}$ complexes; the formation of such intramolecular stacks in aqueous solution [7, 8, 29, 30] is wellknown.

The support of this research by the Swiss National Science Foundation is gratefully acknowledged.

- [1] D. C. Rees, *J. Mol. Biol.* **141**, 323 (1980).
- [2] G. R. Moore, *FEBS Lett.* **161**, 171 (1983).
- [3] N. K. Rogers, G. R. Moore, and M. J. E. Sternberg, *J. Mol. Biol.* **182**, 613 (1985).
- [4] H. Sigel, R. B. Martin, R. Tribolet, U. K. Häring, and R. Malini-Balakrishnan, *Eur. J. Biochem.* **152**, 187 (1985).
- [5] J. G. Watterson, *Biochem. J.* **248**, 615 (1987).
- [6] B. L. Vallee and R. J. P. Williams, *Chem. Brit.* **4**, 397 (1968).
- [7] H. Sigel, *Pure Appl. Chem.* **61**, 923 (1989).
- [8] H. Sigel, *Chimia* **41**, 11 (1987).
- [9] M. Vašák, K. Nagayama, K. Wüthrich, M. L. Mertens, and J. H. R. Kägi, *Biochemistry* **18**, 5050 (1979).
- [10] D. C. Fry, D. M. Byler, H. Susi, E. M. Brown, S. A. Kuby, and A. S. Mildvan, *Biochemistry* **27**, 3588 (1988).
- [11] H. Sigel and D. B. McCormick, *Acc. Chem. Res.* **3**, 201 (1970).
- [12] G. Liang, R. Tribolet, and H. Sigel, *Inorg. Chim. Acta* **155**, 273 (1989).
- [13] G. Liang and H. Sigel, submitted for publication.
- [14] H. Sigel, R. Malini-Balakrishnan, and U. K. Häring, *J. Am. Chem. Soc.* **107**, 5137 (1985).
- [15] G. Liang, R. Tribolet, and H. Sigel, *Inorg. Chem.* **27**, 2877 (1988).
- [16] F. H. Westheimer, *Science (Washington, D.C.)* **235**, 1173 (1987).
- [17] a) L. G. Marzilli, *Prog. Inorg. Chem.* **23**, 255 (1977);
b) F. Y.-H. Wu and C.-W. Wu, *Met. Ions Biol. Syst.* **15**, 157 (1983);
c) H. R. Kalbitzer, *Met. Ions Biol. Syst.* **22**, 81 (1987).
- [18] H. Sigel, in A. V. Xavier (ed.): *Frontiers in Bioinorganic Chemistry*, p. 84, VCH Verlagsgesellschaft, Weinheim (FRG) (1986).
- [19] H. Sigel, in T. D. Tullius (ed.): *Metal-Nucleic Acid Chemistry*, ACS Symposium Series, Washington (DC) (1989), in press.
- [20] S. S. Massoud and H. Sigel, *Inorg. Chem.* **27**, 1447 (1988).
- [21] Y. K. Agrawal, *Talanta* **20**, 1354 (1973).
- [22] R. G. Bates, *Determination of pH: Theory and Practice*, p. 243, J. Wiley and Sons, New York (1973).
- [23] H. R. Mahler and E. H. Cordes, *Biological Chemistry*, p. 131, Harper and Row, New York (1966).
- [24] a) G. Åkerlöf and O. A. Short, *J. Am. Chem. Soc.* **58**, 1241 (1936);
b) F. E. Critchfield, J. A. Gibson (Jr.), and J. L. Hall, *J. Am. Chem. Soc.* **75**, 1991 (1953);
c) G. Åkerlöf and O. A. Short, *J. Am. Chem. Soc.* **75**, 6357 (1953).
- [25] R. Tribolet, R. Malini-Balakrishnan, and H. Sigel, *J. Chem. Soc. Dalton Trans.* **1985**, 2291.
- [26] G. Liang and H. Sigel, results to be published.
- [27] S. S. Massoud and H. Sigel, *Eur. J. Biochem.* **179**, 451 (1989).
- [28] H. Sigel, S. S. Massoud, and R. Tribolet, *J. Am. Chem. Soc.* **110**, 6857 (1988).
- [29] S. S. Massoud and H. Sigel, *Inorg. Chim. Acta* **159**, 243 (1989).
- [30] S. S. Massoud, R. Tribolet, and H. Sigel, to be submitted for publication.