The Colourless 'Chameleon' or the Peculiar Properties of Zn^{2+} in Complexes in Solution.
Quantification of Equilibria Involving a Change of the Coordination Number of the Metal Ion*

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1 Introduction

The metal ion Zn^{2+} plays a crucial role in the active site of over 300 metalloenzymes (*e.g.*[1-3]). However, its role is not limited to catalysis and gene expression: Zn^{2+} also stabilizes the structure of proteins and nucleic acids, preserves the integrity of subcellular organelles, engages in transport processes, and plays important roles in viral and immune phenomena.[1,2] Thus an understanding of its basic coordination properties is desirable. Zn^{2+} coordinated in proteins usually has a tetrahedral or distorted tetrahedral coordination sphere[1,4] with the low coordination number 4. During the course of an enzymatic turnover, this inner-sphere coordination number of catalytically active Zn^{2+} may increase to 5,[4] but to our knowledge coordination number 6 has never been observed for a protein-bound Zn^{2+}.

This situation contrasts with that in low molecular weight complexes: a recent evaluation of 490 crystal structures showed that Zn^{2+} has coordination number 4 in about 58% of the complexes, and coordination numbers 5 and 6 in about 13 and 27%, respectively.[5] The percentages for the related metal ions Co^{2+} and Cd^{2+} are: for the coordination numbers 4, 5, and 6 for Co^{2+}, 13, 10, and 76% (from 1500 structures); and for Cd^{2+} 19, 8, and 56% (from 200 structures; the difference to 100% accounts for other coordination numbers).[5] Although coordination number 6 for Zn^{2+} in the solid state apparently is not as important as for Co^{2+} or Cd^{2+}, Zn^{2+} often has a greater coordination number in low molecular weight complexes than in proteins.

In aqueous solution coordination number 6 is probably even more important for Zn^{2+}: *e.g.*, depending on the concentration, dissolution of $ZnCl_2$ in water leads to $[Zn(H_2O)_6]^{2+}$, $[ZnCl(H_2O)_5]^{+}$, $[ZnCl_2(H_2O)_4]$, and $[ZnCl_4(H_2O)_2]^{2-}$ species.[6] In aqueous 2.88 M $ZnCl_2$ in 9.35 M HCl, or in more diluted aqueous $ZnCl_2$ solutions, upon addition of organic solvents [DMF, CH_3OH, $(CH_3)_2SO$], an equilibrium between octahedral–tetrahedral geometries is suggested.[7]

Evidently, in solution well-defined coordination numbers, especially the lower ones, are difficult to obtain,[8] though by employing well-devised ligands, coordination numbers 4 or 5 can be enforced (*e.g.*, see reference 9). Zn^{2+} is clearly among those metal ions having a most flexible and adaptable coordination sphere: truly a 'chameleon'! However, the switch from one coordination geometry to another cannot easily be studied and quantified in solution as Zn^{2+} is diamagnetic and colourless.

Having noted the peculiar coordinating properties of Zn^{2+} for some time,[3] we recently made a fruitful observation[10] in ternary systems consisting of *N*,*N*-bis(2-hydroxyethyl)glycinate (= Bicinate), imidazole (Im), and a divalent metal ion (M^{2+}), *i.e.* Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, or Cd^{2+}. Bicinate is a derivative of the amino acid glycine (Figure 1), yet owing to its two hydroxyethyl groups, it is potentially a tetradentate ligand. Indeed, for the aforementioned M^{2+} only about 0.5 percent of $M(Bicinate)^{+}$ exist with a twofold coordinated glycinate-type binding mode, whereas in the case of Co^{2+}, Ni^{2+}, and Zn^{2+} about 85 to 90% are bound in a tetradentate mode;[11] in the remaining 10 to 15% only one of the two hydroxyethyl groups binds. Owing to the Jahn–Teller effect, the tetradentate mode drops to about 60% for $Cu(Bicinate)^{+}$. Surprisingly, despite this dominating action of Bicinate as a tetradentate ligand,[11] the ternary $Zn(Bicinate)(imidazole)^{+}$ complex shows a very high stability: the affinity of imidazole toward $Zn(Bicinate)^{+}$ is significantly

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* Abbreviations: Bic^-, abbreviation for Bicinate as used in formula; Bicinate, monoanion of Bicine; Bicine, *N*,*N*-bis(2-hydroxyethyl)glycine; CN, coordination number (*e.g*., Zn^{2+}_{CN6} means Zn^{2+} with coordination number 6); En, ethylenediamine (= 1,2-diaminoethane); Gly^-, glycinate; Ha, histamine; HisMe, histidine methyl ester; Im, imidazole; Nta^{3-}, nitrilotriacetate; Ox^{2-}, oxalate; Ser^-, serinate; UTP^{4-}, uridine 5′-triphosphate.

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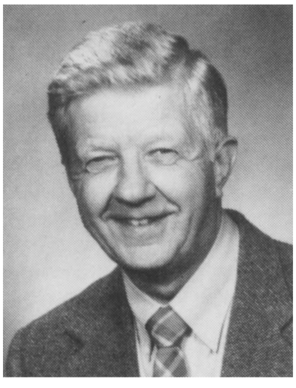

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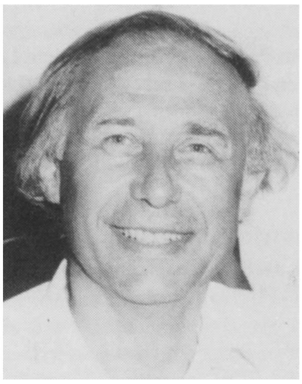

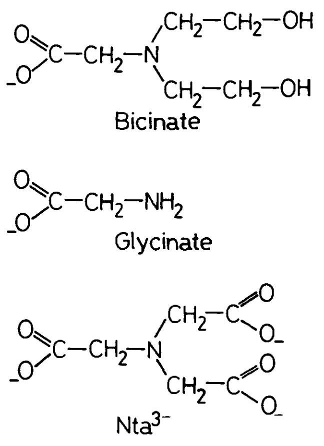

Figure 1 Chemical formula of glycinate, *i.e.* of the anion of the amino acid glycine, as well as of its derivatives *N,N*-bis(2-hydroxyethyl)glycinate (= Bicinate or Bic$^-$) and nitrilotriacetate (Nta^{3-}).

larger than the one toward Zn(aq)$^{2+}$.[10] This may be explained by a reduction in coordination number; *i.e.*, upon coordination of one imidazole to Zn(Bicinate)$^+$ two water molecules (and possibly one hydroxyethyl group) are released from the coordination sphere of Zn^{2+}, giving rise to a reduced coordination number of 5 (or even 4).

Similarly, nitrilotriacetate (Nta^{3-}) is also, like Bicinate, a derivative of glycinate (Figure 1). Considering the iminodiacetate moiety as the central binding core in M(Nta)$^-$ complexes,[12] one calculates that the third acetate residue coordinates in more than 99.8% of the M(Nta)$^-$ species, proving that Nta^{3-} also acts overwhelmingly as a tetradentate ligand. Furthermore, like Zn(Bicinate)(imidazole)$^+$ the Zn(Nta)(imidazole)$^-$ complex shows high stability.[13]

We believe that these observations[10,13] suggest a changing coordination number of Zn^{2+} in certain complexes in aqueous solution.[10] Herein we describe in more detail the connected equilibria and quantify the degree of formation of the species containing Zn^{2+} with various coordination numbers.

2 Evidence for a Reduction of the Coordination Number of Zn^{2+} in the Ternary Complex Formed in the Zn^{2+}–Bicinate–Imidazole System and Relevant Equilibria

The stability of mixed ligand complexes can be quantified by comparison with that of their binary parent complexes; *i.e.*, by finding the position of equilibrium 1:[14,15]

$$M(Bic)^+ + M(Im)^{2+} \rightleftharpoons M(Bic)(Im)^+ + M^{2+} \qquad (1)$$

Its constant is defined by equation 2, and values for it follow from relation 3:[14,15]

$$10^{\Delta \log K_M} = [M(Bic)(Im)^+][M^{2+}]/[M(Bic)^+][M(Im)^{2+}] \qquad (2)$$

$$\begin{aligned}\Delta \log K_M &= \log K^{M(Im)}_{M(Im)(Bic)} - \log K^{M}_{M(Bic)} \\ &= \log K^{M(Bic)}_{M(Bic)(Im)} - \log K^{M}_{M(Im)} \qquad (3)\end{aligned}$$

The definition of the stability constants of the binary and ternary complexes occurring in equation 3 is evident from the following two examples:

$$M^{2+} + Im \rightleftharpoons M(Im)^{2+} \qquad (4a)$$

$$K^{M}_{M(Im)} = [M(Im)^{2+}]/[M^{2+}][Im] \qquad (4b)$$

$$M(Bic)^+ + Im \rightleftharpoons M(Bic)(Im)^+ \qquad (5a)$$

$$K^{M(Bic)}_{M(Bic)(Im)} = [M(Bic)(Im)^+]/[M(Bic)^+][Im] \qquad (5b)$$

Where further identification of $\Delta \log K_M$ (equation 3) is desirable, additional subscripts are given like $\Delta \log K_{Zn/Bic/Im}$, meaning that the value refers to the Zn^{2+}–Bicinate–imidazole system.

According to the general rule for binary complexes, $K_1 > K_2 \ldots$ (*e.g.*, see reference 16), equilibrium 1 would be on its left side; *i.e.*, for $\Delta \log K_M$ (equation 3) *negative* values are expected. Indeed, statistical (st) reasoning[13,14] leads to the same conclusion: a monodentate ligand entering an octahedral (oh) coordination sphere has six possibilities for binding, while only two remain if four are already occupied by Bicinate. As the probability for dissociation is the same, namely 1, in the binary and ternary complex, the statistical value for $\Delta \log K_{st/oh}$ is log (2/6) = − 0.5. For Cu^{2+} the value is more uncertain:[14] assuming a square-planar (sp) or a distorted octahedral (do) coordination sphere one may conclude[13] that the value is log (1/4) or log (1/6) and hence, $\Delta \log K_{st/sp/do} = -0.6$ or -0.8; *i.e.*, $\log K_{st/Cu} \simeq -0.7$ appears as a reasonable estimate.

Comparison of these statistical values, $\Delta \log K_{st}$, with the experimental results[10] $\Delta \log K_{M/Bic/Im} = -0.05, -0.08, -0.11, +0.31$, and -0.50 for the complexes with Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, and Cd^{2+}, respectively, indicates that only the Cd^{2+}/Bicinate/Im system behaves as predicted by the statistics. The ternary complexes with Co^{2+}, Ni^{2+}, Cu^{2+}, and Zn^{2+} are more stable than expected. This observation agrees with the previous evidence[13–15,17] that mixed ligand complexes formed by a metal ion of the second half of the 3*d* series, an O-donor (as Bicinate is, to a large part), and a heteroaromatic N-base with π-accepting properties, like imidazole, are especially stable.[17,18] However, the most unexpected result is the high relative stability of Zn(Bicinate)(Im)$^+$: a *positive* value for Δ log $K_{Zn/Bic/Im}$ is observed, meaning that equilibrium 1 is on its right-hand side. This observation suggests that a reduction of the coordination number of Zn^{2+} occurs upon the coordination of imidazole to Zn(Bicinate)$^+$, as this is the only way to explain the increased complex stability.

3 The Special Properties of Zn(Bicinate)(Im)$^+$ and of Related Ternary Complexes

To assist further comparisons and to decide whether Zn(Bicinate)(Im)$^+$ is an isolated case, we collected the results of Table 1 which include Bicinate, Nta^{3-} (Figure 1), and uridine 5′-triphosphate (UTP^{4-}; Structure I) as multidentate ligands.[10,13,19] At first we compare the values for $\Delta \log K_M$ given in rows 1 and 2: The relative stabilities of the complexes with Co^{2+}, Ni^{2+}, or Cu^{2+} and Nta^{3-}/Im are slightly larger than those with Bicinate and imidazole, probably due to coordination of the negatively charged oxygens of the carboxylate groups of Nta^{3-} (Figure 1), which should make the π-accepting properties of imidazole somewhat more effective (Section 2). However, most importantly, a rather high relative stability is also observed for Zn(Nta)(Im)$^-$, especially if compared with the value for $\Delta \log K_{Co/Nta/Im}$ for the similarly sized Co^{2+} or the statistical value (Section 2).

From rows 1, 2, and 4 of Table 1 it is clear that the relative stability of the ternary Zn^{2+} complex is always pronounced, especially when Cu^{2+} is excluded because of its irregular (tetragonal) coordination sphere, and when emphasis is put on the comparison with Co^{2+} (or Ni^{2+}), which usually prefers a

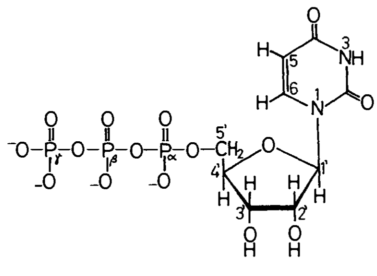

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Table 1 Comparison of the relative stabilities of various mixed ligand complexes in analogy to equilibrium 1 and equations 2, 3 for aqueous solutions at 25 °C and $I = 0.1$ M[a]

No	Equilibrium	$\Delta \log K_M$					Ref.
		Co^{2+}	Ni^{2+}	Cu^{2+}	Zn^{2+}	Cd^{2+}	
1	$M(Bic)^+ + M(Im)^{2+} \rightleftharpoons M(Bic)(Im)^+ + M^{2+}$	− 0.05 ± 0.04	− 0.08 ± 0.01	− 0.11 ± 0.03	0.31 ± 0.04	− 0.50 ± 0.04	10
2	$M(Nta)^- + M(Im)^{2+} \rightleftharpoons M(Nta)(Im)^- + M^{2+}$	0.01 ± 0.02	− 0.01 ± 0.01	0.26 ± 0.01	0.22 ± 0.02		13,19[b]
3	$M(Nta)^- + M(NH_3)^{2+} \rightleftharpoons M(Nta)(NH_3)^- + M^{2+}$	− 0.26 ± 0.04	− 0.20 ± 0.06	− 0.39 ± 0.08	− 0.09 ± 0.09[c]		13
4	$M(UTP)^{2-} + M(Im)^{2+} \rightleftharpoons M(UTP)(Im)^{2-} + M^{2+}$	− 0.36 ± 0.04	− 0.40 ± 0.03	− 0.37 ± 0.02	0.03 ± 0.02	− 0.38 ± 0.03	19
5	$M(UTP)^{2-} + M(NH_3)^{2+} \rightleftharpoons M(UTP)(NH_3)^{2-} + M^{2+}$			− 0.58 ± 0.12	$\leqslant -0.1$	$\leqslant -0.4$	19

[a] The error limits (3 σ) were calculated according to the error propagation after Gauss, they are based on the errors of the stability constants measured for the binary and ternary complexes (*cf* equations 3—5) [b] The values for $\Delta \log K_M$ in this row were calculated according to equation 3 with the stability constants of the ternary $M(Nta)(Im)^-$ complexes given in reference 13 and those of the binary $M(Im)^{2+}$ complexes given in reference 19 (regarding the latter values see also reference 10) [c] Given above is the exact (unrounded) result of the previous experiments, in reference 13 $\Delta \log K_{Zn} \simeq -0.1$ is listed.

regular octahedral sphere (Section 1).[20] That this effect of a relative stability enhancement for the Zn^{2+} complexes is beyond that attributable to the combination of an O-donor and a heteroaromatic π-accepting amine[15,17,18] (Section 2) is evident from a comparison of the data between rows 2 and 3 or 4 and 5. Ternary complexes with ammonia are always less stable than the corresponding ones with imidazole, yet even among the ternary complexes containing NH_3, those with Zn^{2+} show an increased stability.

We are convinced (Section 2) that the explanation for these observations is linked to a varying coordination sphere of Zn^{2+}, which switches easily from coordination number 6 to 4 or 5.[2,8,20] The prime example for this behaviour is the Zn^{2+}–ethylenediamine (En) system, cited in many textbooks (*e.g.*, page 42 in reference 2, page 71 in reference 6*a*, or page 45 in reference 6*b*): the $Zn(En)_2^{2+}$ complex is relatively stable while $Zn(En)_3^{2+}$ is unstable.[21] This difference is explained by attributing to Zn^{2+} in $Zn(En)^{2+}$ an octahedral coordination sphere (but see Section 5) which switches to a tetrahedral one upon coordination of the second En, releasing four water molecules;† binding of the third En requires again an enlargement of the coordination sphere. In contrast, with Co^{2+} the coordination sphere remains octahedral[22] throughout the coordination process with En (for the revised discussion of the Zn^{2+}–En systems see Section 5).

The results of Table 1 may be similarly explained. If Bicinate and Nta^{3-} bind to an octahedral Zn^{2+},[11] and if upon the further binding of imidazole or ammonia the coordination number is reduced to 4 or 5, two water molecules are released upon the coordination of the monodentate ligand and such a switch in the coordination geometry is favoured. Clearly, for Co^{2+}, Ni^{2+}, or Cd^{2+} with their more well-defined octahedral spheres, such a process is less probable.

The analogous explanation holds for the UTP^{4-} systems (Table 1). Considering UTP^{4-} (Structure I) as a tridentate ligand coordinating *via* its triphosphate chain[23] one arrives[14] at the following statistical values: $\Delta \log K_{st/oh} = -0.30$ (*cf.* also reference 19) and $\Delta \log K_{st/Cu} \simeq -0.7$. Comparison with the data of Table 1 indicates a steric hindrance of about 0.1 log unit, at least for $M(UTP)(Im)^{2-}$ with Co^{2+}, Ni^{2+}, and Cd^{2+} (neglecting any promotional effects due to the O-donor/heteroaromatic amine combination[19]). This hindrance is probably due to hydrogen-bonding between coordinated water and oxygens of the also coordinated triphosphate chain which would render the replacement of a water in the coordination sphere of the metal ion by an incoming ligand more difficult. In any case, $Zn(UTP)(Im)^{2-}$ has an increased stability (Table 1).

4 To What Extent is the Coordination Sphere of Zn²⁺ Altered upon Binding of Imidazole or Ammonia to Zn(Bicinate)⁺, Zn(Nta)⁻, or Zn(UTP)²⁻?

At this stage there is no final quantitative answer to this question, yet an estimate is possible. If we define an octahedral sphere with coordination number 6 as 'CN6' and a tetrahedral one as 'CN4', and if we further express our uncertainty about the extent of the reduction of the coordination sphere of Zn^{2+} by 'CN4(5)', indicating thus that a species with coordination number 5 could also be involved, equilibrium 5a and the definition 5b may be rewritten:

$$Zn(Bic)^+ + Im \rightleftharpoons Zn_{CN6}(Bic)(Im)^+ \rightleftharpoons Zn_{CN4(5)}(Bic)(Im)^+ \tag{6a}$$

$$K^{Zn(Bic)}_{Zn(Bic)(Im)} = \frac{[Zn(Bic)(Im)^+]}{[Zn(Bic)^+][Im]} = \frac{[Zn_{CN6}(Bic)(Im)^+] + [Zn_{CN4(5)}(Bic)(Im)^+]}{[Zn(Bic)^+][Im]} \tag{6b}$$

$K^{Zn(Bic)}_{Zn(Bic)(Im)}$ is the measured constant,[10] but there follow two further definitions:

$$K^{Zn(Bic)}_{Zn(Bic)(Im)/CN6} = \frac{[Zn_{CN6}(Bic)(Im)^+]}{[Zn(Bic)^+][Im]} \tag{7}$$

$$K_I = \frac{[Zn_{CN4(5)}(Bic)(Im)^+]}{[Zn_{CN6}(Bic)(Im)^+]} \tag{8}$$

This latter (intramolecular) dimensionless** equilibrium constant K_I is of interest and based on equations 6b, 7, and 8 the corresponding relations 9 and 10a are obtained (in analogy to other considerations on intramolecular equilibria):[11,12,24]

$$K^{Zn(Bic)}_{Zn(Bic)(Im)} = K^{Zn(Bic)}_{Zn(Bic)(Im)/CN6} + K_I \cdot K^{Zn(Bic)}_{Zn(Bic)(Im)/CN6} \tag{9}$$

$$K_I = \frac{K^{Zn(Bic)}_{Zn(Bic)(Im)}}{K^{Zn(Bic)}_{Zn(Bic)(Im)/CN6}} - 1 \tag{10a}$$

$$= \frac{10^{\Delta \log K_{Zn/Bic/Im}}}{10^{\Delta \log K_{Zn/Bic/Im/CN6}}} - 1 \tag{10b}$$

$$= 10^{\Delta\Delta \log K_{Zn}} - 1 \tag{10c}$$

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† It is difficult to predict whether the driving force for the reduction of the coordination number is enthalpic and/or entropic Owing to the opposing effects of replacing six long bonds (0 74 pm) by four short bonds (0 60 pm, see page 28 of reference 20) one may in a first approximation assume that the overall bond energies are similar in both geometries and that therefore the enthalpy change (ΔH) is small, hence, the process appears as entropy-driven due to the release of extra solvent molecules from the metal ion-coordination sphere upon reduction of the coordination number (see also S Ahrland, *Struct Bonding*, 1973, **15**, 167, *Pure Appl Chem*, 1979, **51**, 2019)

** K_I is dimensionless and attributed to an intramolecular equilibrium despite $Zn_{CN4(5)}(Bic)(Im)^+$ and $Zn_{CN6}(Bic)(Im)^+$ being no true isomeric complexes because they differ in the number of coordinated water molecules; yet the concentration of water in these diluted aqueous solutions is of course not significantly altered by this process

Instead of expanding 5a into 6a, equilibrium 1 could have been reconsidered[24] to obtain equation 10b, which may be transformed into 10c with the definition 11

$$\Delta\Delta \log K_{Zn} = \Delta \log K_{Zn\ Bic\ Im} - \Delta \log K_{Zn\ Bic\ Im\ CN6} \qquad (11)$$

The expressions $\Delta \log K_{Zn}$ in equations 10b and 11 are defined in equations 2 and 3, but now also additional subscripts are given for sure identification of the terms

K_I may now be calculated with equations 10a or 10b, provided that values for $K^{Zn(Bic)}_{Zn(Bic)(Im)/CN6}$ or $\Delta \log K_{Zn/Bic/Im/CN6}$ can be obtained It is easier to estimate $\Delta \log K_{Zn/Bic/Im/CN6}$ and therefore equations 10b and 10c are used in the following Of course, $\Delta \log K_{Zn/Bic/Im}$ (equation 11) is already determined (Table 1)

The well-known enhanced stability of mixed ligand complexes formed by a metal ion of the second half of the 3*d* series including Zn^{2+}, an O-donor, and a heteroaromatic N-base with π-accepting properties (see Section 2) should be more pronounced with Co^{2+} than with Zn^{2+}, owing to the completed 3*d* shell of Zn^{2+} Therefore $\Delta \log K_{Co/Bic/Im}$ of the octahedral Co^{2+} may be considered as the *upper limit* for $\Delta \log K_{Zn/Bic/Im/CN6}$ (equation 11) On the other hand, the *lower limit* for $\Delta \log K_{Zn/Bic\ Im/CN6}$ seems to be well represented by the statistical value $\Delta \log K_{st/oh} = -0\ 5$ (Section 2), which is also identical with that determined for $\Delta \log K_{Cd/Bic/Im/}$ (Table 1) This result is comforting because one could argue that the experimental values for the ratios K_2/K_1, *etc* , are usually somewhat smaller than the statistical result due to steric effects and the like (*e g* , see page 70 in reference 6*a* or page 44 in reference 6*b*), however, should this also be true for the systems presently considered, the formation degree of the Zn^{2+}_{CN4} species would be even larger Hence, we conclude that the lower and upper limits for K_I according to equations 8 and 10c may be calculated as well as the percentage of $Zn_{CN4(5)}$(Bicinate)(Im)$^+$ (equation 13) in the (intramolecular) equilibrium 12 (which is a part of equilibrium 6a)

$$Zn_{CN6}(Bic)(Im)^+ \rightleftharpoons Zn_{CN4(5)}(Bic)(Im)^+ \qquad (12)$$

$$\%\ Zn_{CN4(5)}(Bic)(Im)^+ = 100\ K_I/(1 + K_I) \qquad (13)$$

The same reasonings may be applied to the other ternary Zn^{2+} complexes listed in Table 1, for which the general 'intramolecular' equilibrium 14 may be written

$$Zn_{CN6}(A)(B) \rightleftharpoons Zn_{CN4(5)}(A)(B) \qquad (14)$$

This analysis assumes insignificant tendencies toward tetrahedral Zn^{2+} with the first ligand bound – which indeed appears valid at least for Zn(Bicinate)$^+$ [11] However, to the extent that there is significant (> 15%) Zn^{2+}_{CN4} upon binding of the first ligand, including it in the analysis would result in an increase in the calculated K_I and would narrow the difference between the upper and lower limits in Table 2

The calculations summarized in Table 2 show that an equilibrium exists between Zn^{2+}_{CN6} and $Zn^{2+}_{CN4(5)}$ In addition, the values for $\Delta \log K_{Zn}$ (Tables 1 and 2) are such that equilibrium 1 as well as the analogous equilibria in Table 1 are, in general, weakly on the side of the ternary complexes which are formed only between approximately 50 and 60%, based on the total amount of Zn(Bicinate)$^+$, Zn(Nta)$^-$, or Zn(UTP)$^{2-}$ present For Zn^{2+}–Bicinate–imidazole this weakness means that about 60% are present as Zn(Bicinate)(Im)$^+$ and 40% as Zn(Bicinate)$^+$, consequently about two thirds (70 ± 15%, Table 2) of 60% Zn(Bicinate)(Im)$^+$ occur with $Zn^{2+}_{CN4(5)}$ The equilibrium $Zn^{2+}_{CN6} \rightleftharpoons Zn^{2+}_{CN4(5)}$ may shift to either coordination mode depending on the ligands binding to Zn^{2+}, though at this stage too little information is available to identify systematic trends

5 The Old Example '*par Excellence*': Considerations on the Zn^{2+}–Ethylenediamine System

1,2-Diaminoethane or ethylenediamine (En) combines with Zn^{2+} to form 1 1, 2 1, and 3 1 complexes with the observed successive stability constants $\log K^{Zn}_{Zn(En)} = \log K_1 = 5\ 92$, $\log K^{Zn(En)}_{Zn(En)_2} = \log K_2 = 5\ 15$, and $\log K^{Zn(En)_2}_{Zn(En)_3} = \log K_3 = 1\ 86$ (I = 1 M, 25 °C) [21] As indicated in the third paragraph of Section 3, the steep drop in the last constant has long been taken as strong evidence for a tetrahedral disposition of the four nitrogens in the 2 1 complex $Zn(En)_2^{2+}$

Below, we estimate the amount of tetrahedral Zn^{2+}, Zn^{2+}_{CN4}, in the Zn^{2+} En = 1 1 and 1 2 complexes by comparison with the successive En stability constants for the similarly sized Co^{2+} $\log K_1 = 5\ 89$, $\log K_2 = 4\ 83$, and $\log K_3 = 3\ 10$ (I = 1 M, 30 °C) [22] Comparison of the constants for Zn^{2+} and Co^{2+} reveals similar K_1 values, and a stronger K_2 and a much weaker K_3 for Zn^{2+} Without a heteroaromatic amine nitrogen, no synergism among the ligands is expected, hence, we may simply use the Co^{2+}–En system to represent an octahedral coordination mode, M^{2+}_{CN6} [††]

[††] One may add that the statistical values[14] expected for an octahedral coordination sphere and binding of a bidentate ligand are $\log K_2 - \log K_1 = \log(5/2) - \log(12/1) = -0\ 68$ and $\log K_3 - \log K_2 = \log(1/3)(4/5) - \log(5/2) = -0\ 97$ Comparison of these statistical differences with the experimental values obtained for the Co^{2+} system reveals that these are smaller by 0 38 and 0 76 log units respectively This is typical for amines and shows that other factors like steric effects are also important (see *e g* page 44 of reference 6*b*) for this reason the statistical values are not employed in the evaluations given in Section 5

Table 2 Information on the intramolecular equilibria 12 and 14 regarding Zn^{2+}_{CN6} and $Zn^{2+}_{CN4(5)}$ as calculated from equations 6 to 11, together with the estimated percentages in which the $Zn^{2+}_{CN4(5)}$ (equation 13) and Zn^{2+}_{CN6} species occur in aqueous solution at 25 °C and I = 0 1 M

Complex system	limit	$\Delta \log K_{Zn}$[a]	$\Delta \log K_{Zn\ CN6}$	$\Delta\Delta \log K_{Zn}$	K_I	% $Zn^{2+}_{CN4(5)}$		% Zn^{2+}_{CN6}[e]
Zn(Bic)(Im)$^+$	lower	0 31	− 0 05[b]	0 36	1 29	56	70 ± 15[d]	30 ± 15
	upper	0 31	− 0 50[c]	0 81	5 46	85		
Zn(Nta)(Im)$^-$	lower	0 22	0 01[b]	0 21	0 62	38	60 ± 25[d]	40 ± 25
	upper	0 22	− 0 50[c]	0 72	4 25	81		
Zn(Nta)(NH$_3$)	lower	− 0 09	− 0 26[b]	0 17	0 48	32	45 ± 15[d]	55 ± 15
	upper	− 0 09	− 0 50[c]	0 41	1 57	61		
Zn(UTP)(Im)^2	lower	0 03	− 0 36[b]	0 39	1 45	59	60 ± 10[d]	40 ± 10
	upper	0 03	− 0 40[f]	0 43	1 69	63		

Values from Table 1 *cf* equations 2 3 [b] Lower limit regarding K_I (equations 8 10) the value corresponds to $\Delta \log K_{Co}$ of Table 1 as justified in the text of Section 4 it is needed in equations 10 11 Upper limit regarding K_I the value corresponds to $\Delta \log K_{st\ oh}$ and in the case of the Bic system also to $\Delta \log K_{Cd}$ of Table 1 (see also *b*) [d] These estimates are the rounded averages of the lower and upper limits together with also estimated error limits [e] These estimates follow from 100 − % $Zn^{2+}_{CN4(5)}$ [f] The statistical value $\Delta \log K_{st\ oh} = -0\ 30$ was in this case (*cf* also *c*) reduced by 0 1 log unit to account for steric effects (see the last paragraph in Section 3)

5.1 Calculation of the Percentage of Zn^{2+}_{CN4} in En Complexes

The binding of En, or any bidentate ligand, to a hexacoordinate metal ion designated as M_{CN6}, involves three successive equilibria (charges are ignored):

$$M_{CN6} + En \rightleftharpoons M_{CN6}(En) \qquad K_{1/CN6} = [M_{CN6}(En)]/[M_{CN6}][En] \tag{15}$$

$$M_{CN6}(En) + En \rightleftharpoons M_{CN6}(En)_2 \qquad K_{2/CN6} = [M_{CN6}(En)_2]/[M_{CN6}(En)][En] \tag{16}$$

$$M_{CN6}(En)_2 + En \rightleftharpoons M_{CN6}(En)_3 \qquad K_{3/CN6} = [M_{CN6}(En)_3]/[M_{CN6}(En)_2][En] \tag{17}$$

The 1:1 and 2:1 complexes are in (an intramolecular) equilibrium with their four coordinate, M_{CN4}, counterparts:

$$M_{CN6}(En) \rightleftharpoons M_{CN4}(En) \qquad K^{*}_{I/M/En} = [M_{CN4}(En)]/[M_{CN6}(En)] \tag{18}$$

$$M_{CN6}(En)_2 \rightleftharpoons M_{CN4}(En)_2 \qquad K^{**}_{I/M/2En} = [M_{CN4}(En)_2]/[M_{CN6}(En)_2] \tag{19}$$

For such a system the observed stability constants are given by equations 20 to 22, where the right-hand part follows from combinations with equations 15 to 19:

$$K_1 = \frac{[M_{CN6}(En)] + [M_{CN4}(En)]}{[M_{CN6}][En]} = K_{1/CN6}(1 + K^{*}_{I/M/En}) \tag{20}$$

$$K_2 = \frac{[M_{CN6}(En)_2] + [M_{CN4}(En)_2]}{\{[M_{CN6}(En)] + [M_{CN4}(En)]\}[En]} = K_{2/CN6}\frac{(1 + K^{**}_{I/M/2En})}{(1 + K^{*}_{I/M/En})} \tag{21}$$

$$K_3 = \frac{[M_{CN6}(En)_3]}{\{[M_{CN6}(En)_2] + [M_{CN4}(En)_2]\}[En]} = \frac{K_{3/CN6}}{(1 + K^{**}_{I/M/2En})} \tag{22}$$

Owing to the inherent differences in binding strengths between the Zn^{2+} and Co^{2+} complexes, these equations cannot be used directly. However, by taking the ratios of the observed stability constants we normalize for these inherent differences:

$$\frac{K_1}{K_2} = \frac{K_{1/CN6}(1 + K^{*}_{I/M/En})^2}{K_{2/CN6}(1 + K^{**}_{I/M/2En})} \tag{23}$$

$$\frac{K_2}{K_3} = \frac{K_{2/CN6}(1 + K^{**}_{I/M/2En})^2}{K_{3/CN6}(1 + K^{*}_{I/M/En})} \tag{24}$$

$$\frac{K_1}{K_3} = \frac{K_{1/CN6}(1 + K^{*}_{I/M/En})(1 + K^{**}_{I/M/2En})}{K_{3/CN6}} \tag{25}$$

The stability constants, $K_{1/CN6}$, $K_{2/CN6}$, and $K_{3/CN6}$, for the hexacoordinated octahedral complexes are taken from the Co^{2+}–En system. These constants also appear as ratios in equations 23 to 25, as is the case for the constants K_1, K_2, and K_3 attributed to the Zn^{2+}–En system. Any two of the last three equations may be solved simultaneously for $1 + K^{*}_{I/M/En}$ and $1 + K^{**}_{I/M/2En}$.

With the stability constants given in the first two paragraphs of Section 5, we calculate that $K^{*}_{I/Zn/En} = 1.12$ for $Zn(En)^{2+}$, corresponding to 53% $Zn_{CN4}(En)^{2+}$ (*cf.* equation 13), and $K^{**}_{I/Zn/2En} = 7.8$ for $Zn(En)_2^{2+}$, corresponding to 89% $Zn_{CN4}(En)_2^{2+}$. Thus about half of the 1:1 and about 90% of the 2:1 complexes are tetrahedral.

5.2 Conclusions from the Evaluation of the Zn^{2+}–En System

Three main points follow from Section 5.1 and we list these in order of their decreasing importance:

(1) The formulation regarding $M_{CN6} \rightleftharpoons M_{CN4}$ for the Zn^{2+}–En system applies not only to other bidentate ligands, but may easily be generalized to the successive additions of any number of identical ligands to any metal ion in any pair of coordination numbers. The only restriction is that to apply it successfully one must be able to estimate the ratios of the $K_{n/CN6}$ constants or of their analogues.

(2) Our calculations show that for $Zn(En)^{2+}$ about half of the species are octahedral and half tetrahedral (equation 18). This evidence that about 50% of the $Zn(En)^{2+}$ species already exist with a lower coordination number in aqueous solution is an important new insight; previously it was believed (Section 3) that tetrahedral complexes occur only with $Zn(En)_2^{2+}$.

(3) The result of a degree of formation of about 90% for $Zn_{CN4}(En)_2^{2+}$ agrees with the previous assumption (Section 3) that $Zn(En)_2^{2+}$ in aqueous solution is tetrahedral. However, the presence of about 10% of $Zn(En)_2^{2+}$ as an octahedral species is consistent with the formation of $Zn(En)_3^{2+}$ by a large excess of En over Zn^{2+}.

6 Considerations on Some Other Zn^{2+} Systems with Bidentate Ligands

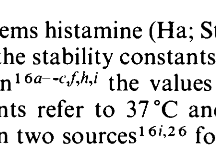
6.1 The Oxalate and Glycinate Systems are Octahedral!

After evaluation of the Zn^{2+} system with the bidentate ethylenediamine, an N-donor forming 5-membered chelates, it seemed appropriate to deal with a bidentate O-donor that also forms 5-membered rings; hence, we considered oxalate (Ox^{2-}). The stability constants given in the literature[16a—c,g,h] for the complexes with Zn^{2+} and Co^{2+} disagree, making it difficult to select the most reliable values; therefore, we averaged all the available constants (close to 25 °C and $I = 0$—2 M) and obtained $\log K^{Zn}_{Zn(Ox)} = 4.30 = \log K_1$ (averaged from 11 values), $\log K^{Zn(Ox)}_{Zn(Ox)_2} = 2.52 = \log K_2$ (10), and $\log K^{Zn(Ox)_2}_{Zn(Ox)_3} = 0.98 = \log K_3$ (3), as well as $\log K^{Co}_{Co(Ox)} = 4.13$ (from 17 values), $\log K^{Co(Ox)}_{Co(Ox)_2} = 2.32$ (12), and $\log K^{Co(Ox)_2}_{Co(Ox)_3} = 1.47$ (1). Comparison shows that the K_1/K_2 ratios are very similar for Zn^{2+} ($10^{1.78}$) and Co^{2+} ($10^{1.81}$), indicating that there is no pronounced tendency towards a tetrahedral geometry in $Zn(Ox)_2^{2-}$; this conclusion agrees with the K_2/K_3 ratios, though their comparison is less meaningful since for $Co(Ox)_3^{4-}$ only a single stability value is available.

A ligand that fits into a comparison with ethylenediamine and oxalate is glycinate (Gly^-).[16a—c,e] Here we could rely on a recent 'Critical Survey of Stability Constants of Complexes of Glycine':[25] $\log K^{Zn}_{Zn(Gly)} = 5.03$, $\log K^{Zn(Gly)}_{Zn(Gly)_2} = 4.20$, and $\log K^{Zn(Gly)_2}_{Zn(Gly)_3} = 2.54$, as well as $\log K^{Co}_{Co(Gly)} = 4.66$, $\log K^{Co(Gly)}_{Co(Gly)_2} = 3.85$, and $\log K^{Co(Gly)_2}_{Co(Gly)_3} = 2.32$ (25 °C; $I = 0.1$—0.2 M). For glycinate the K_1/K_2 and K_2/K_3 ratios are closely similar for both the Zn^{2+} ($10^{0.83}$ and $10^{1.66}$) and Co^{2+} ($10^{0.81}$ and $10^{1.53}$) complexes, suggesting that this bidentate N,O-donor ligand preserves octahedral hexacoordinate Zn^{2+} through all complexes.

6.2 The Zn^{2+}-Histamine System has a Significant Tetrahedral Portion

For biological systems histamine (Ha; Structure II) is of special interest. From all the stability constants listed for its Zn^{2+} and Co^{2+} complexes in[16a—c,f,h,i] the values given in[26] appear the best: these constants refer to 37 °C and $I = 0.15$ M, and the differences between two sources[16i,26] for the $\log K_1$ and $\log K_2$ values are $\leqslant 0.08$ log unit for the Zn^{2+} and Co^{2+} complexes. We therefore use the following averages: $\log K^{Zn}_{Zn(Ha)} = 4.99 = \log K_1$ and $\log K^{Zn(Ha)}_{Zn(Ha)_2} = 4.78 = \log K_2$; $\log K^{Co}_{Co(Ha)} = 4.90$ and $\log K^{Co(Ha)}_{Co(Ha)_2} = 3.54$. The values for the 1:3 complexes are in each case given only once: $\log K^{Zn(Ha)_2}_{Zn(Ha)_3} = 2.28$[26] $= \log K_3$ and $\log K^{Co(Ha)_2}_{Co(Ha)_3} = 2.27$.[16i]



II

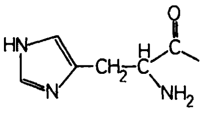
The ratio $K_1/K_2 = 10^{0.21}$ for the Zn^{2+} complexes is much smaller than that of $10^{1.36}$ for the Co^{2+} complexes, while the ratio $K_2/K_3 = 10^{2.50}$ is much larger for the Zn^{2+} species than for those of Co^{2+} with $10^{1.27}$. This comparison suggests a pronounced degree of formation of tetrahedral Zn^{2+} complexes.

Application of the procedure according to equations 23—25 of Section 5.1 leads to a *negative* value for $K^{*}_{\mathrm{I/Zn/Ha}}$ (equation 18), which can at the minimum be zero, meaning that no $\mathrm{Zn_{CN4}(Ha)^{2+}}$ species are formed. This negative result further indicates that something must be wrong. A careful evaluation of the mathematical calculation shows that the negative value for $K^{*}_{\mathrm{I/Zn/Ha}}$ is due to an oversized $\log K_3$; *i.e.*, the stability constant $\log K^{\mathrm{Zn(Ha)_2}}_{\mathrm{Zn(Ha)_3}} = 2.28$ is too large! Indeed, none of the other studies listed in[16a—c,f,h,i] give a stability constant for the $\mathrm{Zn(Ha)_3^{2+}}$ complex; thus the degree of formation of this species must be low. We suggest that unrecognized hydroxo-complex formation led to the above irregular result.

The mathematical evaluation shows further that the upper limit for $\log K_3 = 1.21$; only then $K^{*}_{\mathrm{I/Zn/Ha}}$ turns from a negative value to zero. With this value also the lower limit for equation 19 is obtained; *i.e.*, $K^{**}_{\mathrm{I/Zn/2Ha}} \geqslant 3$. Hence, the degree of formation of $\mathrm{Zn_{CN4}(Ha)_2^{2+}}$ is larger than 75%. Moreover, based on our experience and comparisons of related data we estimate for $\mathrm{Zn(Ha)_3^{2+}}$ the stability constant $\log K^{\mathrm{Zn(Ha)_2}}_{\mathrm{Zn(Ha)_3}} \simeq 0.7 \simeq \log K_3$, a value also in accord with the Zn^{2+}–En system (Section 5). This constant together with the other values listed above and application of equations 23—25 gives the following results: $K^{*}_{\mathrm{I/Zn/Ha}} = 0.48$ and 32% of $\mathrm{Zn_{CN4}(Ha)^{2+}}$, as well as $K^{**}_{\mathrm{I/Zn/2Ha}} = 30$ and 97% of $\mathrm{Zn_{CN4}(Ha)_2^{2+}}$.

6.3 The Zn^{2+}–Histidine Methyl Ester System Confirms the Importance of $\mathrm{Zn}^{2+}_{\mathrm{CN4}}$ Species for Histamine-type Ligands

Considering the importance of the imidazole group as binding site in Zn^{2+} enzymes, we included in our evaluations the bidentate histidine methyl ester (HisMe; Structure III). For the Zn^{2+}–HisMe system the first two stability constants of the Zn^{2+} complexes differ only slightly, $\log K^{\mathrm{Zn}}_{\mathrm{Zn(HisMe)}} = 4.46 = \log K_1$ and $\log K^{\mathrm{Zn(HisMe)}}_{\mathrm{Zn(HisMe)_2}} = 4.20 = \log K_2$;[27] in line with our conclusions at the Zn^{2+}–Ha system (Section 6.2), K_3 is indeterminately small.[27] No experiments with Co^{2+} were conducted, but the same study[27] reports for Ni^{2+} the stability constants $\log K_1 = 6.19$, $\log K_2 = 4.91$, and $\log K_3 = 2.90$. We compare these constants despite the indication that a square-planar $\mathrm{Ni(HisMe)_2^{2+}}$ species might occur to some extent; in fact, for Zn^{2+}–HisMe we aim only for the limiting conditions of the $\mathrm{Zn}^{2+}_{\mathrm{CN4}}$ formation.



III

As before in the Zn^{2+}–Ha system (Section 6.2), and now with Zn^{2+}–HisMe, the value for $K^{*}_{\mathrm{I/Zn/HisMe}}$ is difficult to obtain. For the necessary condition that $K^{*}_{\mathrm{I/Zn/HisMe}} > 0$, it may be shown that relation 26 holds (in fact, this condition was indirectly applied already in Section 6.2 in the arguments about the validity of $\log K^{\mathrm{Zn(Ha)_2}}_{\mathrm{Zn(Ha)_3}} = 2.28$ and the replacement of this value by an estimate):

$$2\log K_1 - \log K_2 \cdot K_3 > 2\log K_{1/\mathrm{CN6}} - \log K_{2/\mathrm{CN6}} \cdot K_{3/\mathrm{CN6}} \tag{26}$$

With condition 26 and using the Ni^{2+} results as reference for $K_{1/\mathrm{CN6}}$, $K_{2/\mathrm{CN6}}$, and $K_{3/\mathrm{CN6}}$, we find $\log K_3 < 0.2$ and $K^{**}_{\mathrm{I/Zn/2HisMe}} > 10$, meaning $\mathrm{Zn}^{2+}_{\mathrm{CN4}}$ must comprise more than 90% of the $\mathrm{Zn(HisMe)_2^{2+}}$ species. These conclusions are consistent both with the experimental difficulty of determining $K_3 = K^{\mathrm{Zn(HisMe)_2}}_{\mathrm{Zn(HisMe)_3}}$ as well as with the results for the Zn^{2+}–Ha system.

6.4 Conclusions from the Evaluation of the Various Zn^{+} Systems Containing Bidentate Ligands

From the results presented in Sections 6.1 to 6.3 it follows:

(1) Bidentate ligands like oxalate or glycinate do not enforce a significant formation degree of tetrahedral $\mathrm{Zn}^{2+}_{\mathrm{CN4}}$ in any of the 1:1 or 1:2 complexes.

(2) The results of Sections 6.2 and 6.3 allow the conclusion that 1:1 complexes of histamine-type ligands exist to about 30% with a $\mathrm{Zn}^{2+}_{\mathrm{CN4}}$ coordination sphere. In the 1:2 complexes the tetrahedral portion increases to more than 90%. Consequently, the stability of 1:3 complexes is low and (if at all) difficult to determine.

(3) A small $\log K_1/K_2$ term requires a large $\log K_2/K_3$ term of a Zn^{2+} system in comparison with a 'normal' set of reference constants for an octahedral system on the right-hand side of the inequality relation 26. This means that the more strongly the 1:2 Zn^{2+} complex goes tetrahedral, the greater the difficulty of adding a third bidentate ligand to yield again a hexacoordinate Zn^{2+}.

7 Estimations on the Extent of $\mathrm{Zn}^{2+}_{\mathrm{CN4}}$ in Some Mixed Ligand Complexes Containing Bidentate Ligands

The stability of any mixed ligand M(A)(B) complex may be quantified analogously to equilibrium 1 (Section 2); its general form (neglecting charges) is

$$\mathrm{M(A) + M(B) \rightleftharpoons M(A)(B) + M} \tag{27}$$

and the corresponding equilibrium constant $10^{\Delta \log K_{\mathrm{M}}}$ (equation 3) may be calculated *via* equation 28:

$$\Delta \log K_{\mathrm{M}} = \log K^{\mathrm{M(A)}}_{\mathrm{M(A)(B)}} - \log K^{\mathrm{M}}_{\mathrm{M(B)}} = \log K^{\mathrm{M(B)}}_{\mathrm{M(B)(A)}} - \log K^{\mathrm{M}}_{\mathrm{M(A)}} \tag{28}$$

Table 3 lists results for mixed ligand complexes formed with histamine (Ha), ethylendediamine (En), and the glycinate-like serinate (Ser^-); *i.e.*, for ligands or ligand-types we have considered in their binary parent complexes in Sections 5 and 6.

Comparison of the equilibrium constants in Table 3 for the complexes containing Ha confirms the well-known discriminating properties and the stability enhancing effect (in the presence of O-donor sites) of an imidazole group, *i.e.*, of a heteroaromatic amine;[15,18] a topic extensively discussed[13—15,17—19] (*cf.* also Sections 2 and 3) and not considered further here. In the present context the increased stability of all the ternary Zn^{2+} complexes compared to the corresponding Co^{2+} complexes is of interest. This is again evidence for the formation of tetrahedral $\mathrm{Zn}^{2+}_{\mathrm{CN4}}$ species!

In view of the difficulties encountered with $\log K^{\mathrm{Zn(Ha)_2}}_{\mathrm{Zn(Ha)_3}}$ for the octahedral $\mathrm{Zn(Ha)_3^{2+}}$ complex (Section 6.2), we attempt here

Table 3 Comparison of the relative stability of various mixed-ligand complexes according to the equilibrium
$$\mathrm{M(A) + M(B) \rightleftharpoons M(A)(B) + M}$$
and the corresponding constant $\Delta \log K_{\mathrm{M}}$ (equation 28) for aqueous solutions at 37 °C and $I = 0.15$ M ($\mathrm{KNO_3}$)[a]

		$\Delta \log K_{\mathrm{M}}$			
A	B	Co^{2+}	Ni^{2+}	Cu^{2+}	Zn^{2+}
Ha	En	− 0.88	− 1.20	− 1.43	− 0.19
Ha	Ser^-	− 0.48	− 0.83	− 0.58	+ 0.17
En	Ser^-	− 0.46	− 0.41	− 0.87	− 0.14

[a] The values were calculated from the stability constants provided by Perrin *et al.*: Co^{2+}, Zn^{2+};[26] Ni^{2+} (D. D. Perrin and V. S. Sharma, *J. Chem. Soc. (A)*, 1968, 446); Cu^{2+} (D. D. Perrin, I. G. Sayce, and V. S. Sharma, *J. Chem. Soc. (A)*, 1967, 1775).

only to obtain an estimate for the degree of formation of $\mathrm{Zn_{CN4}(A)(B)}$ by applying equations analogous to equations 6—11, *i e*, we will proceed as in Section 4 However, we now consider the equilibrium constants of the $\mathrm{Co^{2+}}$ complexes rather as the *upper* limits or even the 'true' values for the $\mathrm{Zn^{2+}_{CN6}}$ complexes assuming, because of the similar size of $\mathrm{Co^{2+}}$ and $\mathrm{Zn^{2+}}$, a comparable steric hindrance among the ligands in the coordination sphere of the two metal ions and that this also applies for the already mentioned 'heteroaromatic amine effect' where appropriate The *lower* limit is most probably well represented by the statistical value (st) for $\Delta\log K_M$ for the coordination of the two different bidentate ligands A and B Ligand B has 12 possibilities for binding to an octahedral (oh) coordination sphere,[14] yet only 5 for binding to an octahedral M(A) species, but the probability for dissociation is, of course, 1 in both cases, hence, $\Delta\log K_{\mathrm{oh/st}} = \log\ (5/1) - \log\ (12/1) = -0\ 38$ We attribute the lower limit to $\Delta\log K_{\mathrm{oh/st}}$ because the statistical ratios for K_2/K_1 (*etc*) are often larger than those found experimentally (*cf* footnote p 86)

The preceding reasoning leads to the results in Table 4 These data are only estimations and should not be overinterpreted because in this evaluation the presence of $\mathrm{Zn^{2+}_{CN4}}$ in any of the binary Zn(A) or Zn(B) complexes is ignored However, considering them would lead to even higher percentages for $\mathrm{Zn_{CN4}(A)(B)}$, hence, the results listed provide strong evidence that in all these mixed ligand complexes equilibrium 14 is operating and that significant portions of the Zn(A)(B) species have a tetrahedral geometry In this sense, these results further generalize the observations described in the preceding sections In addition they indicate that a $\mathrm{N_2O_2}$ donor set as present in Zn(En)(Ox) gives rise to a significant portion of a $\mathrm{Zn^{2+}_{CN4}}$ coordination sphere while this is not the case for the same donor set in $\mathrm{Zn(Gly)_2}$ (Section 6 1) The difference is evidently that in Zn(En)(Ox) the two crucial N-donor sites are within the same ligand and thus lead to a *cis*-type coordination in an octahedral coordination sphere Moreover, the present result for $\mathrm{Zn_{CN4}}$ (En)(Ox) agrees with that obtained in Section 5 for $\mathrm{Zn_{CN4}(En)(H_2O)_2^{2+}}$

8 Imidazole and the $\mathrm{Zn^{2+}_{CN4}}$ Coordination Sphere which Facilitates Hydroxo-Complex Formation

We have already seen in Section 4 that upon its coordination to $\mathrm{Zn^{2+}}$, imidazole (Im) initiates a tetrahedral geometry in mixed-ligand complexes Now we ask What are the properties of the binary $\mathrm{Zn^{2+}}$–imidazole system? For our evaluation we use the following stability constants which refer to 25 °C and $I = 0\ 16$ M [28] $\log K^{\mathrm{Zn}}_{\mathrm{Zn(Im)}} = 2\ 57 = \log K_1$, $\log K^{\mathrm{Zn(Im)}}_{\mathrm{Zn(Im)_2}} = 2\ 36 = \log K_2$, $\log K^{\mathrm{Zn(Im)_2}}_{\mathrm{Zn(Im)_3}} = 2\ 22 = \log K_3$ and $\log K^{\mathrm{Zn(Im)_3}}_{\mathrm{Zn(Im)_4}} = 2\ 01 = \log K_4$,[28a] for the $\mathrm{Co^{2+}}$–Im system the values are $\log K_1 = 2\ 42$, $\log K_2 = 1\ 95$, $\log K_3 = 1\ 58$, and $\log K_4 = 1\ 2$ [28b]

These stability constants yield for the $\mathrm{Zn^{2+}}$ system the ratios $K_1/K_2 = 10^{0\ 21}$, $K_2/K_3 = 10^{0\ 14}$, and $K_3/K_4 = 10^{0\ 21}$, for the $\mathrm{Co^{2+}}$ system they yield $10^{0\ 47}$, $10^{0\ 37}$, and $10^{0\ 38}$ respectively These ratios are significantly smaller throughout for the $\mathrm{Zn^{2+}}$–Im system than for the $\mathrm{Co^{2+}}$ one, clearly indicating a progressing tetrahedral geometry with an increasing amount of imidazole in the coordination sphere of $\mathrm{Zn^{2+}}$ Unfortunately an exact evaluation (*cf* Section 5) is not possible because the $\log K_5$ value for the $\mathrm{Zn^{2+}}$–Im system is not known, though for $\mathrm{Co(Im)_5^{2+}}$ and $\mathrm{Co(Im)_6^{2+}}$ estimates do exist [16b,f]

Rough estimations, however, suggest that $\mathrm{Zn(Im)^{2+}}$ occurs as about 20% $\mathrm{Zn_{CN4}(Im)^{2+}}$, and $\mathrm{Zn(Im)_2^{2+}}$ as about 50% $\mathrm{Zn_{CN4}(Im)_2^{2+}}$, the tetrahedral species is formed to approximately 90% for $\mathrm{Zn(Im)_3^{2+}}$ and to more than 98% for $\mathrm{Zn(Im)_4^{2+}}$ In fact, the latter two degrees of formation are most probably even more pronounced at high ionic strength, as revealed by the following stability constants which refer to 25 °C and $I = 3$ M ($\mathrm{NaClO_4}$) [29] $\log K_1 = 2\ 92$, $\log K_2 = 2\ 01$, $\log K_3 = 3\ 84$, and $\log K_4 = 2\ 64$ Under these conditions the stability of $\mathrm{Zn(Im)_3^{2+}}$ exceeds that of $\mathrm{Zn(Im)^{2+}}$ and $\mathrm{Zn(Im)_2^{2+}}$ by factors of about 8 and 70, respectively, this implies that already the formation degree of $\mathrm{Zn_{CN4}(Im)_3^{2+}}$ is large and that apparently the switch from $\mathrm{Zn^{2+}_{CN6}}$ to $\mathrm{Zn^{2+}_{CN4}}$ occurs mainly upon addition of a further imidazole to $\mathrm{Zn(Im)_2^{2+}}$

This conclusion confirms the observation that under the same conditions (25 °C, $I = 3$ M, $\mathrm{NaClO_4}$) $\mathrm{Zn(Im)_3(H_2O)^{2+}}$ loses a proton with $\mathrm{p}K_a = 8\ 0$ [29 30] This impressive acidification of a $\mathrm{Zn^{2+}}$-bound water by the coordinated imidazoles is of biological significance [30 31] From the $\mathrm{Zn^{2+}}$ complexes and the various $\mathrm{p}K_a$ values assembled in Figure 2 it clearly follows that this acidification is connected with the formation of $\mathrm{Zn^{2+}_{CN4}}$ No ligands with a special structure have to be devised since ligands with a single coordinating atom are enough for this effect, provided that upon their coordination a tetrahedral coordination sphere at $\mathrm{Zn^{2+}}$ is enforced Imidazole is such a ligand, and nature makes plentiful use of this ligating moiety in many $\mathrm{Zn^{2+}}$-enzymes That hydroxo-complex formation and tetrahedrality facilitate each other is also apparent from the tetrahedral $\mathrm{Zn(OH)_3(H_2O)^-}$, which is the principal zincate ion in aqueous solution (see page 598 in reference 6*a* or page 605 in reference 6*b*), though in strongly alkaline media the (also) tetrahedral $\mathrm{Zn(OH)_4^{2-}}$ may be formed [32]

9 Conclusions and Outlook

To our knowledge, this is the first time that increased relative complex stabilities in low-molecular-weight ternary $\mathrm{Zn^{2+}}$ complexes have been explained quantitatively in terms of decreasing coordination numbers of $\mathrm{Zn^{2+}}$ Observation of *positive* values for $\Delta\log K_{\mathrm{Zn}}$ (equations 2, 3) and, correspondingly, the shift of equilibrium 1 to the side of the mixed ligand complex leave no other reasonable explanation Indeed, consideration of the intramolecular equilibria 12 and 14 and estimations for the corresponding species distributions prove that in Zn(Bicina-

Table 4 Information on the intramolecular equilibrium 14 regarding $\mathrm{Zn^{2+}_{CN4}}$ as calculated in analogy to equations 6 to 11, together with the estimated percentages in which ternary species $\mathrm{Zn_{CN4}(A)(B)}$ occur in aqueous solution at 37 °C and $I = 0\ 15$ M

Complex system	limit	$\Delta\log K_{\mathrm{Zn}}$[a]	$\Delta\log K_{\mathrm{Zn\ CN6}}$	$\Delta\Delta\log K_{\mathrm{Zn}}$	K_1	% $\mathrm{Zn^{2+}_{CN4}(A)(B)}$
$\mathrm{Zn(Ha)(En)^+}$	lower	− 0 19	− 0 38[b]	0 19	0 55	35 } 60 ± 30
	upper	− 0 19	− 0 88[c]	0 69	3 90	80
$\mathrm{Zn(Ha)(Ser)^+}$	lower	0 17	− 0 38[b]	0 55	2 55	72 } 75 ± 10
	upper	0 17	− 0 48[c]	0 65	3 47	78
$\mathrm{Zn(En)(Ser)^+}$	lower	− 0 14	− 0 38[b]	0 24	0 74	42 } 45 ± 15
	upper	− 0 14	− 0 46[c]	0 32	1 09	52
Zn(En)(Ox)	lower	0 05[d]	− 0 38[b]	0 43	1 69	63 ⩾ 60

[a] Values from Table 3 [b] Lower limit regarding K_1 the value corresponds to $\Delta\log K_{\mathrm{st\ oh}}$ for bidentate ligands see Section 7 [c] Upper limit regarding K_1 the value corresponds to $\Delta\log K_{\mathrm{Co}}$ of Table 3 as justified in the text of Section 7 [d] This value refers to 25 °C and $I = 1$ M ($\mathrm{KNO_3}$) it is calculated from the stability constants provided by Y Kanemura and J I Watters *J Inorg Nucl Chem* 1967 **29** 1701

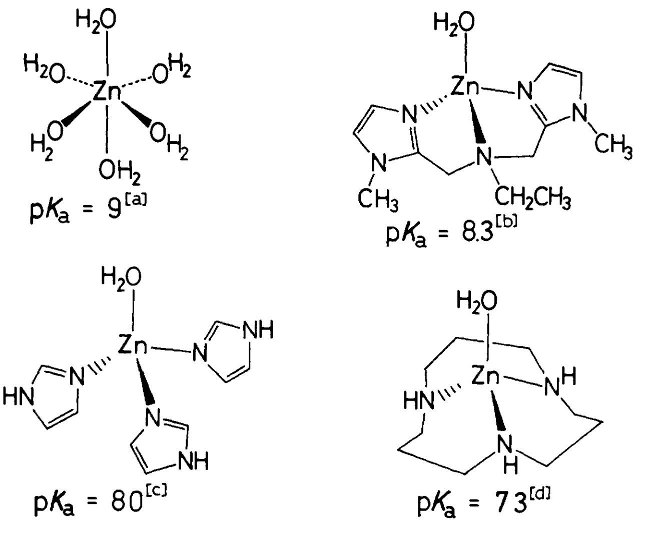


Figure 2 Effect of the coordination sphere on the deprotonation of H_2O bound to Zn^{2+} The pK_a values given refer to the reaction $Zn^{II}(X)(H_2O) \rightleftharpoons Zn^{II}(X)(OH^-) + H^+$ These acidity constants are taken from the following sources [a] $pK_a = 8\ 95 \pm 0\ 15$ is the average of selected values from references 16*a*,*b*,*d* at 25 °C and $I = 0 - 2$ M [b] The above value is from kinetic experiments and refers to a 33% ethanol/H_2O mixture at 25 °C with I = undefined (probably in the order of 0 1 M), potentiometric pH titrations gave $pK_a = 8\ 16 \pm 0\ 10$, from R G Clewley, H Slebocka-Tilk, and R S Brown, *Inorg Chim Acta*, 1989, **157**, 233 We estimate that the corresponding pK_a value in aqueous solution would be lower and close to 7 8 [c] 25 °C and I = 3 M ($NaClO_4$), from references 29, 30 It may be added that at an ionic strength lower than 3 M the pK_a value of $Zn(Im)_3(H_2O)^{2+}$ is most probably somewhat below 8, as is indicated by the pK_a values for $Zn(H_2O)_6^{2+}$ available at various ionic strengths [16a,b] [d] 25 °C and I = 0 1 M ($NaClO_4$), from reference 9*a*

te)(Im)$^+$, $Zn(Nta)(Im)^-$, $Zn(Nta)(NH_3)$, and Zn(UTP)(Im)$^{2-}$ at least two isomers with different coordination numbers of Zn^{2+} are present simultaneously (Table 2)

Moreover, the results described, including those for the binary Zn^{2+}–ethylenediamine, –histamine and other systems (see Sections 5—8), indicate that, in principle, in *any* Zn^{2+} system, in (aqueous) solution, species are present which contain Zn^{2+} with coordination number 6 as well as further species having Zn^{2+} in lower coordination numbers, mainly 4 and with 5 as intermediate Depending on the conditions, especially the ligands present, the intramolecular equilibrium can be largely on the side of 6-fold coordinated Zn^{2+} but also on that of 4-coordinated Zn^{2+}, giving the impression that only a certain (single) Zn^{2+}-coordination sphere occurs in the system Of course, with sterically more rigid ligands like macrocycles certain coordination numbers for Zn^{2+} may be enforced[9] to some degree

However, the common claim that the stereochemistry of a metal ion in an enzyme is 'undoubtedly' controlled by the ligand geometry (*e g* , references 8, 33) is only partly correct Certainly, Zn^{2+} submits readily to structural demands of a ligand,[1] but the present evaluations demonstrate that a tetrahedral Zn^{2+} is also created by flexible and highly adaptable ligands like ethylenediamine or histamine, in fact, the best example here is the monodentate imidazole Overall it appears that the formation of Zn^{2+}_{CN4} is driven by the Lewis basicity of the donor atoms if the coordinating ligand is a strong Lewis base,[34] the coordination number of Zn^{2+} drops and the bond length shortens, *i e* , the metal–ligand bond becomes more of the covalent type This explains why an imidazole group promotes Zn^{2+}_{CN4} formation more effectively than an amino group, and this group more than an O-donor

Considering the adaptability of Zn^{2+}-coordination spheres in biological systems, the results described and their explanations are also meaningful An incoming substrate is favourably bound and also enhances the Lewis acidity of the Zn^{2+} (*cf* also Figure 2) if the coordination number is *reduced* and thus more water (or other bound sites or) molecules are released, in addition this property allows that during a reactive transition a further donor site can easily be bound *without* releasing another group from the coordination sphere As indicated above, the reduction from coordination number 6 to (5 or) 4 is favoured by the participation of N-donor ligands (or sites) This reduction accords with the situation in biological systems where the extremely versatile[18b,35] imidazole group is often involved [1,2,17]

To conclude, the strategies described here for the quantification of the varying coordination numbers of Zn^{2+} in its complexes in aqueous solution may also be applied to other metal ions such as Al^{3+}, Ag^+, and Hg^{2+}, and ions that undergo a change of spin state such as Ni^{2+} Further insights into the 'flexibility' of metal ion coordination spheres in solution are thus to be expected, a topic so far not appropriately considered – possibly due to the lack of methods – in coordination chemistry

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10 References

1 B L Vallee and D S Auld, *Biochemistry*, 1990, **29**, 5647 and 1993, **32**, 6493

2 J J R Frausto da Silva and R J P Williams, 'The Biological Chemistry of the Elements', Clarendon Press, Oxford, 1991, p 299

3 (*a*) 'Metal Ions in Biological Systems', Vol 15, Zinc and Its Role in Biology and Nutrition, ed H Sigel and A Sigel, Marcel Dekker, Inc , New York and Basel, 1983, (*b*) 'Progress in Inorganic Biochemistry and Biophysics', Vol 1 Zinc Enzymes, ed I Bertini, C Luchinat, W Maret, and M Zeppezauer, Birkhauser Verlag, Basel, 1986

4 D W Christianson, *Adv Protein Chem* , 1991, **42**, 281

5 J P Glusker, *Adv Protein Chem* , 1991, **42**, 1

6 (*a*) F A Cotton and G Wilkinson, 'Advanced Inorganic Chemistry', 4th Edn , J Wiley, New York, 1980, p 599, (*b*) 5th Edn , 1988, p 607

7 H B Silber, D Simon, and F Gaizer, *Inorg Chem* , 1984, **23**, 2844, C O Quicksall and T G Spiro, *Inorg Chem* , 1966, **5**, 2232

8 R S Brown, J Huguet, and N J Curtis, *Met Ions Biol Syst* , 1983, **15**, 55

9 (*a*) E Kimura, T Shiota, T Koike, M Shiro, and M Kodama, *J Am Chem Soc* , 1990, **112**, 5805, (*b*) E Kimura, H Kurosaki, T Koike, and K Toriumi, *J Inclusion Phenom Mol Recogn Chem* , 1992, **12**, 377, M Shionoya, E Kimura, and M Shiro, *J Am Chem Soc* , 1993, **115**, 6730, (*c*) A Looney, G Parkin, R Alsfasser, M Ruf, and H Vahrenkamp, *Angew Chem* , 1992, **104**, 57 (*Angew Chem Int Ed Engl* , 1992, **31**, 92), R Alsfasser, S Trofimenko, A Looney, G Parkin, and H Vahrenkamp, *Inorg Chem* , 1991, **30**, 4098

10 L -n Ji, N A Corfu, and H Sigel, *Inorg Chim Acta*, 1993, **206**, 215

11 H Sigel, *Coord Chem Rev* , 1993, **122**, 227

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

13 D Banerjea, T A Kaden, and H Sigel, *Inorg Chem* , 1981, **20**, 2586

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

15 H Sigel, 'Stability, Structure and Reactivity of Mixed Ligand Complexes in Solution', in *Coordination Chemistry – 20*, ed D Banerjea, IUPAC, Pergamon Press, Oxford and New York, 1980, p 27

16 (*a*) L G Sillen and A E Martell, 'Stability Constants of Metal-Ion Complexes', Special Publication No 17, The Chemical Society, London, 1964, (*b*) L G Sillen and A E Martell, 'Stability Constants of Metal-Ion Complexes', Suppl I, Special Publication No 25, The Chemical Society, London, 1971, (*c*) D D Perrin, 'Stability Constants of Metal-Ion Complexes', Part B Organic Ligands, IUPAC Chemical Data Series No 22, Pergamon Press, Oxford, 1979, (*d*) E Hogfeldt, 'Stability Constants of Metal-Ion Complexes', Part A Inorganic Ligands, IUPAC Chemical Data Series No 21, Pergamon Press, Oxford, 1982, (*e*) A E Martell and R M Smith, 'Critical Stability Constants', Vol 1 Amino Acids, Plenum Press, New York, 1974, (*f*) R M Smith and A E Martell, 'Critical Stability Constants', Vol 2 Amines, Plenum Press, New York, 1975, (*g*) A E Martell and R M Smith, 'Critical Stability Constants', Vol 3 Other Organic Ligands, Plenum Press, New York, 1977, (*h*) A E Martell

and R M Smith, 'Critical Stability Constants', Vol 5 1st Suppl , Plenum Press, New York, 1982, (*i*) R M Smith and A E Martell, 'Critical Stability Constants', Vol 6 2nd Suppl , Plenum Press, New York, 1989

17 H Sigel, B E Fischer, and B Prijs, *J Am Chem Soc* , 1977, **99**, 4489

18 (*a*) P R Huber, R Griesser, and H Sigel, *Inorg Chem* , 1971, **10**, 945, (*b*) H Sigel, *Inorg Chem* , 1980, **19**, 1411

19 N Saha and H Sigel, *J Am Chem Soc* , 1982, **104**, 4100

20 R B Martin, *Met Ions Biol Syst* , 1986, **20**, 21

21 J Bjerrum, *Chem Rev* , 1950, **46**, 381

22 J Bjerrum, 'Metal Ammine Formation in Aqueous Solution', P Haase and Son, Copenhagen, 1941 & 1957 (taken from ref 16*a*)

23 (*a*) R B Martin and Y H Mariam, *Met Ions Biol Syst* , 1979, **8**, 57, (*b*) H Sigel, *Eur J Biochem* , 1987, **165**, 65, H Sigel, *Chem Soc Rev* , 1993, **22**, 255

24 B E Fischer and H Sigel, *J Am Chem Soc* , 1980, **102**, 2998, S S Massoud and H Sigel, *Inorg Chim Acta*, 1989, **159**, 243

25 T Kiss, I Sovago, and A Gergely, *Pure Appl Chem* , 1991, **63**, 597

26 D D Perrin and V S Sharma, *J Chem Soc (A)*, 1969, 2060

27 H L Conley, Jr and R B Martin, *J Phys Chem* , 1965, **69**, 2923

28 (*a*) Y Nozaki, F R N Gurd, R F Chen, and J T Edsall, *J Am Chem Soc* , 1957, **79**, 2123, (*b*) R B Martin and J T Edsall, *J Am Chem Soc* , 1958, **80**, 5033

29 W Forsling, *Acta Chem Scand* , 1977, **31A**, 759

30 R B Martin, *Met Ions Biol Syst* , 1979, **9**, 1, see page 29

31 D K Wilson and F A Quiocho, *Biochemistry*, 1993, **32**, 1689

32 N Wiberg, 'Holleman-Wiberg Lehrbuch der Anorganischen Chemie', 91st–100th Edn , W de Gruyter, Berlin and New York, 1985, p 1038

33 R H Prince, *Adv Inorg Chem Radiochem* , 1979, **22**, 349

34 I D Brown, *Acta Cryst* , 1988, **B44**, 545

35 R J Sundberg and R B Martin, *Chem Rev* , 1974, **74**, 471, H Sigel and R B Martin, *Chem Rev* , 1982, **82**, 385, H Sigel, R Tribolet, and O Yamauchi, *Comments Inorg Chem* , 1990, **9**, 305