Quantification of successive intramolecular equilibria in binary metal ion complexes of *N,N*-bis(2-hydroxyethyl)glycinate (Bicinate). A case study

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ABBREVIATIONS

ATP^{4-}	adenosine 5′-triphosphate
Bic^-	abbreviation of Bicinate as used in formula
Bicinate	monoanion of Bicine
Bicine	*N,N*-bis(2-hydroxyethyl)glycine
$(CH_3)_2N \cdot Gly^-$	*N,N*-dimethylglycinate
$(C_2H_5)_2N \cdot Gly^-$	*N,N*-diethylglycinate
Gly^-	glycinate
L^-	bidentate amino acid monoanion (or general ligand)
M^{2+}	divalent metal ion

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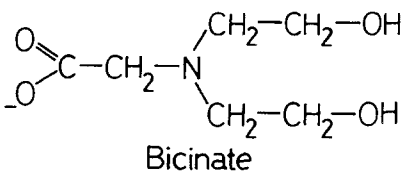
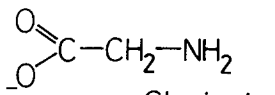


Bicinate



Glycinate

Fig.1. Chemical formula of *N*,*N*-bis(2-hydroxyethyl)glycinate (Bicinate or Bic^-), as well as of its parent compound glycinate (Gly^-).

A. INTRODUCTION

N,*N*-bis(2-hydroxyethyl)glycine, generally known as Bicine, is a very common buffer substance often used in biochemical studies (see, for example, refs. 1–4). Like its parent compound, the amino acid glycine, Bicine is also expected to form metal ion complexes in the monoanionic form. These anions, glycinate and Bicinate, are shown in Fig. 1.

Indeed, the metal ion coordinating properties of Bicinate have long been recognized [1,5]; many stability constants are known [5–12], and the participation of the hydroxy groups in metal ion binding has been suggested [5] and recently confirmed for aqueous solution [11,12] as well as for the solid state [13,14]. Moreover, the formation of mixed ligand complexes involving Bicinate and 1,10-phenanthroline [15] or imidazole [16] is known. Very recently, it was also established that rather stable ternary complexes are formed [12], even between Bicinate and $M(H \cdot ATP)^-$ or $M(ATP)^{2-}$, despite the evident charge repulsion. These few examples demonstrate quite well that Bicinate is a very versatile ligand, and that great care should be exercised in employing this substance as a buffer in the presence of metal ions and possibly also other ligands.

The structure of Bicinate in Fig. 1 immediately reveals that, in principle, all four potential binding sites could coordinate to a metal ion with a tetrahedral or an octahedral coordination sphere. Clearly, the main stability-determining binding core is of a glycinate-type mode and the question arises: to what extent do the hydroxy groups additionally bind to the metal ion?

It is clear that not only one, but also both hydroxy groups of bicinate could coordinate, leading to the equilibrium sequence that is illustrated schematically in Fig. 2. Equilibrium (1) is evidently concentration-dependent, while equilibria (2) and (3) are of an intramolecular type, i.e. they occur between structurally different isomers and are thus concentration-independent. The corresponding isomers in Fig. 2 are designated as $M(Bic)^+_{op}$ (eqn. (1)), $M(Bic)^+_{cl(1)}$ (eqn. (2)) and $M(Bic)^+_{cl(2)}$ (eqn. (3)); hence,

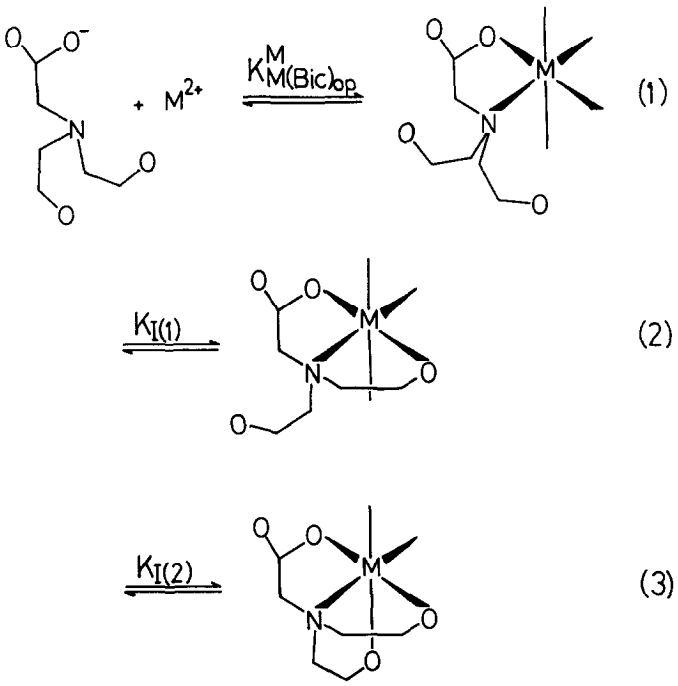


Fig. 2. Schematic representation of the successive formation of the various complexes formed between a divalent metal ion and Bicinate (see also text in Sects. A and E.(ii)). The equilibrium constants for the above equilibria (1)–(3) are defined in eqns. (4)–(6), respectively.

the corresponding equilibrium constants can be defined as follows:

$$K^{M}_{M(Bic)_{op}} = \frac{[M(Bic)^{+}_{op}]}{[M^{2+}][Bic^{-}]} \tag{4}$$

$$K_{I(1)} = \frac{[M(Bic)^{+}_{cl(1)}]}{[M(Bic)^{+}_{op}]} \tag{5}$$

$$K_{I(2)} = \frac{[M(Bic)^{+}_{cl(2)}]}{[M(Bic)^{+}_{cl(1)}]} \tag{6}$$

It should be emphasized that the only constant which is accessible experimentally is

$$M^{2+} + Bic^{-} \rightleftharpoons M(Bic)^{+} \tag{7a}$$

$$K^{M}_{M(Bic)} = \frac{[M(Bic)^{+}]}{[M^{2+}][Bic^{-}]} \tag{7b}$$

$$= \frac{([M(Bic)^{+}_{op}] + [M(Bic)^{+}_{cl(1)}] + [M(Bic)^{+}_{cl(2)}])}{[M^{2+}][Bic^{-}]} \tag{7c}$$

The difficulty lies in eqns. (4)–(6); only knowledge of these equilibrium constants

allows the calculation of the percentages of the various $M(Bic)^+$ isomers (Fig. 2) which occur simultaneously in solution.

At this point, it must be emphasized that the chemical formulae $M(Bic)_{cl(1)}$ and $M(Bic)_{cl(2)}$ already represent the sum of various isomers; e.g. the three donor atoms forming $M(Bic)_{cl(1)}$ may coordinate in a facial or an equatorial way (the latter is shown in equilibrium (2) of Fig. 2). These additional isomeric equilibria will be ignored in the following evaluations (see also Sect. E.(ii)); emphasis is only put on the question of whether *one* or *both* hydroxy groups are bound and this is solely expressed by the formulae $M(Bic)_{cl(1)}$ and $M(Bic)_{cl(2)}$, respectively. Hence, in the following section, an evaluation is attempted of the equilibrium constants for eqns. (4)–(6). In fact, the ligand *N*,*N*-bis(2-hydroxyethyl)glycinate and its complexes with several divalent metal ions are used here simply with the intention of demonstrating by example, how the isomeric complexes of such multidentate ligands and the involved equilibria may be treated.

B. COMPARISON OF THE STABILITIES OF BINARY COMPLEXES FORMED WITH GLYCINATE AND SOME OF ITS DERIVATIVES, INCLUDING BICINATE

The tertiary amino nitrogen and the negatively charged carboxylate group of Bicinate are certainly the crucial binding sites in the formation of metal ion complexes of this ligand (as is also shown in Fig. 2), but the extent of the (partial) involvement of the oxygen atoms of the hydroxyethyl groups remains to be determined, as already indicated (Sect. A). Hence, the question is: is it possible to quantify the extent of participation of the hydroxy group(s) in the binding of metal ions?

To help to answer this question, the equilibrium constants listed in Table 1 have been collected [1,5,8,9,11,12,17–22]; data for glycinate, as well as for its derivatives *N*,*N*-dimethylglycinate [$=(CH_3)_2N \cdot Gly^-$], *N*-*N*-diethylglycinate [$=(C_2H_5)_2N \cdot Gly^-$] and Bicinate are given. The fact that the data refer in part to various ionic strengths is of minor importance as no significant trends are observable. Of course, one might argue about the sources and selection of some of the equilibrium constants, yet in the present case the results and conclusions are hardly affected by this choice (see also Tables 2 and 3 in Sects. D and E(iii), respectively).

There is a further, more serious problem associated with the values of Table 1; i.e. not all pertinent stability constants for the five metal ions listed are known, but the systems involving Mn^{2+} and Zn^{2+} were included in the present considerations due to their biological importance. The data of row 11 are based on the constants given in rows 1–4; with the aid of these constants and those of rows 5–7, those of rows 12 and 13 have been deduced and, in part, estimated. Finally, row 14 contains the equilibrium constants for the Bicinate systems, which are based on rows 8–10. All these "best estimates" listed in rows 11–14, which will be discussed below, refer to 25°C and an ionic strength of 0.1 M. The error limits are estimated to be, in most instances, within ± 0.1 log unit, though in some cases they may extend (at the most) to ± 0.2 log unit.

TABLE 1

Negative logarithms of the acidity constants for monoprotonated glycinate and for some of its derivatives including Bicinate[a], and logarithms of the stability constants of the corresponding complexes with some divalent metal ions (analogous to eqn. (7)) in aqueous solution at 25°C and various ionic strengths (I)

Row no.	Ligand (L^-)	I (M)	pK^H_{HL}	log K^M_{ML} for M^{2+} =					Ref.[b]
				Mn^{2+}	Co^{2+}	Ni^{2+}	Cu^{2+}	Zn^{2+}	
1	Gly^-	0.1	9.57	2.80	4.64	5.78	8.15	4.96	9,17
2	Gly^-	0.1	9.62			5.86	8.38		18
3	Gly^-	0.1	9.68		4.63	5.83	8.27	4.96	19
4	Gly^-	1.0	9.75			5.69	8.30		20
5	$(CH_3)_2N \cdot Gly^-$	0.1	9.80			4.82	7.30		18
6	$(CH_3)_2N \cdot Gly^-$	0.15	9.80			4.77	7.26		21
7	$(C_2H_5)_2N \cdot Gly^-$	0.1	10.47			4.21	6.88		18
8[c]	Bic^-	0.1	8.08[d]	3.18	5.27	6.38	8.15	5.37	5
9	Bic^-	0.1	8.39	3.07	5.30	6.42	8.07	5.37	11
10	Bic^-	1.0	8.33	3.1[e]	5.08	6.02	8.24		12
11	Gly^-	0.1	9.65	2.80	4.63	5.83	8.27	4.96	[f]
12	$(CH_3)_2N \cdot Gly^-$	0.1	9.80	2.0	3.7	4.80	7.28	4.1	[f]
13	$(C_2H_5)_2N \cdot Gly^-$	0.1	10.45	1.5	3.2	4.20	6.85	3.6	[f]
14	Bic^-	0.1	8.25	3.10	5.25	6.30	8.15	5.35	[f]

[a] $K^H_{HL} = [H^+][L^-]/[HL]$.

[b] If nothing else is specified, all values in a row are taken from the corresponding reference.

[c] All values in this row refer to 30°C.

[d] Other values for $pK^H_{H(Bic)}$ are 8.35 (20°C; $I \sim 0.1$ M (?) [1]), 8.22 (25°C; $I = 0.2$ M, $NaClO_4$ from ref. 22 as given in ref. 8), and 8.25 (25°C; $I = 0.5$ M, KNO_3 [12]).

[e] From ref. 1; 20°C; $I \sim 0.4$ M(?).

[f] These values are based on the data given in the rows above as well as on experience; they are considered as the "best estimates" presently available; see also text in Sects. B and D.

To a first approximation, the stability constants of the glycinate complexes (row 11) may be directly compared with those of the *N,N*-dimethylglycinate complexes (row 12) because the basicities of these two ligands are rather similar, despite the small increase by about 0.15 pK unit for *N,N*-dimethylglycinate. It is therefore immediately evident that substitution of the two amino hydrogens in glycinate by methyl groups inhibits complex stability by about 0.8 to 1 log unit due to steric influence. Clearly, replacement of the two methyl groups (row 12) by ethyl groups (row 13) considerably enhances this effect: complex stability decreases further by about another 0.5 log unit, despite an additional increased basicity (by about 0.65 pK unit) of the nitrogen site.

In contrast to the observations summarized in the preceding paragraph, which establish the inhibitory steric effect of alkyl substituents at the amino nitrogen, it is quite evident that the stabilities of the bicinate complexes of Mn^{2+}, Co^{2+}, Ni^{2+} and

Zn^{2+} (row 14), if compared with those of glycinate (row 11) *increase* on average by about 0.4 log unit, despite a considerably *lower* basicity (about −1.4 pK units) of the nitrogen site in bicinate; Cu^{2+} is somewhat special (see Sects. D and E.(ii)), but even here the stabilities of Cu(glycinate)$^+$ and Cu(Bicinate)$^+$ are nearly the same. Hence, these comparisons confirm earlier conclusions [5,11,12] about the importance of the hydroxy groups for the complexing properties of Bicinate in solution.

With these qualitative considerations in mind, it is necessary to develop first a method which allows quantification of the described effect of the hydroxy groups in M(Bicinate)$^+$ complexes. This is done in the following section; only then may the above formulated question be addressed further (see Sects. D and E).

C. QUANTIFICATION PROCEDURE FOR THE COMPLEX STABILITY-ENHANCING EFFECT OF THE HYDROXY GROUPS OF BICINATE

It is safe to assume, as indicated before, that any metal ion coordinated to Bicinate binds to the amino and carboxylate groups (Fig. 2); hence, the additional participation of the hydroxy groups then leads to an intramolecular equilibrium between an isomer with a glycinate-type coordination, which was designated as the "open" $M(Bic)^+_{op}$ isomer (eqn. (1)), and two further isomers in which one or both hydroxy groups coordinate (see eqns. (2) and (3)). As there is, at this point, no way to distinguish between these last two isomers, for the present simply the sum of their concentrations is considered and they are designated as the "closed" $M(Bic)^+_{cl/tot}$ species. Based on these definitions, one may combine equilibria (2) and (3) into

$$M(Bic)^+_{op} \rightleftharpoons M(Bic)^+_{cl/tot} \tag{8}$$

where $[M(Bic)^+_{cl/tot}] = [M(Bic)^+_{cl(1)}] + [M(Bic)^+_{cl(2)}]$.

It is evident that the presence of any closed species will enhance complex stability [23]; this means that the presence of such species will be reflected in the experimentally measured stability constants, $K^M_{M(Bic)}$ (eqn. (7); row 14 in Table 1). Thus, one may define a stability enhancement, E, according to eqn. (9) (for further details see ref. 23):

$$E = \frac{K^M_{M(Bic)} - K^M_{M(Bic)_{op}}}{K^M_{M(Bic)_{op}}}$$

$$= \frac{K^M_{M(Bic)}}{K^M_{M(Bic)_{op}}} - 1 \tag{9}$$

The stability constant $K^M_{M(Bic)_{op}}$ (eqns. (1 and 4)) in eqn. (9) quantifies the stability of the open species in equilibrium (8). It is evident that, in the case where the two equilibrium constants in eqn. (9) become equal, the stability enhancement E becomes zero.

Usually, the so-called stability enhancement factor $(1 + E)$ is considered [23].

This factor follows from eqn. (9) and is given by

$$1 + E = \frac{K^{M}_{M(Bic)}}{K^{M}_{M(Bic)_{op}}} \tag{10}$$

This stability enhancement factor is often also expressed [24–26] as $10^{\log \Delta}$ because it equals the difference between the logarithms of two stability constants:

$$\log \Delta = \log(1 + E)$$

$$= \log K^{M}_{M(Bic)} - \log K^{M}_{M(Bic)_{op}} \tag{11}$$

Hence, provided one can obtain values for $\log K^{M}_{M(Bic)_{op}}$, one is now in the position to describe in a quantitative way, based on eqn. (11), the effect of the hydroxy groups on the stability of M(Bicinate)$^+$ complexes.

D. EVALUATION OF THE STABILITY ENHANCEMENT FACTORS FOR M(BICINATE)$^+$ COMPLEXES

For a calculation of the stability enhancement factor, $10^{\log \Delta}$, according to eqn. (11), it is necessary to determine the stability constant, $\log K^{M}_{M(Bic)_{op}}$, for the open isomer in equilibrium (8). Corresponding determinations are often based on $\log K^{M}_{ML}$ versus pK^{H}_{HL} plots [23,27]; this is possible because straight lines are obtained for families of structurally related ligands. For simple, sterically uninhibited bidentate amino acid monoanions and the resulting complexes with several divalent metal ions, Martin [28] has recently provided the slopes, m, of such linear $\log K^{M}_{ML}$ versus pK^{H}_{HL} plots. These slopes are listed in the second column of Table 2 [23,27,29].

Such a slope together with the stability constant of the corresponding metal ion complex of an amino acid monoanion, which is representative of the glycinate-like binding mode of the open isomer of a M(Bicinate)$^+$ complex, allows calculation of the respective stability constant $\log K^{M}_{M(Bic)_{op}}$ according to the equation

$$\log K^{M}_{M(Bic)_{op}} = \log K^{M}_{ML} - m \cdot \Delta pK_a \tag{12}$$

where $\Delta pK_a = pK^{H}_{HL} - pK^{H}_{H(Bic)}$ and L = bidentate amino acid monoanion.

Clearly, the difficulty is to select the most representative amino acid anion for the glycinate-like binding in M(Bicinate)$^+$. Glycinate itself is a possibility, of course, and the results of the corresponding calculations are listed in the third row of Table 2. However, these values for $\log K^{M}_{M(Bic)_{op}}$ are definitely too large because there is no steric inhibition with glycinate by alkyl substituents at the amino nitrogen, an important effect as already discussed in Sect. B.

The linear plots of $\log K^{M}_{ML}$ versus pK^{H}_{HL} for two families of related ligands which differ by a constant steric effect are parallel to each other, i.e. the straight lines have the same slope [30,31]. Hence, we may use the slopes of column two in Table 2 and the equilibrium data for *N*,*N*-dimethylglycinate (row 12 in Table 1); this leads to the results for $\log K^{M}_{M(Bic)_{op}}$ listed in the fourth column of Table 2. These results are further illustrated in Fig. 3 for the systems with Cu^{2+} and Zn^{2+}, and they also

TABLE 2

Estimation of log $K^{M}_{M(Bic)_{op}}$ (eqn. (4)) for the "open" isomer of M(Bicinate)$^+$ (eqns. (1 and 8)) in which the ligand is bound in a glycinate-type mode for several metal ion systems, together with the logarithm of the stability enhancement factor log Δ (eqn. (11)), which quantifies the contribution of the hydroxy groups of Bicinate to the stability of M(Bicinate)$^+$ complexes ($I = 0.1$ M; 25°C)

M^{2+}	m[a]	log $K^{M}_{M(Bic)_{op}}$ based[b] on			Estimate[c] for log $K^{M}_{M(Bic)_{op}}$	log $K^{M}_{M(Bic)}$[d]	log Δ (eqn. (11))[e]
		Gly^-	$(CH_3)_2N\cdot Gly^-$	$(C_2H_5)_2N\cdot Gly^-$			
Mn^{2+}	0.40	2.24	1.38	0.62	1.00 ± 0.30	3.10 ± 0.10	2.1 ± 0.3
Co^{2+}	0.38	4.10	3.11	2.36	2.74 ± 0.30	5.25 ± 0.15	2.5 ± 0.3
Ni^{2+}	0.35	5.34	4.26	3.43	3.85 ± 0.30	6.30 ± 0.15	2.5 ± 0.3
Cu^{2+}	0.55	7.50	6.43	5.64	6.04 ± 0.30	8.15 ± 0.10	2.1 ± 0.3
Zn^{2+}	0.47	4.30	3.37	2.57	2.97 ± 0.30	5.35 ± 0.10	2.4 ± 0.3

[a]Slopes of the linear log K^{M}_{ML} versus pK^{H}_{HL} plots for the bidentate monoanion systems of α-amino acids [28]. No slope is listed for Mn^{2+} in ref. 28, but experience shows [23,27,29] that the average of the slopes for the Co^{2+}, Ni^{2+} and Zn^{2+} systems is usually very close to that for the corresponding Mn^{2+} system. The above Mn^{2+} value was therefore estimated by forming the average in the indicated way.

[b]Calculated with $pK^{H}_{H(Bic)} = 8.25$ (Table 1) and the data of rows 11, 12 and 13 in Table 1 with eqn. (12) for glycinate, *N*,*N*-dimethylglycinate and *N*,*N*-diethylglycinate, respectively.

[c]Averages of the values given in the two columns to the left (see text in Sect. D); the error limits are estimated.

[d]Values from row 14 in Table 1; the error limits are estimated.

[e]These error limits are based on those given in the two columns to the left; they were calculated according to the error propagation after Gauss.

confirm the unsuitability of M(Gly)$^+$ complexes as a reference for M(Bicinate)$^+$ species. The vertical broken lines in Fig. 3 represent the values for log Δ in eqn. (11), clearly revealing that the stability of the M(Bicinate)$^+$ complexes is much higher than expected for a simple bidentate glycinate-type binding mode.

In Fig. 3, the data pairs for Cu^{2+} and Zn^{2+} complexes with *N*,*N*-diethylglycinate from row 13 of Table 1 are also inserted. As one might expect (see Sect. B), the full circles corresponding to these data are somewhat below the reference lines defined by the *N*,*N*-dimethylglycinate systems, which is a reflection of the somewhat larger steric effect of ethyl substituents compared with methyl groups. Taking this into account, and considering the similarity of the structures of *N*,*N*-bis(2-hydroxyethyl)-glycinate (= Bicinate; Fig. 1) and *N*,*N*-diethylglycinate, one may conclude that the latter ligand is a very suitable representative for the open binding mode of Bicinate (Fig. 2). Therefore, values for log $K^{M}_{M(Bic)_{op}}$ were also calculated on the basis of the *N*,*N*-diethylglycinate data (row 13 of Table 1); these results are listed in the fifth column of Table 2.

Indeed, the steric effect of an ethyl and of a 2-hydroxyethyl substituent is

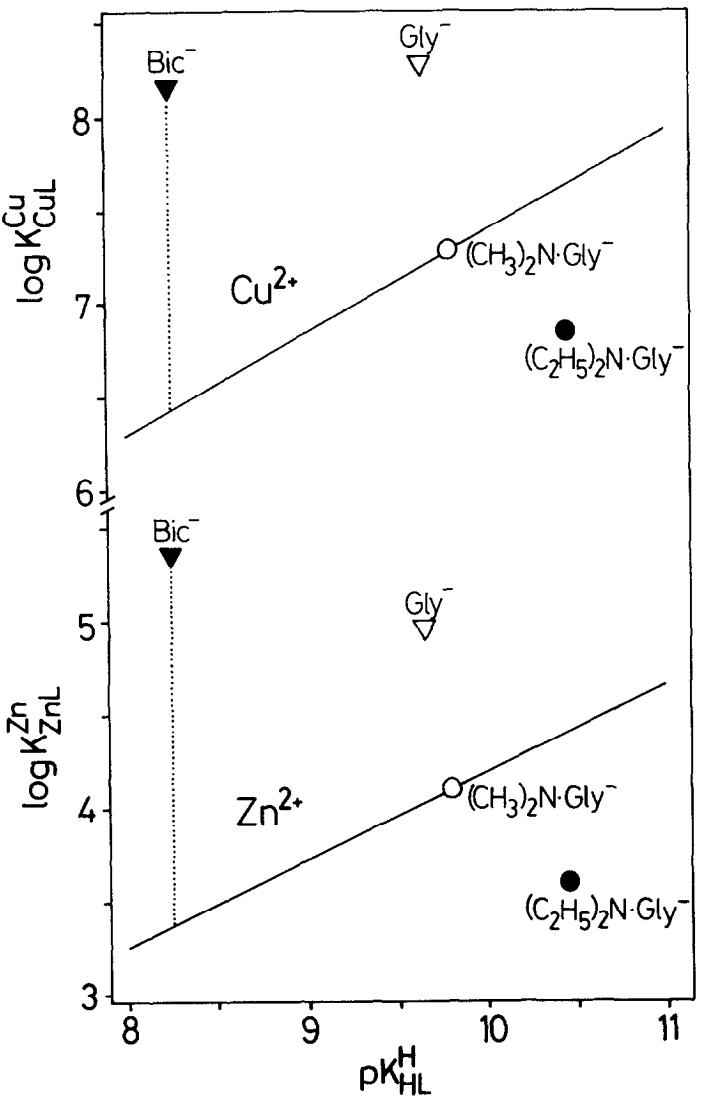


Fig. 3. Relationship between log K^{M}_{ML} and pK^{H}_{HL} for the Cu^{2+} and Zn^{2+} 1:1 complexes of the monoanions of *N*,*N*-dimethyl-substituted amino acids in aqueous solution (25°C; $I = 0.1$ M) based on the straight-line slopes given in Table 2 and the equilibrium constants for the *N*,*N*-dimethylglycinate systems (○, $(CH_3)_2N \cdot Gly^-$) listed in row 12 of Table 1. The points due to the 1:1 complexes formed with Bicinate (▼, Bic^-), glycinate (▽, Gly^-) and *N*,*N*-diethylglycinate (●, $(C_2H_5)_2N \cdot Gly^-$) are given for comparison; these values are from rows 14, 11 and 13 in Table 1, respectively. The vertical broken lines emphasize the stability enhancement observed for the $M(Bic)^+$ complexes on the basis of the $M[(CH_3)_2N \cdot Gly]^+$ species; the lengths of these broken lines correspond to the logarithm of the stability enhancement factor log Δ as defined in eqn. (11).

expected to be very similar; hence, these results (column 5 in Table 2) possibly represent the stability of the $M(Bicinate)^{+}_{op}$ complexes rather well. An argument against the simple use of the *N*,*N*-diethylglycinate data could be that, in this ligand, the two ethyl groups undergo a hydrophobic interaction in aqueous solution and that this leads to a different solvation and makes the ligand more rigid compared with Bicinate (where solvation is favoured due to the presence of the hydroxy groups). Hence, to prevent an overestimation of the steric effect and to be on the safe side regarding the following conclusions about the extent of hydroxy group coordination, the averages obtained for the *N*,*N*-dimethylglycinate and the *N*,*N*-diethylglycinate systems are used as the final values for $\log K^{M}_{M(Bic)_{op}}$ and a comfortable error limit of ± 0.3 log unit has also been added. These estimates are listed in the sixth column of Table 2, and they now allow one finally to calculate, using the data in the seventh column, values for the stability enhancement as expressed by $\log \Delta$ in eqn. (11).

Evidently, the participation of the two hydroxy groups in metal ion binding, which is sterically possible in octahedral (or tetrahedral) coordination spheres (Fig. 2), is very significant; it adds about 2.3 log units to the stability of the glycinate-type binding mode of Bicinate for all five metal ions, including the Cu^{2+} complex. This means that the stability increase for the $Cu(Bicinate)^{+}$ complex is only slightly smaller if at all, than for the complexes with Mn^{2+}, Co^{2+}, Ni^{2+} and Zn^{2+} (Table 2, final column). Considering the inability of the tetragonally distorted Cu^{2+} to bind ligating atoms strongly in an apical position [32], though weak interactions are possible and well known [23,33], this observation may at first sight appear to be surprising. However, it should be recalled (see also Fig. 2) that, in the Jahn–Teller distorted coordination sphere of Cu^{2+}, one of the two hydroxy groups still reaches a strongly coordinating equatorial position while for the other, only a weakly coordinating apical position is available; the average of these interactions then brings the stability increase for $Cu(Bicinate)^{+}$ into the order observed for the other $M(Bicinate)^{+}$ complexes. Moreover, the stability increase for complexes of the same five metal ions with *N*-(2-hydroxyethyl)iminodiacetate due to the presence of the single hydroxy group in this ligand amounts to 0.8–1.5 log units [23]; hence, the stability increase of about 2.3 log units observed now for *two* hydroxy groups is most reasonable.

E. CONCLUSIONS REGARDING THE INTRAMOLECULAR EQUILIBRIA IN M(BICINATE)$^{+}$ COMPLEXES

(i) The mathematical treatment

It is evident that the position of equilibrium (8) is defined by the dimensionless constant $K_{I/tot}$:

$$K_{I/tot} = \frac{[M(Bic)^{+}_{cl/tot}]}{[M(Bic)^{+}_{op}]} \tag{13}$$

Values for $K_{I/tot}$ may be calculated according to eqn. (14) [23,24,27]:

$$K_{I/tot} = \frac{K^{M}_{M(Bic)}}{K^{M}_{M(Bic)_{op}}} - 1 \tag{14}$$

Hence, from eqns. (9) and (13) it follows that $K_{I/tot} = E$, and together with eqn. (11), eqn. (15) results:

$$K_{I/tot} = 10^{\log \Delta} - 1 \tag{15}$$

Recall (see Sect. C) that the "closed" species $M(Bic)^{+}_{cl/tot}$ in equilibrium (8) encompasses, in fact, two isomers (Fig. 2); i.e. one in which a single hydroxy group is coordinated, which was designated as $M(Bic)^{+}_{cl(1)}$, and one in which both hydroxy groups bind to the metal ion, designated as $M(Bic)^{+}_{cl(2)}$ (see also Sect. A). If equilibrium (8) is rewritten in this more detailed form, one obtains the successive equilibria (16), which in fact correspond to equilibria (2) and (3) in Fig. 2.

$$M(Bic)^{+}_{op} \overset{K_{I(1)}}{\rightleftharpoons} M(Bic)^{+}_{cl(1)} \overset{K_{I(2)}}{\rightleftharpoons} M(Bic)^{+}_{cl(2)} \tag{16}$$

The corresponding intramolecular equilibrium constants were already defined in eqns. (5) and (6). Together with the experimentally determined stability constant (eqn. (7c)) and the stability of the open isomer (eqn. (4)), one obtains the relation

$$K^{M}_{M(Bic)} = K^{M}_{M(Bic)_{op}}(1 + K_{I(1)} + K_{I(1)} \cdot K_{I(2)}) \tag{17}$$

From eqns. (13)–(15) and (17), it follows that

$$K_{I/tot} = \frac{[M(Bic)^{+}_{cl(1)}] + [M(Bic)^{+}_{cl(2)}]}{[M(Bic)^{+}_{op}]} = \frac{[M(Bic)^{+}_{cl/tot}]}{[M(Bic)^{+}_{op}]} \tag{18a}$$

$$K_{I/tot} = K_{I(1)} + K_{I(1)} \cdot K_{I(2)} = 10^{\log \Delta} - 1 \tag{18b}$$

Hence, $K_{I/tot}$ can now be calculated while solutions still have to be sought for $K_{I(1)}$ and $K_{I(2)}$.

(ii) Statistical considerations

Assuming a regular octahedral coordination sphere for a certain metal ion and no differences between the two hydroxy groups in their affinity for the metal ion sites, one may make a simple statistical consideration [34] for the relation between $K_{I(1)}$ and $K_{I(2)}$: There are two hydroxy groups at the N-site of Bicinate and for each there are two sterically accessible positions at the metal ion (the third position is always shielded by the other hydroxy group; see Fig. 2), hence four possibilities for the formation of $M(Bic)^{+}_{cl(1)}$ and evidently one possibility for its decomposition; i.e. $K_{I(1)} \propto 4/1$. However, once $M(Bic)^{+}_{cl(1)}$ is *equatorially* formed, only a *single* pathway remains for the formation of $M(Bic)^{+}_{cl(2)}$, yet there are now two possibilities for the reverse reaction; i.e. $K_{I(2)equatorial} \propto 1/2$. Moreover, should $M(Bic)_{cl(1)}$ form via an

apical position, i.e. have a *facial* structure, then there are *two* ways for the formation of $M(Bic)_{cl(2)}$, and of course still two possibilities for its decomposition; i.e. $K_{I(2)facial} \propto 2/2$. Assuming both pathways for the formation of $M(Bic)_{cl(2)}$ are equally likely, then $K_{I(2)} \propto 1.5/2$. Therefore one may conclude, for an octahedral coordination sphere, that $K_{I(2)}/K_{I(1)} = (1.5/2)/(4/1)$ and hence eqn. (19) holds:

$$K_{I(2)} = \frac{1}{4} \cdot \frac{1.5}{2} \cdot K_{I(1)} = \frac{1.5}{8} \cdot K_{I(1)} \tag{19}$$

Combination of eqns. (18b) and (19) gives:

$$\frac{1.5}{8}(K_{I(1)})^2 + K_{I(1)} - K_{I/tot} = 0 \tag{20}$$

If eqn. (20) is solved for $K_{I(1)}$, one obtains:

$$K_{I(1)} = \frac{-1 + \sqrt{1 + 4(1.5/8)K_{I/tot}}}{2 \times (1.5/8)} = \frac{-1 + \sqrt{1 + 0.75K_{I/tot}}}{0.375} \tag{21}$$

Clearly, once $K_{I(1)}$ is calculated, $K_{I(2)}$ follows from eqn. (19).

The coordination spheres of Mn^{2+}, Co^{2+} and Ni^{2+} are usually octahedral [35]. The following comparison indicates that this is, to a first approximation, also true for Zn(Bicinate)$^+$: the difference $\log K^{M}_{M(Bic)} - \log K^{M(Bic)}_{M(Bic)_2} = 1.84$, 2.04 and 2.14 for Co^{2+}, Ni^{2+} and Zn^{2+}, respectively (averages from the values given in refs. 5 and 11). Not surprisingly, the same difference for the Cu^{2+}/Bicinate complexes equals 2.81, which is considerably larger, showing that the second Bicinate to be bound to Cu(Bicinate)$^+$ is disfavoured, in agreement with the tetragonal distorted coordination sphere of Cu^{2+} [32,33] (see also Sect. D).

Therefore, for the special case of Cu(Bicinate)$^+$, it is apparent that $K_{I(2)}$ should be further disfavoured and for eqn. (19) an additional factor of 1/6 is assumed, which leads then to the relation

$$K_{I(2)Cu} \simeq \frac{1}{32} \cdot K_{I(1)Cu} \tag{22}$$

By analogy to eqns. (20) and (21), it follows that

$$K_{I(1)Cu} = \frac{-1 + \sqrt{1 + 0.125K_{I/tot}}}{0.0625} \tag{23}$$

(iii) Results of the calculations. Percentages for $M(Bic)^+_{cl(1)}$ *and* $M(Bic)^+_{cl(2)}$

The values for $\log \Delta$ (eqn. (11)) discussed in Sect. D now allow the calculation of the percentages of $M(Bic)^+_{cl/tot}$, i.e. of the sum of the species shown in equilibria (2) and (3) of Fig. 2, and of $M(Bic)^+_{op}$ (see also eqn. (8)) from eqns. (13)–(15). These results are listed in columns 4 and 5 of Table 3, and in agreement with the large stability

TABLE 3

Intramolecular equilibrium constants (Fig. 2) for the formation of the various isomeric M(Bicinate)$^+$ complexes together with the percentages in which the isomers occur in aqueous solution at 25°C and $I = 0.1$ M[a]

M^{2+}	log Δ (eqn. (11))[a]	$K_{I/tot}$ (eqns. (13)–(15))[b]	$\%M(Bic)^+_{cl/tot}$ (eqns. (8) and (13))[c]	$\%M(Bic)^+_{op}$ (eqns. (8) and (13))[d]	$K_{I(1)}$ (eqns. (2), (5) and (21))[e,f]	$K_{I(2)}$ (eqns. (3), (6) and (19))[e,f]	$\%M(Bic)^+_{cl(1)}$ (eqn. (2))[b,g]	$\%M(Bic)^+_{cl(2)}$ (eqn. (3))[b,h]
Mn^{2+}	2.1 ± 0.3	125 ± 87(29)	99.21 ± 0.55	0.79 ± 0.55	23.29 ± 8.94	4.37 ± 1.68	18 ± 15(5)	81 ± 15(5) [71(24)]
Co^{2+}	2.5 ± 0.3	315 ± 218(73)	99.68 ± 0.22	0.32 ± 0.22	38.41 ± 14.15	7.20 ± 2.65	12 ± 10(3)	88 ± 10(3) [76(25)]
Ni^{2+}	2.5 ± 0.3	315 ± 218(73)	99.68 ± 0.22	0.32 ± 0.22	38.41 ± 14.15	7.20 ± 2.65	12 ± 10(3)	88 ± 10(3) [76(25)]
Cu^{2+}	2.1 ± 0.3	125 ± 87(29)	99.21 ± 0.55	0.79 ± 0.55	49.24 ± 21.34[e]	1.54 ± 0.67[e]	39 ± 32(11)	60 ± 32(11) [56(18)]
Zn^{2+}	2.4 ± 0.3	250 ± 174(58)	99.60 ± 0.28	0.40 ± 0.28	33.95 ± 12.67	6.36 ± 2.38	14 ± 11(4)	86 ± 11(4) [75(25)]

[a] The data in column 2 are taken from the last column in Table 2. All error limits given in this table are based on those in column 2; they were calculated according to the error propagation after Gauss.

[b] The error limits given in parentheses are based on an error limit of ±0.1 log unit for log Δ.

[c] Calculated according to $\%M(Bic)^+_{cl/tot} = 100\ K_{I/tot}/(1 + K_{I/tot})$.

[d] These values follow from $100 - \%M(Bic)^+_{cl/tot}$.

[e] The values for Cu^{2+} were calculated from eqns. (22) and (23); see text in Sect. E.(ii).

[f] Despite the large error limits, two digits are given after the decimal point because the calculations for columns 8 and 9 rest on these data and only then does the sum of the percentages of the various species amount to a value close to 100% (as it has to be).

[g] These percentages were calculated with the values for $K_{I(1)}$ in the following way: $K_{I/tot}$ defines the mol fraction of the sum of the closed species, as well as of the open species (eqn. (18a)); this allows one to calculate with $K_{I(1)}$ the mol fraction of $M(Bic)^+_{cl(1)}$ (eqn. (5)), i.e. of $\%M(Bic)^+_{cl(1)}$.

[h] These values follow from $\%M(Bic)^+_{cl/tot} - \%M(Bic)^+_{cl(1)}$; they may also be calculated with $K_{I(2)}$ (eqn. (6); column 7) and $\%M(Bic)^+_{cl(1)}$, (column 8) but, in this case, the error limits (also calculated via the error propagation after Gauss), not surprisingly, are larger; these limits are given for comparison within the square brackets.

enhancement factors $10^{\log \Delta}$ (Sect. C; Table 2), they show that the M(Bicinate)$^+$ species, in which the hydroxy groups are also involved in metal ion binding, reach a degree of formation of about 99%. However, it should also be emphasized that the species $M(Bic)^+_{op}$, in which the metal ion is coordinated only in a glycinate-type mode, still occurs to a remarkable extent for all five metal ions, even though the degree of formation of this species is small, about 0.5%.

Based on the statistical considerations outlined in Sect. E.(ii), it is possible to estimate the degrees of formation of the species with a single, $M(Bic)^+_{cl(1)}$, as well as with both hydroxy groups, $M(Bic)^+_{cl(2)}$, bound to the metal ion. These results are listed in the last two columns most right in Table 3. The error limits given with these data are rather large; this fact originates in the large errors of $K_{l/tot}$, which continue to $K_{l(1)}$ and $K_{l(2)}$, and consequently also to the percentages of $M(Bic)^+_{cl(1)}$ and $M(Bic)^+_{cl(2)}$. Clearly, the large errors of $K_{l/tot}$ are the result of the ± 0.3 log unit assumed as error limit for the log Δ values.

Indeed, if an error limit of ± 0.1 log unit is taken for log Δ (a value which is well achieved in many instances (see, for example, refs. 26,27 and 36)), then the other limits are also considerably reduced; this is demonstrated for $K_{l/tot}$ (column 3), % $M(Bic)^+_{cl(1)}$ (column 8) and % $M(Bic)^+_{cl(2)}$ (column 9) for which these reduced error limits are listed in parentheses in Table 3. Despite the indicated shortcomings, it is evident that $M(Bic)^+_{cl(1)}$ and $M(Bic)^+_{cl(2)}$ reach degrees of formation of about 10–20% and 80–90%, respectively, for the systems with Mn^{2+}, Co^{2+}, Ni^{2+} and Zn^{2+}. The corresponding values for the Cu(Bicinate)$^+$ system are about 40 and 60%, as here different statistical considerations were employed (Sect. E.(ii)). Of course, the total degree of formation of the closed species, i.e. $M(Bic)^+_{cl/tot}$, is well defined (see column 4 in Table 3), because the stability enhancement due to the binding of the hydroxy groups is large, i.e. log $\Delta > 2$. It may further be added that, in the case of Cu(Bicinate)$^+$ in the solid state, the $M(Bic)^+_{cl(2)}$ species is isolated [13,14].

Finally, in connection with the discussion of the error limits in the preceding two paragraphs and the way in which the values for log Δ were obtained (see Sect. D), the following considerations on Ni(Bicinate)$^+$, as an example, appear to be important. It should be recalled that the values listed for $\log K^{M}_{M(Bic)_{op}}$ in the sixth column of Table 2 are the *averages* of the data calculated for the complexes with *N*,*N*-dimethylglycinate and *N*,*N*-diethylglycinate, and this procedure then leads to values for log Δ (eqn. (11)), which are possibly somewhat too small. If one employs only the *N*,*N*-diethylglycinate data, one obtains for Ni(Bicinate)$^+$: $\log \Delta = \log K^{Ni}_{Ni(Bic)} - \log K^{Ni}_{Ni(Bic)_{op}} = 6.30 - 3.43 = 2.87$. From this latter value, it then follows that $K_{l/tot} = 740$, $K_{l(1)} = 60.21$ and $K_{l(2)} = 11.29$; the species distributions are $Ni(Bic)^+_{cl/tot} = 99.87\%$, $Ni(Bic)^+_{op} = 0.13\%$, $Ni(Bic)^+_{cl(1)} = 8\%$, and $Ni(Bic)^+_{cl(2)} = 92\%$. Hence, this example definitely confirms that the order of the values listed in Table 3 is correct and it further proves that all three complex species shown in Fig. 2 are actually formed.

F. SOME GENERAL CONCLUSIONS

In summary, the presented calculations show that, in the complexes of Bicinate with Mn^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, and Zn^{2+}, more than 99% of the M(Bicinate)$^+$ species also contain one or two bound hydroxyethyl groups. Obviously, a series of successive intramolecular equilibria occurs in M(Bicinate)$^+$ systems: (i) an "open" isomer in which only the amino and carboxylate groups are bound to the metal ion, $M(Bicinate)^+_{op}$, (ii) a "closed" species in which, in addition, one of the two hydroxy groups is linked to the metal ion, $M(Bicinate)^+_{cl(1)}$, and (iii) a species where both hydroxy groups participate in metal ion binding, $M(Bicinate)^+_{cl(2)}$. The approximate degrees of formation for $M(Bicinate)^+_{cl(1)}$ and $M(Bicinate)^+_{cl(2)}$ are 10–20 and 80–90%, respectively; in the case of Cu(Bicinate)$^+$, the first value is somewhat higher and the other lower due to the Jahn–Teller distorted coordination sphere of Cu^{2+}. The "open" isomer, $M(Bicinate)^+_{op}$, mentioned above always occurs in only minor amounts, i.e. with a degree of formation of about 0.5%.

It has been the aim of this study to indicate, by example, how intramolecular equilibria arising in complexes of multidentate ligands may be treated. Such ligands are legion in biological systems. To some extent, *N*,*N*-bis(2-hydroxyethyl)glycinate may even be considered as a very simple model for a protein as it offers for metal ion binding an amino next to a carboxylate group, and the intramolecular equilibria involve the two hydroxy groups. Such weakly coordinating sites as hydroxy, ether, thioether, carbonyl groups, etc., next to stronger binding sites, such as amino, carboxylate or imidazole groups are common in proteins [37]. There can be no doubt that, in enzymic processes, the "off" and "on" of certain binding sites is crucial for the whole catalysis. Needless to say, such intramolecular equilibria involving weakly bound groups easily allow the formation of mixed ligand complexes, e.g. with imidazole [16].

To conclude, the demonstration that M(Bicinate)$^+$ complexes actually exist in aqueous solution in the form of three different isomers, linked by intramolecular equilibria, emphasizes that such complexes must not be viewed as "static" species (an often encountered view). In a way, one could say that such a complex is "breathing", altering its structure all the time within the limits defined by the equilibrium constants.

It is hoped that this article will stimulate further research on successive intramolecular equilibria, an area in coordination chemistry which has so far not received much attention, despite its obvious importance for catalytic and biological processes.

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