Biological Implications from the Stability of Ternary Complexes in Solution.[1] Mixed-Ligand Complexes with Manganese(II) and Other 3d Ions

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Abstract: The change in the stability of ternary complexes containing Mn^{2+} is quantified by $\Delta \log K_{Mn} = \log K^{MnA}_{MnAB} - \log K^{Mn}_{MnB}$, corresponding to the equilibrium MnA + MnB ⇌ MnAB + Mn (eq 7). For mixed-ligand complexes containing 2,2′-bipyridyl (bpy) and malonate, pyrocatecholate, hydrogen triphosphate, ATP^{4-}, or ITP^{4-}, $\Delta \log K_{Mn} = 0.02$ to 0.42, i.e., O donors coordinate at least as well to $Mn(bpy)^{2+}$ than to $Mn(aq)^{2+}$, while for bpy-Mn^{2+}-ethylenediamine (en) $\Delta \log K_{Mn} \leq -1.0$; hence, $Mn(bpy)^{2+}$ favors O over N donors. Both the discrimination and the enhanced stabilities depend on the participation of a heteroaromatic N base like bpy; for en-Mn^{2+}-malonate or -ATP^{4-} $\Delta \log K_{Mn}$ is negative, indicating that eq 7 is on its left side. These mixed-ligand Mn^{2+} systems resemble those with Co^{2+}, Ni^{2+}, Cu^{2+}, or Zn^{2+}; those with Fe^{2+} or Fe^{3+} are also thought to be similar. For S donors and for the nitrogen of a deprotonated amide group, an "oxygen like" behavior in mixed-ligand complexes is deduced. In addition, it is suggested that S ligands may also have π-accepting qualities like those of heteroaromatic N bases; imidazole is shown to belong to the latter class. These low-molecular-weight mixed-ligand systems are compared with compiled data on the coordination spheres of naturally occurring complexes; in these the imidazole/O donor (or "O donor like") combination dominates. This corresponds with the enhanced stabilities and discrimination observed in model systems. Thus selectivity may be influenced by the metal ion and not only by the protein part in, e.g., enzymes, as has been generally suggested. The participation of Co^{2+} and Ni^{2+} in biological systems, the evolution of coordination spheres, and intramolecular ligand–ligand interactions in mixed-ligand complexes are also discussed.

The specific and distinct macroscopic structures occurring in nature are the result of a molecular order and the information stored in molecules; thus the reactions occurring on the molecular level must be highly specific. This specificity and selectivity[2] is partly due to the properties of enzymes, the biological catalysts; many of them contain either "fixed" metal ions or at least need metal ions to be catalytically active.[4] In other words, many enzyme reactions are metal ion dependent, so that dialysis or addition of chelating agents causes loss of activity which is regained if further metal ions are added. Examples include the magnesium-, calcium-, and manganese-activated phosphoryl transferases, copper-activated polyphenol oxidase, and zinc-activated alkaline phosphatase.[5]

The selectivity of such enzymes is often attributed to their protein part,[7] but one wonders what influence the metal ion itself has, especially as it has been shown that the reactions proceed often via higher order complexes and in many cases occur within the coordination sphere of the metal ion.[8,9] The problem may be summarized in the question:[10] What are the driving forces that lead to the right, i.e., the catalytically active, enzyme-metal ion-substrate(s) complexes?

However, the familiarity with metalloenzymes and metal ion activated enzymes tends to divert attention from the dynamic equilibria involving the many other metal-complexing species that are present in biological fluids and living tissues.[3,5] As in the mammalian body the total ligand concentration greatly exceeds the metal content: in the living tissues and fluids the various complexing species compete for the different metal ions present. Under these conditions mixed-ligand complex formation is to be expected,[5,11] and ternary complexes[10,12-14] have been implicated in the storage and transport of metal ions itself and of active substances through membranes.[11,15] Taking everything together, one is not surprised anymore about Wood's[16] conclusion: "If you think that biochemistry is the organic chemistry of living systems, then you are misled; *biochemistry is the coordination chemistry of living systems*".

As mixed-ligand complexes play such a central role it is worthwhile to assemble information on their formation[17-19] and stability[11-15] and on the mutual influence of two ligands bound to the same metal ion. Among the ternary complexes containing 3d ions, those with Cu^{2+} are by far the best studied,[13,14] but for Co^{2+}, Ni^{2+}, and Zn^{2+} systems some data are also available.[12,13,15,20-24] It is becoming evident that for certain ligand combinations a discriminating behavior as well as an increased stability of the complexes is observed. As the data available for ternary Mn^{2+} complexes[18,22,23,25] were too limited to permit any generalization, we have now systematically studied their stability. These results together with earlier ones for the other metal ions allow conclusions about the "self-organizing" qualities that are inherent even in mixed-ligand systems of low molecular weight and which are evidently important in biological systems.

Experimental Section

Materials and Apparatus. Pyrocatechol and malonic acid (both purissimum) were obtained from Fluka AG, Buchs, Switzerland. Sodium tripolyphosphate,[26] ethylenediamine dihydrochloride,[27] the other materials, and the apparatus were as described before.[1]

Determination of Equilibrium Constants. These were measured by automatic potentiometric pH titrations (25 °C; $I = 0.1$, $NaClO_4$),[28] titrating 25 mL of aqueous solutions of the reactants under N_2 with 0.1 N NaOH. Some data from earlier work were also used: the acidity constants of 2,2′-bipyridyl and the stability constants of its binary Mn^{2+} complexes,[29,30] $K^{MnA}_{MnA_2}$ for ethylenediamine[31] and pyrocatecholate,[32-34] as well as some acidity constants.[35-37] Those which significantly influence the calculation of the stability constants were from our own earlier work[28,38] and were confirmed in this study.

The concentrations used for the redetermination of the acidity constants[34] of $H_2(en)^{2+}$ were $[H_2(en)^{2+}] = 1.5$–2.4×10^{-3} M. The stability constants of the binary Mn^{2+} complexes were measured under the following conditions: $[H_2(en)^{2+}] = 2.4 \times 10^{-3}$ M and $[Mn(ClO_4)_2] = 2.4$ or 3.6×10^{-2} M; $[H_2(Mal)] = 1.2 \times 10^{-3}$ M and $[Mn^{2+}] = 1.2$ or 2.4×10^{-2} M; $[H_2(Pyr)] = 1.5 \times 10^{-3}$ M and $[Mn^{2+}] = 1.2$ or 3.6×10^{-2} M. The conditions for the ternary systems were $[H_2(en)^{2+}] = 2 \times 10^{-3}$ M, $[Mal^{2-}] = 1$–1.4×10^{-2} M, and $[Mn^{2+}] = 1$–2×10^{-2} M; $[H_2(Mal)] = 1.2$ or 2.4×10^{-3} M, [bpy] = 2.4–4.8 × 10^{-3} M, and $[Mn^{2+}] = 4.8 \times 10^{-3}$ M; $[H_2(Pyr)] = 1.5$–3×10^{-3} M, [bpy] = 6×10^{-3} M to 1×10^{-2} M, and $[Mn^{2+}] = 6 \times 10^{-3}$ M; $[H_2(en)^{2+}] = 2.4 \times 10^{-3}$ M, [bpy] = 8×10^{-3} M to 1.6×10^{-2} M, and $[Mn^{2+}] = 1.2$–2.4×10^{-2} M. The calculations for the binary and ternary systems were carried out as described before.[28] Under our experimental conditions the formation of MAB_2 or MA_2B is negligible; this was confirmed by Daniele et al.[39a] and can also be seen from the distribution curves of the complex species in dependence

Table I. Negative Logarithms of the Acidity Constants of Ligands and Logarithms of the Stability Constants of Their Binary Mn^{2+} Complexes (25 °C; I = 0.1, $NaClO_4$)

Ligand[k]	$pK^{H}_{H_2A}$	pK^{H}_{HA}	Log K^{Mn}_{MnA}	Log $K^{MnA}_{MnA_2}$
bpy	−0.2[a]	4.47[b]	2.62[b]	2.00[b]
en	7.15 ± 0.01	10.10 ± 0.02	2.74 ± 0.06	2.10[c]
Mal^{2-}	2.62[d]	5.30[d]	2.72 ± 0.03	
Pyr^{2-}	9.32[d]	13.00[e]	7.97 ± 0.02	5.7[f]
HTP^{4-}	2.6[g]	5.50 ± 0.01	4.31 ± 0.02	
ATP^{4-}	4.06[h,i]	6.42[j]	4.70 ± 0.02	
ITP^{4-}	2.1[i]	6.45 ± 0.01	4.66 ± 0.02	

[a] Reference 30. [b] Reference 29. [c] Reference 31 (25 °C; I = 1.4). [d] Reference 28. [e] Reference 35. [f] Reference 32. [g] Reference 36. [h] Reference 37. [i] Reference 38a (37 °C; $I \sim 0.3$). [j] Reference 38b. [k] Reference 34.

Table II. Logarithms of Stability Constants of Ternary Mn^{2+} Complexes Together with the Values of Δ Log K_{Mn} (25 °C; I = 0.1, $NaClO_4$)

Ligand[e]					
A	B	Log β^{Mn}_{MnAB}	Log K^{MnB}_{MnBA}	Log K^{MnA}_{MnAB}	Δ log K_{Mn}
en	Mal^{2-}	5.3 ± 0.2	2.58	2.56	−0.2
bpy	Mal^{2-}	5.36 ± 0.07	2.64	2.74	0.02
bpy	Pyr^{2-}	11.01 ± 0.03	3.04	8.39	0.42
bpy	en	≤4.3	≤1.6	≤1.7	≤−1.0
bpy	$H(TP)^{4-}$	7.23 ± 0.03	2.92	4.61	0.30
bpy	ITP^{4-}	7.40 ± 0.03	2.74	4.78	0.12
bpy	ATP^{4-}	7.35 ± 0.04	2.65[d]	4.73	0.03
en	ATP^{4-}	6.6 ± 0.2	1.9[a] / 1.80 ± 0.08[b]	3.8[c]	−0.9[c]

[a] Calculated according to procedure I as described in ref 26. [b] Calculated with procedure II of ref 26. [c] Average from the results of procedures I and II. [d] This value agrees well with the one determined by Hague and Martin.[18b] [e] Reference 34.

on pH given in Figure 4 of the work of Scharff and Genin;[25] within the first pH unit in which MAB is significantly formed, $[MAB_2]$ and $[MA_2B]$ are negligible.

The stability constant of Mn(en)$(ATP)^{2-}$ was determined from solutions in which $[Mn^{2+}] = [ATP^{4-}] = 1.9$ or 2.4×10^{-2} M and $[H_2(en)^{2+}] = 1.8 \times 10^{-3}$ M. The data were evaluated by procedures I and II as described earlier.[26]

The constants of the systems containing ATP^{4-}, ITP^{4-}, or $H(TP)^{4-}$ were determined as described,[1] although a twofold excess of bpy was sometimes used, and the reaction solutions had only a volume of 25 mL; i.e., the concentrations were twice those given in ref. 1.

The equilibrium constants were calculated from at least six (in average ten) independent titration curves. The range of error given in the tables is three times the standard error of the mean.

Results and Discussion

Mixed-Ligand Systems Containing Mn^{2+}. As an increased stability of the ternary complexes containing a heteroaromatic N base and an O donor ligand has been observed[12-14,21-24,39-41] for Co^{2+}, Ni^{2+}, Cu^{2+}, and Zn^{2+}, we used the same combinations of ligands for the study of the ternary Mn^{2+} complexes. The acidity constants of the ligands and the stability constants of their binary Mn^{2+} complexes are given in Table I. The stability constants of the corresponding mixed-ligand systems are defined by eq 1-3 and are given in Table II. The overall stability constant β^{M}_{MAB}, which was computed, is related to the constants K^{MA}_{MAB} and K^{MB}_{MBA} by eq 4 and 5.

$$M + A + B \rightleftharpoons MAB \qquad \beta^{M}_{MAB} = [MAB]/[M][A][B] \tag{1}$$

$$MA + B \rightleftharpoons MAB \qquad K^{MA}_{MAB} = [MAB]/[MA][B] \tag{2}$$

$$MB + A \rightleftharpoons MAB \qquad K^{MB}_{MBA} = [MAB]/[MB][A] \tag{3}$$

$$\log K^{MA}_{MAB} = \log \beta^{M}_{MAB} - \log K^{M}_{MA} \tag{4}$$

$$\log K^{MB}_{MBA} = \log \beta^{M}_{MAB} - \log K^{M}_{MB} \tag{5}$$

One way to quantify the stability of ternary complexes of the kind studied here is according to eq 6[12,14,42]

$$\Delta \log K_M = \log K^{MA}_{MAB} - \log K^{M}_{MB} = \log K^{MB}_{MBA} - \log K^{M}_{MA} \tag{6}$$

i.e., by comparing the difference in stability, Δ log K_M, e.g., for the reaction between $Mn(bpy)^{2+}$ or $Mn(aq)^{2+}$ and pyrocatecholate. In addition, Δ log K_M is identical with the constant for equilibrium 7.

$$MA + MB \rightleftharpoons MAB + M \tag{7}$$

Since more coordination positions are available for bonding by the first ligand than for the second one (eq 6), Δ log K_M is expected to be *negative*,[43] and the statistical value for the coordination of two different bidentate ligands to an octahedral (=oh) coordination sphere is Δ log $K_{oh} = -0.4$.[14]

The value of Δ log K_{Mn} for the en-Mn^{2+}-Mal system (cf. Table II) is the same as this statistical value within experimental error, while for bpy-Mn^{2+}-Mal Δ log K_{Mn} is about zero, suggesting a stabilizing effect of the heteroaromatic N base. This is confirmed by the Δ log K_{Mn} values of bpy-Mn^{2+}-ATP (+0.03) and en-Mn^{2+}-ATP (−0.9). Comparison with the bpy-Mn^{2+}-H(TP) system,[34] in which stacking cannot occur, indicates that the increased stability of Mn(bpy)$(ATP)^{2-}$ is not due to an intramolecular stacking[1,44] between bipyridyl and the purine moiety of ATP. Leong[22] obtained the same result for bpy-Mn^{2+}-3-oxoglutarate: Δ log K_{Mn} = log $K^{Mn(bpy)}_{Mn(bpy)(OGA)}$ − log $K^{Mn}_{Mn(OGA)}$ = 1.18 − 0.90 = +0.28.[23] It should be noted that positive values for Δ log K_M mean that equilibrium 7 is shifted toward the right; hence, ligands with O donors coordinate preferably with the binary $Mn(bpy)^{2+}$ complex compared to the $Mn(aq)^{2+}$ ion.

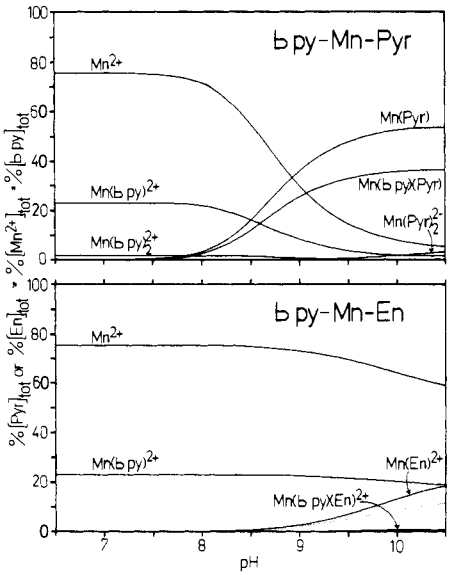

Figure 1. Effect of pH on the concentrations of the species in an aqueous solution (I = 0.1; 25 °C) of Mn^{2+}, 2,2'-bipyridyl, and pyrocatecholate (upper part) or ethylenediamine (lower part: $[Mn(bpy)_2^{2+}] \leq 1.7\%$; $[Mn(en)_2^{2+}] \leq 1.3\%$), given as the percentage of the total $[Mn^{2+}]$, [bpy], and [Pyr] or [en]; computed with the constants of Tables I and II for 10^{-3} M reactant concentrations. As the stability constant for $Mn(bpy)(en)^{2+}$ is an upper limit (Table II), the same is true for its calculated concentration. The dotted line represents $[Mn(bpy)(en)^{2+}]$ under the above conditions but assuming $\Delta \log K = +0.4$, which gives then "$\log \beta^{Mn}{}_{Mn(bpy)(en)} = 5.76$" (cf. text).

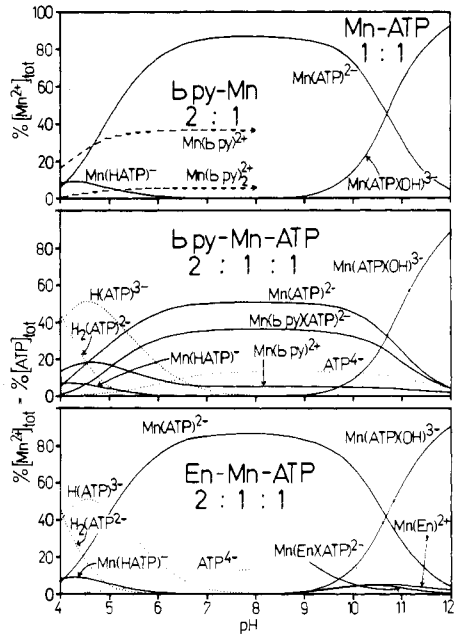

Figure 2. Effect of pH on the concentrations of the species in aqueous solutions (I = 0.1; 25 °C) of binary and ternary Mn^{2+} systems, given as the percentage of the total $[Mn^{2+}]$; computed with the constants of Tables I and II and $\log K^{Mn}{}_{Mn(HATP)} = 2.39$ (cf. ref 37). The dotted lines represent the free ATP species and the solid ones the ATP complexes; twofold protonated ATP complexes do in this pH range not occur.[47] Upper part: Mn^{2+} and 2,2'-bipyridyl [dashed lines; $[Mn^{2+}]_{tot} = 10^{-3}$ M, $[bpy]_{tot} = 2 \times 10^{-3}$ M; $[Mn(bpy)_3^{2+}] < 0.11\%$ calculated with $\log K^{Mn(bpy)_2}{}_{Mn(bpy)_3} = 1.1$, cf. ref 29] and Mn^{2+} and ATP ($[Mn^{2+}]_{tot} = [ATP]_{tot} = 10^{-3}$ M). Middle part: ATP, Mn^{2+}, and 2,2'-bipyridyl [$[ATP]_{tot} = [Mn^{2+}]_{tot} = 10^{-3}$ M, $[bpy]_{tot} = 2 \times 10^{-3}$ M; $[Mn(bpy)_2^{2+}] < 2.1\%$; $[Mn(bpy)(HATP)^-] \leq 2.3\%$ calculated with $\log K^{Mn(bpy)}{}_{Mn(bpy)(HATP)} = 2.39$, cf. ref 48]. Lower part: ATP, Mn^{2+}, and ethylenediamine [$[ATP]_{tot} = [Mn^{2+}]_{tot} = 10^{-3}$ M, $[en]_{tot} = 2 \times 10^{-3}$ M; $[Mn(en)_2^{2+}] < 1.1\%$; $[Mn(en)(HATP)^-]$ negligible traces only, calculated with $\log K^{Mn(en)}{}_{Mn(en)(HATP)} = 1.5$, cf. ref 48].

The discriminating qualities of $Mn(bpy)^{2+}$ are also evident from the systems with pyrocatecholate or ethylenediamine: With OL $\Delta \log K_{Mn}$ is positive (+0.42; Table II) while only an upper limit ($\Delta \log K_{Mn} \leq -1.0$) could be determined with NL. This is also reflected in the formation of the complex species as a function of pH (Figure 1). In the bpy-Mn^{2+}-Pyr system the ternary complex reaches ~36.5% of $[Mn^{2+}]_{tot}$, and in the bpy-Mn^{2+}-en system only ~0.5%.

The low concentration of $Mn(bpy)(en)^{2+}$ (Figure 1) is not mainly due to the lower absolute size of the constants $\beta^{Mn}{}_{MnAB}$ and $K^{MnA}{}_{MnAB}$ (see Table II) of this system, compared with those of bpy-Mn^{2+}-Pyr,[45] but due to the different relative magnitudes of the constants within these two systems. Indeed, if $\Delta \log K_{Mn}$ for bpy-Mn^{2+}-en were +0.4, $\log \beta^{Mn}{}_{Mn(bpy)(en)}$ would be 5.76 and the concentration of this ternary complex would be about 13.5% (dotted line in the lower part of Figure 1), i.e., about one-third of [Mn(bpy)(Pyr)]. Thus, the concentration of the ternary complex depends more on the position of equilibrium 7 (and on that of eq 8, see later) than on the absolute size of the other constants listed in Tables I and II.

This is even more evident for the systems bpy-Mn^{2+}-ATP and en-Mn^{2+}-ATP: The constants of the binary complexes $Mn(en)^{2+}$ and $Mn(bpy)^{2+}$ are about the same,[46] but the formation degrees of the ternary complexes are very different (middle and bottom parts of Figure 2). Hence, the discriminating qualities of the Mn^{2+} complex with a heteroaromatic N base regarding NL or OL can also be looked at beginning with Mn(OL) (cf. eq 6). Thus $Mn(ATP)^{2-}$ discriminates between the two amines which have about the same coordination tendency toward $Mn(aq)^{2+}$; the heteroaromatic N base is now strongly favored, i.e., by a factor of about 10 corresponding to the difference between the $\Delta \log K_M$ values.

It is also worthwhile comparing the binary systems (upper part of Figure 2) with the corresponding mixed-ligand systems (middle and bottom parts). The concentration of $Mn(ATP)^{2-}$ drops from about 87% of $[Mn^{2+}]_{tot}$ in the binary system to about 50% if 2,2'-bipyridyl is present as well, due to the formation of $Mn(bpy)(ATP)^{2-}$ which reaches about 36% of $[Mn^{2+}]_{tot}$ (or of $[ATP]_{tot}$). The concentration of the latter ternary species is nearly the same as that of $Mn(bpy)^{2+}$ under corresponding conditions ($[Mn^{2+}]_{tot} = 10^{-3}$ M, $[bpy]_{tot} = 2 \times 10^{-3}$ M) in the binary system; i.e., ATP converts $Mn(bpy)^{2+}$ predominantly into the ternary complex. On the contrary, in the binary Mn^{2+}-en system $Mn(en)^{2+}$ reaches about 40% of $[Mn^{2+}]_{tot}$, while in the corresponding ternary system $Mn(en)(ATP)^{2-}$ is formed only to about 4.7% (cf. bottom part of Figure 2). As a consequence, the concentrations of the ATP complexes in en-Mn^{2+}-ATP (bottom part) are about the same as those in Mn^{2+}-ATP (upper part). Again the discriminating qualities of $Mn(ATP)^{2-}$ are clearly seen.

Comparison of the Ternary Mn^{2+} Systems with Those Containing Co^{2+}, Ni^{2+}, Cu^{2+}, or Zn^{2+} and Some Tentative Conclusions. In the preceding section the stability of ternary complexes was quantified by $\Delta \log K_M$ (eq 6 and 7). The statistical value of $\Delta \log K_{oh}$ for a regular octahedral (oh) coordination sphere is −0.4. For the distorted octahedron (do) of $Cu(aq)^{2+}$ and two different bidentate ligands, the statistical value was deduced as $\Delta \log K_{do/Cu} \simeq -0.9$.[14] However, besides $\Delta \log K_M$ the "disproportionation" equilibrium 8 can be used to quantify the stability of ternary complexes.

$$MA_2 + MB_2 \rightleftharpoons 2\,MAB \qquad X = [MAB]^2/[MA_2][MB_2] \tag{8}$$

The corresponding constant X may be calculated with eq 9.

$$\log X = 2 \log \beta^{M}{}_{MAB} - (\log \beta^{M}{}_{MA_2} + \log \beta^{M}{}_{MB_2}) = (\log K^{MB}{}_{MBA} - \log K^{MA}{}_{MA_2}) + (\log K^{MA}{}_{MAB} - \log K^{MB}{}_{MB_2}) \tag{9}$$

Table III. Examples for the Discriminating Qualities of M(2,2'-Bipyridyl)$^{2+}$, Expressed by the Stability of the Ternary Complexes Formed with Ethylenediamine or Pyrocatecholate, i.e., by the Values due to Δ Log K_M (Eq 6 and 7) and Log X (Eq 8 and 9) (25 °C; I = 0.1, $NaClO_4$)[a]

A——M^{2+}—bpy		Δ log K_M	Log X
en	Mn^{2+}	≤ -1.0	<0[b]
	Co^{2+}	−0.27	0.68
	Ni^{2+}	−0.18	0.68
	Cu^{2+}	−1.29	1.10
	Zn^{2+}	−0.49	0.36
Pyr	Mn^{2+}	0.42	3.7
	Co^{2+}	0.76	4.11
	Ni^{2+}	0.36	3.71
	Cu^{2+}	0.43	6.15
	Zn^{2+}	−0.01	2.98

[a] With the exception of the data of the Mn^{2+} systems (see Table II) the results are taken from ref 21. [b] With the data of Tables I and II one obtains log $X \leq -0.86$; we conclude log $X < 0$.

The statistical value for X is 4; i.e., log X = 0.6.[49,50]

The advantages and disadvantages arising from the use of Δ log K_M or log X have been discussed.[14,42] Here it is enough to note that for the calculation of log X (eq 9) the constants of the binary 1:2 parent complexes must be known and these are often not available, e.g., for nucleotides and peptides. However, for the following examples of ternary complexes formed by simple bidentate ligands the values of Δ log K_M and log X lead to the same conclusions.

It can be seen from Table III that the discriminating qualities of $Mn(bpy)^{2+}$ fit into the general picture. The ternary complexes formed with pyrocatecholate are considerably more stable than those with ethylenediamine for all these metal ions. In general, the combination of a heteroaromatic N base with an O donor gives ternary complexes of increased stability,[13,21-24] an observation not made with N ligands.[13,21] Roughly speaking, the values for X of bpy-M^{2+}-en are in the statistically expected order, while those of bpy-M^{2+}-Pyr are by 2 to 6 orders of magnitude larger. In addition, the Δ log K_M and log X values for "mixed" N/O ligands, like glycinate[21] or *o*-aminophenolate,[51] are about midway between those for pure O and N donors.[13,14] In contrast, *aliphatic* amine-M^{2+} complexes, like $M(en)^{2+}$, show no discriminating qualities.[13,14,20,21]

There are unfortunately no comparable studies so far on the behavior of Fe^{2+}. However, from the features evident for Mn^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, and Zn^{2+} it may be surmised that the stability of ternary complexes containing Fe^{2+} (or Fe^{3+}, which is electronically equivalent to Mn^{2+}) is also governed by the same rules.

A heteroaromatic N base is essential for the high stability of a ternary complex.[10,12,13,21-24,28,39-41] This was attributed to π back-bonding from the metal ion to the aromatic ligand.[12,21,24,28,52,53] This is in accordance with the stabilities of the ternary complexes formed by Cu(Pyr) with 2,2'-bipyridyl, 4-(2'-pyridyl)imidazole, 2-picolylamine, 4-aminomethylimidazole, or ethylenediamine for which log X = 6.15, 5.47, 4.64, 3.46, and 2.65, respectively (eq 8 and 9); Δ log K_{Cu} = +0.43, +0.11, −0.11, −0.35, and −0.76 (eq 6 and 7).[52] With decreasing π-acceptor qualities of the N base ternary complex stability decreases; the imidazole group is still able to enhance complex stability, though not quite as effectively as a pyridyl group. As similar results for Δ log K_{Cu} were obtained[54] in a corresponding series with AMP^{2-}, this shows that there is no real difference between the coordination behavior of phenolate and phosphate groups. Indeed, enhanced stability in ternary complexes has been observed for all the important O donors occurring in nature, i.e., carboxylates, phosphates, and phenolates (Table II).[13,14]

Table IV. Comparison of the Stability Constants of the Ternary Nitrilotriacetate-M^{2+}-Imidazole Complexes with the Corresponding Data of the Binary M^{2+}-Imidazole Complexes

M^{2+}	Log $K^{M(Nta)}{}_{M(Nta)(Im)}$[a]	Log $K^{M}{}_{M(Im)}$[b]	Δ log K_M[c]
Co^{2+}	2.35	2.37	−0.02
Ni^{2+}	3.01	3.08	−0.07
Cu^{2+}	4.35	4.24	0.11
Zn^{2+}	2.73	2.57	0.16

[a] 25 °C; I = 0.1, $NaClO_4$; the data are from Israeli and Saulnier.[62] [b] 25 °C; I = 0.1-0.2; the values are the average of the constants listed in ref 43. [c] The absolute size of these values may be somewhat questionable as they were calculated from the results of several authors, but it is clear that the Δ log K_M values are around zero.

Knowledge on the biologically important S donors is still very incomplete. Studies of systems with 2,2'-bipyridyl, Cu^{2+} or Zn^{2+}, and the bidentate tetrahydrothiophene-2-carboxylate or carboxymethyl ethyl sulfide (CH_3CH_2-S-CH_2-COO^-) or related thioethers have shown that for both metal ions Δ log K_M is about zero.[55,56] The stabilities of the ternary complexes formed by 2,2'-bipyridyl, Ni^{2+} or Zn^{2+}, and salicylate or thiosalicylate are also similar,[57] suggesting that R-S^- behaves about like R-O^-. Hence, we may conclude that S donors resemble O donors in mixed-ligand complexes with heteroaromatic N bases. However as sulfur has also π-acceptor qualities,[56] S ligands could possibly also play a bipyridyl-like role.

Another biologically important binding site is the deprotonated amide group, -C(O)N^--, often observed in peptide complexes. Though a number of such ternary Cu^{2+} complexes have been studied,[13-15,58] the coordinating qualities of the deprotonated amide nitrogen are difficult to assess, because of steric restrictions in all these systems. However, as the deprotonated amide group is isoelectronic with the carboxylate group, "O-like" qualities may again be surmised.[59]

There is one other aspect to be considered here. The combination of a heteroaromatic N base and of an O donor within *one* ligand should lead to an "intramolecular mixed-ligand" complex owning also the described properties. Indeed, this is true, for example, for copper(8-hydroxyquinolinate)$_2$ as was detected by its EPR parameters and electronic spectra.[53] Comparison of stability data also indicates a surprisingly high stability for Cu(8-HQ)$_2$: for the 2,2'-bipyridyl-Cu^{2+} system Δ log $K^*_{Cu/bpy}$ = log $K^{Cu(bpy)}{}_{Cu(bpy)_2}$ − log $K^{Cu}{}_{Cu(bpy)}$ = −2.40,[29b] for pyrocatecholate-Cu^{2+} Δ log $K^*_{Cu/Pyr}$ = −2.89,[28] while for the 8-hydroxyquinoline-Cu^{2+} system Δ log $K^*_{Cu/8\text{-}HQ}$ = −0.9.[43] Such relatively small differences are also observed[43] for the 8-hydroxyquinoline systems with Mn^{2+} (−1.1), Fe^{2+} (−0.7) and Fe^{3+} (−1.0), Co^{2+} (−1.2), Ni^{2+} (−1.0), and Zn^{2+} (−1.0), but *not* with Ca^{2+} (−1.4), Sr^{2+} (−2.6), or Pb^{2+} (−2.6), which is reasonable, as these latter metal ions have no π-electron-donating qualities. Such intramolecular combinations of binding sites are, of course, also available in proteins.

Mixed-Ligand Complexes with Special Reference to Biological Systems. As the imidazole moiety of histidine is the most important heteroaromatic N base residue in biological systems,[61] apart from the N donor sites in purines and pyrimidines, we would like to reassess the stability increasing effect of imidazole. In Table IV the coordination of imidazole to M(nitrilotriacetate)$^-$ is compared with that to $M(aq)^{2+}$; indeed, the Δ log K_M values are around zero. Moreover, the increased stability becomes unequivocal if compared with the statistical (st) value, Δ log K_{st} = −0.5.[63] Similar conclusions[13]

Table V. Enhanced Stability of Some 2,2′-Bipyridyl-M^{2+}-Nucleotide Complexes, Expressed by $\Delta \log K_M$ (25 °C; $I = 0.1$, $NaClO_4$)[a]

	$\Delta \log K_M$				
M^{2+}	AMP^{2-}	IMP^{2-}	ATP^{4-}	ITP^{4-}	UTP^{4-} [g]
Mn^{2+}			0.03[d]	0.12[d]	0.01
Co^{2+}	0.03[b,c]		−0.07[c]	−0.08[f]	0.10
Ni^{2+}			−0.40[c]	−0.29[f]	−0.13
Cu^{2+}	0.50[b,c]	0.22[b,e]	0.53[c]	0.04[f]	0.36
Zn^{2+}	0.00[b,c]		0.05[c]	0.02[f]	0.15

[a] For comparison: with HPO_4^{2-}, $\Delta \log K_{Co} = 0.0$, $\Delta \log K_{Cu} = 0.4$, $\Delta \log K_{Zn} = 0.0$; [b,c] with $H(TP)^{4-}$, $\Delta \log K_{Mn} = 0.30$. [d] [b] Determined in water containing 10% dioxane. [c] Reference 38b. [d] Cf. Table II. [e] Reference 44. [f] Reference 1. [g] Reference 72.

follow from data[64] on ternary glycylglycinate-M^{2+}-imidazole complexes (M^{2+} = Ni^{2+}, Cu^{2+}, or Cd^{2+}). The results[20] for histamine-M^{2+}-ethylenediamine, histamine-M^{2+}-serinate, and ethylenediamine-M^{2+}-serinate, where M^{2+} = Co^{2+}, Ni^{2+}, Cu^{2+}, or Zn^{2+}, also confirm[13,21] the stability increasing effect *and* the discriminating qualities of the imidazole group in ternary complexes. The observation[65] that adenosine forms a more stable complex with Ni^{2+} triphosphate than with $Ni(aq)^{2+}$ may also be noted here especially as the 3d ions may coordinate to N(7) of the imidazole part of the purine moiety.[66-71]

As nucleotides are important substrates in biological reactions and as many of them are metal ion dependent,[8,69-71] the data of Table V have been assembled. Stability enhancement due to heteroaromatic N bases is confirmed; except with Ni^{2+}, for most of the ternary M(bpy)(nucleotide) complexes $\Delta \log K_M$ is even positive (eq 6 and 7). As expected, strongly negative values for $\Delta \log K_M$ are observed if bipyridyl is replaced by ethylenediamine (see Table II) or alaninate: e.g., in the alaninate-M^{2+}-ATP systems with Mn^{2+}, Cu^{2+} or Zn^{2+}, the $\Delta \log K_M$ values are −1.31, −1.53, and −0.80, respectively.[26] It should be noted that the differences in stability of these ternary complexes, i.e., the discrimination factors, are between about 7 and 70.

Possible Biological Implications. Taking all these results together, a number of observations and open questions regarding metal ions in biological systems may at least tentatively be understood.

(1) The results give clear evidence that in ternary complexes with the mentioned 3d metal ions the first ligand to coordinate influences the kind of the second one; thus even low-molecular-weight complexes exhibit "self-organizing" qualities resulting from cooperative effects[53,73] induced by the ligand donors within the coordination sphere of the metal ion which forms the higher order complex.[14] This sheds some light on questions about the selectivity of metal ion interactions in biological systems, e.g., the one: Why does the *right* substrate (that can be converted by a special enzyme) coordinate to the *right* enzyme-metal ion complex or the *right* enzyme to the *right* substrate-metal ion complex?[74]

(2) Knowing the preferred donor atom combinations which lead to enhanced stability, we once concluded:[13] "One starts to understand why mixed-ligand complexes are so widely used in nature, and one is tempted to predict that in many naturally occurring mixed-ligand complexes an imidazole group together with a ligand having O donors is involved" [10,12,73] The coordination spheres of many more naturally occurring metal ion complexes are now known. Besides iron-sulfur[77,78] and hemoproteins,[79] or proteins containing Ca^{2+},[77] an impressive number of 3d ion systems have been studied (see Table VI). It is amazing to see that in nearly all the systems the imidazole/O donor (or at least "O donor-like") combination occurs. Superoxide dismutase seems to be an exception, but here the two metal ions Cu^{2+} and Zn^{2+} are bridged by an imidazol*ate* moiety and one of the axial directions of copper is widely open to solvent access.[100,102] The only other seemingly exception is the second Zn^{2+} of alcohol dehydrogenase with its four-coordinated cysteine sulfurs (but as discussed, S can be a σ-electron donor and a π-electron acceptor; see also footnote *l* in Table VI). In addition, one must be aware that crystal and solution structures may be somewhat different, as indicated for concanavalin A[61] and especially for carboxypeptidase A.[109]

(3) As expected, there is evidence that the same principles outlined in paragraph (2) also hold for naturally occurring low-molecular-weight complexes. Serum albumin is the major transporting agent for Cu^{2+} in the blood and there is an equilibrium between the albumin and amino acid bound Cu^{2+}. The ternary complex, albumin-Cu^{2+}-histidinate, plays an important role in the transfer of Cu^{2+} from a macromolecule to a low-molecular-weight complex, which can then be readily transported across biological membranes.[61,96,97] The most important of these low-molecular-weight complexes is Cu(histidinate)(threoninate) (Table VI).[89,96,97] Indeed, the rather high stability[13,61,95] of such ternary histidinate-Cu^{2+}-amino acid complexes is also evident from thin-layer chromatography[114a] and paper electrophoresis.[114b] This is in accordance with a mathematical "metal-ligand model for blood plasma"; the calculations[5] showed that in the low-molecular-weight fraction Cu^{2+} and Zn^{2+} are mainly present as mixed-ligand amino acid complexes containing histidinate. Moreover, in Cu(histidinate)$_2$ itself one histidinate is coordinated to the equatorial plane of Cu^{2+} in a histamine-like and the other in a glycine-like manner.[61]

(4) As the combination of a heteroaromatic N base moiety and of an O (or an "O-like") donor strongly dominates in naturally occurring complexes of low and high molecular weight, the question arises: Have these more stable combinations gradually been achieved in the systems by evolution, or did they "survive" evolution due to their relatively high stability? Tentatively, we feel that the latter possibility is more likely, because even at the prebiotic stage the more stable complexes were certainly formed, and the favored ligand combinations are the same in vitro and in vivo.

(5) In this connection an additional evaluation of the data in Tables III and V should be made. The values of $\Delta \log K_M$ and of $\log X$ do not follow the Irving-Williams series,[115] although this *is* followed by the overall stability constants $\beta^M{}_{MAB}$ of the ternary complexes.[12,13,21-23,38b] In particular the mixed-ligand complexes containing Ni^{2+} are less favored than those of the neighboring elements. This seems to be a general feature if a heteroaromatic N base and an O donor are involved.[12,13,21-23,38b,72] Maybe the relative instability of the mixed-ligand complexes of Ni^{2+} and its restricted availability[116] are reasons why this metal ion does not occur[9,87,117] widely in biological systems. It is also surprising that no Co^{2+} system is listed in Table VI as this ion may be artificially substituted into Zn^{2+} enzymes with retention of the activity,[102] and the mixed-ligand Co^{2+} complexes are relatively stable (Tables III and V).[12,13,21-24] The reason is probably the high sensitivity of Co^{2+} toward oxidation to inert Co^{3+} complexes when coordinated to several N donors.[118] This probably makes Co^{2+} in general unsuitable for biological systems, but a transcarboxylase containing tightly bound Zn^{2+} and Co^{2+} has been reported.[119a] Its specific activity appears to be proportional to the sum of the Zn + Co content of the enzyme; the total metal content (Co + Zn + Cu) is two metal ions per biotin residue.[119b] The main biological exceptions are certainly "the cobaltous porphyrin structures in the corrinoids (vitamin B_{12}) which represent highly specialized adaptations",[120] in

Table VI. Compilation of Binding Sites in Naturally Occurring Metal Ion Complexes of 3d Ions[a]

Enzyme/protein amino acid	M^{n+}	Binding sites 1	2	3	4	5	6	Methods/comments	Ref
Concanavalin A[b]	Mn^{2+}	O(Glu)	O(Asp)	O(Asp) N(Im)	N(Im) N(Im)?	H_2O/Glx	H_2O	X-Ray Titration/chem modification/dialysis	80 81/61
Pyruvate kinase	Mn^{2+} (Mg^{2+}?)	N(Im)	O(phosphate/substrate)					NMR/chem modification	82/8b
Hemerythrin[c]	Fe^{2+}/Fe^{3+}	Four N(Im) and two O(Tyr) are bound to two Fe						X-Ray (5.5-Å resolution) Chem modification	83 84
Transferrin[d] (conalbumin is closely related)	Fe^{3+}	O(Tyr)	O(Tyr)	O(Tyr)	N(Im)	N(Im)	HCO_3^-	Chem studies (Tyr role more emphasized, His involvement more uncertain)	85, 86, 61
Urease (jack bean)	Ni^{2+}	S(Cys)						Chem modification	87/9
Ceruloplasmin[e]	Cu^{+}/Cu^{2+}	N(Im) moieties and sulfhydryl groups						Chem modification/ titration	88/61
Hemocyanin[f]	Cu^{2+}	Cu(II)-O_2^{2-}-Cu(II) and Cu-N(Im) bonds						Spectroscopic expt	90
Plastocyanin (bean)[g]	Cu^{2+}	N(Im)	S(Cys)	N(Im)	N^-(deprot amide)			Spectroscopic expt	92
L-Histidinate and L-threoninate[h]	Cu^{2+}	N(Im)	NH_2(His)	O(Thr)	NH_2(Thr)	O(His)[i]	H_2O	X-Ray[i] Rather stable in aq soln	94 95/13, 61
Albumin (human)	Cu^{2+}	NH_2 (Asp)	N^-(amide, Ala)	N^-(amide, His)	N(Im)			Chem studies	98/61, 89
Galactose oxidase	Cu^{2+}	N_2O_2 square-planar system with a protein sulfur in one axial site/N(Im) seems to be involved						Spectral studies, chem modification	99
Superoxide dismutase[j]	Cu^{2+} Zn^{2+}	N(Im) N(Im)	N(Im) N(Im)	N(Im) N(Im)	N(Im) O(Asp)			X-Ray	100
Insulin	Zn^{2+}	N(Im)	N(Im)	N(Im)	H_2O	H_2O	H_2O	X-Ray	103
Thermolysin[b]	Zn^{2+}	N(Im)	N(Im)	O(Glu)	H_2O			X-Ray	104/102
Alkaline phosphatase (*E. coli*)	Zn^{2+}	Three N(Im) appear to be involved and O(phosphate/substrate)						Photooxidation EPR of Cu^{2+} enzyme ^{31}P NMR	105 106/102 102
Carbonic anhydrase[k]	Zn^{2+}	N(Im)	N(Im)	N(Im)	H_2O			X-Ray	107/102
Carboxypeptidase A	Zn^{2+}	N(Im)	O(Glu)	N(Im)	H_2O O(carbonyl/substrate)			X-Ray X-Ray	108/102 108/102
		O(phenolate of Tyr) coord to Zn^{2+} in soln						Chem, spectrosc, kinetic data	109
Alcohol dehydrogenase	Zn^{2+} Zn^{2+}	S(Cys) S(Cys)	S(Cys) S(Cys)	N(Im) S(Cys)	H_2O[l] S(Cys)			X-Ray[l]	110

[a] Im = imidazole moiety of a histidyl residue; the abbreviations of the amino acid residues are those generally used. [b] There is in addition Ca^{2+} present, which is coordinated to O donors. [c] Oxygen carrying nonheme protein.[61] [d] Conalbumin is closely related in its binding sites with transferrin.[61] [e] Half of the copper atoms are in the cupric form and the other half in the cuprous state.[89] [f] Oxygen carrying "Cu(I)" protein.[61,91] [g] ^1H NMR studies of plastocyanins from spinach and a blue-green alga "suggest that the imidazole groups of two histidine residues are liganded directly to the copper. . . . The single copper of plastocyanin may exist in either the oxidized Cu(II) or reduced Cu(I) form".[93] [h] Present in human serum and in equilibrium with albumin bound Cu^{2+}.[89,96,97] [i] The distance of the O(carbonyl) atom of L-histidine from Cu^{2+} is 2.58 Å, compared with only 1.97 Å for the O(carbonyl) atom of L-threonine; moreover, the O(carbonyl) atom of histidine is in an irregular axial position [angle N(amino)-Cu-O(carboxyl) = 68.3°]. Thus, it seems that this latter bond is not very strong, and in aqueous solution the axial position (at least in equilibrium) may well be occupied by water. [j] The active site contains an imidazolate bridged Cu^{2+}-Zn^{2+} moiety;[101] one of the axial directions of the Cu is quite wide open to solvent access.[102] [k] Human carbonic anhydrase B and C are two isoenzymes which differ in the position of the third histidine residue.[102] [l] There is evidence that this (i.e., the first) Zn^{2+} with its coordination sphere is at the active site and that H_2O is replaced by an O of alcohol/aldehyde in the active enzyme.[102,111] The function of the second Zn^{2+} is not clear; it appears there is no direct functional role in catalysis or in maintaining the secondary or quarternary structure.[102]

contrast to structural units which simply survived evolution as suggested in paragraph (4).

(6) A more general point should finally be added. The factors which favor the formation of mixed-ligand complexes have been discussed,[14] but it seems worthwhile mentioning here that the presence of polydentate ligands in biological systems leads to situations in which there are more binding sites than available coordination positions at the metal ion; and this situation may drastically change the properties of a system. Indeed, displacement of ligand groups during the formation of a ternary complex is quite common,[13,14] and new properties may thus be achieved. These include the formation of protonated species that cannot be formed in either of the corresponding binary complexes[58,121] and also possible interactions between the ligands coordinated to the same metal ion. Such an interaction may result in the formation of covalent bonds, e.g., Schiff base formation between an amino acid and pyruvate leading to a ligand of higher denticity and thus to an increased stability of the "ternary" complex, compared with the binary complexes of the simple ligands,[122,123] or it may result in the formation of noncovalent but still rather specific intramolecular interactions. Among these are ionic[124] or hydrogen bond[125] formation, aromatic ring stacking,[26,38a,44] and hydrophobic interactions.[126] All these interactions do, of course, provide additional tools for the specificity which is so important for biological systems.

To conclude, it is becoming clear that much of the specificity and many of the properties so far only associated with metal-ion complexation in macromolecular biological systems are inherent already in relatively simple coordination compounds. The principles of coordination chemistry will thus allow an increasing understanding of the structural and dynamic features involved in biological processes. This conclusion agrees with the view recently given by Zerner et al.[9] about a related problem, namely "that catalysis by metalloenzymes may be a reflection of the chemistry of the metal ion itself"

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