# On the Discriminating Behavior of Metal Ions and Ligands with Regard to Their Biological Significance

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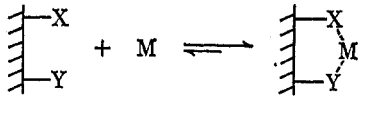
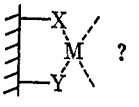
The significance of metal ions in biological systems is currently obvious. For example, the functioning of many enzymes is metal ion dependent.[1,2] Enzymes contain metal ions at their active sites, for example, $Zn^{2+}$ in dihydroorotase,[3] carboxypeptidase, and carbonic anhydrase, $Mn^{2+}$ in isocitric dehydrogenase and malic enzyme, and $Mg^{2+}$ in enolase and a variety of kinases.[4] Other enzymes appear to require the presence of an ion, frequently a monovalent one such as $Na^+$, $K^+$, or $NH_4^+$, for stabilization of the particular conformation responsible for maximal catalytic activity.[4]

Recently, Gillard[5] summarized the possible reactions at an active site of a metal ion potentiated enzyme in the following way. (a) The metal ion may induce, by coordination, a specific "lock" geometry of the apoprotein metal binding site so that only certain substrates are able to become attached to the framework produced. (b) The metal ion may activate a bond or bonds of the substrate (or the protein) through coordination. This is quite feasible, since it may well be that the metal binding site of the apoprotein has a constant geometry, whether or not the metal ion is present. (c) The metal ion may induce by coordination a specific "key" geometry of the substrate so that it will fit the "lock" of the apoprotein specifically.

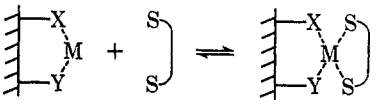
Besides the question about the functions of the metal ion, there are others such as: which are the factors that determine the coordination of metal ions to ligands, for example, in biological fluids or enzyme–metal ion–substrate complexes? For the special case of the latter, some of the general questions[6] that arise can be formulated in the following way. (I) What are the reasons for the "right" metal ion coordinating at the "right" enzyme (or substrate)?




(II) How great is the coordination tendency of the remaining coordination positions of such a bound metal ion? Does the first coordinated ligand influence the type of ligand which may be coordinated at further coordination sites?




(III) Why does the "right" substrate, *i.e.*, the substrate that can be converted to products by a special enzyme, coordinate to the "right" enzyme–metal ion complex (or the "right" enzyme to the "right" substrate–metal ion complex)?




All these questions are closely connected with each other and can be summarized in one question: what are the control mechanisms that determine the coordination and coordination tendency of metal ions? This article is an attempt to answer this question as far as our current understanding and available space allow.

**Kind of Metal Ions and Their Availability.** Metal ions that all living organisms require are sodium, potassium, magnesium calcium, manganese, iron, cobalt, copper, and zinc. In addition, there are small quantities of vanadium, chromium, molybdenum, niobium, and cadmium in particular organisms.[7] According to Williams,[8] these metal ions can conveniently be divided with regard to their functional difference: the heavy metal ions, with the possible exception of manganese, have more or less fixed chemical neighbors[9] in a biological system, while sodium, potassium, magnesium, and calcium are usually mobile as cations.

The availability of metal ions was discussed by Williams.[8] The abundance restricts the available metals to those of atomic number below 40. Among these, the insolubility of the metal hydroxides makes aluminum

(1) M. Dixon and E. C. Webb, "Enzymes," Green and Co., London, 1964.
(2) B. L. Vallee and J. E. Coleman, *Compr. Biochem.*, **12**, 165 (1964).
(3) E. G. Sander, L. D. Wright, and D. B. McCormick, *J. Biol. Chem.*, **240**, 3628 (1965).
(4) H. R. Mahler and E. H. Cordes, "Biological Chemistry," Harper and Rowe, New York, N. Y., 1966.
(5) R. D. Gillard, *Inorg. Chim. Acta Rev.*, **1**, 69 (1967).
(6) H. Sigel, *Chimia* (Aarau), **21**, 489 (1967).
(7) C. L. Comar and F. Bronner, Ed., "Mineral Metabolism," Vol. I and II, Academic Press, New York, N. Y., 1960.
(8) R. J. P. Williams, *Endeavour*, **26**, 96 (1967).
(9) B. G. Malmström and J. B. Neilands, *Annu. Rev. Biochem.*, **33**, 331 (1964).

and titanium virtually unavailable. Furthermore, the virtual absence of nickel and chromium from living things probably results from the special stability of these cations in the octahedral binding provided by soil silicates. The usually irregular geometry of protein chelating sites lowers the stability of the protein complexes of these cations, so that their distribution between soil and living things favors the soil.[8]

**The Irving–Williams Series.** Oxygen, nitrogen, and sulfur are the donor atoms of greatest biological interest. For complexes, formed between ligands containing these donor atoms and the divalent metal ions of the second half of the first transition series, with a given ligand, the stability is in the order predicted by Irving and Williams:[10] $Mn^{2+} < Fe^{2+} < Co^{2+} < Ni^{2+} < Cu^{2+} > Zn^{2+}$. This sequence can be somewhat extended by including the ions $Ba^{2+}$, $Ca^{2+}$, $Sr^{2+}$, and $Mg^{2+}$ (*cf.* ref 2).

Shown in Figure 1 are the log stability constants, log $K^{M}_{ML}$ ($K^{M}_{ML}$ = [ML]/[M][L]),[11,12] for the 1:1 complexes of oxalic acid, glycine, ethylenediamine, mercaptoacetic acid, and mercaptoethylamine and the metal ions $Ba^{2+}$ through $Zn^{2+}$. For all the complexes with these ligands, the above-mentioned sequence is fulfilled. A comparison of the stability of the complexes with oxalic acid, glycine, and ethylenediamine is informative. With $Fe^{2+}$ the complexes of these ligands show about the same stability. For the metal ions on the right side of $Fe^{2+}$ in Figure 1,[13] *viz.*, $Co^{2+}$, $Ni^{2+}$, $Cu^{2+}$, and $Zn^{2+}$, nitrogen is the preferred binding atom, while for the metal ions on the left, *viz.*, $Ba^{2+}$, $Sr^{2+}$, $Ca^{2+}$, $Mg^{2+}$, and $Mn^{2+}$, oxygen is preferred. As a result of this behavior, the values for the complexes with the "mixed" ligand, glycine, lie between those of ethylenediamine and oxalic acid (Figure 1). Thus, it is obvious that different metal ions preferentially bind to different ligand atoms and, furthermore, the qualities of several donor atoms can be accumulated in one ligand leading to a "mixed" quality. Biological systems are no exception to this statement.

The relatively flat shape of the curve representing the stabilities of the oxalic acid complexes (Figure 1) is generally found with oxygen ligands. This behavior is especially marked for phosphate ligands, like adenosine 5′-mono-, di-, and triphosphate, where the difference in stability between different metal ions and a given ligand is relatively small; sometimes it is even hard to decide if the Irving–Williams sequence is still followed.[14–16] From Figure 2 it can be seen that the order of stability for the phosphate complexes is $Ba^{2+} \sim Sr^{2+} \sim Ca^{2+} \sim Mg^{2+} < Mn^{2+} \sim (Fe^{2+}) \sim Co^{2+} \sim Ni^{2+} < Cu^{2+} > Zn^{2+}$. It is of interest that this quality is independent of the number of phosphate groups which form one ligand. Furthermore, the phosphate groups are the stability-determining factors of the ligands, *i.e.*, the nucleic acid base has no significant influence,[16–18] as can be seen from the constants given in Figure 2[14,16,19–22] for the $HPO_4^{2-}$, methyl phosphate, and hydrogen triphosphate complexes. This quality may be one of the reasons why phosphate ligands occur and can be used in nature in widely different reactions and together with a great variety of different metal ions.

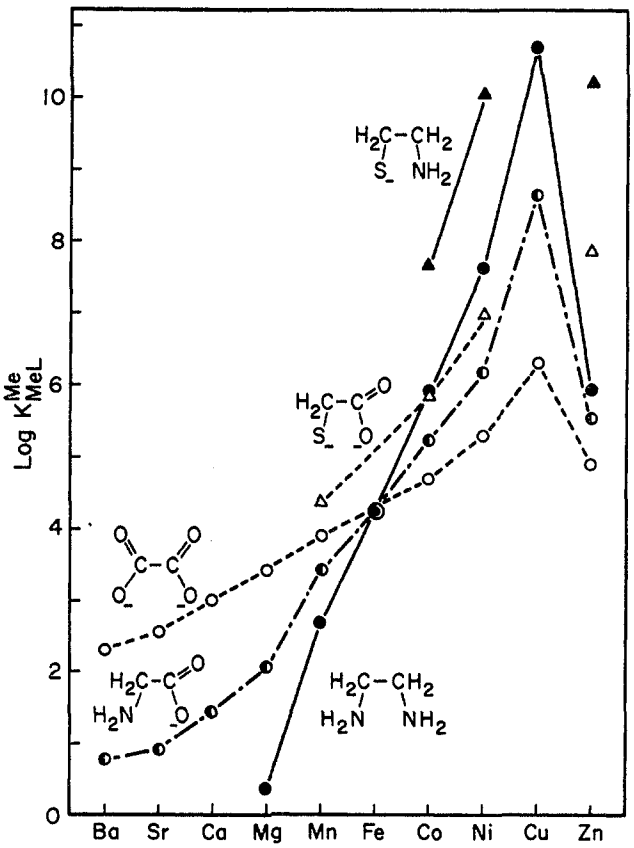



Figure 1. Logarithms of the stability constants for the 1:1 complexes[13] between $Ba^{2+}$ through $Zn^{2+}$ and the bidentate ligands oxalic acid, glycine, ethylenediamine, mercaptoacetic acid, and mercaptoethylamine.

(10) H. Irving and R. J. P. Williams, *Nature*, **162**, 746 (1948); *J. Chem. Soc.*, 3192 (1953). For the background of the Irving-Williams series, *cf.* L. E. Orgel, "An Introduction to Transition-Metal Chemistry: Ligand-Field Theory," Methuen and Co., Ltd., London, 1961.

(11) Abbreviations used: metal ion, M; Ligand, L; adenosine 5′-mono-, 5′-di-, and 5′-triphosphate, AMP, ADP, and ATP; the 5′-triphosphates of inosine, guanosine, cytosine, uridine, and thymidine, ITP, GTP, CTP, UTP, and TTP; acetylacetone, Acac; 2,2′-bipyridyl, Bipy; ethylenediamine, En; glycine anion, Gly; histamine, Ha; imidazole, Im; oxalic acid dianion, Ox; 1,2-propanediamine, PA; 1,3-propanediamine, PDA; pyrocatechol dianion, Pyr; 5-sulfosalicylic acid trianion, SSal; serine anion, Ser.

(12) For the determination of stability constants see, *e.g.*, F. J. C. Rossotti and H. S. Rossotti, "The Determination of Stability Constants," McGraw-Hill Book Co., Inc., New York, N. Y., 1961; H. L. Schläfer, "Komplexbildung in Lösung," Springer-Verlag, Berlin, Göttingen, Heidelberg, 1961.

(13) L. G. Sillén and A. E. Martell, "Stability Constants of Metal Ion Complexes," Special Publication No. 17, The Chemical Society, London, 1964.

(14) M. M. Taqui Khan and A. E. Martell, *J. Amer. Chem. Soc.*, **88**, 668 (1966).

(15) D. D. Perrin and V. S. Sharma, *Biochim. Biophys. Acta*, **127**, 35 (1966).

(16) H. Sigel, K. Becker, and D. B. McCormick, *ibid.*, **148**, 655 (1967).

(17) E. Walaas, *Acta Chem. Scand.*, **12**, 528 (1958).

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(20) M. M. Taqui Khan and A. E. Martell, *J. Amer. Chem. Soc.*, **84**, 3037 (1962).

(21) H. Brintzinger, *Helv. Chim. Acta*, **48**, 47 (1965).

(22) G. Anderegg, *ibid.*, **48**, 1712 (1965).

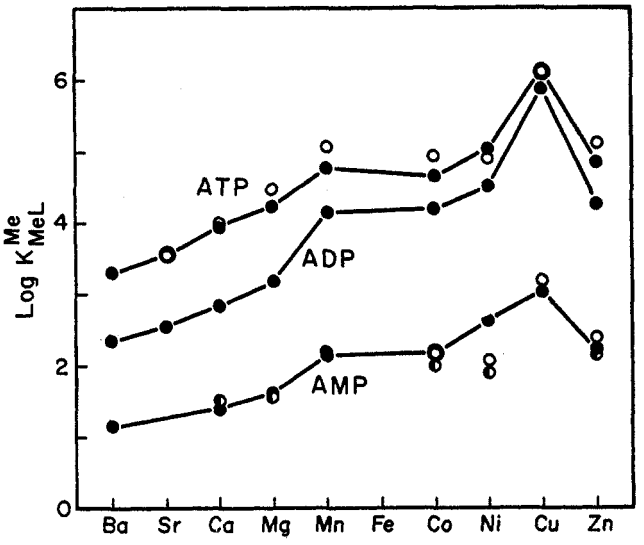


Figure 2. Logarithms of the stability constants for the 1:1 complexes between $Ba^{2+}$ through $Zn^{2+}$ and the nucleotides AMP,[19] ADP,[20] and ATP;[14] for comparison, the values of $HPO_4^{2-}$ (○),[16] methyl phosphate (◐),[21] and hydrogen triphosphate (○)[22] are also given.

One of the possible reasons for the relative equality in stability of the mentioned phosphate complexes may be the formation of inner sphere and outer sphere complexes, *i.e.*, complex formation with the "free" or hydrated metal ion.[23] The important thing to note is that the complexing abilities of metal ions toward nitrogen and probably also sulfur donors (Figure 1) are very different, while the complexing abilities toward oxygen donors (Figures 1 and 2) are more constant.

**Pearson's Hard and Soft Rule.** "The principle of hard and soft acids and bases," a rule suggested by Pearson,[24] is: "Hard acids prefer to bind to hard bases and soft acids prefer to bind to soft bases." Examples of hard bases are $H_2O$, $OH^-$, $F^-$, $PO_4^{2-}$, $SO_4^{2-}$, $NH_3$, and soft bases are $R_2S$, $RS^-$, $CN^-$, CO; hard acids are $H^+$, $Li^+$, $Na^+$, $K^+$, $Mg^{2+}$, $Ca^{2+}$, $Sr^{2+}$, $Mn^{2+}$, $Fe^{3+}$, and soft acids are $Cu^+$, $Hg^+$, $Hg^{2+}$, $Cd^{2+}$; borderline bases are $C_5H_5N$, $Br^-$, $N_2$; and borderline acids are $Fe^{2+}$, $Co^{2+}$, $Ni^{2+}$, $Cu^{2+}$, $Zn^{2+}$.

The shape, *i.e.*, slope, of the curves resulting from the Irving–Williams sequence (*cf.* Figure 1) can be considered, at least in part, as a result of the hard and soft qualities of the donor atoms and the metal ions. For example, the softness will decrease in the series $CH_3^-$, $NH_2^-$, $OH^-$, and $F^-$, while going across the 3d transition series from $Ca^{2+}$ to $Zn^{2+}$ the elements become softer as a consequence of the increasing number of d electrons.[24] In other words, nitrogen donors are considered to be somewhat softer than oxygen donors; therefore, the curve with ethylenediamine has a greater slope than that with oxalic acid. Hence, the softer are the donor atoms, the steeper the slope of the curve (Figure 1).

It is also obvious that a sulfur donor in a protein will be a better ligand for the 3d metal ions, $Mn^{2+}$ through $Zn^{2+}$, than for the earth alkali ions, $Mg^{2+}$ through $Ba^{2+}$; the same is true for the imidazole group. In addition, the hard $Mn^{2+}$ will complex with hard oxygen donors better than with soft sulfur donors, while the borderline $Cu^{2+}$ will complex well with both donors.[25,26] For example, the $Mn^{2+}$ and $Cu^{2+}$ 1:1 complexes with hydroxyacetic acid are both more stable than expected on the basis of the basicity of the carboxylic acid group alone as a result of the participation of the hydroxy group which leads to a chelate, but with S-carboxymethyl ethyl mercaptan, an increased stability is found only with $Cu^{2+}$, which shows that the interaction between $Mn^{2+}$ and the thioether group is weak.[25]

According to Pearson,[24] pyridine is borderline. The same can be assumed for the "pyridine" nitrogen in imidazole, and nucleic acid bases. Thus, the borderline metal ions, $Cu^{2+}$ (*cf.* ref 27, 28) and $Cd^{2+}$ (*cf.* ref 28), prefer to bind to the bases of DNA, while the hard ions, $Mg^{2+}$ (*cf.* ref 27, 28), $Li^+$, $Na^+$, and $K^+$ (*cf.* ref 27), prefer the phosphates.

**Competition between Metal Ions and Protons.** Since most metal ion coordinators are also proton bases, the degree of complex formation for a special case will depend not only upon the magnitude of the stability constant of the complex but also upon the pH of the solution. One way to take into account the competition between protons and metal ions for a given binding site is to calculate "standardized stability values," $X^{M}_{ML}$, which are no longer universal constants and are correct only for the pH for which they are calculated.[29] The relationship for monobasic ligands among the stability constant of the complex, the acidity constant of the ligand, and the pH of the solution is given by eq 1.

$$X^{M}_{ML} = K^{M}_{ML} \frac{1}{1 + [H^+]/K^{H}_{HL}} \qquad (1)$$

In the upper half of Figure 3, the stability constants of the $M^{2+}$ 1:1 complexes with acetic acid, ammonia, and imidazole are given.[13,30] The carboxylate, the amino, and the imidazole groups are important as possible binding sites in proteins. Therefore, it is informative to compare the influence of pH on these three isolated binding sites; this is done for pH 7 according to eq 1 in the lower part of Figure 3. Due to the great differences in basicity,[13] the stabilities of the

(23) It is suggested that with $Cu^{2+}$ and $Zn^{2+}$ the inner-sphere, and with $Mn^{2+}$ and $Co^{2+}$, the outer-sphere complexes are predominant.[21] For an equilibrium study with $Ni^{2+}$ between these two species, *cf.* H. Brintzinger and G. G. Hammes, *Inorg. Chem.*, **5**, 1286 (1966).

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(26) D. B. McCormick, H. Sigel, and L. D. Wright, *Biochim. Biophys. Acta*, **184**, 318 (1969).

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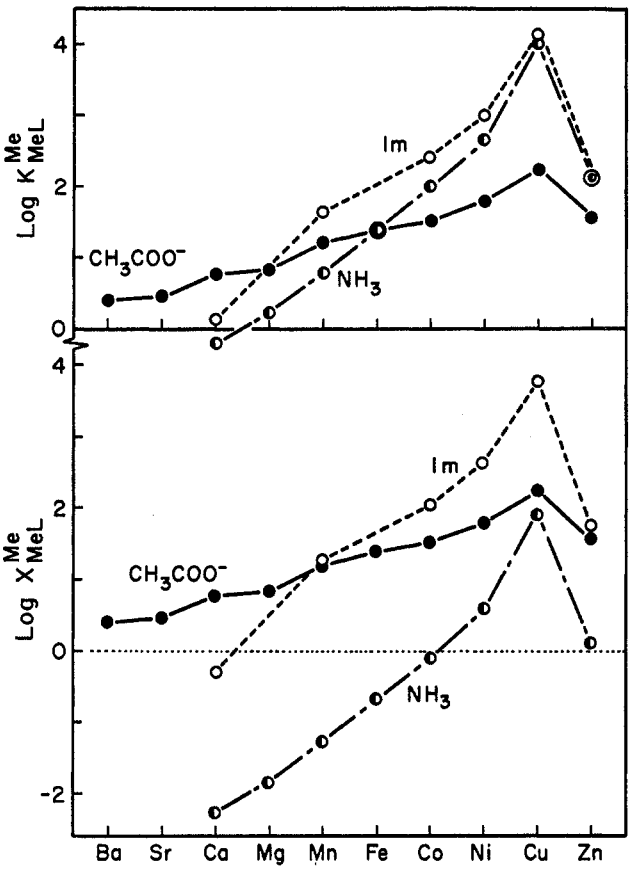



Figure 3. Logarithms of the stability constants, log $K^{M}_{ML}$ (upper part), for the 1:1 complexes between $Ba^{2+}$ through $Zn^{2+}$ and the monobasic ligands acetic acid,[13] ammonia,[13] and imidazole ($Ca^{2+}$, $Mg^{2+}$, $Co^{2+}$ *cf.* ref 13; others ref 30), and the logarithms of the "standardized stability values," $X^{M}_{ML}$ (lower part), calculated according to eq 1 for the same systems.

imidazole complexes are only slightly influenced, while those of the ammonia complexes are influenced very strongly. This shows that the imidazole group is, under physiological conditions, a much more effective binding site than the amino group. Since acetic acid is completely deprotonated at pH 7, there is no competition between protons and metal ions. It is of interest to note that at pH 7 both the imidazole and the carboxylate groups show about the same coordination tendency toward the biologically significant metal ion, $Mn^{2+}$, and that those toward $Mg^{2+}$, $Fe^{2+}$, and $Zn^{2+}$ are probably not much different. Thus, under these conditions, it is easy to shift the equilibria and favor the one or the other binding site by small changes in pH, or even ionic strength, hydrophobic interactions, etc.; for enzyme actions, these matters are surely important.

The coordination tendency of the essential phosphate ligands is only very slightly influenced by the competition of the proton at pH $\geq 7$. These groups, therefore, are available for coordination with nearly all metal ions. This is not true for binding sites which contain, for example, the SH group. Because the $pK_A$ of this latter group is very high,[18] the competition of the proton is very strong; therefore, only metal ions with a high coordination tendency toward this group, like $Cu^{+}$, can coordinate. The same is true for phenolic hydroxyl groups and, *e.g.*, $Fe^{3+}$.

That the influence of the proton is able to favor one or the other binding site within the same molecule, depending on the pH of the solution, can be shown in the following examples: adenosine 5′-monophosphate $N^1$-oxide is an "ambivalent" ligand; it offers to metal ions two different binding sites which cannot complex simultaneously, *viz.*, the phosphate group ($pK_A$ = 6.13) and the *o*-amino N-oxide group ($pK_A$ = 12.49).[19] Since the acidity constants of the two groups are quite different, the coordination of a metal ion to one or to the other binding site is strongly pH dependent. Thus, for example, affinity of the phosphate group for $Ni^{2+}$ is about 1.6 log units greater at low pH values (*cf.* Figure 4; calculated according to eq 1) than that of the *o*-amino N-oxide group; *i.e.*, under these conditions, $Ni^{2+}$ is bound practically completely to the phosphate group. Increasing pH favors the metal ion affinity of the *o*-amino N-oxide group more than that of the phosphate group: at pH 7.7, the affinity of both groups is equal, and at higher pH values the coordination tendency of the *o*-amino N-oxide group dominates strongly. A similar behavior is observed for $Mn^{2+}$, $Co^{2+}$, and $Zn^{2+}$, where the intersections between log $X$ curves occur at pH values of about 8.9, 7.8, and 6.9, respectively.[19] In contrast, the log $X$ curves for $Cu^{2+}$ show no intersection (Figure 4); $Cu^{2+}$ is bound to the *o*-amino N-oxide group in the whole pH range.

The acidity constants[31] of the ambivalent ligand, inosine 5′-monophosphate $N^1$-oxide,[32] are of similar order. Therefore, the influence of the pH is much less, and the metal ions in ML are distributed more equally to the two binding sites.[32] Of interest in this connection is that the formation of macro chelates[33] in the metal ion complexes of ATP, ITP, GTP, CTP, UTP, and TTP is influenced by the competition of the protons for the nucleic acid bases.[34] Furthermore, the acidity constants and, therefore, the metal ion affinities of these bases are different;[34] this surely results in different complex structures at a given pH.

**Ligand Structure and Stereoselectivity.** The importance of kind and quality of donor atoms in ligands for complex formation was discussed, at least in part, in the previous sections; however, of similar importance is the "frame" of the ligand. It is well known that five- and six-membered chelate rings are stabler than those with a smaller or greater number of atoms in the ring. How incisive such matters can be becomes obvious from the following examples. In the $Cu^{2+}$–glycinamide 1:1 complex, the amide group is deprotonated at pH approximately 7 ($pK^{H}_{CuHL}$ = 7.01), forming a five-membered chelate.[35] In the $Cu^{2+}$–β-alaninamide

(31) Phosphate group: $pK_A$ = 6.31; *o*-hydroxy-N-oxide group: $pK_A$ = 5.43 (*cf.* ref 32).
(32) H. Sigel, *Helv. Chim. Acta*, **48**, 1513, 1519 (1965).
(33) Coordination of the metal ion to the phosphate group and nucleic acid base at the same time.
(34) H. Sigel, *Eur. J. Biochem.*, **3**, 530 (1968).
(35) H. Sigel, *Angew. Chem.*, **80**, 124 (1968); *Angew. Chem. Int. Ed. Engl.*, **7**, 137 (1968).

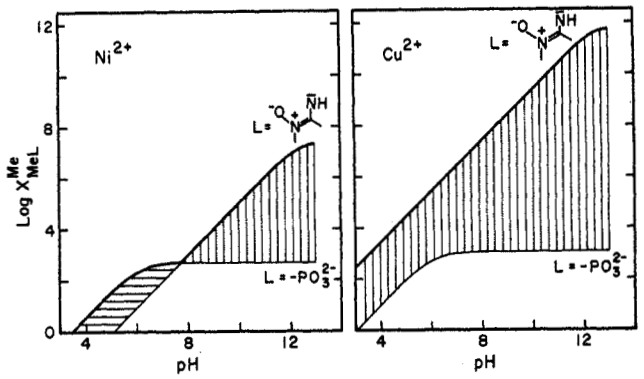
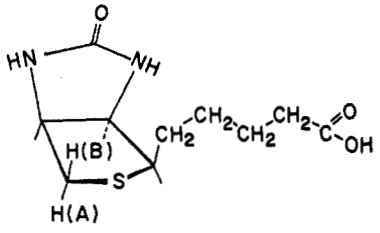



Figure 4. Metal ion affinities (standardized stability values, log $X^{M}_{ML}$) of the *o*-amino N-oxide binding site and the phosphate binding site of adenosine 5′-monophosphate $N^1$-oxide at different pH values for $Ni^{2+}$ and $Cu^{2+}$. Calculated according to eq 1 with the values of ref 19.

system, where a six-membered ring has to be formed, no such deprotonation occurs in the physiological pH range.[36] The $Cu^{2+}$ systems with oxalic acid monoamide or N-acetylglycine also show no deprotonation of the amide group under these conditions.[36] This demonstrates that the proper kind of second donor atom and a very specific ligand structure are necessary for the deprotonation of the amide group in $Cu^{2+}$ complexes. With regard to $Cu^{2+}$–proteins, it is worth noting that there is evidence that in the $Cu^{2+}$–acetylglycylhistidine complex the amide group can be deprotonated by forming a six-membered chelate with the "pyridine" nitrogen of the imidazole group.[37] Also of interest in this connection is that from the bivalent 3d metal ions, besides $Cu^{2+}$, only $Ni^{2+}$ (*cf.* ref 38) can deprotonate the amide group, while with adenosine 5′-monophosphate $N^1$-oxide,[19] $Mn^{2+}$, $Co^{2+}$, $Ni^{2+}$, $Cu^{2+}$ (*cf.* Figure 4), and $Zn^{2+}$ can form complexes which are deprotonated at the amino group.

Another example which demonstrates the significance of the "frame" of a ligand on the structure of the resulting complexes is that of *d*-biotin shown below. By




means of nmr spectroscopy, it was shown[39] that $Mn^{2+}$ interacts with the sulfur atom of the tetrahydrothiophene ring in a very stereospecific way; the interaction occurs from below the plane of the tetrahydrothiophene ring (*cf.* formula), since the signals of proton H(A) but not H(B) are broadened.

In enzymes and other proteins, the end groups provide for the coordination of metal ions, and possibly for ions like $Cu^{2+}$ the amide groups may so function. However, probably more important for the active sites of enzymes which coordinate metal ions are the binding sites created within a protein by the occurrence of trivalent amino acids. The latter possess, besides the amino and carboxylic acid groups which are tied up in a protein by the formation of peptide bonds, a third function which remains available for the coordination of metal ions. The potential trivalent amino acids can be divided into those that offer a strong third binding site and those that offer only a weak one. To the first group belong cysteine, histidine, lysine, arginine, glutamic acid, and aspartic acid which have as their third functions the sulfhydryl, imidazole, amino, guanido, and carboxylate groups, respectively. To the second, much less investigated, group belong methionine, S-methylcysteine, serine, and threonine which have the thioether and hydroxy groups as their potential third binding sites. The strong binding sites of the first group have often been suggested as essential for the active site of an enzyme. The same, however, can be assumed for the weak binding sites of the second group. From investigation of $\alpha$-oxy- and $\alpha$-thio-substituted carboxylic acids,[25,40] it is known that hydroxy, ether, and thioether groups can coordinate to metal ions. Also, it has been shown for some amino acid complexes that the third function, a thioether or hydroxy group, participates in complex formation.[26]

Such weak interactions often do not manifest themselves in the stability of complexes, *i.e.*, the questioned group coordinates only about as strongly as did the dislodged water molecule, and therefore no visible gain of free energy occurs. Nevertheless, from the complexes with *d*-biotin,[39] adenosine 5′-triphosphate,[16,18,41] and other nucleotides,[34] it is known that such interactions can be strong enough to create a specific structure. In this connection, the formation of outer sphere complexes, the chemistry of which recently was summarized by Beck,[42] should also be mentioned: the interactions with the second coordination sphere of a metal ion are usually weak, but again they may be sufficient to induce specific structures.

The significance of such low-symmetry coordination spheres in enzymes has lately been discussed by Williams[8] and Vallee and Williams,[43] and the features of metal ion complexes with amino acids and peptides (of low molecular weight), their stereoselectivity, and reactivity, by Gillard.[5]

**Formation of Ternary (Mixed) Complexes.** Ternary complexes, *i.e.*, complexes formed by a metal ion and two different ligands, can be considered as models for enzyme–metal ion–substrate complexes. Investiga-

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(39) H. Sigel, D. B. McCormick, R. Griesser, B. Prijs, and L. D. Wright, *Biochemistry*, **8**, 2687 (1969).
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**Table I**
**Stabilities of Binary and Ternary $Cu^{2+}$ Complexes and Their Respective Stability Differences, $\Delta \log K$ (*cf.* eq 4)**

| No. | Equilibrium | $\log K^{Cu}_{CuL}$ | $\log K^{CuA}_{CuAL}$ | $\Delta \log K$ | Ref |
|---|---|---|---|---|---|
| a | $Cu^{2+} + Pyr^{2-} \rightleftharpoons Cu(Pyr)$ | 13.96 | | | |
| | $Cu(Bipy)^{2+} + Pyr^{2-} \rightleftharpoons Cu(Bipy)(Pyr)$ | | 14.39 | +0.4[a] | 48 |
| b | $Cu^{2+} + Gly^{-} \rightleftharpoons Cu(Gly)^{+}$ | 8.27 | | | |
| | $Cu(Bipy)^{2+} + Gly^{-} \rightleftharpoons Cu(Bipy)(Gly)^{+}$ | | 7.88 | −0.4 | 46 |
| c | $Cu^{2+} + En \rightleftharpoons Cu(En)^{2+}$ | 10.44 | | | |
| | $Cu(Bipy)^{2+} + En \rightleftharpoons Cu(Bipy)(En)^{2+}$ | | 9.15 | −1.3 | 48 |
| d | $Cu^{2+} + Pyr^{2-} \rightleftharpoons Cu(Pyr)$ | 13.96 | | | |
| | $Cu(En)^{2+} + Pyr^{2-} \rightleftharpoons Cu(En)(Pyr)$ | | 13.20 | −0.8 | 49 |
| e | $Cu^{2+} + SSal^{3-} \rightleftharpoons Cu(SSal)^{-}$ | 9.41[b] | | | |
| | $Cu(Bipy)^{2+} + SSal^{3-} \rightleftharpoons Cu(Bipy)(SSal)^{-}$ | | 9.86 | +0.5 | 44 |
| f | $Cu^{2+} + SSal^{3-} \rightleftharpoons Cu(SSal)^{-}$ | 9.04[b] | | | |
| | $Cu(NH_3)_2^{2+} + SSal^{3-} \rightleftharpoons Cu(NH_3)_2(SSal)^{-}$ | | 7.85 | −1.2 | 50, 6 |
| g | $Cu^{2+} + Gly^{-} \rightleftharpoons Cu(Gly)^{+}$ | 8.33 | | | |
| | $Cu(NH_3)_2^{2+} + Gly^{-} \rightleftharpoons Cu(NH_3)_2(Gly)^{+}$ | | 7.10 | −1.2 | 6, 50, 51 |
| h | $Cu^{2+} + SSal^{3-} \rightleftharpoons Cu(SSal)^{-}$ | 8.91[b] | | | |
| | $Cu(Gly)^{+} + SSal^{3-} \rightleftharpoons Cu(Gly)(SSal)^{2-}$ | | 7.71 | −1.2 | 6, 51 |
| i | $Cu^{2+} + HPO_4^{2-} \rightleftharpoons Cu(HPO_4)$ | 3.4 | | | |
| | $Cu(Bipy)^{2+} + HPO_4^{2-} \rightleftharpoons Cu(Bipy)(HPO_4)$ | | 3.8 | +0.4 | 16[c] |
| j | $Cu^{2+} + AMP^{2-} \rightleftharpoons Cu(AMP)$ | 3.22 | | | |
| | $Cu(Bipy)^{2+} + AMP^{2-} \rightleftharpoons Cu(Bipy)(AMP)$ | | 3.72 | +0.5 | 16[c] |
| k | $Cu^{2+} + ATP^{4-} \rightleftharpoons Cu(ATP)^{2-}$ | 6.38 | | | |
| | $Cu(Bipy)^{2+} + ATP^{4-} \rightleftharpoons Cu(Bipy)(ATP)^{2-}$ | | 6.91 | +0.5 | 16 |

[a] L'Heureux and Martell[44] determined $\Delta \log K = 0.36$. [b] The different values for $\log K^{Cu}_{CuL}$ in systems e, f, and h are mainly due to the different values of $pK_{A/3}$ used by the different authors for the calculation of the stability constants. Since the influence of $pK_{A/3}$ on the calculation of the stability constants is the same for the binary and ternary complexes, $\Delta \log K$ is independent of $pK_{A/3}$. [c] These values were determined in water containing 10% dioxane.

tions of the stability of ternary complexes may result in some general knowledge about the formation and function of metalloenzymes as biological mixed complexes. With regard to enzyme–metal ion–substrate complexes and our questions II and III, it is informative to compare the stability of the binary complex (eq 2) with that of the ternary complex (eq 3). The

$$M + B \rightleftharpoons MB$$
$$K^{M}_{MB} = \frac{[MB]}{[M][B]} \quad (2)$$
$$MA + B \rightleftharpoons MAB$$
$$K^{MA}_{MAB} = \frac{[MAB]}{[MA][B]} \quad (3)$$

difference in stability between the binary and ternary complexes (eq 4) is a way to characterize the tendency toward formation of ternary complexes.[6] Since

$$\Delta \log K = \log K^{MA}_{MAB} - \log K^{M}_{MB} = \log K^{MB}_{MBA} - \log K^{M}_{MA} \quad (4)$$

more coordination positions are available for bonding of the first ligand to a given multivalent metal ion than for the second ligand, the stability constant for the formation of a 1:1 complex is usually greater than that for the 1:2 complex,[13] *i.e.*, $\log K^{M}_{ML} > \log K^{ML}_{ML_2}$, and one expects to find negative values for $\Delta \log K$, according to eq 4. The difference, $\log K^{ML}_{ML_2} - \log K^{M}_{ML}$, is about −0.5 log unit for monodentate ligands and about −1 to −2 log units for bidentate ligands.[13]

In Table I the $\Delta \log K$ values for several $Cu^{2+}$ systems are given. A surprising fact is that positive $\Delta \log K$ values occur, *i.e.*, the ternary complexes are more stable than the binary ones. This is true for all systems (*cf.* a, e, and i–k in Table I) which contain 2,2′-bipyridyl and a ligand with oxygen atoms as donors, such as, for example, phosphate and carboxylate groups.[6,16,44,45] The O ligand is essential for a positive $\Delta \log K$ value, as can be contrasted with the 2,2′-bipyridyl–$Cu^{2+}$–ethylenediamine system (c) which has $\Delta \log K = -1.3$. The "mixed" ligand, glycine, containing an O and N donor, forms a mixed complex with $Cu^{2+}$–bipyridyl (b); $\Delta \log K = -0.4$ is between those observed for the ternary systems, 2,2′-bipyridyl–$Cu^{2+}$–O donor and 2,2′-bipyridyl–$Cu^{2+}$–N donor. This discriminating behavior of the $Cu^{2+}$–2,2′-bipyridyl 1:1 complex toward other ligands seems to be a general feature[6,35,46,47] and is of relevance to our questions II and III.

Investigation of the ethylenediamine–$Cu^{2+}$–pyrocatechol system (d) shows that not each ternary N-ligand–$Cu^{2+}$–O-ligand system has a positive $\Delta \log K$ value. An essential condition is obviously the $\pi$ system of the nitrogen ligand;[6,45,46,48,49] that is also suggested from the results obtained with systems e through h.

Since one might expect that the imidazole group has

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**Table II**
**Stability Increasing Effect of the Imidazole Group: Comparison of the Stability of Some Ternary Complexes (*cf.* eq 5 and 6)**

| No. | Equilibrium | log $X^a$ | $I$ | Temp, °C | Ref |
|---|---|---|---|---|---|
| a | $Cu(Bipy)_2^{2+} + Cu(Pyr)_2^{2-} \rightleftharpoons 2Cu(Bipy)(Pyr)$ | 6.15 | 0.1 | 25 | 48, 49 |
| b | $Cu(Ha)_2^{2+} + Cu(Pyr)_2^{2-} \rightleftharpoons 2Cu(Ha)(Pyr)$ | 4.86 | 0.1 | 25 | 49 |
| c | $Cu(Ha)_2^{2+} + Cu(Ser)_2 \rightleftharpoons 2Cu(Ha)(Ser)^+$ | 2.95 | 0.15 | 37 | *b* |
| d | $Cu(Ha)_2^{2+} + Cu(En)_2^{2+} \rightleftharpoons 2Cu(Ha)(En)^{2+}$ | 1.54 | 0.15 | 37 | *b* |
| e | $Cu(En)_2^{2+} + Cu(Pyr)_2^{2-} \rightleftharpoons 2Cu(En)(Pyr)$ | 2.65 | 0.1 | 25 | 49 |
| f | $Cu(En)_2^{2+} + Cu(Ox)_2^{2-} \rightleftharpoons 2Cu(En)(Ox)$ | 1.3 | 1.0 | 25 | *c* |

[a] Statistical value: log $X$ = 0.6 (*cf.* ref 53 and 54). [b] The values were calculated according to eq 6, using the results of Perrin, Sayce, and Sharma.[55] [c] Calculated according to eq 6 with the values given by Schaap and McMasters;[56] values determined by other authors for this equilibrium are 1.4 (*cf.* ref 57), 1.1 (*cf.* ref 54), and 1.0 (*cf.* ref 53).

bipyridyl-like qualities, and since the former often has been suggested as a binding site for metal ions in proteins, it is of interest to compare the results for some mixed complexes containing the imidazole group as binding site. The data of some ternary $Cu^{2+}$ systems containing histamine as an imidazole ligand are given in Table II. In this case, eq 5 was used for the characterization of the stability of the ternary complexes.[50] Log $X$ can be calculated according to eq 6.

$$MA_2 + MB_2 \rightleftharpoons 2MAB$$

$$X = \frac{[MAB]^2}{[MA_2][MB_2]} \qquad (5)$$

$$\log X = 2 \log \beta^M{}_{MAB} - (\log \beta^M{}_{MA2} + \log \beta^M{}_{MB_2}) \qquad (6)^{52}$$

A comparison of systems a through e of Table II shows that the imidazole group has indeed a stability-increasing effect. Systems b through d demonstrate that the $Cu^{2+}$–histamine 1:1 complex forms stabler ternary complexes with O donors than with N donors. In addition, from systems a, b, e, and f, it becomes obvious that the $\pi$ system not only of the N ligand but also of the O ligand has an influence on the stability. This shows that cooperative effects occur between the ligands in the coordination sphere of the same metal ion.[48,49]

The acidifying qualities of $Cu^{2+}$, bound in a $Cu^{2+}$–2,2′-bipyridyl 1:1 complex, are still quite strong;[6,47] thus, the amide group in 2,2′-bipyridyl–$Cu^{2+}$–glycinamide[35] is deprotonated ($pK^H{}_{Cu(Bipy)HL}$ = 7.71). This observation suggests that the same might be possible in ternary $Cu^{2+}$ complexes of $Cu^{2+}$ proteins containing an imidazole group as binding site.

Another point that should be mentioned here is the creation of "new" and specific structures by formation of ternary complexes. For example, in the $Cu^{2+}$–ATP complex, the metal ion coordinates to the nucleic acid base and the $\beta$- and $\gamma$-phosphate groups forming a macro chelate.[18,58] Through the formation of the mixed 2,2′-bipyridyl–$Cu^{2+}$–ATP complex (*cf.* Table I) the base is pushed out of the coordination sphere.[16] The same is true for the ternary 2,2′-bipyridyl–$Cu^{2+}$ nucleotide complexes with ITP, GTP, UTP, and TTP,[34] as can be seen from the deprotonation of the nucleic bases: free base ($pK^H{}_{HL} \sim 9.5$), $Cu^{2+}$ complex ($pK^H{}_{CuHL} \sim 7.7$), and ternary $Cu^{2+}$ complex ($pK^H{}_{Cu(Bipy)HL} \sim 9.0$) (*cf.* ref 34). It can be assumed that such matters are of importance for the organization and reorganization in enzyme–metal ion–substrate complexes during and after the reaction.

Ternary complexes containing $Cu^{2+}$ are relatively well investigated. Much less is known about other metal ions. With $Co^{2+}$, $Ni^{2+}$, and $Zn^{2+}$, the differences between binary and ternary complexes seem to be not so significant as with $Cu^{2+}$ (*cf.* ref 6, 16, 45). However, before one will be able to draw definite conclusions over these and other metal ions, much more work will be necessary.

**Polarity of Solvents and Ionic Strength.** A factor of great influence on the stability of complexes is the nature of the solvent. The acidity constants of both N and O ligands are very strongly dependent upon the polarity of the solvent; however, the basicity of N ligands decreases as the polarity of the solvent decreases. The change in the stability of complexes is in accord with the change in basicity, but with N ligands the stability decreases only slightly as the polarity of the solvent decreases, while with O ligands the increase in stability is about the same as that of the basicity as the polarity decreases.[6,13,16]

The ternary complexes, too, are influenced by a change in the polarity of the solvent. The stability of acetylacetone–$Cu^{2+}$–ethylenediamine and acetylacetone–$Cu^{2+}$–8-quinolinol increases as the polarity of the solvent (water–acetone mixtures)[59] increases. If the formation of the ternary complex is connected with a change in charge, compared with the two binary complexes, the solvent dependence is greater ($Cu(Acac)_2 + Cu(En)_2^{2+} \rightleftharpoons 2\ Cu(Acac)(En)^+$).[59] The

(52) $\beta^M{}_{ML_2} = [ML_2]/([M][L]^2)$; $\beta^M{}_{MAB} = [MAB]/([M][A][B])$. For the determination of stability constants of ternary complexes, computers are an essential tool; *cf.*, *e.g.*, ref 48 and 51, and I. G. Sayce, *Talanta*, **15**, 1397 (1968); D. D. Perrin and V. S. Sharma, *J. Chem. Soc., A*, 446 (1968).

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**Table III**
**Dependence of the Stability of Ternary Complexes (eq 5 and 6) on the Ionic Strength[a]**

| Cu(En)(PA) | | Cu(En)(PDA) | | Cu(PDA)(PA) | | Cu(PA)(SSal)[d] | |
|---|---|---|---|---|---|---|---|
| $I$ | log $X$ | $I$ | log $X$ | $I$ | log $X$ | $I$ | log $X$ |
| →0 | 0.31[b] | →0 | 0.98[b] | →0 | 1.15[b] | →0 | 3.94 |
| 0.057 | 0.28[c] | 0.054 | 0.97[c] | 0.054 | 1.14[c] | 0.017 | 2.87 |
| 0.558 | 0.29[c] | 0.556 | 1.15[c] | 0.556 | 1.20[c] | 0.100 | 1.83 |
| 1.057 | 0.23[c] | 1.057 | 1.20[c] | 1.055 | 1.17[c] | 0.550 | 0.57 |
| 2.057 | 0.53[c] | 2.056 | 1.59[c] | 2.055 | 1.48[c] | 2.000 | −0.25 |

[a] $I$ was adjusted with $NaClO_4$; temperature 25°; values taken from Näsänen, *et al.*[60-62] [b] Values from ref 60. [c] Values calculated according to eq 6 with the results from ref 61. [d] Values from ref 62.

influence of solvent on the structure and stereoselectivity of complexes was further discussed by Gillard.[5]

Another way to influence the water activity is to change the ionic strength (*cf.* Table III). If there is no change in the charge between the binary complexes, the influence on the stability of the mixed complex is small, besides the fact that the stability increases somewhat with increasing ionic strength, *i.e.*, decreasing water activity. However, this influence is considerable when changes of charge occur, as in the equilibrium $Cu(PA)_2^{2+} + Cu(SSal)_2^{4-} \rightleftharpoons 2Cu(PA)(SSal)^-$ (Table III). In this case, the stability of the ternary complex decreases enormously with increasing ionic strength.

It is reasonable to assume that changes in the polarity of biological fluids and especially in regions near enzymes and their active sites can occur, for example, through the creation of a structured water region, etc.[43] Thus, it seems possible that the features described in this section are "tools" used by nature in favoring one or the other side of a given equilibrium.

## General Conclusions

In the previous sections, we discussed the factors that influence the coordination tendency of metal ions and ligand groups. A well-balanced control of these factors within nature allows coordination at the "right" metal ion with the "right" ligand or ligands. A given kind of donor attracts a special group of the available metal ions more than others.[63] The remaining possible partners for coordination can be further selected by the more subtle use of changes in polarity or pH, the creation of asymmetric coordination positions which are not equally suitable for all metal ions, and the influence of two kinds of ligands on each other within the coordination sphere of the same metal ion. These subtle qualities are provided by proteins which can offer different donor atoms and originate special symmetries, create hydrophobic regions, and influence the water structure. Since metal ions are widely used in nature as catalysts, the factors mentioned have, in part, to be changed during the reactions to guarantee recycling of functional enzyme.

That two different ligands in the coordination sphere of the same metal ion influence each other was emphasized by the mentioned investigations over the stability of ternary complexes. The same is evident from kinetic studies. The $Cu^{2+}$-catalyzed decomposition of hydrogen peroxide is strongly dependent on the kind of ligand bound to the metal ion.[64] Also, the rate of the metal ion catalyzed decarboxylation of acetonedicarboxylate, oxaloacetate, or dimethyloxaloacetate is increased by coordination of the metal ion to an aromatic ligand, *e.g.*, 2,2′-bipyridyl, compared with the aqueous metal ions, while no enhancement is observed with aliphatic amines.[65] Moreover, the hydrolysis of diisopropyl fluorophosphate is catalyzed through several $Cu^{2+}$ complexes, among which the $Cu^{2+}$–2,2′-bipyridyl 1:1 complex is especially effective; in addition, the complexes of L-histidine, 1,10-phenanthroline, and imidazole are better catalysts than that of glycine.[66] These examples show that a protein can exert a direct influence on the rate of a reaction not only through the kind of metal ion it binds but also through the kind of donor groups it provides for the binding of the metal ion.

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