barrier is not observed with the tripodal ligands even though the approximately tetrahedral arrangement of the tripod is expected to be maintained in both oxidation states. *We conclude that rearrangement from tetragonal to tetrahedral does not make a significant contribution to the structural barrier. We propose instead that the important factor is the change of coordination number on reduction*, which in the present case requires the loss of coordinated water molecules.

A second specific feature of the ligands in this paper is the presence of sulfur donor atoms. These too do not have any dramatic effect on the rate of electron transfer or exchange.

Comparison to Copper Proteins. The "blue" or type 1 copper proteins have intrigued chemists because of their unusual physical properties in the Cu(II) state. Crystal structure studies[5b,c] have now shown that the copper site in these proteins (plastocyanin and azurin) is four-coordinate, with two histidine nitrogen donor atoms and two sulfur donor atoms from cysteine and methionine side chains. Presumably this arrangement has some functional value to the proteins in their role in electron transport. It has been argued that the sulfur donor atoms are necessary to obtain the "high" redox potentials of the copper proteins (plastocyanin, 0.35 V; azurin, 0.33 V). The $E°$ values for $Cu(peas)^{2+/+}$ and $Cu(pdto)^{2+/+}$ are in fact considerably higher than those of the proteins, even though they have the same N_2S_2 donor set. Other features of the protein—the four-coordinate, tetrahedral coordination[2,22] and the protein envelope around the copper site[2,23] —are expected to further increase the potential of the proteins relative to those of small copper complexes, so that the protein redox potentials appear to be abnormally *low* rather than high compared to those of model compounds. In part this is because the thiol sulfur of cysteine will produce a lower potential than the thioether sulfur donors of the models.[24] Also, the considerable effect of chelate ring size on the redox potentials of copper complexes does suggest that strain within the coordination sphere of the copper proteins may be important in determining their redox potentials. Whatever the reasons for the low potentials, it is apparent that sulfur donors are not necessary to obtain potentials as high or higher than those of plastocyanin and azurin.

It might be argued instead that the sulfurs have a kinetic role. The results in this paper do not support this argument, however, at least not for thioether sulfur.

Electron exchange rate constants have been derived for plastocyanin and azurin from reactions with inorganic oxidants and reductants[5d,25] and cytochromes.[5d,26] For good reagents the exchange rate constants fall in the range 10^3–10^5 M^{-1} s^{-1}. This would suggest that these proteins have a kinetic advantage over model systems such as those in this paper of from 10 to 10^5 M^{-1} s^{-1} and that the structural barrier is lower in the copper proteins by 5–20 kJ mol^{-1}. As the protein shell will decrease the rate of electron transfer by reducing access to the copper center, the kinetic advantage of the copper structure in the protein may be even greater than suggested by these figures. The structural barrier in the blue copper proteins may therefore be quite small. This observation is consistent with the conclusion above—that the main factor contributing to the slow rate of electron transfer is the change in coordination number accompanying electron transfer—since the coordination number does not change on oxidation or reduction in the proteins.

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Registry No. $Cu(pdto)^{2+}$, 64685-82-1; $Cu(pdto)^{+}$, 68378-73-4; $Cu(pmas)^{2+}$, 88854-98-2; $Cu(pmas)^{+}$, 76682-77-4; $Cu(peas)^{2+}$, 72077-09-9; $Cu(peas)^{+}$, 72077-07-7; $Ru(NH_3)_4bpy^{2+}$, 54194-87-5; $Co(tpy)_2^{2+}$, 18308-16-2; $Ru(NH_3)_5py^{2+}$, 21360-09-8; cyt *c*(II), 78690-22-9.

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New Multidentate Ligands. 22. *N,N'*-Dipyridoxylethylenediamine-*N,N'*-diacetic Acid: A New Chelating Ligand for Trivalent Metal Ions

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The synthesis and study of a new sexadentate ligand, *N,N'*-dipyridoxylethylenediamine-*N,N'*-diacetic acid (PLED), and of its proton and metal ion affinities are described. The ligand has two phenolate donors attached to a nitrogen heterocyclic ring to impart specificity for trivalent metal ions such as those of Ga(III), In(III), and Fe(III). The ligand has higher overall basicity than analogous phenolic sexadentate ligands containing hydroxybenzyl in place of pyridoxyl groups. Potentiometric studies of the protonation constants of the ligand and its metal chelates are reported. Stability constants of the metal chelates of Cu(II), Ni(II), Co(II), Zn(II), Fe(III), Ga(III), and In(III) ions are reported and are compared with those of analogous ligands. A comparison of the affinities of PLED and human serum protein transferrin for gallium(III) shows that PLED may compete successfully in vivo with transferrin for this metal ion.

Introduction

Frost et al.[1b] and Anderegg and L'Eplattenier[2] have reported the high stability of the Fe(III) chelate of *N,N'*-ethylenebis-[2-(*o*-hydroxyphenyl)glycine], EHPG (**1**), to be due to the high affinity of Fe(III) for the two phenolate groups present in the ionized ligand and to the orientation of these groups so as to permit their participation in chelate-ring formation. However, the structure of EHPG is such that steric hindrance may interfere somewhat with simultaneous participation in metal ion coordination by all six donor groups (two basic nitrogens, two carboxylate groups, and the two phenolate groups), with the result that the two (axial) carboxylate groups are displaced

(1) (a) Abstracted in part from a dissertation to be submitted by Christina H. Taliaferro to the faculty of Texas A&M University in partial fulfillment of the requirements for the degree of Doctor of Philosophy. (b) Frost, A. E.; Freedman, H. H.; Westerback, S. J.; Martell, A. E. *J. Am. Chem. Soc.* **1958**, *80*, 530.
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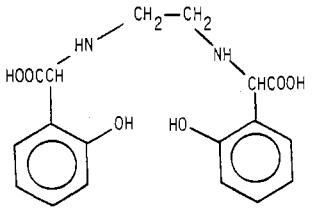
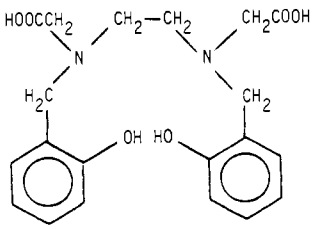


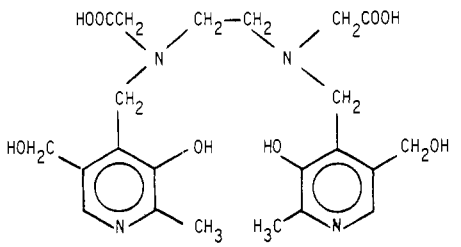
EHPG (1)

somewhat from their most favorable octahedral positions about the central metal ion. For this reason, *N,N'*-bis(2-hydroxybenzyl)ethylenediamine-*N,N'*-diacetic acid, HBED[3] (**2**), was



HBED (2)

synthesized so as to present a more favorable arrangement of the same donor groups. The experimental data confirmed the fact that the Fe(III) chelate of HBED is extremely stable ($K_{ML} = 10^{39.68}$). This paper describes the synthesis and quantitative study of a new ligand, *N,N'*-dipyridoxylethylenediamine-*N,N'*-diacetic acid, PLED (**3**), analogous to HBED, with pyridine



PLED (3)

rings derived from vitamin B_6, in order to increase solubility and lower the toxicity of the ligand for its potential application as a radiopharmaceutical involving Ga(III) and In(III). The analogous ligands HBED and EHPG and their esters have proved to be effective in test animals for the removal of iron overload,[4] thus demonstrating the usefulness of aromatic hydroxyl groups in the design of chelating agents having high affinity for trivalent metal ions.

Experimental Section

***N,N'*-Dipyridoxylethylenediamine-*N,N'*-diacetic Acid.** Pyridoxal hydrochloride (2.0 g, 9.83×10^{-3} mol) was suspended in 10 mL of MeOH. To this were added NaOH (0.39 g as a 50% solution) and ethylenediamine (0.3 g, 4.91×10^{-3} mol). After the mixture was stirred at room temperature for ~10 min, a bright yellow precipitate formed and was collected: yield 2.0 g; mp 218–219 °C. NMR in Me_2SO-d_6: δ 9.07 (s, 2 H, N=CH), 7.9 (s, 2 H, ArN=CH (aromatic)), 4.63 (s, 4 H, $ArCH_2O$), 4.05 (s, 4 H, NCH_2CH_2N), 2.52 (m, 4 H, CH_2OH), 2.38 (s, 6 H, $ArCH_3$). This material is the Schiff base *N,N'*-dipyridoxylideneethylenediamine.

The Schiff base was catalytically reduced to the amine with hydrogen and a Pt/C catalyst. The product *N,N'*-dipyridoxylethylenediamine had the following NMR spectrum (in D_2O): δ 2.53 (s, 6 H, $ArCH_3$), 2.78 (s, 4 H, NCH_2CH_2N), 3.75 (s, 4 H, NCH_2Ar), 4.45 (s, 4 H, $ArCH_2OH$), 7.62 (s, 2 H, ArH).

To a solution of the ethylenediamine derivative (3.95 g) and NaOH (5.24 g) in 40 mL of water was added dropwise a solution containing bromoacetic acid (3.03 g) and NaOH (3.5 g). The resulting mixture was stirred at ~90 °C for 2 h and then cooled in an ice bath and saturated with HCl gas. A colorless precipitate formed and was collected: 7.25 g; mp >300 °C NaCl/NaBr. The filtrate was concentrated "in vacuo" to give ~7 g of yellow solid. This was dissolved in hot methanol, and on standing, a yellow solid formed: 0.66 g; IR 1660 cm^{-1} (N–CO– indicative of lactam). The filtrate was again concentrated "in vacuo", and the residue was treated with 95% EtOH. A pale yellow solid was collected: yield 1.67 g; mp ~220–230 °C. NMR in D_2O/NaOD: δ 7.7 (s, 2 H, ArH), 4.52 (m, 4 H, $ArCH_2OH$), 3.83 (m, 4 H, $ArCH_2N$), 3.27 (m, NCH_2CO_2H), 2.8 (m, 4 H, NCH_2CH_2N), 2.65 (s, 6 H, $ArCH_3$). This material is the desired product *N,N'*-dipyridoxylethylenediamine-*N,N'*-diacetic acid (PLED). Anal. Calcd for $C_{22}H_{30}N_4O_8 \cdot 3HCl \cdot 2NaBr \cdot NaCl \cdot 2H_2O$: C, 29.75; N, 6.31; H, 4.20. Found: C, 30.23; N, 6.56; H, 4.20.

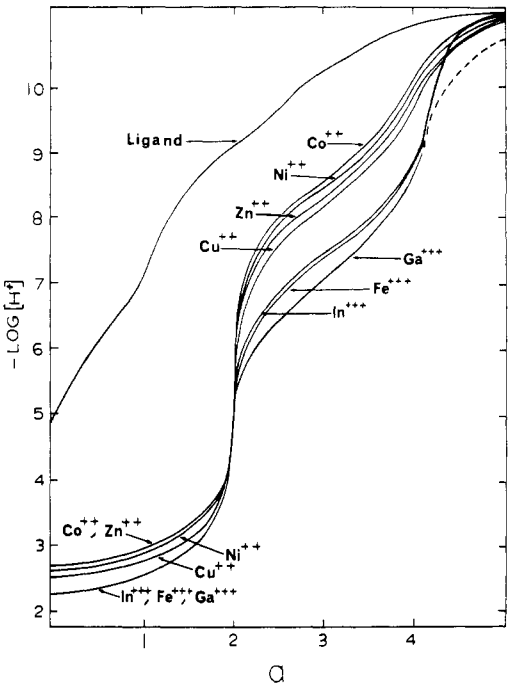


Figure 1. Potentiometric equilibrium curves of PLED in the absence and presence of 1:1 molar ratios of metal ion to ligand. a = moles of base added per mole of ligand present; μ = 0.100 M (KCl); t = 25.0 °C; concentrations of ligand and metal ions $\cong 2.00 \times 10^{-3}$ M.

Potentiometric Equilibrium Measurements. Potentiometric measurements of PLED in the absence and presence of metal ions were carried out with a Corning Digital pH meter equipped with glass and calomel reference electrodes calibrated with standard aqueous HCl solution to read –log [H^+] directly. The temperature was maintained at 25.00 ± 0.05 °C, and the ionic strength was adjusted to 0.100 by the addition of KCl. The concentrations of the experimental solutions were 2.000×10^{-3} M in metal ions and ligands. In most cases potentiometric measurements were carried out on systems containing molar equivalents of an appropriate metal salt and the most acidic form of the ligand that is readily accessible in high purity. Additional acid may be added initially if important functional groups of the ligand are not protonated in the species employed. Small increments of standard base are then added at a concentration equivalent to the ionic strength of the solution (in this work, 0.100 M) to minimize changes in ionic strength in the course of the experiment. The reacting species were studied at concentrations (2×10^{-3} M) low enough that they did not significantly contribute to the ionic strength of the medium. For successful determination of the stability constant of the initial complex, it is essential that it be appreciably dissociated to the free metal ion (at least about 50%) at the beginning of the determination, i.e., at the lowest pH measured. Further, in order to maintain the linearity of the emf–hydrogen ion concentration calibration, it is essential that the free [H^+] and [OH^-] concentrations be limited to a very small fraction of that of the supporting electrolyte. This limitation restricts the –log [H^+] measurements to the range 2–12.

Since the Ga(III), In(III), and Fe(III) chelates are completely formed even at low pH, their formation constants could not be calculated from the normal potentiometric titration curve. Therefore, in addition to the 1:1 metal–ligand system titrations shown in Figure 1, ligand–ligand competition titrations[5] were also performed by using

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Table I. Protonation Constants and Metal Chelate Stability Constants of *N,N'*-Dipyridoxylethylenediamine-*N,N'*-diacetic Acid (PLED) and Related Ligands (t = 25.0 °C; μ = 0.100 M (KCl))

	log *K*			
equil quotient	PLED[a]	HBED[3]	EHPG[1]	EDTA[9]
[HL]/[L][H]	11.10	12.60[a]	11.68	10.17
[H_2L]/[HL][H]	10.68	11.00	10.24	6.11
[H_3L]/[H_2L][H]	9.54	8.44	8.64	2.68
[H_4L]/[H_3L][H]	7.21	4.72	6.32	1.95
[H_5L]/[H_4L][H]	5.73	2.53[a]		1.5
[H_6L]/[H_5L][H]	3.31	1.74[a]		
[H_7L]/[H_6L][H]	2.57			
[CuL]/[Cu][L]	19.91	23.69[a]	23.94[b]	18.70
[CuHL]/[CuL][H]	8.81	8.49[a]	8.04	3.0
[CuH_2L]/[CuHL][H]	7.98	5.04[a]	4.98	
[NiL]/[Ni][L]	18.92	19.31	19.66	18.52
[NiHL]/[NiL][H]	9.23	8.51	7.63	3.2
[NiH_2L]/[NiHL][H]	8.40	6.45	6.03	
[CoL]/[Co][L]	16.87	19.89		16.26
[CoHL]/[CoL][H]	9.29	7.77		3.0
[CoH_2L]/[CoHL][H]	8.46	5.56		
[ZnL]/[Zn][L]	16.61	18.37	16.80	16.44
[ZnHL]/[ZnL][H]	8.85	8.27	7.74	3.0
[ZnH_2L]/[ZnHL][H]	8.22	5.99	6.64	
[FeL]/[Fe][L]	36.91	39.68	33.9	25.0
[FeHL]/[FeL][H]	7.49			1.3[b]
[FeH_2L]/[FeHL][H]	6.59			
[InL]/[In][L]	36.89	39.66[a]	33.0[a]	24.9
[InHL]/[InL][H]	7.96			1.5[b]
[InH_2L]/[InHL][H]	6.68			
[GaL]/[Ga][L]	36.35	39.57[c]	33.6	21.70
[GaHL]/[GaL][H]	7.31			1.8[b]
[GaH_2L]/[GaHL][H]	6.38			

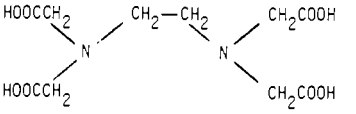
[a] Present work. [b] 200 °C. [c] Reference 5.

a 1:1:1 molar ratio of the metal ion, PLED, and a reference ligand with a known metal chelate formation constant. A good reference ligand for the In(III) and Fe(III) systems was found to be EDTA. Since the formation constant of the $GaEDTA^-$ chelate is about 3 log units lower than the formation constants of the $FeEDTA^-$ and $InEDTA^-$ chelates, it was necessary to use a reference ligand with a higher Ga(III) formation constant. Triethylenetetraminehexaacetic acid (TTHA)[6] was found to be a suitable reference ligand for the Ga(III) system.

Computations. The proton association constants for PLED were determined by using the Fortran computer program PKAS.[7] The stability constants of the normal metal chelates, the protonated metal chelates, and the hydrolyzed metal chelates were determined by using the Fortran computer program BEST.[8] The input for BEST consists of entering the components and their concentrations, the initial estimates of the equilibrium constant for each species thought to be present in terms of these solution components, and finally the potentiometric equilibrium data determined experimentally. The program sets up simultaneous mass–balance equations for all the components present at each increment of base added and, with initial assumptions for the equilibrium constants, solves for the concentration of each species present and calculates the pH at each data point. Equilibrium constants are varied automatically in order to effect a minimization in the sum of the squares of differences between the calculated and observed values of $-\log [H^+]$, thus giving ultimately a close approximation of the original potentiometric equilibrium curve, the concentrations of the individual solution species at each data point, and the associated equilibrium constants for metal chelate formation, protonation, and deprotonation.

Results and Discussion

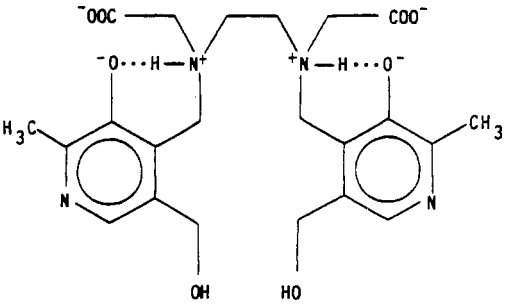
The results of the equilibrium determinations (Figure 1) on PLED are presented in Table I, along the comparable data for the analogous phenolic ligands HBED and EHPG. Data for the well-known parent chelating agent EDTA (**4**) are



EDTA (4)

included for comparison. It is interesting to note that the highest pK of PLED, which is tentatively assigned to an aliphatic amino group, is lower than those of HBED and EHPG. However, the remaining donor groups are considerably more basic than those of the two analogues. The additional aromatic nitrogens of PLED results in an overall basicity of the ligand ($\sum \log K^H \cong 50$), which is much higher than those of its analogues ($\sum \log K^H \cong 39$, 37, and 22 for HBED, EHPG, and EDTA, respectively).

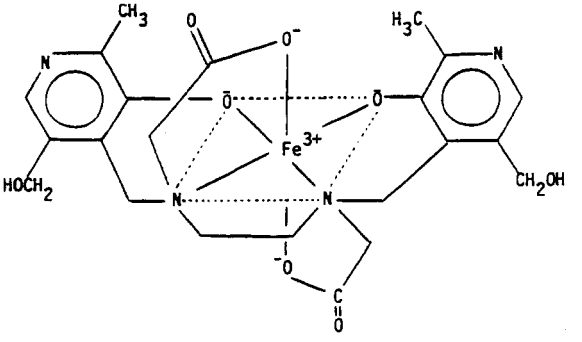
On the basis of the analogy with HBED, the first protonation of the completely dissociated ligand would reasonably be expected to occur at one of the aliphatic amino groups, as indicated by **5**. The implications of the higher basicity of



possible hydrogen bonding in diprotonated PLED (**5**)

PLED for the prediction of its metal ion affinities are dependent on the identities of the donor groups that coordinate the metal ion and assignment of the protonation constants to specific binding sites of the ligand. Such microscopic information is not yet available and will require additional (spectroscopic) studies. NMR measurements of this system are in progress and will be reported later.

It is interesting that log K for the completely deprotonated iron(III) chelate is 2.77 log units below that of HBED and 3.0 log units above that of EHPG. If one assumes that the binding sites involved in metal ion coordination are the carboxylate, tertiary aliphatic nitrogen, and phenolate groups, with total basicities comparable to those of HBED, this result is not surprising. Without detailed microscopic constants it is not possible to assign relative metal ion affinities to the donor groups indicated in **6**, because the proton affinities of the



Fe(III)–PLED chelate, FeL^- (**6**)

phenolate groups are strongly influenced by the pyridine nitrogens, both in the free ligand and in its metal complexes.

The stability of the Fe(III) chelate of PLED would probably have been still higher but for the steric repulsions arising from

(5) Harris, W. R.; Martell, A. E. *Inorg. Chem.* **1976**, *15*, 713.
(6) Motekaitis, R. J.; Martell, A. E. *Inorg. Chem.* **1980**, *19*, 1646.
(7) Motekaitis, R. J.; Martell, A. E. *Can. J. Chem.* **1982**, *60*, 168.
(8) Motekaitis, R. J.; Martell, A. E. *Can. J. Chem.* **1982**, *60*, 2403.
(9) Martell, A. E.; Smith, R. M. "Critical Stability Constants"; Plenum Press: New York, 1974, Vol. I; 1982, Vol. V.

Table II. pM and log K_{ML} Values for Gallium(III) and Indium(III) Chelates Calculated for 1 μM Metal Ion and 10 μM Ligand at pH 7.4

	Ga(III)		In(III)	
ligand	pM	log K_{ML}	pM	log K_{ML}
HBED	30.8	39.57	30.9	39.66
PLED	28.2	36.35	29.2	36.89
EHPG	26.1	33.6	25.5	33.0
TTHA	23.9	28.21	23.3	26.75[b]
DTPA	21.2	24.3	25.6	29.0
EDTA	19.9	21.7	23.1	24.90
transferrin[a]	21.3	20.3 (K_1*) 19.3 (K_2*)		

[a] Calculated for 5 mM $NaHCO_3$. [b] Value determined in this investigation; other constants obtained from Table I or ref 9.

the substituents, primarily the 2-methyl groups, on the pyridine rings. The specific arrangements of donor groups in **5** is not supported by experimental evidence at present, but there can be no doubt about the identities of the donor groups involved in 6-coordination of metal ions. Also, molecular models show that the coordination sphere is considerably more crowded and constrained in the PLED chelates than the corresponding complexes of HBED because of the substituents of the aromatic rings of the former.

The high formation constants of the Cu(II) chelate of PLED relative to those of Ni(II), Co(II), and Zn(II) are probably due to lower steric hindrance in the coordination sphere (which contains only four strong coordinate bonds), thus allowing the basic donor groups of the ligand to come more fully into play in determining the stability constant of the metal chelate.

An important difference between the chelates of PLED and those of the other phenolic ligands is the existence of protonated chelates at neutral and low pH. Coordination sites for two additional protons are provided by the pyridine nitrogens, thus allowing protonation without the breaking of coordinate bonds of the metal ion. The thermodynamic stabilities of the chelates are of course reduced considerably by such protonation even though coordinate bonds are not broken. Protonation reactions of this nature would certainly strongly influence the effectiveness of this ligand in complexing metal ions in biological systems. However, it is seen that the adjustment for this effect through the calculation of the formation constants of the protonated chelates will give high metal ion affinities.

It is of interest to note the similarities of the high stability constants of Ga(III), In(III), and Fe(III) ions for all three phenolic ligands HBED, PLED, and EHPG. The similar coordination geometries and charges of the ligands and metal ions make this result seem entirely reasonable. The stabilities of the Fe(III) and In(III) chelates of EDTA are also quite similar, but that of Ga(III) is somewhat weaker. The reason for this difference in the behavior of EDTA is not immediately obvious.

Table II shows a comparison of the gallium(III) and indium(III) binding affinities of six synthetic ligands and of the human serum protein transferrin, which contains two metal binding sites. Gallium binding to transferrin involves the release of protons and the concomitant binding of bicarbonate. Thus K_n* is a conditional constant, defined as

$$K_n^* = [Ga_nTr]/[Ga][Ga_{n-1}Tr]$$

valid only for pH 7.4 and 5 mM bicarbonate.[10]

Stability constants by themselves do not provide directly comparable numbers for measuring total ion sequestering abilities of a series of ligands, and therefore they were used

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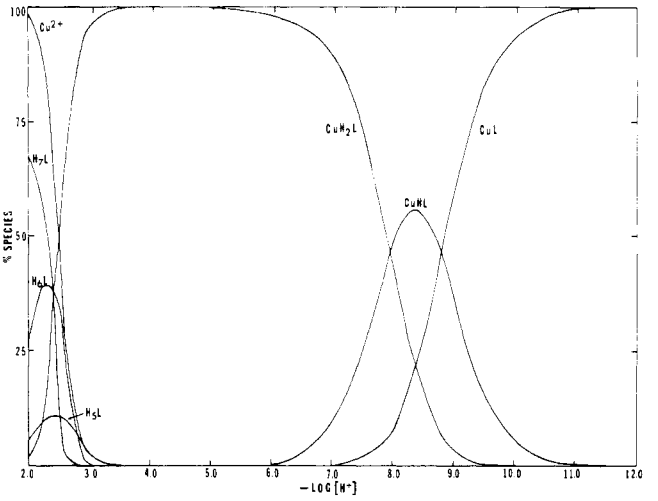

Figure 2. Distribution of species as a function of −log [H+] in a system containing a 1:1 molar ratio of PLED to Cu(II). μ = 0.100 M (KCl); t = 25.0 °C; concentration of ligand and metal ion = 2.00 × 10^{-3} M.

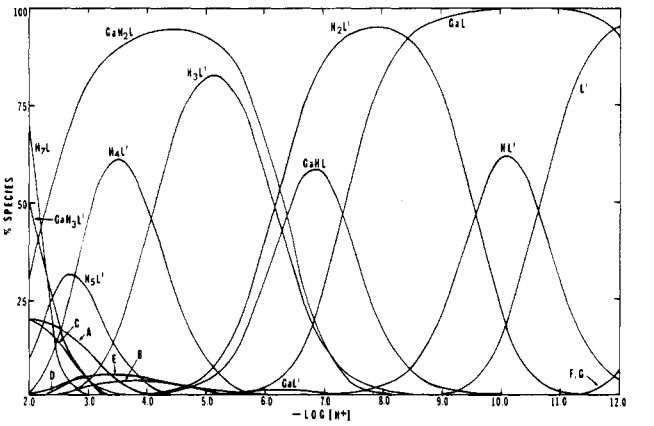

Figure 3. Distribution of species as a function of −log [H+] in a system containing equimolar concentrations of Ga(III), TTHA, and PLED. μ = 0.100 M (KCl); t = 25.0 °C; concentrations of metal ion and ligands ≅ 2.00 × 10^{-3} M; L = PLED, L′ = TTHA; A = GaH_2L', B = GaHL′, C = H_6L', D = H_6L, E = H_5L, F = L, G = $Ga(OH)_4^-$.

to calculate pM values (pM = −log [M]), where [M] is the concentration of the free aquo metal ion that would be present at equilibrium in solution of 1 μM gallium(III) and 10 μM ligand at pH 7.4. The advantage of comparing pM values rather than stability constants is that the pM values reflect the influence of ligand basicity, chelate protonation and hydrolysis, dilution effects, and differences in metal–ligand stoichiometries. The larger the pM value, the higher is the affinity of that ligand for the metal ion under the specified conditions. The relative order of stability may vary if a different set of conditions (concentration, pH, $[HCO_3^-]$) is used to calculate the pM values. The results in Table II indicate that the synthetic ligand with phenolate donor groups (HBED, PLED, and EHPG) are much stronger gallium(III) and indium(III) chelating agents than is the natural iron(III) carrier transferrin.

Thus, HBED is the most effective chelating agent known for gallium(III) and indium(III), with pM values over 9 long units greater than the transferrin pM value. Such high pM values indicate that the phenolate ligands may compete in vivo with transferrin for gallium. However, kinetic studies still need to be done in order to check whether these thermodynamically highly favored reactions will also be kinetically favored and therefore be of physiological importance.

The species distribution curves for the 1:1 Cu(II)–PLED system, illustrated By Figure 2, provide a typical picture of

the succession of complexes formed by all of the metal ions listed in Table I. While it would seem logical to assign the protonated sites of the protonated complexes to uncoordinated phenolic donor groups and to consider the stepwise displacement of these protons to be due to stepwise involvement of the phenolate donors in coordination, as was the case for Cu(II)-HBED,[2] the situation here is probably entirely different. Since the first coordination step in the Cu(II)-PLED system involves displacement of four protons from the ligand to give a neutral species having the formulation CuH_2L (with the protons probably residing on the pyridinium nitrogens), it seems that, in contrast to the behavior of HBED, both aliphatic amino nitrogens and the phenolate groups are involved initially in Cu(II) ion coordination. Easier access to the phenolate groups may be due in this case to their lower proton affinity (compared to HBED) because of the inductive electron-withdrawal effects of the protonated pyridinium groups.

A more complicated system involving competition between TTHA and PLED for Ga(III) is illustrated by Figure 3. Above $-\log [H^+] = 4$, the distribution pattern for Ga(III)-PLED species is analogous to that of Cu(II) in Figure 2, with displacement of the curves to lower pH in accordance with the higher stabilities of the Ga(III) chelates. At the lowest pH indicated, above 70% of the Ga(III) is complexed by TTHA, while 30% is in the initial PLED form GaH_2L. The relative concentration of the latter rises rapidly to a maximum at pH 4 but never reaches 100% because of a residual low concentration of monoprotonated and completely deprotonated TTHA chelates that finally disappear above pH 7.

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Copper(II) Complexes of 3,8-Dimethyl-4,7-diazadeca-3,7-dienediamide in Solution

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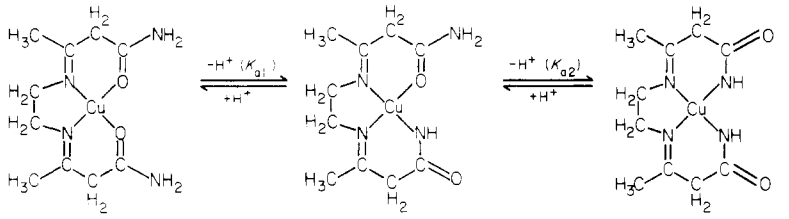
The complexes formed between copper(II) and 3,8-dimethyl-4,7-diazadeca-3,7-dienediamide (DDA) in solution at different pHs have been investigated by means of potentiometric titration, spectrophotometry, and electron spin resonance spectroscopy. The combined evidence from the three techniques established unequivocally that the equilibria



A B C

exist in solution at room temperature with equilibrium constants $pK_{a_1} = 7.35 \pm 0.01$ and $pK_{a_2} = 8.76 \pm 0.01$, respectively. Species A absorbs at λ_{max} 624 nm with ϵ_{max} 105.4 cm^{-1} M^{-1} and shows five superhyperfine lines in its ESR spectrum whereas species C absorbs at 522 nm with ϵ_{max} 72.9 cm^{-1} M^{-1} and shows nine superhyperfine lines in its ESR spectrum.

It is well-known that certain amide groups, including naturally occurring peptides, can coordinate to copper(II) ions in two fashions, with or without deprotonation of the amide nitrogen.[1] While it is relatively easy to establish the site of coordination in the former case because such coordination invariably results in complexes with characteristic purple color (biuret test) that persist in solution as well as in the solid state so much so that X-ray crystallographic results of single crystals may be extrapolated to species in solution with confidence, it is much more difficult to establish unequivocally the structure of the species in solution prior to deprotonation. This is reflected by the fact that although there is a general consensus that the site of coordination in the complexes prior to deprotonation is the carbonyl oxygen,[1-5] there are nevertheless persistent claims in the literature that the intact amide nitrogen can serve as the coordinating site.[6-9] The situation arises because hitherto there has been no experimental method that can distinguish definitively between the two alternative modes of binding of such species in solution. Despite the fact that all complexes whose solid-state structures have been determined crystallographically invariably show that the coordination is through the carbonyl oxygen,[10] there remains the possibility that such results may not be directly applicable to species in solution. Spectrophotometric studies provide strong supporting evidence of carbonyl oxygen coordination but are

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