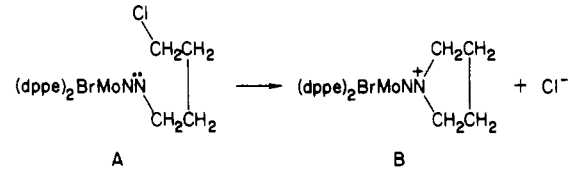
of the reaction (eq 6). Formation of the (2-methylheptyl)diazenido complex involves reaction between the 2-octyl radical and the molybdenum(I) complex $MoBr(N_2)((S,S)$-Chiraphos$)_2$ (eq 8). That this step in the reaction is slow has been shown by studies of the reaction of 6-bromo-1-hexene with $Mo(N_2)_2(dppe)_2$.[11,20] Therefore, the 2-octyl radical will have completely equilibrated before alkylation occurs (eq 8). Again kinetic resolution is observed. Excess of one diastereomeric product over the other (we cannot say which predominates from these data) arises because ΔG^* is different for the interaction of each of the two faces of the prochiral trigonal carbon radical with the chiral molybdenum(I) radical (asymmetric synthesis[22]).

Hydrogenolysis of the mixture of diastereomeric alkyldiazenido complexes ($NaBH_4/C_6H_6/CH_3OH$, 100 °C)[11] resulted in the formation of 2-aminooctane that was totally racemized.

There is an interesting contrast between the difficulty of alkylating $MoBr(NNC_4H_9)(dppe)_2$, for example, and the apparent ease with which cyclization occurs to form cyclic dialkylhydrazido complexes. The reaction of 1-bromo-4-chlorobutane with $Mo(N_2)_2(dppe)_2$ is assumed to involve first alkylation of coordinated N_2 by the alkyl bromide end of the molecule and second an intramolecular alkylation of the carbon-bound nitrogen atom resulting in cleavage of the carbon–chlorine bond. This order of alkylation is supported by previous work[20] and the occurrence of chloride rather than bromide (or a mixture) as the counterion. It seems clear that the facile cyclization of A to B is the result of anchiomeric assistance with the transition state being stabilized by



the chelating effect of the five-membered ring. The presence of four of the phenyl groups of the two dppe ligands around the $NNCH_2$–group will cause considerable steric hindrance toward intermolecular secondary alkylation. This can be demonstrated by comparing the reactions of $Mo(N_2)_2(dppe)_2$ and $Mo(N_2)_2(Et_2PCH_2CH_2PEt_2)_2$ with bromomethane. Under similar conditions, the former complex produces the methyldiazenido complex $MoBr(NNMe)(dppe)_2$ whereas the latter directly forms the dimethylhydrazido complex $[MoBr(NNMe_2)(Et_2PCH_2CH_2PEt_2)_2]Br$.[8] A more dramatic effect of anchiomeric assistance is demonstrated by the rapid cyclization of 1,4-dibromopentane to form a product in which both a primary and a secondary carbon atom are attached to the same nitrogen atom.

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New Multidentate Ligands. 23. Chelating Tendencies of Octadentate Diamido Diamino Tetraacetic Acids

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Protonation constants and metal ion binding constants are reported for five octadentate chelating ligands: *N,N'*-bis(2-aminoethyl)oxalamide-*N'',N''',N'''',N''''*-tetraacetic acid (BAOTA), *N,N'*-bis(2-aminoethyl)malonamide-*N'',N''',N'''',N''''*-tetraacetic acid (BAMTA), *N*-(glycylglycyl)-1,2-diaminoethane-*N',N'',N''',N''''*-tetraacetic acid (GGENTA), *N,N'*-diglycyl-1,2-diaminoethane-*N'',N''',N'''',N''''*-tetraacetic acid (DGENTA), and *N,N'*-diglycyl-1,4-diaminobutane-*N'',N''',N'''',N''''*-tetraacetic acid (DGBNTA). Synthetic procedures for the four new ligands (BAOTA, BAMTA, GGENTA, and DGBNTA) are described. All of these ligands, including the one previously reported (DGENTA), contain two amido groups and two terminal iminodiacetate groups and differ mainly in the arrangement and spacing of the amido groups. Differences in protonation constants and metal ion binding constants are interpreted in terms of constitutional and steric effects resulting from differences in ligand structures. The tendencies of the amide groups to coordinate metal ions through proton displacement are sensitive to the steric arrangements of these groups and to the participation of iminodiacetate groups in metal coordination. Microscopic information on metal ion binding sites of the ligands are inferred from visible and infrared absorption spectra of the ligands and metal complexes in aqueous solution. Potentiometric results with iron(III) ion suggest amide proton displacement with BAOTA and GGENTA, but results with zinc ion are less conclusive.

Introduction

Amide groups in polypeptides have been extensively studied, and their metal-binding tendencies are greatly increased by proton displacement.[1] Direct evidence for this was obtained from infrared spectra for the Cu(II) and Ni(II) complexes in aqueous solution[2-4] and from X-ray crystallographic studes for Cu(II)–polyglycines in the solid state.[5,6] Few coordination chemists have taken advantage of the coordination tendencies of deprotonated amide groups in the design of selective multidentate ligands. Several reports have been published on the chelating tendencies of diglycylethylenediamine and its derivatives.[7-11] Several reports have described the incorporation of the oxalamido linkage with ad-

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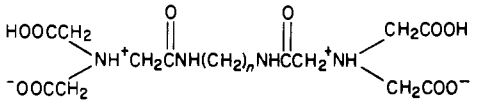
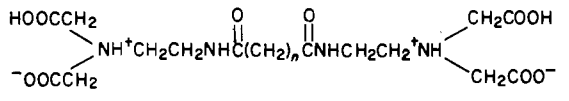
ditional coordinating groups.[12-19] Similar ligands have employed the malonamide function to provide two coordinating amido groups.[12,13] Additional structural variations have been achieved by carboxymethylating the terminal amino groups of polyglycines.[20] Several macrocyclic polyamines containing amido groups have been reported to undergo complex formation with amido proton displacement.[21,22] Apparently the only amide-containing multidentate ligands containing a relatively large number of auxiliary donor groups are the octadentate ligands reported by Motekaitis and Martell,[23] which contain two amido, two amino, and four carboxylate donor groups.

The purpose of the present research is to study the effects on metal binding affinities of structural variations of two amide groups, relative to each other, and to two iminodiacetate donors, in octadentate ligands. The ability of metal ions to displace amido protons and thus produce metal chelates of higher stability is greatly influenced by the steric arrangement of all functional groups in the multidentate ligands.

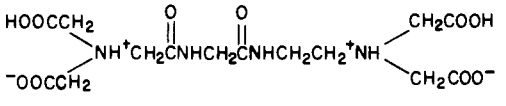
The ligands studied for this purpose are *N,N'*-diglycyl-1,2-diaminoethane-*N'',N'',N''',N'''*-tetraacetic acid (DGENTA) (**1**), *N,N'*-diglycyl-1,4-diaminobutane-*N'',N'',N''',N'''*-tetraacetic acid (DGBNTA) (**2**), *N,N'*-bis(2-aminoethyl)oxalamide-*N'',N'',N''',N'''*-tetraacetic acid (BAOTA) (**3**), *N,N'*-bis(2-aminoethyl)malonamide-*N'',N'',N''',N'''*-tetraacetic acid (BAMTA) (**4**), and *N*-(glycylglycyl)-1,2-aminoethane-*N',N',N'',N''*-tetraacetic acid (GGENTA) (**5**).



1, DGENTA ($n = 2$)
2, DGBNTA ($n = 4$)



3, BAOTA ($n = 0$)
4, BAMTA ($n = 1$)



5, GGENTA

These ligands and their chelates with Cu(II), Ni(II), and Co(II) were studied by potentiometry, visible absorption spectrophotometry, and infrared absorption spectrophotometry.

Experimental Section

Reagents used were of the highest purity available.

Synthesis of Ligands. ***N,N'*-Diglycylethylenediamine (DGEN)** was prepared by the method of Cottrell and Gill,[24] in which chloroacetyl chloride is added to ethylenediamine in chloroform, the product is separated, recrystallized from absolute ethanol, and treated with ammonium carbonate and ammonia, and the final product is separated and recrystallized from aqueous ethanol.

***N,N'*-Bis(2-aminoethyl)oxalamide (BAO)** was prepared by slowly adding benzyl chloroformate and 4 M NaOH simultaneously to a 10-fold excess of ethylenediamine in 60% methanol, extracting the product with ether, adding diethyl oxalate, separating the precipitate, dissolving the precipitate in trifluoroacetic acid, adding hydrobromic acid in acetic acid, filtering the precipitate, and recrystallizing the product from 50% ethanol. Anal. Calcd for $C_6H_{14}N_4O_2 \cdot 2HBr$: C, 21.43; H, 4.74; N, 16.67; Br, 47.62. Found: C, 21.66; H, 4.85; N, 16.56; Br, 46.30.

***N,N'*-Diglycyl-1,2-diaminoethane-*N'',N'',N''',N'''*-tetraacetic acid (DGENTA)** was prepared from DGEN and bromoacetic acid as described by Motekaitis and Martell.[23]

***N,N'*-Diglycyl-1,4-diaminobutane-*N'',N'',N''',N''''*-tetraacetic acid (DGBNTA)** was prepared by C. Y. Ng in a manner analogous to that given for DGENTA.[23] Anal. Calcd for $C_{16}H_{26}N_4O_{10}$: C, 44.24; H, 6.03; N, 12.90. Found: C, 44.23; H, 5.89; N, 12.76.

***N,N'*-Bis(2-aminoethyl)oxalamide-*N'',N'',N''',N'''*-tetraacetic acid (BAOTA)** was prepared by slowly adding diethyl oxalate to a 10-fold excess of ethylenediamine, separating the product and adding a slight excess of bromoacetic acid, and then adding 10 M NaOH, acidifying with HCl, separating the product, and purifying with Dowex 50W-X8 ion-exchange resin. Anal. Calcd for $C_{14}H_{22}N_4O_{10}$: C, 41.38; N, 5.46; N, 13.79. Found: C, 41.28; H, 5.44; N, 13.61.

***N,N'*-Bis(2-aminoethyl)malonamide-*N'',N'',N''',N'''*-tetraacetic acid (BAMTA)** was prepared from diethyl malonate in a manner similar to that employed for BAOTA except that the final ion-exchange step was omitted. Anal. Calcd for $C_{15}H_{24}N_4O_{10} \cdot H_2O$ (with Na, 15.50; Cl, 24.33): C, 24.73; H, 3.60; N, 7.69. Found: C, 24.96; H, 3.49; N, 7.19.

***N*-(Glycylglycyl)-1,2-diaminoethane-*N',N',N'',N''*-tetraacetic acid (GGENTA)** was prepared by slowly adding glycylglycine ethyl ester hydrochloride to a 10-fold excess of ethylenediamine, separating the product, and adding acetic acid groups as with BAOTA.

The nitrogen content was found to be low, but the position of the major peaks in the NMR spectra and the potentiometric data indicate that GGENTA is the major component and that any impurities present are inert with respect to the reactions under investigation.

NMR Measurements. Ligand solutions of about 0.05 M concentration were prepared by dissolving the ligand in D_2O (a trace of NaOD was added to dissolve DGENTA and BAOTA), evaporating the solution to dryness in a Rotovap, redissolving the residue in D_2O, reevaporating, redissolving, adding a trace of sodium 3-(trimethylsilyl)propanesulfonate (DSS) for a reference peak, and making strongly basic with 40% NaOD shortly before measuring. Spectra were measured on a Varian XL-200 FT NMR spectrometer at 21 °C by Trish Klahn of the Texas A&M University NMR Center.

Potentiometric Measurements. Potentiometric equilibrium measurements were made by adding standardized KOH solutions of about 0.1000 M concentration to 0.0015–0.0019 M ligand or ligand plus metal ion solutions. The 50.00-mL experimental solutions were maintained at 25.0 °C and protected from the air with humidified, prepurified nitrogen. The KOH solutions were prepared from "carbonate-free" concentrates and delivered with a 10-mL Metrohm E274 piston buret with an elongated, constricted tip under the surface of the solutions. The pH of the solutions was measured with a Beckman Research pH meter with E-2 glass and calomel electrodes. The electrodes were calibrated to measure hydrogen ion concentration by titrating a 0.01000 M HCl solution before each equilibrium run. Metal ion solutions were standardized by titration with ethylenediaminetetraacetic acid (EDTA) solutions to the murexide end point in ammonia-buffered solutions. All solutions were prepared from freshly boiled, double-distilled water and were maintained at 0.100 ionic strength with KNO_3. The CO_2 content of the base solution was estimated periodically from the Gran plot of a 0.0100 M HCl titration.[25] Freshly prepared base solutions had 0.5–1.0% CO_2. The value of pK_w can be calculated from these titrations, and the average value was found to be 13.792 ± 0.007.

Stability constants were calculated on a Digital Equipment Corp. VAX11/780 computer with the methods previously described.[26]

Preliminary Potentiometric Work. In order to check the potentiometric measurements and calculation procedures, EDTA, DGEN, and BAO were measured potentiometrically. The results were in good agreement with previous work. The protonation constants obtained are as follows: for EDTA, log K_1H = 10.19 and log K_2^H = 6.13;[27,28] for DGEN, log K_1^H = 8.23 and log K_2^H = 7.48;[7] for BAO, log K_1^H = 9.23 and log K_2^H = 8.48.[13] The stability constants obtained for the 1:1 complexes with cobalt(II) ions were log K_1 = 3.23 for DGEN[7] and log K_1 = 2.78 for BAO.

Visible Absorption Spectral Measurements. Ligand solutions of about 0.005 M concentration were prepared by dissolving the solid ligand and

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Table I. NMR Spectra of Ligands[a]

ligand	pD (pH + 0.4)	δ (rel to DSS)	rel integration	type peak[b]	assignt	no. of protons
DGENTA	13.5	3.38	3.8	s	$-NCH_2CH_2N-$	4
		3.32	4.1	s	$-NCOCH_2N-$	4
		3.23	8.0	s	$-NCH_2CO_2^-$	8
DGBNTA	13.3	3.32	4.1	s	$-NCOCH_2N-$	4
		3.23	12.2	s	$-NCH_2CO_2^-$	8
				m	$-N(CH_2)_4N-$	4
		1.54	3.8	m	$-N(CH_2)_4N-$	4
BAOTA	13.4	3.32	2.0	t $^1/_2A_2B_2$	$-NCH_2CH_2N-$	4
		3.21	4.3	s	$-NCH_2CO_2^-$	8
		3.16	6.0	s		
		2.73	2.0	t $^1/_2A_2B_2$	$-NCH_2CH_2N-$	4
		2.61	6.6	m		6
BAMTA	13.4	3.28	4.0	t $^1/_2A_2B_2$	$-NCH_2CH_2N-$	4
		3.22	7.9	s	$-NCH_2CO_2^-$	8
		3.16	0.7	s	$-NCOCH_2CON-$	1
		2.72	4.0	t $^1/_2A_2B_2$	$-NCH_2CH_2N-$	4
GGENTA	13.2	3.33	5.0	s	$-NCOCH_2N-$	2
		3.30		t $^1/_2A_2B_2$	$-NCH_2CH_2N-$	2
		3.25		s	$-NCOCH_2NCH_2CO_2^-$	4
		3.22	8.8	s	$-CONCH_2CH_2NCH_2CO_2^-$	4
		3.20		s	$-CONCH_2CON-$	2
		2.70	1.9	t $^1/_2A_2B_2$	$-NCH_2CH_2N-$	2

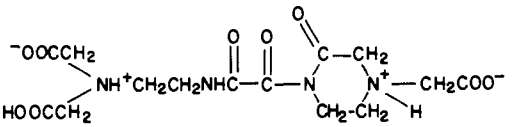
[a] 0.05 M solutions in D_2O at 21 °C. [b] Key: s = singlet; t = triplet; m = multiplet.

metal salt in 0.1000 M KCl solutions and then placing them in a potentiometric cell, the electrodes of which had been calibrated with an 0.0100 M HCl solution. Appropriate quantities of 1.000 M KOH were added with a 2.000-mL micrometer buret, and a portion of the solution was transferred to a 1.000-cm spectral cell and after measurement returned to the titration cell. Spectra were recorded on a Cary 14 recording spectrophotometer.

Infrared Spectral Measurements. Solutions containing 0.1000 M ligand were prepared by dissolving the solid ligand in D_2O, adding appropriate quantities of metal salt solutions in D_2O and 0.970 M NaOD, and transferring a portion to a 0.0150-cm spectral cell. Solutions were protected from air by rubber septum stoppers and transferred with 1.00-mL hypodermic syringes. Spectra were measured on a Beckman IR-9 spectrophotometer.

Results

NMR Spectra. NMR spectra of the ligands are presented in Table I. The ligand DGENTA shows the expected three singlets corresponding to symmetrically substituted ethylenediamine, glycyl, and acetate protons. For DGBNTA the glycyl and acetate proton singlets and a pair of multiplets for the symmetrically substituted 1,4-butylenediamine were observed. One multiplet occurs at the same position as the acetate proton resonance. The ligand BAOTA shows the acetate proton singlet, a pair of triplets for the unsymmetrically substituted ethylenediamine, and in addition another singlet and a weakly split pair of doublets. The last two resonances are not found at lower pH and apparently show the effects of the formation of a cyclic amide structure produced by condensation of an acetate group with an amide group, as indicated by **6**. The homologous ligand BAMTA shows the



6, BAOTA lactam

acetate singlet, the ethylenediamine pair of triplets, and the malonamide methylene proton singlet. The integration of the last resonance shows that it is only half of the expected value. Apparently one of the malonamide methylene protons has exchanged with the D_2O solvent. The ligand GGENTA shows the first glycyl singlet superimposed on one of the ethylenediamine triplets, two acetate singlets reflecting the different environments of the two ends of the molecule, the second glycyl singlet, and the other ethylenediamine triplet.

Potentiometric Equilibria. An example of the primary equilibrium data obtained for these ligands is illustrated by Figure 1, which consists of potentiometric equilibrium curves for the ligand BAOTA and for the formation of its mononuclear and binuclear Cu(II), Ni(II), and Co(II) chelates.

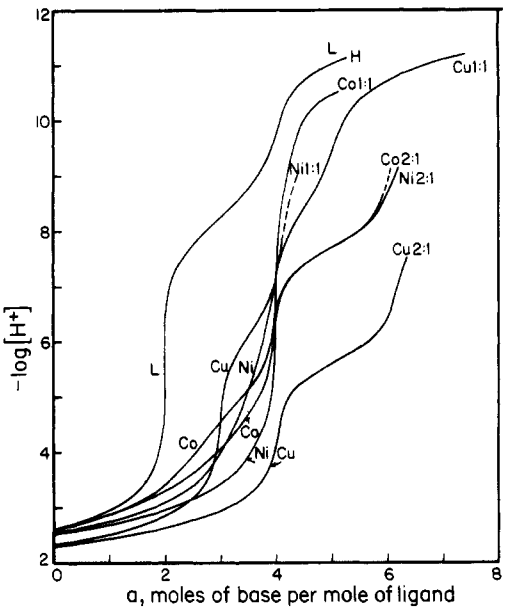


Figure 1. Potentiometric equilibrium curves for BAOTA alone (L) and for 1:1 and 2:1 molar ratios of metal ions to BAOTA with varying amounts of 0.0996 M KOH; 0.001 60 or 0.003 20 M metal ion, and 0.001 60 M BAOTA (in 0.100 M KNO_3 at 25.0 °C).

Proton Complexes. Potentiometric equilibrium measurements of DGENTA with added standard base gave an equilibrium curve similar to the one published previously[23] with a loss of two protons from carboxyl groups in the acidic region and two more from amino groups in the basic region. DGBNTA, BAOTA, and BAMTA have similar curves, as would be expected from their symmetrical structures. However, with GGENTA two protons are lost in the acidic regin but the amino protons are lost in a stepwise sequence because of the reduced basicity of the glycyl amino relative to the more isolated amino group separated from an amido group by the ethylene bridge. The calculated protonation constants are given in Table II. DGENTA values are near those previously reported.[23]

log K_1 and log K_2 reflect the addition of a proton to amino groups with the larger values of 8.6 and 8.7 for the first protonation

Table II. Protonation Constants and Stability Constants[a]

metal ion	equilibrium quotient	DGENTA	DGBNTA	BAOTA	BAMTA	GGENTA
H^+	$HL/H\cdot L$	7.24	7.06	8.60	8.67	8.7
	$H_2L/HL\cdot H$	6.11	6.14	7.87	7.97	6.2
	$H_3L/H_2L\cdot H$	2.60	2.55	2.61	2.70	2.9
	$H_4L/H_3L\cdot H$	1.89	1.95	2.01	2.03	2.1
Co^{2+}	$ML/M\cdot L$	8.85	8.58	10.63	10.05	11.0
	$MHL/ML\cdot H$	5.23	5.42	4.90	5.46	4.6
	$M_2L/ML\cdot M$	5.26	5.17	2.84	3.41	2.9
	$M_2L/M_2(H_{-2}L)\cdot H^2$			14.92		
Ni^{2+}	$ML/M\cdot L$	9.95	10.00	11.36	11.59	11.3
	$MHL/ML\cdot H$	5.00	4.59	5.10	5.14	5.2
	$M_2L/ML\cdot M$	5.80	5.81	4.30	4.16	4.2
	$M_2L/M_2(H_{-2}L)\cdot H^2$			15.48		
Cu^{2+}	$ML/M\cdot L$	11.22	10.4	12.06	12.48	11.7
	$MHL/ML\cdot H$	4.64	5.07	6.42	5.60	4.8
	$ML/M(H_{-1}L)\cdot H$	9.63	9.90		9.41	7.2
	$M(H_{-1}L)/M(H_{-2}L)\cdot H$	9.84			10.79	
	$ML/M(H_{-2}L)\cdot H^2$			18.77		
	$M_2L/ML\cdot M$	6.05	7.40	6.92	6.48	5.7
	$M_2L/M_2(H_{-1}L)\cdot H$	7.32	7.19		6.40	6.0
	$M_2(H_{-1}L)/M_2(H_{-2}L)\cdot H$	7.56	9.00		8.03	13.3
	$M_2(H_{-2}L)/M_2(H_{-3}L)\cdot H$	8.28	9.59			
	$M_2(H_{-3}L)/M_2(H_{-4}L)\cdot H$		10.77			
	$M_2L/M_2(H_{-2}L)\cdot H^2$			11.37		

[a] log K at 25.0 °C; ionic strength 0.100 M (KNO_3).

constant and 7.9–8.0 for the second protonation constant, showing their greater isolation from the amido groups by an ethylene bridge rather than a methylene bridge. The higher value for log K_1 with DGENTA as compared to that of DGBNTA and the small increases as one goes from BAOTA to BAMTA to GGENTA probably reflect decreased influence on the basic amino nitrogen by the closest amido group because of its increased secondary electronic interactions with the more remote amido group.

log K_3 and log K_4 involve the addition of protons to carboxylate groups. The trends for log K_3 in the two kinds of ligands generally approximate those of log K_1 and are probably indirectly due to the same trends.

Metal Complexes. Cobalt(II) and Nickel(II) Equilibria. With a 1:1 molar ratio of metal ion to ligand, the potentiometric measurements with each of the ligands showed inflections with three protons removed, and again with four protons removed, which indicate the formation of MHL and ML species. In each case, after four protons were removed, the measurements showed a slow drift to lower values, indicating a slow equilibrium which probably involves the approach of precipitation or perhaps a rearrangement of coordinated groups to a more stable conformation.

With 2:1 molar ratios, the curves had inflections with four protons removed, indicating formation of the M_2L species. Precipitate formation occurred as more base was added, except in the case of BAOTA. With BAOTA there was no precipitation until after a second inflection with six protons removed, indicating the formation of an $M_2(H_{-2}L)$ species. Apparently BAOTA loses two amide protons concurrently at a lower pH than the other ligands, and the greater coordination tendency of this ligand postpones the onset of precipitation as further base is added.

Copper(II) Equilibria. The potentiometric curves for 1:1 molar ratios of copper(II) ion with DGENTA, DGBNTA, and BAMTA have inflections after three, four, and six protons, which indicate the presence of MHL, ML, and $M(H_{-2}L)$ species. With BAOTA and GGENTA the curves are similar except that the last inflection was after five protons, indicating an $M(H_{-1}L)$ species.

With 2:1 molar ratios of metal ion to ligand, each potentiometric equilibrium curve is different. With BAOTA the curve is similar to the Co(II) and Ni(II) curves, showing M_2L and $M_2(H_{-2}L)$ species with the latter forming at a lower pH than with Co(II) and Ni(II). With BAMTA the inflections after four, five, and six protons indicate stepwise loss of amide protons from M_2L. With DGENTA the inflections are at four and seven protons lost, indicating M_2L and $M_2OH(H_{-2}L)$ species with a probable hydroxy bridge as indicated previously.[23] The DGBNTA curve has inflections at four, five, and about eight protons, which indicates M_2L, $M_2(H_{-1}L)$, and $M_2(OH)_2(H_{-2}L)$ species.

Iron(III) Equilibria. In the course of potentiometric equilibrium measurements on solutions with a 1:1 molar ratio of iron(III) ion to DGENTA or BAMTA, precipitates were observed, indicating that the complexes formed are not sufficiently stable to suppress hydrolysis of iron(III). With DGBNTA there was an inflection after the loss of four protons, indicating formation of an ML species, but after about five protons there was a slow drift in the measurements, and an inflection after about 7.5 protons indicates rearrangement to polynuclear complexes. With BAOTA the inflections occur after four and six protons, thus indicating the formation of ML and $M(H_{-2}L)$ species. The color of the solution between the two inflections is golden yellow, which supports the assignment of the $M(H_{-2}L)$ species. With GGENTA there were inflections after five and six protons, which suggests $M(H_{-1}L)$ and $M(H_{-2}L)$ species. There was a precipitate from three to five protons and after six protons, but between five and six the precipitate disappeared and the solution was observed to have a golden yellow color.

Zinc(II) Equilibria. Because of the ease of amide proton loss with BAOTA, potentiometric measurements were made in the presence of zinc ions to see if this metal ion can displace amide protons. With a 2:1 molar ratio of zinc ions to BAOTA, there were inflections after 4 protons and 5.5 protons to indicate formation of M_2L and polynuclear species involving proton displacement, presumably from one or more amide groups.

Species Distribution. As an example of how the stability and protonation constants in Table II may be used to determine the concentrations of individual species as a function of pH or of other concentration variables, percent formation vs. pH plots are presented in Figures 2 and 3 for solutions with 2:1 and 1:1 molar ratios of Cu(II) to DGBNTA, respectively. The 2:1 system is fairly simple, with initial formation of a binuclear complex in which no amide deprotonation has occurred. Above pH 7, the deprotonated complexes predominate, with successive deprotonation and hydroxo complex formation as the pH is increased, until an additional four equiv of base is consumed. The 1:1 system (Figure 3), however, is surprisingly complex, with initial formation of the M_2L species along with protonated chelates and protonated free ligand. In the middle pH range the 1:1 complex is predominant but is converted at higher pH to amide-deprotonated forms and binuclear complexes having increasing degrees of deprotonation, with the concomitant release of free ligand.

Visible Spectra. Visible absorption spectra of the copper(II) complexes of the diamido tetraacetic acid ligands gave broad peaks

Table III. Visible Absorption Spectra[a]

ligand	MHL^-	ML^{2-}	$M(H_{-1}L)^{3-}$	$M(H_{-2}L)^{4-}$	M_2L	$M_2(H_{-1}L)^-$	$M_2(H_{-2}L)^{2-}$	$M_2OH(H_{-2}L)^{3-}$	$M_2(OH)_2(H_{-2}L)^{4-}$
				Complexes with Cu^{2+}					
DGENTA	770 (49)	650 (46)	702 (70)	772 (82)	775 (54)			721 (86)	
DGBNTA	772 (55)	672 (36)	725 (61)	749 (83)	772 (57)	772 (60)			753 (81)
BAOTA	725 (63)	667 (83)	658 (139)	636 (139)	724 (72)		668 (153)		
BAMTA	718 (72)	671 (55)		658 (92)	724 (73)	693 (87)	676 (91)		
GGENTA	770 (55)	658 (54)	635 (99)		745 (88)	732 (63)	677 (68)	642 (74) (ppt)	
				Complexes with Ni^{2+}					
BAOTA		360 (9)			380 (8)		374 (30)		
		599 (4)			637 (3)		605 (10)		
		920 (4)			925 (2)		909 (25)		
				Complexes with Co^{2+}					
BAOTA		~480 sh (9)			~480 sh (4)		483 (41)		
		518 (28)			516 (10)		~520 sh (11)		
		630 (9)			~610 (2)				

[a] Spectral peak maxima in nm with molar absorptivities in parentheses.

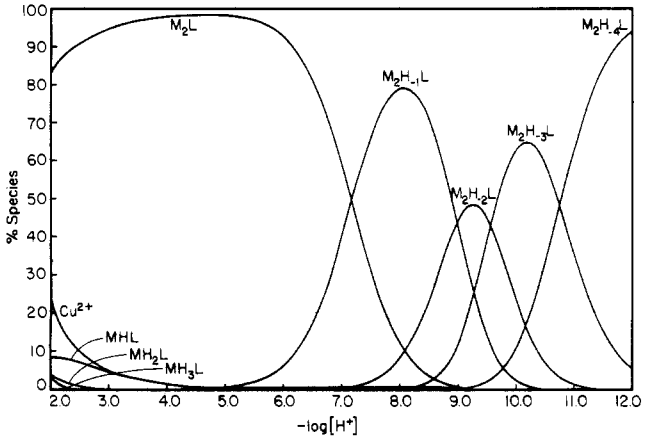


Figure 2. Species distribution as a function of pH for 2:1 copper(II) DGBNTA complexes (0.003 60 M copper(II) ion and 0.001 80 M DGBNTA).

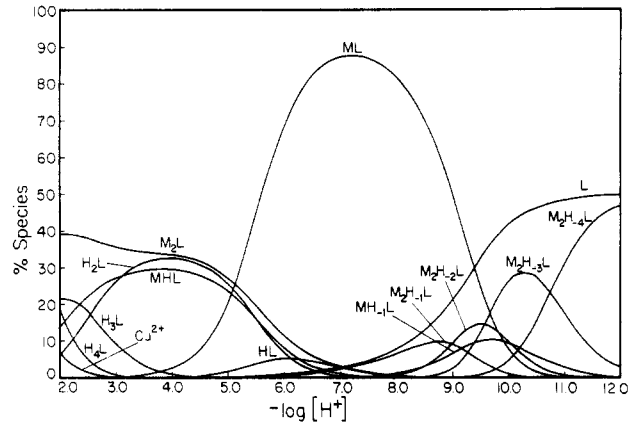


Figure 3. Species distribution as a function of -log [H^+] for 1:1 copper(II) DGBNTA complexes (0.001 80 M copper(II) ion and 0.001 80 M DGBNTA).

with maxima from 635 to 775 nm and molar absorptivities of 36–153 cm^{-1} M^{-1}. These absorption bands are due to d–d transitions and are composites of at least three components. The position of the maxima for each chemical species is in accord with Billo's[29] empirical observation that even in tetragonal complexes the average ligand field in a mixed complex MA_nB_{4-n} is the weighted average of the ligand fields of the complexes MA_4 and MB_4. Spectra were measured for pH values corresponding to integral degrees of neutralization starting with three protons for

(29) Billo, E. J. *Inorg. Nucl. Chem. Lett.* **1974**, *10*, 613.

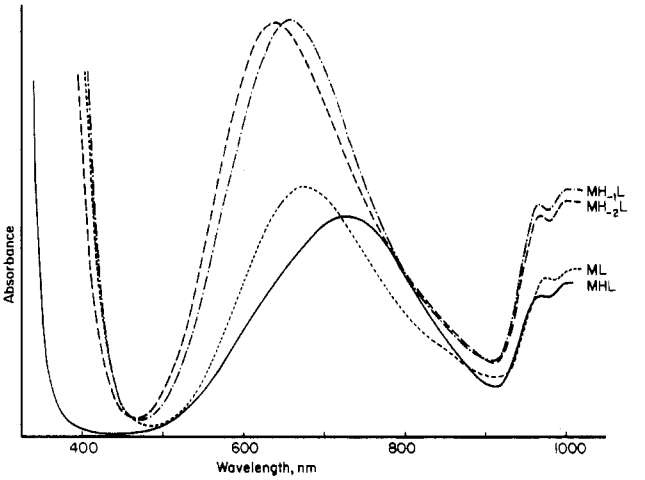


Figure 4. Visible absorption spectra of 1:1 copper(II) BAOTA complexes at a = 3 (MHL), 4 (ML), 5 ($MH_{-1}L$), and 6 ($MH_{-2}L$) (a = mol of base/mol of ligand; 0.0050 M copper(II) ion and 0.0050 M BAOTA).

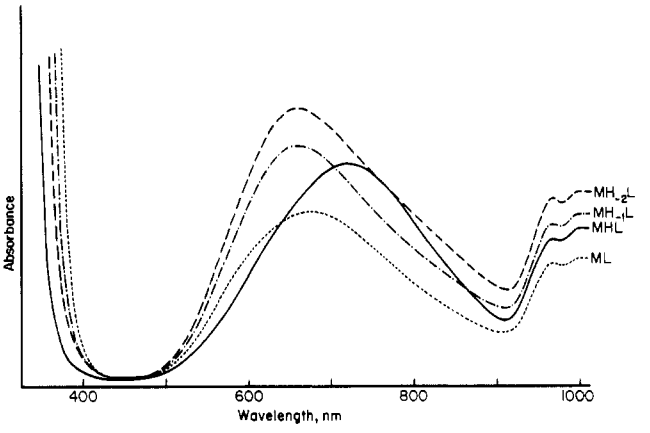


Figure 5. Visible absorption spectra of 1:1 copper(II) BAMTA complexes at a = 3 (MHL), 4 (ML), 5 ($MH_{-1}L$), and 6 ($MH_{-2}L$) (a = mol of base/mol of ligand; 0.0050 M copper(II) ion and 0.0050 M BAMTA).

1:1 molar ratios (MHL^-). In general, as protons are moved, ligand field strength increases and the peaks appear at shorter wavelength and increase in absorptivity. Results are shown in Table III and examples in Figures 4 and 5.

Spectra for nickel(II) complexes of BAOTA gave three peaks near 900, 600, and 400 nm with molar absorptivities of 2–9 cm^{-1} M^{-1}. These spin-forbidden d–d transitions are consistent with the three-band spectra normally found in octahedral Ni(II) complexes having ML and M_2L formulations. Removal of protons from the 2:1 complex shifts the peaks to lower wavelength and increases

Table IV. Infrared Carbonyl Frequencies (cm^{-1})

ligand	species	N^+-CONH-a[a,b]	N^+-CONH-	-CONH-a	-CONH-	N^+-CO_2^-	N-CO_2^-[c]
DGENTA	H_2L^{2-}	1678 (2)[d]				1640 (4)[d]	
	L^{4-}			1640 (2)[d]			1597 (4)
DGBNTA	H_2L^{2-}		~1670 sh (2)			1640 (4)	
	L^{4-}				1635 (2)		1598 (4)
BAOTA	H_2L^{2-}			1671 (2)		1637 (4)	
	L^{4-}			1667 (2)			1594 (4)
BAMTA	H_2L^{2-}				buried (2)	1641 (4)	
	L^{4-}				1632 (2)		1596 (4)
GGENTA	H_2L^{2-}	~1675 sh (1)		buried (1)		1639 (4)	
	L^{4-}			~1643 sh (2)			1600 (4)

[a] N^+ represents $R_2NH^+CH_2$-. [b] -a represents $RCONHCH_2$-. [c] N represents R_2NCH_2-. [d] Numbers in parentheses are numbers of carbonyls per molecule that produce the observed IR absorption.

Table V. Infrared Carboxylate Frequencies for Copper(II) Complexes in D_2O (cm^{-1}) for Composite Peak Maxima

ligand	species	4-CO_2M	3-CO_2M	2-CO_2M	1-CO_2M	no CO_2M
DGENTA	$CuHL^-$	1638				
	CuL^{2-}			1618		
	$CuOH(H_{-1}L)^{4-}$					1602
	Cu_2L	1638				
	$Cu_2OH(H_{-2}L)^{3-}$					1600
DGBNTA	CuL^{2-}			1619		
	$CuOH(H_{-1}L)^{4-}$					1600
	Cu_2L		1625			
	$Cu_2(H_{-1}L)^-$			1618		
	$Cu_2(OH)_2(H_{-2}L)^{4-}$					1600
BAOTA	CuL^{2-}		1622			
	$Cu(H_{-1}L)^{3-}$				1610	
	$Cu(H_{-2}L)^{4-}$					1598
	Cu_2L	1638				
	$Cu_2(H_{-2}L)^{2-}$				1612	
BAMTA	CuL^{2-}		1626			
	$Cu(H_{-2}L)^{4-}$					1599
	Cu_2L	1637				
	$Cu_2(H_{-1}L)^-$		1632			
	$Cu_2(H_{-2}L)^{2-}$			1618		
GGENTA	CuL^{2-}			1617		
	$Cu(H_{-1}L)^{3-}$				1611	
	Cu_2L		1625			
	$Cu_2(H_{-1}L)^-$				1610	
	$Cu_2(H_{-2}L)^{2-}$					1602

their intensities. The considerable intensification of the peaks in $Ni_2(H_{-2}L)^{2-}$ species is associated with square-planar Ni(II) geometry of this type of complex.

Spectra for cobalt(II) complexes with BAOTA give two overlapping peaks at 518 and 480 nm with molar absorptivities of 4 and 28 M^{-1} cm^{-1} and a weak peak at about 630 nm. Since for Co(II) spectra the relationships between spin state, geometry, and the nature and number of donor groups are interlocked, no simple correlations based on positions and absorptivities of absorption maxima can be made at this point. Removal of protons from the 2:1 complex changed the relative magnitudes of the peaks and increased the absorptivities of both. The intensities of the 2:1 complex peaks increased slowly with time, but this phenomenon was not further investigated in the present study. Results for nickel(II) and cobalt(II) are shown in Table III.

Infrared Spectra. The peak positions in the asymmetric carbonyl stretching region (1550–1750 cm^{-1}) for the ligand studied in this research are given in Table IV. As neighboring protons are removed, the carbonyl frequencies are displaced to lower energy. Except for the amido carbonyl groups of BAOTA and BAMTA, the displacement is 36 ± 2 cm^{-1} for amido carbonyl groups and 44 ± 2 cm^{-1} for carboxylato carbonyl groups. The small displacement of the BAOTA and BAMTA amido carbonyl groups, about 4 cm^{-1}, is due to their more isolated positions relative to those of the protonated amines. The GGENTA peaks were broader than those of the other ligands and overlapped.

The spectra of the copper(II) chelates also showed broadening with considerable overlap, which makes the assignment of component peaks difficult, and only the position of the peak maximum is given in Table V. An example of the type of IR spectra obtained for the metal chelates is given in Figure 6. The peak position is apparently dominated, at least to a first approximation, by carboxylato groups because of their greater number. As base

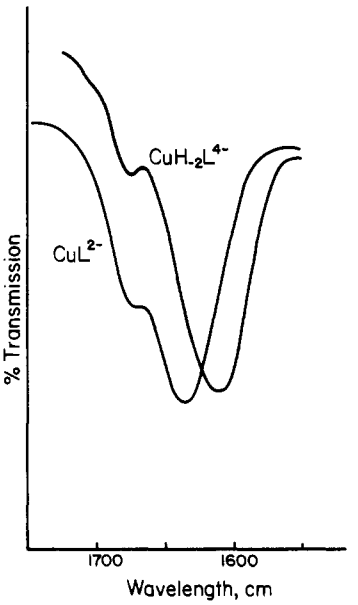


Figure 6. Infrated spectra of the copper(II) BAOTA complex in the asymmetric carbonyl stretching region, before and after displacement of amide protons.

Table VI. Carbonyl Frequencies for BAOTA Complexes (cm^{-1})

complex	—HNC(=O)—CNH—	—C(=NH)—	—CO$_2^{-a}$—CNM—	-CO$_2^-$
H_2L^{2-}	1671		1637	
L^{4-}	1667			1594
Cu_2L	~1675 sh	1637		
$Cu_2(H_{-2}L)^{2-}$		~1635 sh	1612	
Ni_2L	~1673 sh	~1637 sh	1612	
$Ni_2(H_{-2}L)^{2-}$		~1639 sh	1605	
Co_2L	~1668 sh		1622	
$Co_2(H_{-2}L)^{2-}$		~1637 sh	1608	
Zn_2L	~1668 sh		1620	
$Zn_2(H_{-2}L)^{2-}$		~1637 sh	1608	
Ca_2L	~1668 sh	~1640 sh	1608	
$Ca_2(OH)_2L^{2-}$		~1641 sh	1602	

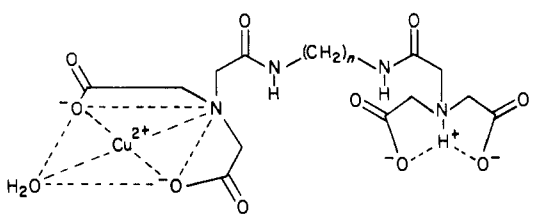
[a] N$^+$ represents $R_2HN^+CH_2-$ or $R_2NM^{2+}CH_2-$.

is added to the chelates to remove protons, the peak maximum is displaced to lower energy, which indicates that the carboxylato groups are being displaced from the copper(II) and have greater net negative charge with concomitant lower carbonyl bond order. The composite peak position is apparently modified in some cases by the peak position of the amido groups, such as the composite peak of the M_2L species of DGBNTA, which is at lower energy (1625 cm^{-1}) than is observed with DGENTA, BAOTA, and BAMTA (1638 cm^{-1}). This was not expected because of the similarity in structure. The difference is perhaps due to the lower energy of the amido groups in metal-free solutions. Deprotonated (i.e., metal-coordinated) amido groups have carbonyl IR frequencies at energy lower than those of protonated amido groups, an effect that apparently counterbalances the effect of a bound carboxylate in the more basic solutions.

Because of the relative ease of displacing amido protons in BAOTA 1:2 chelates, the spectra with several metal ions were measured and the results with those of the ligand alone are presented in Table VI. Estimates for the position of shoulders are also given. Apparently the other metal ions have less interaction with carboxylate groups than does copper(II). The order of decreasing interaction seems to be Cu > Co ≈ Zn > Ni > Ca.

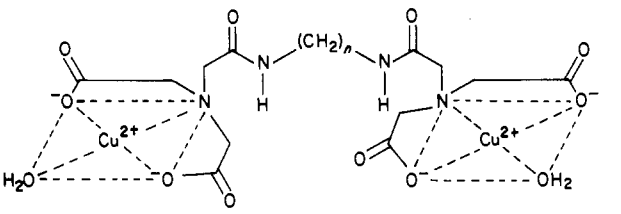
Discussion

DGENTA and DGBNTA are homologues, differing only in the number of methylenes between the two amide groups. The visible absorption spectra for the copper(II) complexes of these two ligands are identical within experimental error in position of the peak maximum (772 ± 1 nm), molar absorptivity (54 ± 2), and general peak shape for both 1:1 molar ratios after three protons are neutralized ($CuHL^-$) and 2:1 molar ratios after four protons are neutralized (Cu_2L). These similarities are in agreement with the interpretation that each copper(II) ion is coordinated to only one end of the molecule in all four species by identical sets of coordinating groups. These species probably involve coordination of the copper(II) by one iminodiacetate moiety, as indicated by formulas **7** and **8**.



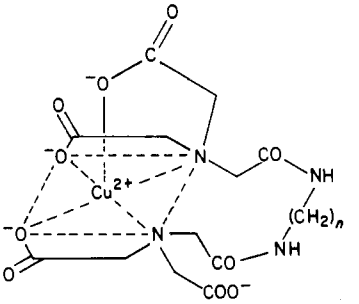
7, suggested coordinate bonding in DGENTA and DGBNTA complexes, $CuHL^-$

When base is added to the $CuHL^-$ species, one proton is removed with a log K value of about 4.8 (4.64 for DGENTA and 5.07 for DGBNTA). Comparison with the analogous ligand triglycine-*N*,*N*-diacetic acid (3GDA),[20] in which the end coordinated to the copper(II) ion is identical with those of the DGENTA and DGBNTA complexes, indicates that one would



8, suggested coordinate bonding in DGENTA and DGBNTA complexes, Cu_2L

not expect an amido or an amino proton to be removed at this pH. The terminal peptide carboxyl proton of 3GDA has a log K of 3.55 with a proton on the iminodiacetate end and a value of 3.42 with a copper(II) ion coordinated to the iminodiacetate group, which shows that the copper(II) ion has only a small effect on the acidity of the terminal carboxyl group. Also, the amido proton of 3GDA is removed with a log K of 6.91. If a similar situation exists with DGENTA and DGBNTA, the terminal amino proton should be removed at about pH 6.0 and the amido proton at about pH 6.9. Obviously the ease of ionization of one of these protons in DGENTA and DGBNTA is greatly increased in these ligands and is probably due to the coordination of the copper(II) ion to the other end of the molecule, thus contributing to the loss of the terminal amino proton as the hydrogen ion concentration decreases. This ionization is accompanied by a large shift of the visible absorption peak (120 and 110 nm, respectively) toward higher energy, which suggests stronger copper(II) ion bonding because of the addition of another nitrogen to the coordination sphere.[29] The shift in the infrared carbonyl bands indicates decreased carboxylato copper coordination, which is also compatible with formula **9**.

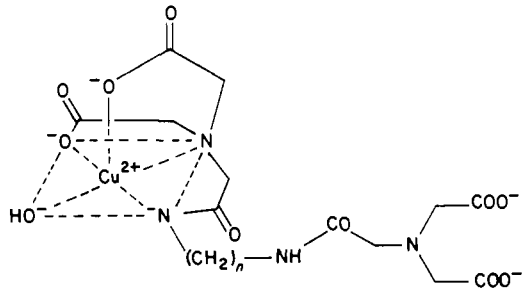


9, suggested coordinate bonding in DGENTA and DGBNTA chelates, CuL^{2-}

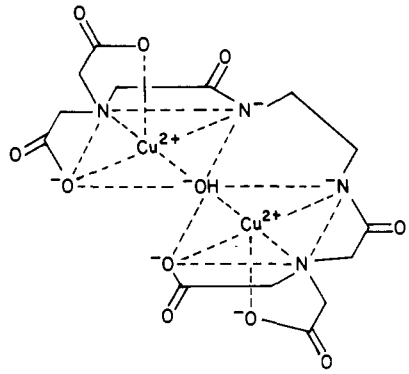
The CuL^{2-} species of these two ligands lose two protons with the addition of base at pH 9–10. The two protons could be from the two amide groups, from one amide and one coordinated water, or from two coordinated waters. If two amido protons are lost, the ligand should encircle the copper(II) ion and furnish two amino nitrogens and two amido nitrogens for coordination accompanied by an increased "chelate effect" to strongly coordinate the copper(II) ion. A large shift to higher energy of the visible absorption peak as with DGEN[7] would be expected, but instead there is a shift to lower energy, which should indicate weaker bonding. Also, the visible spectrum for this species with DGENTA resembles that of the $Cu_2OH(H_{-2}L)^{3-}$ species with DGENTA, which indicates that similar copper(II) coordination is present in both complexes. Consequently, the latter complex would involve only one copper(II) ion and the other copper(II) ion would then be uncomplexed, with hydrolysis and precipitation. However, no precipitate was observed.

If the two protons came from two coordinated waters, the visible spectrum should be essentially unchanged because of continued involvement of two amino nitrogens in coordination with the copper(II) ions, and this also was not observed.

The loss of one amido proton and one water proton is more compatible with the observed visible spectral change because of the replacement of an amino nitrogen by a amido nitrogen in the copper(II) coordination plane. Also the position of the infrared carbonyl peak is the same as that of the $Cu_2(OH)(H_{-2}L)^{3-}$ species, which is compatible with the probable coordinate bonding given in formula **10**.



10, suggested coordinate bonding in high-pH mononuclear DGENTA and DGBNTA chelates, $CuOH(H_{-1}L)^{4-}$

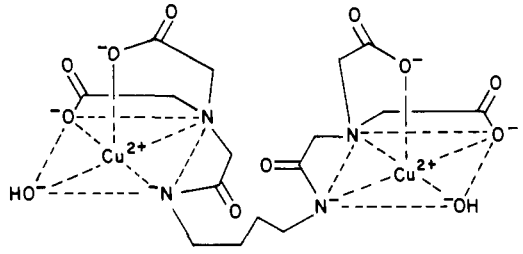


11, suggested coordinate bonding in the high-pH binuclear DGENTA chelate, $Cu_2OH(H_{-2}L)^{3-}$

The M_2L species of DGENTA loses three protons at a pH of 7–9 to form a species with visible and infrared spectra similar to that of $CuOH(H_{-1}L)^{4-}$, which is comparable with the $M_2OH(H_{-2}L)^{3-}$ coordinate bonding arrangement suggested in the earlier paper[23] and shown in formula **11**.

The Cu_2L species of DGBNTA loses one proton at a pH of about 7.2 and three more at a pH of 9–10. The coordinate bonding in the first species is not certain because the visible spectral change is very small and not compatible with the loss of an amido proton. However, since the pK seems rather low for the loss of a coordinated water proton, the formation of a bridging hydroxide between two metal ions is suggested in this case.

The final binuclear Cu(II) chelate of DGBNTA at high pH has visible and infrared spectra similar to that of the $CuOH(H_{-1}L)^{4-}$ species, which is compatible with the overall loss of two amido protons and two coordinated water protons as shown in **12**.

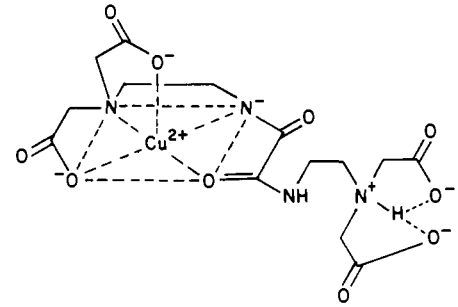


12, suggested coordinate bonding in the high-pH binuclear DGBNTA chelate, $Cu_2(OH)_2(H_{-2}L)^{4-}$

With DGENTA the two copper ions are close enough to be coupled by a hydroxy bridge at high pH, but the greater distance between Cu(II) ions in the DGBNTA complexes results in the formation of a less stable bridge, which is disrupted at high pH to form a species similar to **12**.

BAOTA and BAMTA are nearly isostructural except for the methylene group between the amides in BAMTA. The visible absorption spectra are the same per mole of copper(II) ion in wavelength (723 ± 2 nm) and molar absorptivity (70 ± 4 cm^{-1} M^{-1}) for both $CuHL^-$ and Cu_2L species. The infrared carbonyl spectra of the DGENTA $CuHL^-$ and Cu_2L species have the same peak maximum position as with these Cu_2L species, which supports the conclusion that the coordinate bonds in these chelates are essentially the same. Thus it seems that for all four species the Copper(II) ion is bonded to only one end of these ligands by iminodiacetate moieties in a similar manner as with DGENTA and DGBNTA, which may be represented by formulas similar to **7** and **8**.

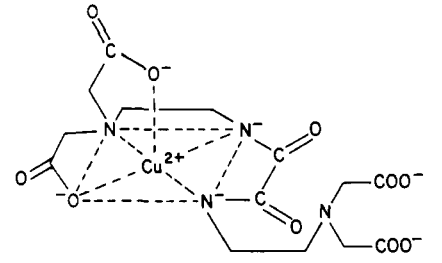
With the addition of base to $CuHL^-$ one proton is removed at pH 6.4 from BAOTA and at pH 5.6 from BAMTA. This is accompanied by a strong shift of the visible absorption peak toward higher energy (56 nm for BAOTA and 53 nm for BAMTA), indicating stronger coordination about the copper ion. This proton comes from an amido group. The position of the visible peak for BAOTA is the same as for its $Cu_2(H_{-2}L)^{2-}$ species, and the position of the peak for BAMTA is close to that of its $Cu_2(H_{-2}L)^{2-}$ species, which indicates that the copper binding involves only one end of the molecule and consequently that an amido proton is lost. The infrared carbonyl peak position is more compatible with amido proton loss than with the amino proton loss. The formula with an amido proton lost was suggested by Griesser and Fallab[13] for the analogous diamido diamine BAO. The suggested coordinate bonding is similar to that shown by **10** for DGENTA, with additional protonation at the uncoordinated, terminal iminodiacetate group. Stronger than usual carbonyl oxygen coordination, aided by the planarity of the –NHCOCONH function, would favor the arrangement of coordinate bonds as illustrated by **13**.



13, suggested coordinate bonding in mononuclear BAOTA and BAMTA chelates, CuL^{2-}

With BAOTA another proton is removed at pH 8.4 with only a small shift in the visible peak maximum toward higher energy (9 nm). This proton could be from the amino group or the second amido group. The second amido proton loss is not compatible with the small visible peak shift since this would suggest a large shift, and the infrared carbonyl peak shift is compatible with the freeing of a carboxylate, as in **10** and **12**, but with one less Cu(II) than the latter.

Above pH 10 BAOTA–Cu(II) has a shift of the visible peak toward higher energy, which suggests that another proton has been lost, but the inflection in the potentiometric equilibrium curve was obscured by base leveling. This proton is probably an amido proton because of the significant shift in the visible peak maximum (22 nm) from the $Cu(H_{-1}L)^{3-}$ position. The probable coordinate bonding is shown in formula **14**.

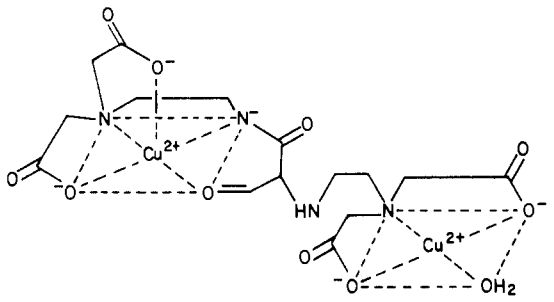


14, suggested coordinate bonding in the high-pH form of mononuclear BAOTA chelate, $CuH_{-2}L^{4-}$

BAMTA loses two protons from the ML^{2-} species at pH 8–10 accompanied by a small shift of the visible peak maximum toward higher energy. The two protons are probably one amino proton and one coordinated water proton because of the relatively small

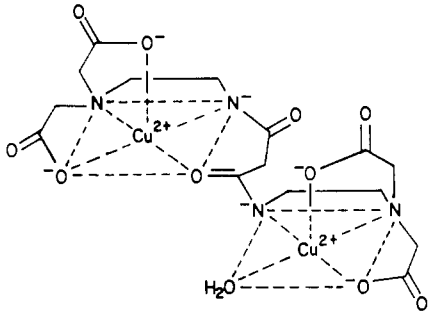
visible peak shift (12 nm) and its relative position to that of the $Cu_2(H_{-1}L)^-$ species as discussed later. The infrared carbonyl spectrum is compatible with this, and the structure is probably similar to formula **10**.

The Cu_2L species of BAMTA loses one proton at a pH of 6.1 accompanied by a significant visible absorption shift to higher energy (30 nm). This is probably an amido proton, and the resultant coordination induces steric restraints to the ionization of the other amido group. The visible absorption peak position for this species (693 nm) is the average (691 nm) within experimental error of that for Cu_2L (723 nm), which is the same as one end of this molecule, and that for $Cu(H_2L)^{4-}$ (658 nm), which is probably the same as the other end of the molecule. The suggested arrangement of coordinate linkages is represented by **15**.



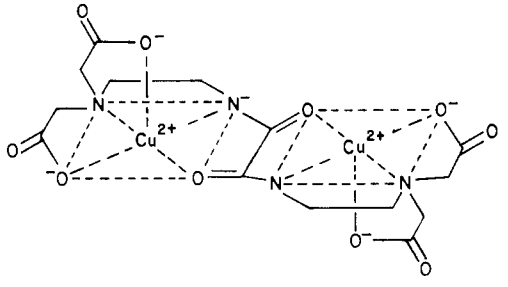
15, suggested coordinate bonding in the binuclear BAMTA chelate, $Cu_2(H_{-1}L)^-$

The binuclear Cu(II) BAMTA chelate loses a second proton at pH 8.1 with a shift of the visible peak (17 nm), which implies that an amido proton is lost. The peak position is near (5 nm) that of the CuL^{2-} species, which suggests that similar coordinating groups are involved. Since two copper(II) ions are involved, the observed shift is consistent with one copper ion the same as in CuL^{2-} and the other displaced 10 nm to lower energy, resulting in an average shift of 5 nm in the composite (combined) absorption band. These results and the infrared carbonyl peak position are consistent with **16**.



16, suggested coordinate bonding in the binuclear BAMTA chelate, $Cu_2(H_{-2}L)^{2-}$

The Cu_2L species of BAOTA loses two protons at pH 5–6 with a strong visible absorption shift (55 nm), which suggests the loss of two amido protons. The position of the peak (668 nm) is the same as that for the ML^{2-} species, which implies the same coordinating groups. The suggested bonding modes are illustrated in formula **17**.



17, suggested coordinate bonding in the binuclear BAOTA chelate, $Cu_2(H_{-2}L)^{2-}$

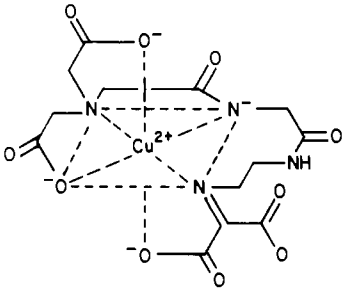
The same arrangement of coordinate bonds was found in the crystal structure of the binuclear Cu(II) chelate of *N,N,N',N'*-tetramethylbis(2-aminoethyl)oxamide.[30]

BAOTA in its Cu_2L complexes was the most efficient ligand among those studied for ease of amido proton ionization. Copper(II) ion displaces the amido protons between pH 5 and 6 while nickel(II) and cobalt(II) ions easily displace them between pH 7 and 8. Apparently this is due to a favorable steric configuration of the oxalamide portion of the molecule, which allows essentially simultaneous deprotonations by both metal ions to form a binuclear complex centered on the oxalamide moiety. Another important factor is the proximity of both carbonyl groups to the amido nitrogens of this ligand.

The fact that the $CuHL^-$ species of GGENTA has the same visible absorption spectra as DGENTA and DGBNTA shows that the copper(II) ion is on the end of the molecule having a structure analogous to that shown in **7**. The M_2L species is halfway between the corresponding complexes of DGENTA and BAOTA in the position and magnitude of its visible absorption peak as would be expected with a copper(II) ion on each end of the molecule in a manner analogous to the coordinate bond arrangement in **8**, but with different orientations of the amide groups.

The addition of base to $CuHL^-$ removes one proton at pH 5.2, and there is a large shift to higher energy of the visible peak (112 nm). The shift is similar in magnitude to that for DGENTA and DGBNTA and probably indicates that a structure similar to **9** for the corresponding DGENTA and DGBNTA complexes is formed.

CuL^{2-} loses one proton at pH 6.9 with another shift to higher energy of the visible peak (23 nm). The magnitude of the shift suggests that an amido proton is lost, and since glycylglycylglycine-*N,N*-diacetic acid (3GDA), which has the same structure as the glyglyglycyl end of this molecule, loses its first amido proton at the same pH, it is probably the first glycylamide proton that is lost here to give the arrangement shown in formula **18**.



18, suggested coordinate bonding in the intermediate-pH mononuclear GGENTA chelate, $Cu(H_{-1}L)^{3-}$

The binuclear chelate Cu_2L loses one proton at about pH 7.2 with a small shift to higher energy of the visible peak (13 nm). The position of the peak is halfway (732 nm) between those of the Cu_2L species of DGENTA (772 nm) and the $Cu_2(H_{-1}L)^-$ species of BAMTA (693 nm) and suggests that one copper(II) ion has the same bonding as in Cu_2L (same as DGENTA) and the other copper(II) ion has the same bonding atoms as in BAMTA. If one copper coordination has not changed and the observed peak is at an average position for the two copper ions, then the actual visible peak shift of the other copper(II) ion is double the observed value (i.e., 26 nm).

The amido proton displaced would be on the ethylenediamine end of the molecule because the visible spectrum indicates that the glycylglycyl moiety is not changed and because BAOTA, which has donor groups similar to the ethylenediamine end of GGENTA, displaces an amido proton more easily than DGENTA, which involves groups similar to those in the glycyl moiety of GGENTA. The corresponding coordinate bonding arrangement suggested for this species is shown in formula **19**.

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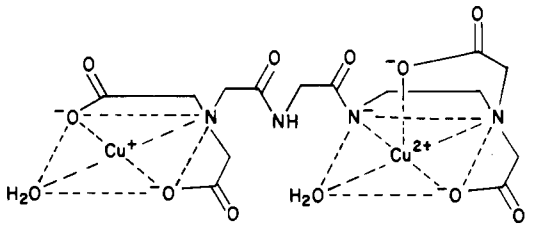

19, suggested coordinate bonding in the binuclear GGENTA chelate at intermediate pH, $Cu_2(H_{-1}L)^-$

$Cu_2(H_{-1}L)^-$ loses one proton at about pH 8.1 with a significant shift of the visible peak toward higher energy (55 nm). The position of the visible peak is the same as that of the corresponding BAMTA species and suggests that both species have the same bonding modes in the coordination sphere. The suggested structure is analogous to that of **16**, with the coordination spheres of the Cu(II) ions occupied by two deprotonated amido nitrogens, four acetates, two tertiary amino nitrogens, and possibly one amido carbonyl oxygen.

Adding another equivalent of base produces a precipitate ($Cu(OH)_2$), and the visible peak of the complex in solution is shifted toward higher energy (35 nm) to a position near that of the $Cu(H_{-1}L)^{3-}$ species. This probably indicates that the more weakly bonded copper(II) ion is released from the ligand through hydrolysis and precipitation and the ligand wraps around the other copper ion to form the $Cu(H_{-1}L)^{3-}$ species.

Amide Deprotonation by Fe(III) Coordination. It is appropriate at this point to add a comment about the tendency of the Fe(III) ion to promote amide deprotonation with concomitant coordination of negative amido nitrogen donors. It has been stated that in general Fe(III) does not deprotonate amide groups of coordinated ligands.[32] However, there are now three clear examples in which precisely such a reaction takes place to form very stable Fe(III) chelates. The first such ligand to be reported is ethylenediamine-*N*,*N*'-bis(acetylglycine)-*N*,*N*'-diacetic acid (EDDAG-DA), described by Motekaitis and Martell.[23,33] The remaining examples are found in the present work, as described under Results. The ability of the multidentate ligands EDDAG-DA, BAOTA, and GGENTA to coordinate Fe(III) via amide deprotonation is due in part to the presence of additional coordinating donor groups, which serve to prevent hydrolysis and separation of ferric hydroxide at low and intermediate pH until the pH is increased to a sufficiently high value to assist proton removal and make the negative amido nitrogens available for metal ion coordination.

Analysis of Equilibrium Constants and Some Generalizations on Amide Coordination. The data in Table II demonstrate that the coordination of a metal ion is influenced by amide groups in several ways. It has been established previously that a neighboring amino group has its basicity reduced and consequently is less strongly coordinated to the metal ion because of the inductive effect of the amido carbonyl group. This is illustrated by the first protonation constant of the polyglycines: glycine (9.57), glygylglycine (8.07), glycylglycylglycine (7.89), glycylglycylglycylglycine (7.87). (Unless otherwise stated, quoted protonation and stability constants are taken from Martell and Smith.[27]) The first amide reduces the value of log *K* by 1.50, the second more distant one 0.18, and the third one 0.02. The first protonation constant of β-alanylglycine (9.41) compared to that of glycylglycine shows an increase of 1.34 as the amide is displaced further from the amino group by an additional methylene group. A similar effect on the basicity of a neighboring carboxylate group is also observed.

The inductive effect of the amide function on metal ion coordination tendencies of adjacent donor groups is reversed when the amido group becomes deprotonated through metal ion coordination. Since this requires metal ion coordination of the deprotonated group, the adjacent functions are usually also coordinated, and changes in the basicity are not available as probes to measure this effect.

A neighboring bonding group such as an amine causes the amido proton to be more easily lost because of the inductive electron-withdrawal effect resulting from coordination of adjacent donors close enough to the amide to form five- or six-membered rings. Copper(II) ion precipitates (pH 5–7) before it displaces a proton from an isolated amide group, but glycylglycine loses an amide proton with a $-\log K$ value of 4.07 and β-alanylglycine shows similar behavior (4.05). With glycylglycine the proton loss is also aided by formation of a chelate ring involving the carboxylate group. The fact that this is an important driving force for amide deprotonation is indicated by the fact that increasing the ring size to glycyl-β-alanine (4.60) and then to glycyl-4-aminobutanoic acid (6.62), with greatly decreased chelate effects, show corresponding increasing reluctance for amide deprotonation. In the latter example the amino group keeps the copper ion in solution as the pH is increased until the amido proton is displaced. Steric effects interfere with amido proton displacement, as in glycylisoleucine (4.71), or aid, as in isoleucylglycine (3.26). Inserting the *N*,*N*-diacetic acid group in glycylglycine to give 2GDA counteracts the effect of the terminal carboxylate for amide proton displacement (6.61) because the new carboxylates occupy the adjacent bonding positions on the copper(II) ion. A general observation in this work and in the previous study by Motekaitis and Martell[23] is that strong coordination of metal ions by aminoacetate functions decreases the tendency of the metal ion to displace protons from, and coordinate with, adjacent amide groups.

The distance and relative orientation of a second amide can influence the ionization of an amido proton. With DGENTA and DGBNTA a direct comparion of the amido proton ionization is not possible because the CuL^{2-} species are very stable, the amido ionization is inhibited, and the pH must be raised to higher values to remove the amido proton. However, the positions of the visible absorption peaks of DGENTA relative to DGBNTA are significantly displaced toward higher energy for CuL^{2-} (22 nm), $Cu(H_{-2}L)^{4-}$ (27 nm), and $Cu_2OH(H_{-2}L)^{3-}$ compared to that for $Cu_2(OH)_2(H_{-2}L)^{4-}$ (32 nm). The fact that $CuHL^-$ and Cu_2L species do not show these relative differences in spectral peak positions suggests that these effects are related to the distance between the first and second amide groups and do not significantly involve the bound terminal amino groups. With $Cu(H_{-2}L)^{4-}$ and the binuclear species which have bound deprotonated amido groups, the effect of the second amide is apparently very significant on the bound amide, while the difference in the CuL^{2-} species may perhaps be explained by a chelate effect having a smaller chelate ring involving the two amide groups.

With BAOTA there is only a small enhancement, compared to that for BAMTA, as indicated by the displacement of the visible absorption peaks of CuL^{2-} (4 nm) and $Cu_2(H_{-2}L)^{2-}$ (8 nm). In considering the effects of spacing of the amide groups on their tendencies to undergo proton displacement on coordination, it is best to consider each group to be the complete –CONH– unit, spaced by one or more saturated carbon atoms.

With GGENTA the amide proton ionization takes place at p*K* 7.23, which is higher than that of BAOTA and BAMTA and seems to indicate an inhibition to the ionization by the second amide group. The more restricted ionization of amide protons as glycyl groups are added to 2GDA (6.61) through to 3GDA (6.91) and to 4GDA (7.04), and also the series from glycylglycine (4.07) through to glycylglycylglycine (5.11) and then to glycylglycylglycylglycine (5.40) support this conclusion. The Cu_2L species give a less ambiguous measure of the effect of a second amido on amido proton ionization since the other end of the molecule is bound to another metal ion and cannot coordinate to the first metal ion. GGENTA and BAMTA, with the second amido group separated from the first one by one $-CH_2-$, lose their first amide protons less easily (p*K*'s 6.03 and 6.40, respectively) than BAOTA, with adjacent amide carbonyls (p*K* 5.6). This trend is also found with DGBNTA, in which the second amido group is four saturated carbon atoms away (7.19) and DGENTA, with the second amido group two saturated carbon atoms away (7.32).

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GGENTA has less tendency to lose its amide proton than the other ligands considering the distance between the amido groups, which indicates that the relative orientation of the two amides, similar to that in peptide linkages, seems to restrict this ionization. When two carbonyls are adjacent to each other, as in BAOTA, the proton ionization of the second amide group is considerably enhanced.

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Registry No. **1**, 29725-86-8; **2**, 95193-07-0; **3**, 95156-15-3; **4**, 95193-06-9; **5**, 95216-33-4; DGEN, 5663-60-5; BAO, 4312-20-3; Fe, 7439-89-6; Zn, 7440-66-6; Co, 7440-48-4; Ni, 7440-02-0; Cu, 7440-50-8; glycylglycine ethyl ester hydrochloride, 2087-41-4.

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Kinetic Studies on Electron-Transfer Reactions of *Brassica oleracea* Cytochrome *f* with Inorganic Oxidants of Varying Charge (5- to 3+)

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Electron-transfer reactions of *Brassica oleracea* cytochrome *f* with a range of inorganic oxidants (charges 5- to 3+) have been studied. Rate constants (25 °C, M^{-1} s^{-1}), I = 0.10 M (NaCl), which are invariant for any one oxidant over the pH range 6.5–8.0, and activation parameters $\Delta H^{\ddagger}$ (kcal mol^{-1}) and $\Delta S^{\ddagger}$ (cal K^{-1} mol^{-1}), are as follows: $[(CN)_5FeCNCo(CN)_5]^{5-}$, 4.9×10^5, -3.2, -42.9; $[Co(dipic)_2]^-$, 9.4×10^4, 2.3, -28.1; $[Co(bpy)_2(O_2CMe)_2]^+$, 16.8, 14.2, -5.4; $[Co(phen)_3]^{3+}$, 3.5×10^5, 10.9, 3.5. The rate constant for the $[Fe(CN)_6]^{3-}$ oxidation of *B. oleracea* cytochrome *f* is in good agreement with that previously reported for *Brassica komatsuna*: 1.7×10^5, -0.9, -38. First-order rate constants (25 °C) for the 5- oxidant (present in large excess) show a nonlinear dependence on oxidant concentration consistent with association K (4.1×10^3 M^{-1}) prior to electron transfer: k_{et} (122 s^{-1}) at pH 7.5. Although the protein (pI 5.5) is negatively charged at pH 7.5, the effect of temperature and ionic strength on K implicates a positively charged binding site on cytochrome *f*. The redox-inactive complexes $[Zr(C_2O_4)_4]^{4-}$, association constant K_B = 530 M^{-1}, and $[Mo(CN)_8]^{4-}$, K_B = 490 M^{-1}, exhibit competitive inhibition for the 5- oxidant at 25 °C, I = 0.10 M (NaCl). The effect of pH (4.0–9.5) on the $[Co(phen)_3]^{3+}$ reaction, pK_a's 5.1 and 9.7, mirrors effects observed for $[Fe(CN)_6]^{3-}$, suggesting that there is a common binding site, protonation of which influences rate constants according to the charge on the oxidant. Activation and thermodynamic parameters are compared to those previously obtained for reactions of other metalloproteins.

Introduction

Cytochrome *f* is a single heme *c*-type cytochrome (mol wt ~33 000) found in the chloroplasts of higher plants, where it is bound to the thylakoid membrane. It was first reported by Hill and Scarisbrick[1] and has been isolated from parsley,[2,3] spinach,[4-6] and tobacco leaves[7] in an oligomeric form. More recently, the protein has been isolated in a monomeric form, with no apparent tendency to aggregate from Japanese radish,[8] charlock,[9] and cabbage,[9,10] all of which are members of the family Cruciferae. Implications of recent sequenching information have been considered.[11] Cabbage leaves are the source of protein for studies describe in this paper. Cytochrome *f* has a reduction potential of ~360 mV and isoelectronic point (charlock) of 5.5.[9] Its function is to transfer electrons from the plastoquinone pool to plastocyanin in the photosynthetic electron-transport chain between photosystems II and I. No X-ray crystal structure information is yet available.

Although fairly extensive studies on the in vivo function of cytochrome *f* have appeared,[12] the properties of purified cytochrome *f* (and in particular its reactivity) have to date been comparatively neglected. Takabe et al.[13] have studied the electron-transfer reaction of *Brassica komatsuna* cytochrome *f* with $[Fe(CN)_6]^{3-}$. Here we report investigations on the reactions of *Brassica oleracea* cytochrome *f* with five inorganic oxidants of varying charge (5- to 3+) including $[Fe(CN)_6]^{3-}$, in order to assess effects of pH, ionic strength, and temperature. The pattern of behavior that emerges is of interest compared to that of other cytochromes and as a preliminary to studies on the reaction of cytochrome *f* with plastocyanin. The competitive inhibition, which has been identified with two redox-inactive complexes, is also of interest in this latter context.

Experimental Section

Preparations. Protein. Cytochrome *f* from Durham early-spring cabbage (*B. oleracea*) was purified by the literature method,[9,10] with modifications as indicated. Reagent grade chemicals and singly distilled water were used throughout. All chromatographic separations were carried out at ~4 °C. Batches of destalked frozen leaves (950 g) were homogenized with 0.2 M Na_2HPO_4/10 mM EDTA (150 cm^3) and butan-2-one (1500 cm^3) at -20 °C in a 1-gal Waring blender for 60 s at medium speed. The upper green phase was poured off after allowing the homogenate to settle in a beaker. The lower phase was squeezed through two layers of muslin and centrifuged for 10 min at 10 000 rpm (4 °C). The resulting lower orange aqueous phase was removed by aspiration.

Cytochrome *f* was precipitated from the aqueous extract by addition of 1.3 vol of acetone cooled to -20 °C. The precipitate was collected by centrifugation at 10 000 rpm for 10 min (4 °C); the reddish pellets were suspended in a minimum volume of 40 mM Na_2HPO_4/2 mM EDTA and centrifuged at 10 000 rpm for 10 min (4 °C). The clear reddish supernatant was desalted on a Sephadex G-25 (coarse) column (50 × 8.0 cm), equilibrated with 1 mM sodium phosphate buffer at pH 7.5. The eluate was applied to a 10 × 5 cm (diethylamino)ethylcellulose (DE52) column equilibrated with 1 mM sodium phosphate buffer pH 7.5. The cytochrome was eluted with 50 mM sodium phosphate buffer pH 7.5 (this column separates out a considerable amount of brown material). The

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