Complexes of Vitamin B_6. 23. Interaction of Some Tertiary Ligating Amino Acids with the Binary Complexes of Ni(II) or Cu(II) and Pyridoxamine

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Binary and ternary complexes involved in the systems pyridoxamine (PM), glutamic acid (Glu), or aspartic acid (Asp) plus Ni(II) or Cu(II) were studied in solution at 0.15 M ionic strength and 25 °C by pH-metric, absorpiometric, and polarographic techniques. The differential pulse polarographic technique was used to study the reduction properties of these complexes. EPR was used to confirm the presence of binary and ternary complex species in the Cu(II) systems. The stoichiometry and formation constants of the binary and ternary complex species were determined by the SUPERQUAD program. Ternary species of the types 1:1:1:*r* and 1:2:1:*r* (PM/Glu or Asp/M/H) were found to exist in the pH range ∼3 to ∼10.

Introduction

Pyridoxamine (PM), a vitamin B_6 compound (Figure 1), interacts with several metal ions in solution to form metal ion complexes of different stabilities.[1,2]

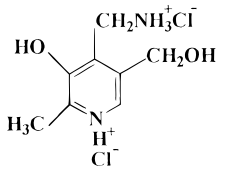
The binary PM−metal ion complexes have been shown to react with other mono- and bidentate ligands in solution to form ternary as well as quaternary complexes.[1,3−7] In most cases protonated species have been shown to exist in a wide pH range. However, the reaction of tridentate ligands has not been studied with the binary complexes of pyridoxamine.

Aspartic (Asp) and glutamic (Glu) acids (Figure 1), amino acids of possibly tridentate coordination nature, were selected to study their reaction with PM binary metal complexes.[6]

The importance of such studies lies in the fact that Asp and Glu are widely distributed in many biological systems, for example, in protein[8] and plasma,[9] and may compete for metal ions with other ligands, such as vitamin B_6 compounds, especially pyridoxamine. Besides, any experimental approach to answer questions concerning the biological action of chemical species depends on the accurate measurement of their concentrations in complex matrixes. Moreover, the states of these metal complexes under the reducing effect of most biological media should be deciphered. The transformation of these species from one form to another is essential for better understanding of their role in biological systems.

Experimental Section

Materials. The ligands pyridoxamine dihydrochloride (PM·2HCl; >99, Fluka, AG), L-aspartic acid (Asp; Puriss, Koch-Light Labs.), and L-glutamic acid (Glu; Riedel-De-Haen, AG) were pure chemicals and used without further purification. Stock solutions of aspartic and glutamic acids were dissolved in HCl (0.02 M) at twice their concentration (0.01 M). A stock solution of pyridoxamine dihydrochloride was dissolved in fresh deionized water (0.10 M). All the stock solutions were kept in a cool dark refrigerator. The ionic strengths *I*, of all the titration solutions were kept at 0.15 M NaCl. The titrant was carbonate-free 0.10 M NaOH (Merck ampules) which was 0.15 M in NaCl. The concentration of the titrant (0.100_8 M) was checked by acid−base titration against standard potassium hydrogen phthalate (0.10 M).



Pyridoxaminedihydrochloride

HOOC - CH_2 - CH_2 - CH(NH_2) - COOH

Glutamic acid

HOOC - CH_2 - CH(NH_2) - COOH

Aspartic acid

Figure 1. Structures of the ligands pyridoxamine (PM), glutamic acid (Glu), and aspartic acid (Asp).

The stock solution concentrations (0.10 M) of $CuCl_2$ (BDH) and $NiCl_2$ (BDH) were standardized by complexometric EDTA titration, as previously reported.[6] Different ratios of the metals to the ligands were used in the potentiometric study (Table 1).

Measurements. The potentiometric titrations were carried out using a Metrohm titrator, model 716 DMS Titrino, equipped with Metrohm glass and calomel electrodes. The titration cell was thermostated at $T = 25$ °C using a Caron circulator. Highly purified (humidified) nitrogen (99.999%) was purged above and below the surface (used as an additional stirring component) of the titrated solution. A pilot experiment was done to test for the attainment of the equilibrium state after the successive additions of 0.04 mL of the titrant to the titrand. It was found that the time of equilibrium attainment did not exceed 5 s (dictated by the constancy of the pH) under the experimental conditions

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Table 1. Analytical Concentrations Used in the Potentiometric Titrations[a] of the Binary and Ternary Complex Formation Involving Pyridoxamine (PM), Aspartic Acid (Asp), or Glutamic Acid (Glu) and Cu(II) or Ni(II) and the pH Range Used in the SUPERQUAD Calculation

(a) Binary Complexes

T_{Asp} ($\times 10^3$ M)	$T_{Cu\ or\ Ni}$ ($\times 10^3$ M)	pH range (Cu(II) system)	pH range (Ni(II) system)
1	1	3.05−5.20	3.39−5.21
2	1	2.73−6.68	2.85−4.80
4	2	2.97−6.83	2.54−6.32
3	1	2.59−6.48	2.69−5.04
T_{Glu} ($\times 10^3$ M)	$T_{Cu\ or\ Ni}$ ($\times 10^3$ M)	pH range (Cu(II) system)	pH range (Ni(II) system)
1	1	3.07−5.07	3.09−8.19
2	1	2.78−6.73	2.77−8.50
4	2	2.53−6.98	2.53−9.34
3	1	2.62−6.55	2.66−9.24
3	1.5	2.62−6.85	
2	2	2.79−5.10	

(b) Ternary Complexes

T_{PM} ($\times 10^3$ M)	$T_{Glu\ or\ Asp}$ ($\times 10^3$ M)	$T_{Cu\ or\ Ni}$ ($\times 10^3$ M)	pH range (PM−Asp−Cu(II) system)	pH range (PM−Asp−Ni(II) system)	pH range (PM−Glu−Cu(II) system)	pH range (PM−Glu−Ni(II) system)
1	1	1	2.92−4.99	2.92−5.35	2.95−8.93	2.90−9.96
2	2	2	2.65−5.09	2.68−9.66	2.67−8.98	2.98−9.99
1	2	1	2.86−4.95	2.88−4.96	2.87−8.91	
2	1	1	2.67−4.94	2.68−6.63	2.73−9.08	

[a] Titration runs were made at least in duplicate.

used. The titrator, however, was adjusted to a 40 s time interval after each successive addition of the titrant.

The ionic strength was adjusted to 0.15 M by the proper addition of NaCl as a 2 M stock solution.

The electrode system was calibrated by three buffers of pH's 4.01, 7.00, and 10.00. It was also calibrated by acid−base titration of standard 0.10 M HCl versus 0.10 M NaOH at the ionic strength 0.15 M and 25 °C. The differences between measured pH and caculated pH values before the end point were in the range 0.013−0.030 (the average was ∼0.016) log unit. These differences correspond to the combined effects of the glass electrode junction potential and the activity coefficient of hydrogen ions at 0.15 M ionic strength and at T = 25 °C. The direct pH-meter readings were used to calculate the protonation constants (these are called Bronsted constants[17,18]). Their positive logarithms given for aqueous solutions at I = 0.15 M (NaCl) and at T = 25 °C may be converted into the corresponding concentration constants by subtracting ∼0.016 n (where n is the coefficient of hydrogen in the complex species) log unit from the listed log β values of the protonated species of the binary and ternary metal complexes in this work.

The data were acquired automatically by the 716 DMS Titrino, which was controlled by an on-line IBM-compatible personal computer. The data were further processed by the SUPERQUAD program.[11] The pH range was ∼2.0 to ∼10.0.

The EPR solution spectra of Cu(II) systems (in the pH range ∼2 to ∼10) were obtained at room temperature (∼23 °C) on an X-band (9.86 GHz) Brucker ECS 106 EPR spectrometer operating at 100 kHz modulated frequency with a modulated amplitude of 10.115 G, in the magnetic field range 2700−3700 G. The solution concentration was 0.005 M for T_{Cu} and 0.010 M for T_{PM}, T_{Glu}, or T_{Asp}. The pH's of the solutions were adjusted by anhydrous Na_2CO_3 at pH values < 5 and with dilute NaOH solution at pH values > 5.

The spectrophotometric measurements were taken for solutions of different pH values of 0.01 M metal ion concentration with twice as much ligand concentrations (0.02 M), using a Cary 5 spectrophotometer, in the wavelength range 470−900 nm at ∼25 °C.

The polarographic measurements were obtained by using a Metrohm polarecord E506 provided with a dropping mercury indicator electrode, a saturated calomel reference electrode, and a platinum-wire electrode. The settings of the polarographs were as follows: the voltage range was either 0.0 to −0.5 or −0.6 to −1.6 V (for Cu(II) and Ni(II) systems, respectively), the pulse amplitude was −40 mV, the drop time was 1 s, the recorder speed was 0.5 mm/s, and the temperature of the cell was room temperature (∼23 °C). The pH range was ∼2.0 to ∼11.0. The solution was deoxygenated by passing pure humidified nitrogen gas through the cell before taking the differential pulse polarograms (DPPs) and above the solution in the cell when the DPP was taken. For the binary and the ternary systems the concentrations of PM, Asp, and Glu (0.005 M) were five times those of the metal ions (0.001 M).

Calculations. The stoichiometries and formation constants were simultaneously determined by the SUPERQUAD[10] program. The procedures used to select the correct equilibrium models were similar to those previously published.[11]

Results and Discussion

(A) Potentiometric Study: (i) Binary Systems. Although the binary systems of Asp (or Glu)−Cu(II) or Ni(II) have been the subject of many research papers, it has been found that disagreement exists on the species types among different reports. This may primarily be explained as due to the experimental conditions used as well as the formation of unseen faint precipitates in slightly alkaline solutions. Of course, the formation of precipitates is dependent on the ligand to metal ion ratios, being greater for small ratios. Tables 2−4 depict the values of the protonation constants of the ligands PM, Asp, and Glu and their binary formation constants with Ni(II) or Cu(II). Some of these constants have been re-evaluated under the experimental

Table 2. Reported Protonation Constants of the Ligands

system	stoichiometry coefficients l	p	q	r	log β	ref	I, T[a]
PM−H	1	0	0	1	10.41		
	1	0	0	2	18.56	1	0.15, 37
	1	0	0	3	22.06		
Asp−H	0	1	0	1	9.71	13	0.12, 25
	0	1	0	2	13.66		
	0	1	0	3	15.53		
Glu−H	0	1	0	1	9.67	13	0.12,25
	0	1	0	2	13.95		
	0	1	0	3	16.25		

[a] I = ionic strength. T = temperature (C°).

Table 3. Formation Constants of Various Ni(II) Binary Complex Species: $(L)_{l\ or\ p}(Ni)_q(H)_r$

system Ni(II)−L	stoichiometry coefficients l	p	q	r	log β^a ($\pm\sigma$) this work	reported log β ref, T, I
PM−Ni(II)	1	0	1	0		6.46 ref 1, 37, 0.15
	1	0	1	1		12.20
	2	0	1	0		10.52
	2	0	1	2		28.14
Asp−Ni(II)	0	1	1	0	7.09 (0.14)	7.14 ref 12, 25, 0.12
	0	1	1	1	12.79 (0.13)	11.19
	0	2	1	4	32.84 (0.13)	
	0	3	1	2	31.86 (0.16)	
	0	3	1	4	42.00 (0.12)	
	0	2	1	0	12.66 (0.08)	12.43
Glu−Ni(II)	0	1	1	0	6.06 (0.04)	5.52
	0	1	1	1	12.33 (0.13)	
	0	1	1	−1	−3.21 (0.10)	
	0	2	1	0	10.33 (0.05)	9.87 ref 12, 25, 0.12
	0	2	1	2	24.58 (0.09)	
	0	2	1	4	33.51 (0.08)	
	0	3	1	4	42.33 (0.07)	
	0	3	1	0		12.51

[a] SUPERQUAD σ and χ^2 never exceed 1.5 and 20, respectively. The data points were not less than 320 points. The pH range was 2.5−7.1 and 2.5−9.3 for the Ni(II)−Asp and Ni(II)−Glu systems, respectively.

Table 4. Formation Constants of Various Cu(II) Binary Complex Species: $(L)_{l\ or\ p}(Cu)_q(H)_r$

system Cu(II)−L	stoichiometry coefficients l	p	q	r	log β^a ($\pm\sigma$) this work	reported log β	ref
PM−Cu(II)	1	0	1	0		10.81	1
	1	0	1	1		17.22	
	1	0	1	2		21.34	
	2	0	1	0		17.47	
	2	0	1	1		25.46	
	2	0	1	2		32.54	
	2	0	1	3		38.91	
Asp−Cu(II)	0	1	1	0	9.04 (0.14)	8.41	14
	0	1	1	1	12.86 (0.06)	12.44	
	0	2	1	0	15.86 (0.07)	15.89	
	0	2	1	1	21.37 (0.10)	20.10	
	0	2	1	2	25.60 (0.03)	24.02	
	0	1	1	2		14.35	
	0	2	1	4		28.34	
Glu−Cu(II)	0	1	1	0	8.43 (0.02)	8.25	14
	0	1	1	1	12.43 (0.05)	12.34	
	0	2	1	0	15.00 (0.04)	14.93	
	0	2	1	2	24.81 (0.05)	29.85	
	0	1	1	−1	0.96 (0.15)		
	0	1	1	2		14.80	
	0	2	1	1		19.62	

[a] SUPERQUAD σ and χ^2 never exceed 1.10 and 25, respectively. The data points were not less than 330 points, and the pH range was 2.5−7.0.

conditions of this work. It is clear that complex species of ratios of 3:1 (L/M; where L stands for PM, Asp, or Glu ligands and M for Ni(II) or Cu(II)) are not obtained for the

Table 5. Formation Constants of Metal Ternary Complex Species: $(PM)_l(Asp\ or\ Glu)_pM_qH_r$

system	stoichiometry coefficients l	p	q	r	log β^a ($\pm\sigma$) this work	SUPERSQUAD σ, χ^2, pH range, and no. of data points
PM−Asp−Cu(II)	1	1	1	1	25.94 (0.06)	0.3, 19.6, 2.7−5.1, 270
	1	1	1	2	31.18 (0.07)	
	1	1	1	3	35.00 (0.08)	
	1	1	1	4	38.37 (0.07)	
	1	2	1	3	43.55 (0.06)	
	1	2	1	4	47.67 (0.08)	
PM−Glu−Cu(II)	1	1	1	0	17.15 (0.05)	1.81, 18.6, 2.7−9.0, 427
	1	1	1	1	24.62 (0.04)	
	1	1	1	−1	7.75 (0.08)	
	1	2	1	1	29.46 (0.17)	
	1	2	1	2	37.40 (0.06)	
	1	2	1	4	46.93 (0.12)	
PM−Asp−Ni(II)	1	1	1	1	22.04 (0.05)	1.41, 20.7, 2.7−9.7, 285
	1	1	1	2	29.49 (0.05)	
	1	1	1	3	34.50 (0.07)	
	1	1	1	4	38.22 (0.06)	
	1	2	1	0	19.62 (0.10)	
	1	2	1	3	41.66 (0.17)	
	1	2	1	4	47.46 (0.09)	
PM−Glu−Ni(II)	1	1	1	0	12.09 (0.05)	0.69, 6.2, 2.8−10.0, 320
	1	1	1	1	21.41 (0.06)	
	1	1	1	2	28.94 (0.07)	
	1	1	1	3	35.76 (0.06)	
	1	1	1	4	39.80 (0.05)	
	1	2	1	1	25.16 (0.07)	
	1	2	1	3	41.58 (0.07)	
	1	2	1	4	48.04 (0.07)	

Cu(II) systems. However, some Ni(II) binary systems, Asp and Glu systems in particular, exhibit this type of species: an observation which emphasizes the octahedral nature of Ni(II) over Cu(II) metal ions. Although, protonation of some species of Ni(II) complexes may deprive the Ni(II) systems of this property. The PM systems do not show such species, although a wider pH range than that in the Asp and Glu systems was used. This has been rationalized as due to the steric factor exhibited by PM on metal complexation. The nonprotonated PM complexes with Cu(II) are more stable than those of Asp and Glu (∼100 times more stable) (Table 4). This reflects the role of the extra π-character in the bonding of PM with Cu(II). However, this π-character is not confirmed in the Ni(II) systems (Table 3).

The formation constants of the complexes of Asp are larger than those of Glu of identical stoichiometries due to the difference in the size of the chelating ring. On the other hand, comparing the formation constants of glycine (Gly)[1] with Ni(II) and Cu(II) with those of Asp and Glu, one finds that the latter systems are more stable by at least one log unit (Tables 3 and 4). This again emphasizes the effect of the tridentate nature of Asp and Glu.

(ii) Ternary Systems. (See Supporting Information Figures 1−4 (the distribution of the ternary species as a function of pH).) Table 5 details the formation constants and stoichiometries of the ternary species in this work. The constants stand for the following equilibrium reaction:

$$l\mathrm{PM}^- + p(\mathrm{Asp}^{2-}\ \text{or}\ \mathrm{Glu}^{2-}) + q\mathbf{M}^{2+} + r\mathrm{H}^+ \rightleftarrows (\mathrm{PM}_l(\mathrm{Asp\ or\ Glu})_p\mathbf{M}_q\mathrm{H}_r)^z \quad (1)$$

where l, p, q and r are the stoichiometric coefficients (M is either Ni(II) or Cu(II), and z ($= -2q + r - l - 2p$) is the charge of the complex species). The important feature of Table 5 is that the ternary complexes are mostly protonated except for a very few cases. Moreover, the highly protonated species for Ni(II) and Cu(II) have formation constants approximately equal in magnitude (e.g. 1:2:1:4 species) whether the second ligand is Asp or Glu. The ternary

Table 6. Summary of the Calculated Values of log K_1 and log K_2 Whenever the Binary Formation Constants Are Available

	log K_1[a]		log K_2[a]	
species	Ni(II)	Cu(II)	Ni(II)	Cu(II)
$[(PM)(Asp)]^-$				
$[(PM)(Asp)MH]^0$	2.75	−0.32		10.56
	2.79	2.27		13.04
$[(PM)(Asp)MH_2]^+$	4.50	1.10	18.18	13.96
		0.80		15.53
				19.29
$[(PM)(Asp)MH_3]^{2+}$		0.80		15.23
				14.09
				16.09
$[(PM)(Asp)MH_4]^{3+}$			33.08	
$[(PM)(Glu)M]^-$	−0.43	−2.09		1.83
$[(PM)(Glu)MH]^0$	3.15	−1.03		15.00
	2.62	0.38		
$[(PM)(Glu)MH_2]^+$	3.05		19.41	
			22.78	
$[(PM)(Glu)MH_3]^{2+}$				
$[(PM)(Glu)MH_4]^{3+}$			26.88	

[a] Some of the species have more than one value of log K_1 or log K_2, depending on the involved protonated type of the binary species; for example, for [(PM)(Asp)CuH] there are two possibilities available: (i) $\log K_1 = \log \beta_{1111} - \log \beta_{1011} - \log \beta_{0110}$ and (ii) $\log K_1 = \log \beta_{1111} - \log \beta_{1010} - \log \beta_{0111}$.

complex enhancement for the 1:1:1:k (k = 0−4) can be realized if the following equilibrium reactions are examined:

$$\mathbf{M}(PM)H_i + \mathbf{M}(\text{Asp or Glu})H_j \rightleftarrows (PM)(\text{Asp or Glu})\mathbf{M}H_k;\ K_1 = \beta_{111k}/\beta_{101i}\,\beta_{011j}$$
where $k = i + j$ (charges are omitted for clarity) (2)

$$\mathbf{M}(PM)_2H_i + \mathbf{M}(\text{Asp or Glu})_2H_j \rightleftarrows 2(PM)(\text{Asp or Glu})\mathbf{M}H_k;\ K_2 = \beta^2{}_{111k}/\beta_{201i}\beta_{021j} \quad (3)$$

(for k = 0, 1, >1 the complex is negatively charged, neutrally charged, and positively charged, respectively).

Table 6 lists the values of log K_1 and log K_2 whenever enough information about the pertinent binary formation constants is available. Statistically, log K_1 should be greater than −0.6 and log K_2 should be greater than +0.6 for ternary complex enhancement.[6] The values shown in Table 6 mostly abide with this argument except for those for a few monoprotonated and unprotonated species. Although the ligand species in the 1:1:1:(0 or 1) complexes are considered bidentate, however, those involved in the species 1:1:1:(2 or 3 or 4) are not. The statistical values of log K_1 and log K_2 cannot be equally used for them, as for the former species, where tetragonal coordination of the metal should be assumed. The location of protons on different sites of the complex species cannot be simply predicted with the exception of the pyridinic nitrogen of PM, where it seems that it does not act as ligating site for Ni(II) and Cu(II) metal ions especially in the presence of other chelating sites. It has been noticed that the species other than 1:1:1:*i* only involve one more Asp or Glu molecule rather than more than one PM (Table 6), that is 1:2:1:*i* (PM/(Asp or Glu)/Cu(II)/H). In such a case Asp or Glu may act as a monodentate or bidentate ligand or a mixture of them. Of course, in the highly protonated species the monodentate state is preferable (e.g. in the case of 1:2:1:4). The preference of two Asp or Glu molecules by the ternary complex rather than two PM may imply that the latter molecule exerts a steric hindrance in the presence of the former ligands.

(B) Polarographic Study: (i) Cu(II) Binary and Ternary Systems. (See Supporting Information Figures 5−9 (representative polarograms of Cu(II) and Ni(II) systems). The differential pulse polarogram (DPP) of the $CuCl_2$ solution at pH ∼ 3.0 shows two cathodic peaks at E_{max} (the potential at maximum faradaic current (δ_i)) of −64 and −220 mV and one peak with negative δ_i at -348 mV. These peaks have been previously described as a result of the stepwise reduction of Cu^{2+} to Cu^+ and Cu^+ to Cu^0.[15] The stepwise reduction was attributed to the formation of stable chloro complexes of Cu(I). The peak with $-\delta_i$ may be due to the adsorption phenomena of the reduced species of the Cl^-−Cu(II) system. The addition of PM to Cu(II) solutions in the ratio 5:1 caused the loss of one of the cathodic DPP peaks (at −220 mV). Besides, the cathodic peak at −64 mV shifted to lower potential (∼ −80 mV) in the pH range 3.0−4.5 with a decrease in δ_i as the pH was increased. The peak with $-\delta_i$, on the other hand, was monotonically shifted toward more negative potential as the pH was increased with a decrease in δ_i. Three more cathodic peaks appeared at the pH's ∼4.5, ∼7.2, and ∼9.0 at −168, −316, and −80 mV, respectively. They were regularly shifted toward more negative potentials as the pH was increased. The stepwise reduction of Cu^{2+} to Cu^0 in chloride media almost disappears in the presence of PM at pH > 5.7, where complex formation of Cu(II) with PM is predominant. The single peak observed in the pH range ∼5.7−6.8 is likely due to a two-electron-transfer process, indicating that PM−Cu(I) complexes do not have the same stabilities as those of the Cl−Cu(I) system. However, as the pH increased beyond pH 7.0, another peak was observed at more negative potential which may show further stability for a PM−Cu(I) complex species. There is a possibility for the disproportionation of the PM−Cu(I) complex to produce back PM−Cu(II) complexes in addition to the free metallic Cu. The appearance of a peak at −80 mV at pH > 9.0 may be attributed to the formation of hydrolyzed complex species, leading to the liberation of hydroxocupric species which have reduction properties similar to those of the Cl^-−Cu(II) system. Figure 2 shows the effect of the pH's on the last three cathodic peaks and the single adsorption peak, E_A. The inflections in the curves indicate the transition stage from one species to another at different pH's. At least three species may be predicted from these inflections. The DPPs of Asp−Cu(II) and Glu−Cu(II) are similar to those of $CuCl_2$ at pH < 4.5, exhibiting the cathodic and adsorption peaks, except that the latter was shifted to more positive potentials. The δ_i values of these peaks decreased as the pH was increased. However, both systems are different in some aspects above pH 5.0. The Glu system lost the adsorption peak while the Asp system did not. However, at pH > 10 another adsorption peak appeared in the Asp system. In addition, a different clear cathodic peak emerged at pH 5.0 in the Glu system which showed an increase in δ_i as the pH increased with little shift in E_{max} toward more negative potentials (∼38 mV from pH 5.0 to 11.0). In the Asp system, the new cathodic peak was actually a split peak which was shifted more toward negative potentials than that in the Glu system. The same argument with respect to the PM−Cu(II) system can also apply to the Glu−Cu(II) system at pH < 9.0. However, the situation is different with respect to the Asp−Cu(II) system. Here again Asp−Cu(II) shows probably combined reduction and adsorption properties similar to those of the Cl^-−Cu(I) species, yet at more

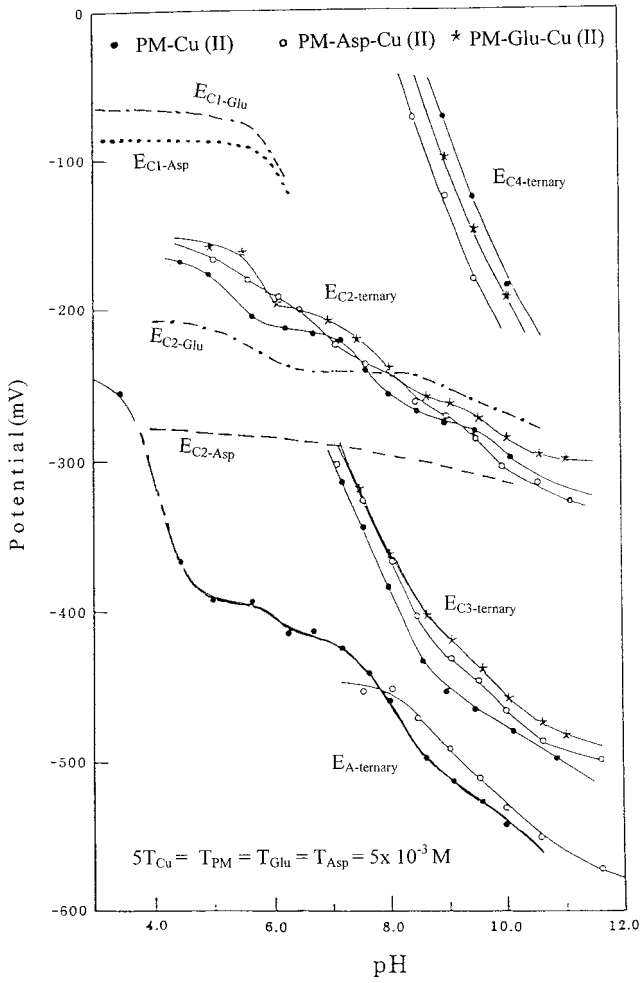

Figure 2. Variation of potential (mV) as a function of pH for the binary and ternary systems.

negative potential. The DPPs of both systems manifest the effect of the ligands on the reduction properties of Cu(II), although no drastic change has been observed as a function of pH. On the other hand, the ternary systems PM−Asp−Cu(II) or PM−Glu−Cu(II) showed similar polarograms to those for the PM−Cu(II) system with the exception of slight positive shift in E_{max} of the second and third cathodic peaks as the pH was increased (Figure 2). The shift was greater in the case of the PM−Glu−Cu(II) system than that of the PM−Asp−Cu(II) system, which proves the destabilization of the Cu(II) oxidation state in some species of the ternary systems. This finding may also point to an increase in the positive character of the ternary complexes over those of the PM−Cu(II) binary species. However, there is an indication of the stabilization of the Cu(II) oxidation state for some ternary species found at pH ≥ 8.5, as illustrated by the shift of the fourth E_{max} to lower potential with respect to that for the PM−Cu(II) system (Figure 2).

(ii) Ni(II) Binary and Ternary Systems. The Ni(II) E_{max} at −1044 mV (at pH ∼ 6.93 and 0.15 M NaCl) was lost completely from the DPP in the range 0 to −1300 mV when PM of 5 times the concentration of Ni(II) (=5 × 10^{-3} M) was used in the pH range ∼2.0−7.5 under identical experimental conditions. There was no indication whatsoever of a Ni(II) peak even if the concentration of PM was decreased to half the concentration of Ni(II) (= 1 × 10^{-3} M). This strange behavior cannot be simply explained in light of the Cu(II) behavior with PM, which forms more stable complexes than Ni(II). Although PM−Ni(II) showed no DPP in the pH range 2.0−7.5 in the 0 to −1300 mV potential range under the experimental conditions of this work, yet the Asp− or Glu−Ni(II) systems had a clear DPP in the −800 to −1300 mV potential range, in the pH range 2.4−10.2. There was no considerable shift in the E_{max} for Ni(II) as pH was increased from ∼2.4 to 4.6 in the Asp−Ni(II) system. However, a decrease in δ_i was observed as pH increased, indicating a drop in the free concentration of Ni(II) as the complex formation reaction proceeded. At pH > 5, three overlapped ill-defined cathodic peaks appeared in the potential range −1020 to −1140 mV which correspond to the reduction properties of the various binary species. Besides, another broad cathodic peak of irregular shape appeared at ∼1240 mV. As the pH increased from ∼5.0 to ∼8.0, all the cathodic peaks in this potential range gradually vanished. On the other hand, the Glu−Ni(II) system was little different from the Asp−Ni(II) system in that there was a shift in the E_{max} of the cathodic peak of Ni(II) toward more positive potential (from −1040 to −970 mV) as the pH was increased from 2.5 to 6.0. Above pH 6.0 a new overlapped ill-defined broad cathodic peak appeared. In addition, a possible adsorption peak also emerged. The cathodic and adsorption (negative current) peaks disappeared at pH > 8.0 with the appearance of a new cathodic peak at E_{max} ∼ −1260 mV. Surprisingly, the ternary systems of PM−Glu (or Asp)−Ni(II) did not show any of the Ni(II) cathodic or adsorption peaks exhibited by the binary complexes of the systems Asp− or Glu−Ni(II), a conclusion which emphasizes the effect of PM on the Ni(II) reduction properties shown earlier in the PM−Ni(II) system.

(C) UV−VIS−NIR Absorption Spectral Study. Figures 3 shows the spectra of the binary and ternary Cu(II) systems at given pH's. The spectra of the ternary systems are quite different from those of the binary systems, emphasizing the formation of the former in solution. Figure 4 shows the variation of absorption as a function of pH at 750 nm for the binary and ternary systems. The absorbance behavior for the ternary systems is dissimilar from that of the binary systems under the same conditions, especially at pH > 4.0, confirming the results obtained from the potentiometric studies. Although precipitation is encountered in the Asp binary systems, the presence of PM facilitates to a certain limit the solubility of the ternary system.

(D) EPR Spectral Study of Cu(II) Systems: (See Supporting Information for figures showing the EPR spectra of the binary and ternary Cu(II) systems.) ***(i) PM−Cu(II) System.*** The EPR spectra of Cu(II) (at 0.15 M ionic strength) consist of one broad signal at 3209 G with the g_{av} factor equal to 2.193 at pH < 6.0. This broad line indicates no Cu(II) nuclear spin−electron spin coupling, especially, in the presence of weakly complexing media. The broad signal disappears at pH > 6.2, probably due to the formation of antiferromagnetic polymeric species. However, the EPR of Cu(II) in the presence of PM is different. It shows three unequal signals at pH < 4.5 and five signals (∼ symmetric of unequal intensities) in the pH range 4.5−5.9.

The coupling constant is ∼61 G. These facts manifest the delocalization of the Cu(II) unpaired electron over the PM moiety (in the localized form Cu(II) nuclear spin−electron spin coupling leads to four signals of equal intensities). The three signals are attributed to the delocalization of the Cu(II) unpaired electron on one PM molecule, indicating the formation of (1:0:1:i) species where the ligation is through the nitrogen of the amino group and the oxygen of the phenoxy group. On the other hand, the

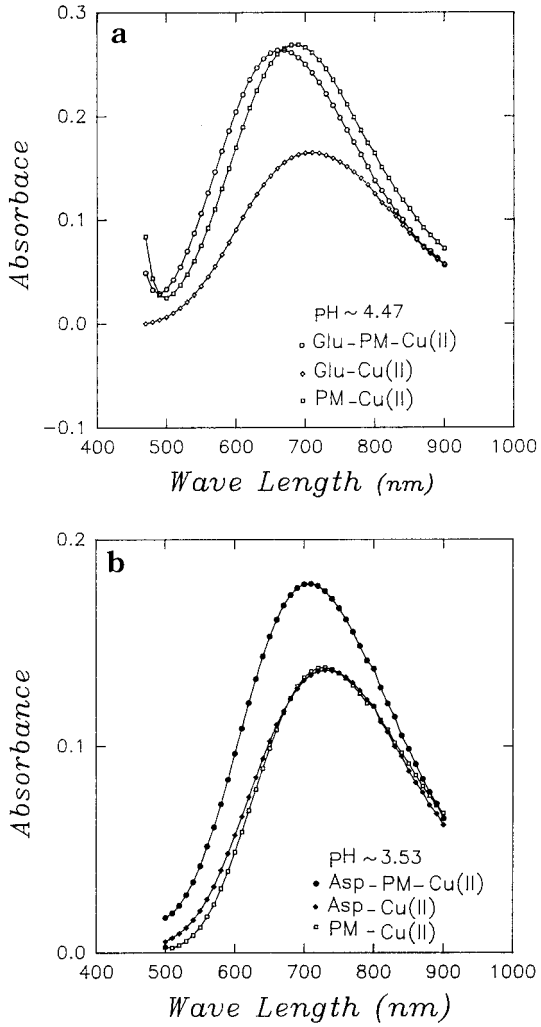

Figure 3. Spectra of the binary and ternary systems at given pH's. (a) PM−Glu−Cu(II); (b) PM−Asp−Cu(II).

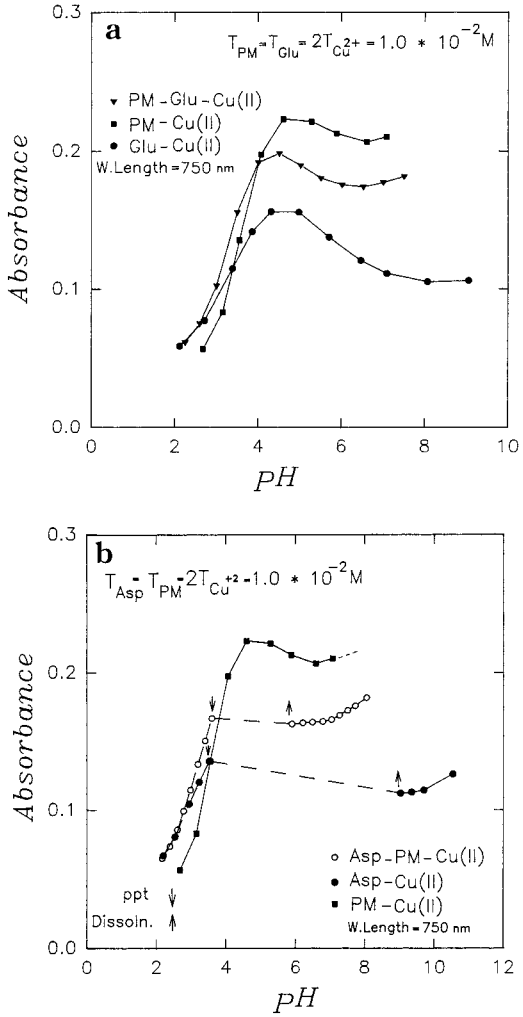

Figure 4. Variation of absorption as a function of pH at 750 nm for the binary and ternary systems of (a) PM−Glu−Cu(II) and (b) PM−Asp−Cu(II).

five signals are attributed to the delocalization of the Cu(II) unpaired electron over two PM molecules, indicating the formation of (2:0:1:*i*) species.

In the pH range ~6.0 to ~7.6, the symmetry in the signal pattern is lost. However, the number of signals is still five, probably due to the loss in planarity of the complex with the ligating atoms by the formation of hydroxy species. Above pH 7.6 almost one signal appears with small overlapped signals, indicating the loss of N nuclear spin−electron spin coupling, possibly due to the hydrolysis of the metal complex.

(ii) Asp−Cu(II) or Glu−Cu(II) Systems. The EPR spectra of Cu(II) in the presence of Asp or Glu are similar in many respects. At pH < 3.7, four signals of unequal intensities are shown, corresponding to a combination of free Cu(II) ion and 0:1:1:*i* species (PM/Asp or Glu/Cu(II)/*i*H). The three extra signals are probably due to the delocalization of the unpaired electron over the Asp or Glu ligands where ligation is through the nitrogen of the amino group and the oxygen of the carboxylic group.

In the pH range 3.7−4.9, five signals of unequal intensities are observed. This is probably due to the formation of 0:2:1:*i* species where the delocalized unpaired electron of Cu(II) takes its place over the two nitrogen atoms of the two Asp or Glu ligands. However, at pH > 4.9, only four signals are observed. This may be explained as due to the involvement of the second carboxylate group in bonding with partial loss of complete delocalization of the Cu(II) unpaired electron on the two nitrogen atoms of the two Asp or Glu molecules or may be attributed to the hydrolysis (formation of hydroxy species) of the complexes with considerable loss of bonding with the nitrogen atoms of Asp or Glu.

The plots of d*I*/d*H* (the first derivative of EPR intensity (*I*) with respect to the magnetic field strength (*H*)) versus pH at ~3367 G (the positive peak of the free Cu(II) EPR) show significant differences among Asp−Cu(II), PM−Cu(II), and Glu−Cu(II) binary systems, (Figure 5). Usually, there is a sharp increase in d*I*/d*H* as the pH increases from 2.0 to 3.5. The (d*I*/d*H*)−pH dependence is however tremendously different among the three binary systems after pH 3.5, being decreased as the pH increased in the pH range 3.5−4.5 for the Asp−Cu(II) system, in the pH range 3.5−8.0 for the PM−Cu(II) system, and in the pH range 7.0−8.0 for the Glu−Cu(II) binary systems. These differences can be attributed to partial precipitation, which may be accompanied by the loss of paramagnetism.

However, Glu− or Asp−Cu(II) systems show an increase in d*I*/d*H* in the pH range 8.0−10.0, in the Glu−Cu(II) system, and in the pH range 4.5−6.0, in the Asp−Cu(II) system, which are attributed to the formation of new ligation centers probably through the O atoms rather than the N atoms.

(iii) PM−Asp− or −Glu−Cu(II) Ternary Systems. The EPR spectra of the ternary systems are similar in

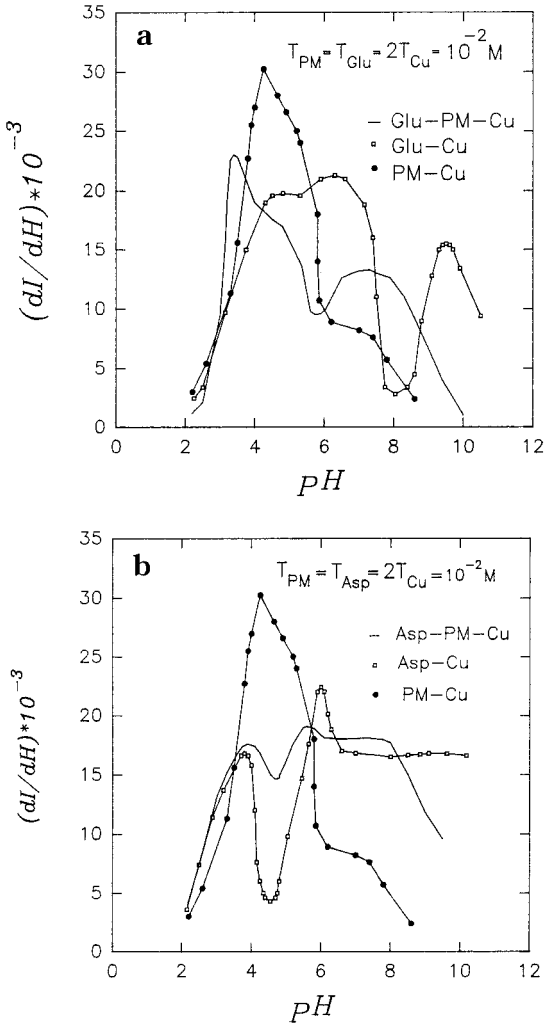

Figure 5. Dependence of d*I*/d*H* as a function of pH at ~3367 G for the binary and ternary systems of (a) PM−Glu−Cu(II) and (b) PM−Asp−Cu(II).

many respects to those of the binary systems Asp or Glu or PM−Cu(II) except that the intensity dependences on pH (e.g. at ~3367 G) are different (Figure 5). This observation indicated that the spin concentration of the ternary species differs appreciably from that of the binary species. In addition, the spin concentration of the PM−Asp−Cu(II) ternary system differs from that of the ternary system PM−Glu−Cu(II). The following conclusions can be drawn:

(a) The decrease in d*I*/d*H* at pH ~ 4.0 in the Asp−Cu(II) and PM−Cu(II) systems are much less in the ternary system PM−Asp−Cu(II), indicating that ternary species are less hydrolyzed; that is the replacement of N atom ligation with O atom ligation is less at pH < 8.0 (Figure 5a). This is confirmed by the higher solubility of the ternary species.

(b) The spin concentration of the ternary species PM−Glu−Cu(II) is different from those of the Glu−Cu(II) binary system in the pH range ~3.0−6.0 (Figure 5a). This behavior indicates more involvement of O atom ligation than N atom ligation; that is, the species involve the carboxylate group of Glu.

(c) The spin concentration dependence on pH of the ternary species PM−Asp−Cu(II) (Figure 5b) is similar in pattern to that of Asp−Cu(II) in the pH range ~2.0−5.5 although the minimum in intensity at pH ~ 4.5 is greater in the case of the ternary species, which reflects the difference in the type of the ligating atoms in both systems; that is, the PM−Asp−Cu(II) system involves less O-ligating atoms than that of Asp−Cu(II).

(d) The spin concentration of the Asp−Cu(II) system is different from that of the Glu−Cu(II) system, especially in the pH range ~3.5−6.5. It is almost constant in the Glu−Cu(II) system, indicating more involvement of N atom ligation in the latter system.

Supporting Information Available:

Figures showing the distribution of the ternary species as a function of pH, representative polarograms at various pH's for the binary systems, and EPR spectra of the binary and ternary Cu(II) systems. This material is available free of charge via the Internet at http://pubs.acs.org.

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