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Pteridine-Containing Ternary Copper(II) Complexes as Pterin Cofactor–Metal Binding Models. Structures, Solution Equilibria, and Redox Activities

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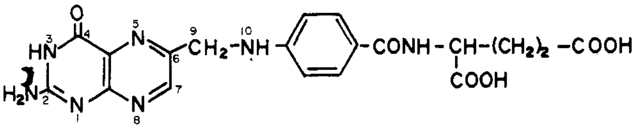
The structures and properties of the ternary Cu(II) complexes Cu(DA)(Pte), where DA = 2,2′-bipyridine (bpy), 1,10-phenanthroline (phen), or ethylenediamine (en) and Pte = folic acid (FA), lumazine (LM), or related compounds, have been investigated by spectroscopic, potentiometric, and electrochemical methods. The pteridine nucleus of FA and LM in the ternary complexes has been concluded to coordinate to Cu(II) through the oxygen and the nitrogen (N(5)) atom. The electronic absorption and ESR spectra indicate that the complexes Cu(DA)(FA) with DA = bpy or phen in aqueous solution have square-pyramidal structures with at least three nitrogens in the coordination plane, while Cu(en)(FA) has a planar structure. The stability constants for Cu(DA)(LM) determined at 25 °C and $I = 0.1$ (KNO_3) revealed that the ternary species predominate at neutral–alkaline pH in the 1:1:1 Cu(II)–DA–LM systems. Alkaline solutions (pH >10) of Cu(DA)(FA) with DA = bpy exhibit a spectral change due to oxidative cleavage of the side-chain $-CH_2-NH-$ group of FA, giving a ternary complex containing pterin-6-carboxylate (PC), Cu(bpy)(PC)·$5H_2O$, as crystals under aerobic conditions. A similar spectral change was observed for Cu(phen)(FA) but not for Cu(en)(FA). The Cu(DA)(FA) and Cu(DA)(LM) systems with DA = bpy and phen have been shown by cyclic voltammetry to have a higher reduction potential, $E_{pc} = -0.20$ and -0.26 V (vs SCE), respectively, than that for DA = en (-0.32 V), which indicates that the side-chain oxidation of FA is effected by the central metal ion and a π-accepting ligand as well as the structure of the ternary complex formed.

Introduction

Natural pteridine derivatives such as folic acid (FA, **1**) and biopterin (2-amino-6-(1,2-dihydroxypropyl)-4-hydroxypteridine)



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are important cofactors in biological redox reactions.[2] Phenylalanine hydroxylase and other aromatic amino acid hydroxylases, which catalyze the hydroxylation of aromatic rings by molecular oxygen and are essential for the synthesis of neurotransmitters, require iron and tetrahydrobiopterin for their activity.[2] Very recently phenylalanine hydroxylase from *Chromobacterium violaceum*[3] has been reported to involve copper in place of iron,[4] and complex formation between the copper and the cofactor has been indicated by ESR spectra.[5] The prosthetic group of oxomolybdoenzymes (Mo-co) also has a pterin (2-amino-4-hydroxypteridine) derivative proposed to be bound to molybdenum.[6,7] Since pterins as cofactors in biological redox reactions are thus associated with metal ions and contribute to electron transfer, information on pterin–metal interactions may give clues to the roles of metal ions in utilization of dioxygen and concomitant electron transfer in relevant enzymatic reactions.

With these points in mind, we studied the structures and properties of ternary complexes containing Cu(II), diamines (DA), and FA and related compounds (Pte). The complex formation between FA and metal ions have been studied by several groups of investigators. Albert concluded from potentiometric studies that pteridines coordinate to Cu(II) through the O(4) and N(5) atoms in the manner similar to 8-hydroxyquinoline,[8] while tetrahydrofolate has been reported to bind with Pd(II) through N(5) and N(10).[9] Fridman et al. made a spectroscopic study of the ternary Cu(II)–glycinate–FA complex and proposed a structure where Cu(II) interacts with the glutamate moiety of FA.[10] Because of the electron deficiency of the pterin nucleus, the α-carbon of the 6-substituent is activated to undergo various redox reactions; FA is known to be oxidized at this carbon to give pterin-6-carboxylic acid (PC) by alkaline permanganate[11] and pterin-6-carboxaldehyde by aqueous hydrogen peroxide.[12] In the course of the present study we found that the ternary system Cu(bpy)(FA) exhibits a unique color change in water at pH >10 due to redox reactions that give a complex of PC, Cu(bpy)(PC)·$5H_2O$.

In this report we describe the results of potentiometric, spectral, and redox investigations on the ternary systems with emphasis on metal ion–pterin ring interactions and the effect of π-accepting ligands.

Experimental Section

Materials.[13] FA, bpy, phen, and en were purchased from Nakarai Chemicals, and ABG, oxine, and meox were from Tokyo Kasei Kogyo. PC was prepared according to the literature[14] by the oxidative degradation of FA with an acidic chlorate solution. Copper(II) nitrate trihydrate was used throughout the experiments. All reagents used were of analytical grade or of the highest grade available, and distilled and deionized water was used throughout.

Isolation of Cu(bpy)(PC). An aqueous solution (pH ~11) containing equimolar amounts of $Cu(NO_3)_2$, bpy, and FA was kept at room temperature under aerobic conditions for 2 weeks. Green needles separated from the reaction mixture. Recrystallization from hot water gave the complex, Cu(bpy)(PC)·$5H_2O$, as green needles. Anal. Calcd for $C_{17}H_{11}N_7O_3Cu·5H_2O$: C, 39.65; H, 4.11; N, 19.04. Found: C, 40.22; H, 4.22; N, 18.88. PC was identified by comparison with the authentic sample after isolation from the complex through a DEAE cellulose column.

Spectral Measurements. Absorption and circular dichroism (CD) spectra were measured with a Hitachi 330 recording spectrophotometer

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(13) The following abbreviations were used: FA, folic acid; Pte, FA-related compounds; LM, lumazine (=2,4-dioxopteridine); ABG, (*p*-aminobenzoyl)glutamic acid; PC, pterin-6-carboxylic acid; bpy, 2,2′-bipyridine; phen, 1,10-phenanthroline; en, ethylenediamine; oxine, 8-hydroxyquinoline; meox, 2-methyl-8-hydroxyquinoline.

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Table I. Stability Constants, log β_{pqrs}, for $Cu_p(DA)_q(LM)_r(H)_s$ at 25 °C and I = 0.1 M(KNO_3)[a]

stability const	species, *pqrs*	DA			LM
		en	bpy	phen	
log β_{pqrs}	0011				11.423 (4)
	0012				19.120 (4)
	1011				15.45 (3)
	1022				30.58 (9)
	0101	9.976[b]	4.503[b]	4.95[c]	
	0102	17.148[b]			
	1100	10.523[b]	8.10[b]	9.25[c]	
	1200	19.505[b]	13.44[b]	16.00[c]	
	1110	17.86 (2)	16.17 (1)	17.29 (1)	
	1111	25.25 (1)	23.51 (2)	24.65 (2)	
log β_{1111}(calcd)	1111	25.99	23.57	24.72	
log β_{stat}	1111	25.34	22.31	23.59	
log β(calcd)	1111	24.97	22.97	24.12	
Δ(log β)	1111	–0.09	1.20	1.06	
log X	1111	0.42	3.00	2.72	

[a] Values in parentheses denote estimated standard deviations. [b] Taken from ref 17a. [c] Taken from ref 17b.

and a JASCO J-500C spectropolarimeter, respectively, for aqueous solutions (2–5 mM) of Cu(DA)(Pte) at various pH values. Electron spin resonance (ESR) spectra (X-band) were recorded for the corresponding solutions at 77 K on a Varian E109 or a JEOL JES FX-1 ESR spectrometer.

pH Titrations. The pH titrations were carried out for binary systems Cu(II)–LM and ternary systems Cu(II)–DA–LM at 25 °C and I = 0.1 M (KNO_3) under a nitrogen atmosphere. The concentrations of the samples were 2 mM with respect to Cu(II), which was used as the complexes of DA's, $Cu(en)SO_4{\cdot}2H_2O$, $Cu(bpy)(NO_3)_2{\cdot}3H_2O$, and $Cu(phen)(NO_3)_2{\cdot}0.25H_2O$. The pH values were measured with an Orion 901 ion analyzer or a Beckman pHI 71 pH meter both equipped with a Beckman 39314 glass electrode and an Orion 91-02-00 double-junction reference electrode. The pH meter was calibrated with the NBS standard buffer solutions (pH 4.008, 7.413, and 9.180 at 25 °C). Duplicate or triplicate titrations were made for each system, a total of 300–400 data being collected. The stability constant β is defined by eq 1 (charges are omitted), where p, q, r, and s denote the moles of Cu(II), DA, LM, and

$$p\mathrm{Cu} + q\mathrm{DA} + r\mathrm{LM} + s\mathrm{H} \xrightleftharpoons{\beta_{pqrs}} \mathrm{Cu}_p(\mathrm{DA})_q(\mathrm{LM})_r(\mathrm{H})_s$$

$$\beta_{pqrs} = \frac{[\mathrm{Cu}_p(\mathrm{DA})_q(\mathrm{LM})_r(\mathrm{H})_s]}{[\mathrm{Cu}]^p[\mathrm{DA}]^q[\mathrm{LM}]^r[\mathrm{H}]^s} \qquad (1)$$

proton (H), respectively, in the complex. The log β_{pqrs} values were calculated by the method of nonlinear least-squares using the computer program SUPERQUAD[15] with the aid of a FACOM M-170F computer at the Kanazawa University Information Center. The pH meter readings (pH_m) were converted to the hydrogen ion concentrations by the relationship $[H^+] = 10^{-pH_m}/0.855$.[16] The pK_a values of DA's and the stability constants of their binary complexes were taken from the literature.[17]

Electrochemical Measurements. Cyclic voltammograms were obtained with an assembly composed of a Hokuto Denko HA501 potentiostat and an HB104 function generator. A glassy-carbon disk (diameter = 3 mm) and a platinum plate were used as a working electrode and a counter electrode, respectively, and a TOA saturated calomel electrode (SCE) was used as the reference electrode with a 1% agar salt bridge (0.1 M KNO_3). Measurements were made at 25 °C under an argon atmosphere for aqueous solutions (2 mM) of the Cu(DA)(FA) and Cu(DA)(LM) systems with KNO_3 or $NaClO_4$ (0.1 M) as a supporting electrolyte.

Measurement of Dioxygen Consumption. The dioxygen consumed in the course of the oxidation reaction of FA with $Cu(bpy)^{2+}$ was measured volumetrically at 27 °C for an aqueous solution (30 ml, pH 11.5) containing equimolar amounts (17 mM) of $Cu(NO_3)_2$, bpy, and FA.

Results and Discussion

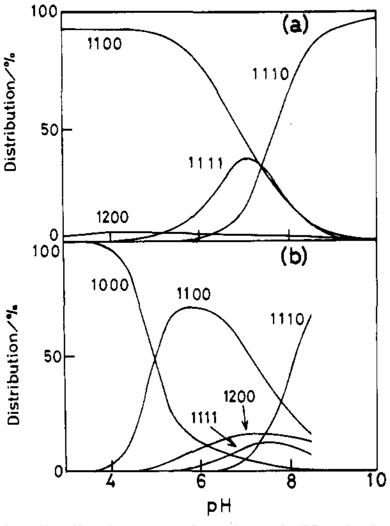

Figure 1. Species distributions as a function of pH calculated for 1 mM solutions of 1:1:1 Cu(II)–bpy–LM (a) and 1:1:1 Cu(II)–en–LM (b) mixtures. Species are indicated on the curves as *pqrs*. The distributions were not calculated for the alkaline region of curve b, where precipitation occurred.

Solution Equilibria. Because of the insolubility of FA at low pH, the complex formation reactions have been investigated for the binary and ternary systems involving LM as a substitute for FA. The systems 1:2 Cu(II)–LM and 1:1:1 Cu(II)–en–LM gave precipitates at pH ~6.7 and 7.7–8.0, respectively. The stability constants calculated from the titration data are listed in Table I, where the values estimated theoretically from eq 2, 3,[18] and 4[19]

$$\log \beta_{1111}(\mathrm{calcd}) = \log \beta_{1011} + \log \beta_{1100} \qquad (2)$$

$$\log \beta_{\mathrm{stat}} = \tfrac{1}{2}(\log \beta_{1022} + \log \beta_{1200}) + \log 2 \qquad (3)$$

$$\log \beta(\mathrm{calcd}) = \log \beta_{1011} + \log \beta_{1100} + \sum^{i}\sum^{j}\delta_{ij}X_i(\mathrm{DA})\;Y_j(\mathrm{LM}) \qquad (4)$$

are also included. In these equations, δ_{ij} is the ligand interaction term, which allows for the effect of the donor atom X_i of DA on the donor atom Y_j of LM, and X_i(DA) and Y_j(LM) are the numbers of X_i and Y_j, respectively. Preferential formation of ternary complexes may be evaluated by Δ(log β) and log X[20] defined as follows:

$$\Delta(\log \beta) = \log \beta_{1111} - \log \beta_{\mathrm{stat}} \qquad (5)$$

$$\log X = 2 \log \beta_{1111} - (\log \beta_{1022} + \log \beta_{1200}) \qquad (6)$$

The stability constants for the ternary complexes are best estimated by eq 2, which apparently indicates that there is no through-metal ligand–ligand interaction between DA and LM. However, the Δ(log β) and log X values clearly show that the ternary complexes with DA = bpy or phen are preferred more than expected on the statistical ground. Equation 4, which accommodates the electronic effects of the combination of ligand atoms around the central metal ion by the empirical parameters, failed to predict the stability constants, indicating that there is a stabilizing factor for the complex formation. This suggests that the 4-OH group coordinates to Cu(II) in the phenolate form, because, according to Sigel,[20] aromatic nitrogens as π-acceptors are favorably combined with phenolate or carboxylate oxygens as π-donors around the central metal ion. The species distributions as a function of pH calculated for 1:1:1 Cu(II)–DA–LM systems (DA = bpy and en) from the stability constants in Table I show that the ternary species 1111 and 1110 predominate at pH >8 and that they are less predominant in the Cu(en)(LM) system (Figure 1).

Spectral Properties. The spectra were measured for 1:1:1 Cu(II)–DA-Pte systems at pH 5.5–10 in order to avoid the mentioned insolubility of FA and facile hydrolysis of its binary complex (Table II). The d–d absorption peaks for the Cu(DA)(FA) systems were observed at 638 and 880 nm for DA = bpy and at 655 and 880 nm for DA = phen at pH ~9.5, indicating

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Table II. Absorption and CD Spectral Data for Cu(DA)(Pte) Systems in Water

		absorption		CD	
system	pH	λ_{max}/nm	ϵ/Cu^{-1} cm^{-1}	λ_{max}/nm	$\Delta\epsilon$/Cu^{-1} cm^{-1}
Cu(bpy)(FA)	8.0	650	56	640	0.030
	9.0	643	56	625	0.017
	9.4	638	56	615	0.011
		880	21		
Cu(phen)(FA)	8.5	655	60	605	-0.008
	9.5	655	67	666	-0.006
		880	28		
Cu(bpy)(LM)	7.0	640	48		
	8.0	625	76		
	9.0	615	65		
Cu(en)(LM)	7.0	620	50		
	8.0	565	70		
	9.0	580	73		
Cu(bpy)(ABG)	8.1	623	61	675	0.012
	9.0	617	64	670	0.004
	9.6	617	62	[a]	[a]
Cu(phen)(ABG)	5.5	686	44	703	0.038
Cu(en)(ABG)	6.5	635	42	730	-0.007
Cu(bpy)(meox)	[b]	650	74		
		366[c]	663		

[a] Not observed. [b] Measured in methanol. [c] Shoulder.

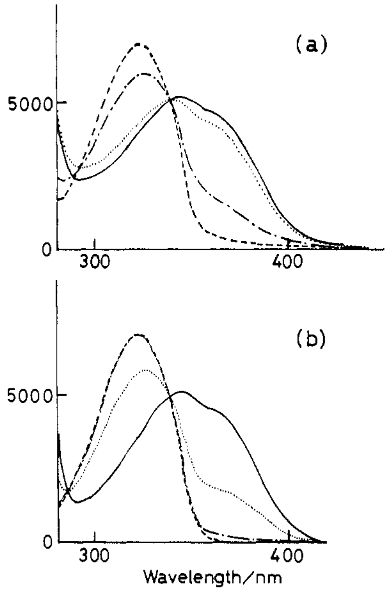

Figure 2. Absorption spectra of Cu(en)(LM) (a) and LM (b) at pH 6.0 (---), 8.0 (--), 9.0 (···), and 10.0 (—).

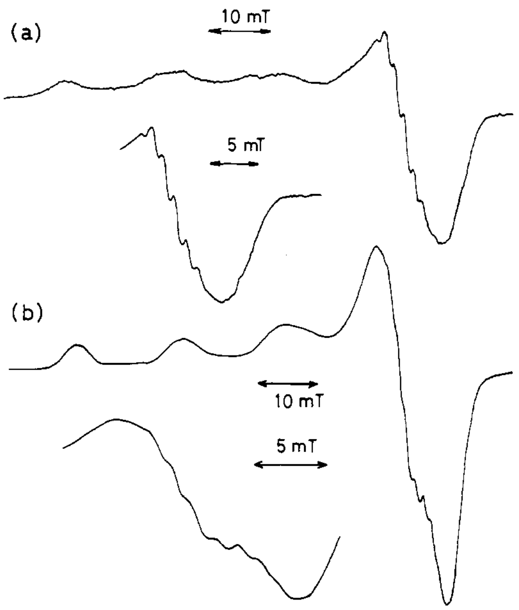

Figure 3. ESR spectra of Cu(bpy)(FA) at pH 9.9 (a) and Cu(bpy)(LM) at pH 9.0 (b) at 77 K. Conditions: (a) microwave frequency, 9.158 GHz; microwave power, 10 mW; modulation, 0.32 (0.25) mT; (b) microwave frequency, 9.272 GHz; microwave power, 1 mW; modulation, 0.63 (0.63) mT.

Table III. ESR Parameters for Cu(bpy)(Ptc) at 77 K

system	pH	$g_\perp$	$g_\parallel$	$\lvert A_\parallel\rvert$/mT	$\lvert A_N\rvert$/mT
Cu(bpy)(FA)	9.9	2.07	2.26	17.0	1.4
			2.30	14.8	
Cu(bpy)(LM)	9.0	2.05	2.21	14.4	1.4
Cu(bpy)(oxine)	[a]	2.06	2.26	17.0	1.4
Cu(bpy)(meox)	[a]	2.07	2.28	14.8	1.3

[a] Measured in methanol.

that the complexes have a pentacoordinate structure.[21] The glutamate moiety of FA and ABG is probably involved in the complex formation at favorable pH, because a weak CD peak was observed for the corresponding systems in the d–d region at neutral–weakly alkaline pH. However, the peak for Cu(bpy)(FA) decreased in magnitude with the pH increase. This implies that part of Cu(II) is bound at the glutamate moiety at neutral pH, whereas at higher pH it is mainly bound at the pterin ring. Because the d–d peak of Cu(bpy)(ABG) at 617 nm at pH ≥9 is very similar to that observed for Cu(bpy) at the same pH, ABG is most probably removed from the Cu(II) coordination site in alkaline solution, which is supported by the disappearance of the CD peak. The ternary system Cu(en)(LM), which lacks the ABG moiety, shows a d–d band centered at 580 nm at pH 9.0, indicating a 3N1O chromophore consistent with the coordination by the pterin ring. Figure 2 illustrates that the shift of the peak at 320 nm to give two overlapping peaks at ~340 and ~360 nm due to ionization of the OH groups is more apparent at lower pH (~6) in the ternary systems Cu(DA)(LM) than in the system with LM alone (pH ~8). This ionization is concomitant with the formation of ternary species 1111 and 1110 as seen from Figure 1. Similar spectral changes have been observed for the interactions of Cu(II) and other metal ions with the flavin ring, which incorporates the lumazine structure.[22]

Comparison of the spectra of Cu(DA)(FA) with the spectrum of Cu(bpy)(meox) suggests that the pterin ring of FA coordinates to Cu(II) through the phenolate O(4) and the N(5) atom; although the Cu(DA)(FA) systems (DA = bpy or phen) showed strong absorption bands at 400 nm due to the pterin ring, the difference spectrum obtained by subtracting the spectra for Cu(bpy) and FA from the spectrum of Cu(bpy)(FA) gave a peak at 400 nm, which is assigned to the phenolate $O^- \rightarrow Cu(II)$ charge transfer (CT) by referring to the spectrum of Cu(II)–bpy–meox showing the CT peak at 366 nm as a shoulder. The coordination through O(4) and N(5) by FA and LM is supported by the same conclusion drawn for binary metal–Pte complexes.[8]

The ESR spectra of axial symmetry were obtained for Cu(bpy)(FA), Cu(bpy)(LM), and related systems (Figure 3 and Table III). The spectrum for Cu(bpy)(FA) at pH 9.9 (Figure 3a) shows the copper hyperfine structures due to two species with $g_\parallel$ = 2.26 and 2.30, the $|A_\parallel|$ values being 17.0 and 14.8 mT, respectively. From $g_\parallel > g_\perp = 2.11$, the Cu(II) in the above complex is concluded to have a $d_{x^2-y^2}$ ground state.[21] The two species indicated by the copper hyperfine structures may arise from different structures or from the products of the oxidation reactions described below. The system Cu(bpy)(LM) shows a spectrum for a single species with $g_\parallel$ = 2.21, $g_\perp$ = 2.05, and $|A_\parallel|$ = 14.4 mT (Figure 3b). Both spectra have seven nitrogen superhyperfine

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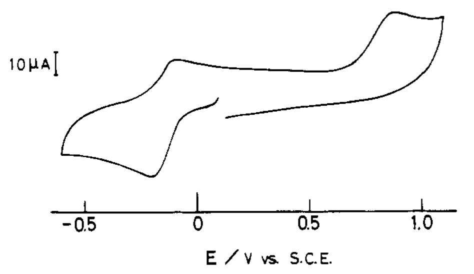
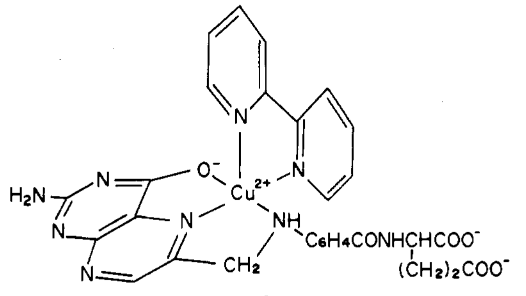


Figure 4. Cyclic voltammogram for Cu(bpy)(FA) in 0.1 M $NaClO_4$ (pH 9.5) at 25 °C. Scan rate: 100 mV s^{-1}.

structures in the $g_\perp$ region, which is compatible with three coordinated nitrogen atoms from bpy and FA or LM in the coordination plane. The spectra exhibited by Cu(bpy)(oxine) and Cu(bpy)(meox) have parameters similar to those of Cu(bpy)(FA). Interestingly the ESR parameters reported for a pterin adduct of Cu-containing phenylalanine hydroxylase are $g_\parallel = 2.27$ and $|A_\parallel| = 166 \times 10^{-4}$ cm^{-1} (15.7 mT),[5] suggesting that the copper chromophore of the enzyme has a 3N1O coordination.[23]

Structures of the Ternary Complexes. Solution equilibria of the ternary systems show that the species 1111 and 1110 predominate in the neutral–alkaline region. From the additional d–d absorption peak observed at 880 nm and suggestive of a pentacoordinate structure, we infer that Cu(bpy)(FA) has the square-pyramidal structure **2**, where FA coordinates to Cu(II)



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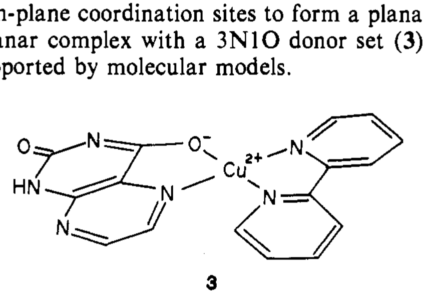
as a tridentate ligand through its O(4), N(5), and side-chain nitrogen (N(10)) atoms, forming two consecutive five-membered rings. The structure is reasonably supported by space-filling models, and as reported for Cu(Gly·Gly)(phen)[24] (Gly·Gly = glycylglycine), bpy probably occupies one equatorial and one axial position. On the other hand, the d–d absorption peak for Cu(en)(LM) and the ESR spectrum with a rather small $|A_\parallel|$ value for Cu(bpy)(LM) indicate that both ligands in Cu(DA)(LM) share the in-plane coordination sites to form a planar or a distorted[25] planar complex with a 3N1O donor set (**3**), which is further supported by molecular models.



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Electrochemistry of Ternary Complexes. Electrochemical properties of the ternary systems were studied by cyclic voltammetry in 0.1 M KNO_3 or $NaClO_4$ (Table IV).[26] Figure 4 shows the cyclic voltammogram for Cu(bpy)(FA), indicating a quasi-reversible one-electron reduction with $\Delta E_p = E_{pa} - E_{pc} = 100$ mV.

Table IV. Cyclic Voltammetric Data for Cu(DA)(Pte) in Aqueous Solution at 25 °C[a]

system	pH	E_{pc}/V	E_{pa}/V	ΔE/mV	electrolyte (0.1 M)
Cu(bpy)(FA)	9.5	-0.20	-0.10	100	$NaClO_4$
Cu(phen)(FA)	9.5	-0.24	-0.10	140	KCl
Cu(en)(FA)	9.0	-0.32	...		KNO_3
Cu(bpy)(LM)	7.0	-0.16	-0.05	110	KNO_3
Cu(phen)(LM)	7.0	-0.25	...		KNO_3
Cu(en)(LM)	7.0	-0.30	...		KNO_3

[a] Scan rate, 100 mV s^{-1}; potentials are reported vs SCE.

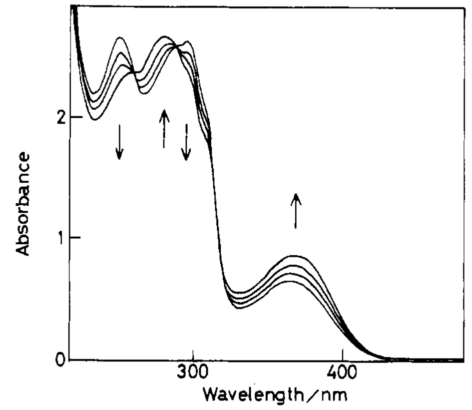

Figure 5. UV spectral change with time of Cu(bpy)(FA) at pH 11.5 and room temperature. Conditions: 1:1:1 Cu(II)–bpy–FA mixture (0.4 mM); path length, 2 mm; time interval, 3 h.

The E_{pc} values for the ternary systems with aromatic DA's are higher than those with DA = en, which may be ascribed to stabilization of the Cu(I) state by π-back-donation from Cu(I) to the aromatic rings of DA through coordinated nitrogens. The Cu(DA)(LM) systems have slightly higher E_{pc} values than Cu(DA)(FA). Comparison of the observed and calculated cathodic peak currents I_{pc}[27] of Cu(bpy)(FA) at various scan rates supported a one-electron reduction process. The system Cu(en)(LM) showed a broad reduction curve with $E_{pc} = -0.30$ V, corresponding to the Cu(II)/Cu(I) reduction process, but no Cu(I)/Cu(II) oxidation was observed. In the presence of dioxygen, the Cu(bpy)(FA) system exhibited no Cu(I)/Cu(II) oxidation process, suggesting that the Cu(I) species suffers from oxidative decomposition.

Oxidative Cleavage of the FA Side Chain Due to Redox Reactions of Cu(bpy)(FA) in Alkaline Solution. The side chain of FA is known to be susceptible to oxidations by alkaline permanganate solution and hydrogen peroxide to give PC[11] and pterin-6-carbaldehyde,[12] respectively, by the oxidative cleavage of the C(9)–N(10) bond. The color of an alkaline solution containing a 1:1:1 Cu(II)–bpy–FA mixture (pH >10) changed from green ($\lambda_{max} = 633$ nm) to red, and the red solution turned green ($\lambda_{max} = 610$ nm) again when allowed to stand in the open air or when O_2 was bubbled into the red solution. The color change strongly suggested redox reactions, and Cu(bpy)(PC)·$5H_2O$ was actually isolated as crystals from the green solution after the mixture was allowed to stand at room temperature for 2 weeks. In the course of the ultraviolet spectral changes observed for Cu(bpy)(FA) in the open air, a peak appeared at 280 nm and the absorbances at 252 and 295 nm decreased with a concomitant increase at ~370 nm (Figure 5). Isosbestic points at 262, 289, and 318 nm imply that the reaction mixture is mainly composed of two absorbing species. The peaks at 280 and 370 nm correspond well with the 279- and 378-nm peaks respectively exhibited by Cu(bpy)(PC) at pH 8.0. An analogous spectral change was also observed for Cu(phen)(FA). In this connection, the spectrum for Cu(en)(FA) remained unchanged for over 2 weeks and no redox reaction took place in this system. In the absence of dioxygen the spectral change of Cu(bpy)(FA) was smaller, but there was

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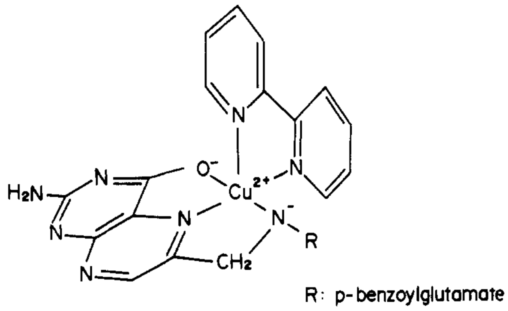
an increase of absorption at 370–450 nm, where a new band centered at 435 nm was detected by measuring the difference spectra. Since the Cu(I) complexes of bpy and related ligands are reported to have a metal-to-ligand CT (MLCT) band around this wavelength,[28] the 435-nm peak is reasonably assigned to MLCT and serves as evidence for the occurrence of the Cu(I) state in the reaction process.

When shaken for 200 min under O_2 at 27 °C, an alkaline solution (pH 11.5) of Cu^{II}(bpy)(FA) absorbed ca. 0.4 mol of O_2/mol of Cu. Because the green → red color change occurs under N_2, the redox reaction leading to the Cu(I) complex takes place irrespective of the presence of O_2. Formation of pterin-6-carbaldehyde in the red solution[29] suggests that O_2 may be used for conversion of the aldehyde to PC, which may also be formed in part by the Cannizzaro reaction of the aldehyde.

The redox reaction observed for Cu(DA)(FA) (DA = bpy or phen) requires high pH (>10), which indicates that the reaction is initiated by deprotonation probably from the coordinated NH group (**4**). Rapid electron transfer may then occur from the resulting N^- to Cu(II), giving the Cu(I) complex with an unpaired electron on N(10). The radical species and other Cu(I) species possibly formed from this radical and the Cu(II) species may undergo subsequent reactions with O_2 and/or H_2O to give Cu^{II}(bpy)(PC). A similar mechanism involving amine deprotonation has been proposed for oxidation of ligands to Schiff bases in Co(II) complexes by O_2, where deprotonation of the coordinated NH group by base is of prime importance for initiation of the reaction.[30-32] ESR spectra of the reaction mixture failed to show the presence of radical species under the conditions used. This may be due to very short lifetimes of the intermediates expected for the reaction, and further studies along this line will be necessary.



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Concluding Remarks and Biological Relevance. The pteridine nucleus of FA and LM coordinates to Cu(II) through the O(4) and N(5) atoms to form ternary complexes Cu(DA)(Pte) where DA = bpy, phen, or en. A recent X-ray study of a molybdenum–xanthopterin complex revealed the bonding through the O(4), N(5), and O(6) atoms of xanthopterin,[33] which supports the present and earlier[8] conclusions for FA. The CD spectra indicated that Cu(II) binding by the ABG moiety of FA probably occurs in acid solution. A pentacoordinate square-pyramidal structure has been concluded from the electronic absorption spectrum of Cu(bpy)(FA), whose ESR spectrum has the nitrogen superhyperfine structures in the $g_\perp$ region corresponding to three coordinated nitrogen atoms. The oxidative cleavage of the C(6)–N(10) bond of FA in Cu(bpy)(FA), which finally gives PC as Cu(bpy)(PC), can be interpreted as due to the stabilization of the Cu(I) state by coordinated aromatic amines and the distortion in the coordination plane. This is supported by the reversible cyclic voltammograms for Cu(DA)(FA) (DA = bpy or phen) with a one-electron-reduction process at higher E_{pc} values than those for square-planar complexes and is further confirmed by the electrochemical properties of Cu(en)(FA), which in contrast shows an irreversible reduction process at low E_{pc} and fails to undergo any redox reactions and accompanying spectral changes.

These findings support the view that the Cu(II) ion in copper-containing phenylalanine hydroxylase directly interacts with the pterin ring of the cofactor in the mechanism of the enzyme reaction[5] and suggest that the other donor atoms are most probably the pyridine nitrogens from two imidazole rings because of the stability and redox activity of the Cu(II) center with the resulting donor set. It should be mentioned in this connection that very recently McCracken et al.[34] have concluded from an electron spin-echo spectroscopic study of the hydroxylase and model complexes that two imidazoles are bound to the active site copper(II). This is in perfect agreement with the present conclusion and very interesting in view of the combination of the donor groups that are required in both natural and model systems.

Studies on the redox reactions exhibited by Cu(DA)(FA) and structures and solution equilibria of related complexes are in progress.

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Registry No. **2**, 116405-45-9; **3**, 116405-46-0; Cu(bpy)(PC), 116405-44-8.

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