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Pteridine-Containing Ternary and Quaternary Complexes as Models for Metalloenzyme-Pterin Cofactor-Substrate Association. Structure of Ternary Copper(II)–2,2′-Bipyridine–Lumazine Complex and Successful Equilibrium Study of a Quaternary Copper(II) System

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Abstract: Structures and stabilities of pteridine-containing ternary and quaternary Cu(II) complexes have been studied by synthetic, spectroscopic, potentiometric, and X-ray diffraction methods. A ternary complex involving 2,2′-bipyridine (bpy) and lumazine (LM = 2,4-dioxopteridine), $[Cu(bpy)(LM)](NO_3)\cdot H_2O$, and quaternary complexes involving bpy, pterin-6-carboxylate (PC), and an additional ligand, imidazole (im), ammonia, and N_3^-, $[Cu(bpy)(PC)(im)]\cdot 5H_2O$, $[Cu(bpy)(PC)(NH_3)]\cdot 2.5H_2O$, and $K[Cu_2(bpy)_2(PC)_2(N_3)]\cdot 7H_2O$, were isolated as crystals. $[Cu(bpy)(LM)](NO_3)\cdot H_2O$ crystallizes in the orthorhombic space group $Pna2_1$ with four molecules in a unit cell of dimensions a = 11.437 (3), b = 23.849 (4), and c = 6.680 (1) Å. The geometry around the Cu(II) ion is slightly distorted square-pyramidal, with the two nitrogen atoms of bpy, the N(5) atom of LM, and the N(1) atom of the nearest neighboring LM in the equatorial plane and with the O(4) atom of LM at the apical position. The two LM ligands coordinated to Cu(II) are both perpendicular to the equatorial plane. The stability constants for the ternary complexes Cu(L)(PC), where L refers to bpy, 1,10-phenanthroline (phen), 2,2′,6′,2″-terpyridine (terpy), ethylenediamine (en), and diethylenetriamine (dien), were determined by pH titration at 25 °C and I = 0.1 M (KNO_3). The stability of Cu(L)(PC) increases in the order of L, bpy < terpy ~ phen < en < dien, which coincides with the stability sequence of Cu(L). The ternary species Cu(L)(PC) predominates in neutral to weakly alkaline regions with a distribution peak at pH 6–8 for 1 mM solutions. The stability constant for Cu(bpy)(PC)(im) was successfully determined to be 17.57, and the constant for the step $Cu(bpy)(PC) + im \rightleftarrows Cu(bpy)(PC)(im)$ was found to be 3.74, which is larger than expected for im bound to the fourth coordination site on statistical grounds. From comparison of the stability constants as well as the spectral data and the present and previous structural evidence the pterin ring of PC in Cu(L)(PC) in solution was concluded to be perpendicular to the Cu(II) coordination plane. Formation of the quaternary complexes Cu(bpy)(PC)(X) suggests a molecular arrangement around the central Cu atom in enzyme–pterin cofactor–substrate intermediates possibly formed in the reaction of Cu-containing phenylalanine hydroxylase.

Introduction

Pterins (= 2-amino-4-oxopteridines) are a class of heterocyclic compounds with unique structures and biological functions. The chemistry of pterins originates from the discovery of yellow pigments in butterfly wings, and some derivatives are now recognized as cofactors playing important roles in the synthesis of neurotransmitters and nucleic acids.[1] Folic acid is a pterin derivative containing a glutamyl moiety and essential for the biosynthesis of purine and pyrimidine bases.[2] Methotrexate which is a widely used anticancer drug has the structure similar to folic acid and inhibits such biosynthesis.[3]

Much attention has recently been paid to pterin cofactors in the actions of oxo-transferases and pterin-dependent hydroxylases.[4] The molybdenum cofactor of xanthine oxidase and other oxomolybdenum enzymes has been reported to involve a pterin moiety containing a molybdenum ion bound to the side-chain thiolate groups of molybdopterin.[4,5] Tetrahydropterins such as tetrahydrobiopterin, on the other hand, are known to be essential for phenylalanine hydroxylase (PAH) and other aromatic amino acid hydroxylases,[6] which catalyze the hydroxylation of the aromatic ring by molecular oxygen in the presence of a reduced pterin cofactor and are essential for the synthesis of neurotransmitters such as dopamine. Although it is well-known that iron and copper are essential for the activity of PAH from mammals and *Chromobacterium violaceum*,[6c,7] respectively, and the active site structures and modes of metal–pterin cofactor interactions in the enzymatic reactions have been studied,[8] details of the structures and reaction mechanisms remain to be established. The amino acid sequencing, cloning, and expression that have been reported very recently[6e] suggest two conserved histidines as the copper ligands previously proposed[8b] and will give valuable information.

Interest in the coordinating ability of the pterin ring prompted us to study the reactions between folic acid and copper(II) complexes, where we observed oxidative cleavage of folic acid side chain at the C(6) atom to give pterin-6-carboxylate (PC) isolated as $[Cu(bpy)(PC)(H_2O)]\cdot 3H_2O$ (bpy = 2,2′-bipyridine).[9] X-ray crystal structure analysis of the ternary complex revealed a unique

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octahedral structure with PC coordinating to Cu(II) through the N(5) atom at an equatorial position and 4-oxo and 6-carboxylate oxygens at the axial positions (Figure 1).[10] An interesting feature of this complex indicated by our preliminary electron spin resonance (ESR) measurements is that the coordinated water molecule can be easily replaced by imidazole (im) to give a quaternary complex, Cu(bpy)(PC)(im),[10] which may be regarded as a model for the metalloenzyme–cofactor–substrate complex possibly formed at the metal center of Cu-containing PAH. A recent kinetic study of recombinant tyrosine hydroxylase showed that the substrate tyrosine is bound to the metalloenzyme–pterin adduct to form a metal–enzyme protein–cofactor–substrate complex.[11] On the other hand, 5,6,7,8-tetrahydro-6,7-dimethylpterin, which is known to be active as a cofactor, has been reported to bind with Cu(II) of bacterial PAH.[8c] In view of the paucity of information on the metal–pterin interaction in the hydroxylation reactions by PAH, we studied the structures and solution equilibria of ternary and quaternary systems involving PC and lumazine (LM = 2,4-dioxopteridine) by synthetic, spectroscopic, potentiometric, and X-ray diffraction methods as a step toward elucidation of the hydroxylation mechanism which may involve ternary and quaternary complex formation. In this paper we describe the synthesis and solution equilibrium analysis of ternary and quaternary systems, Cu^{II}–L–PC (L = bpy, phen, en, dien, terpy (vide infra)) and Cu^{II}–bpy–PC–X (X = im, NH_3, N_3^-), respectively, and the molecular structure determination of a ternary lumazine complex Cu(bpy)(LM) showing a copper(II)–pteridine binding mode. To our knowledge, the Cu–bpy–PC–im system is the first successful example of quaternary Cu(II) complexes analyzed by potentiometry.

Experimental Section

Materials. 2,2′-Bipyridine (bpy), 1,10-phenanthroline (phen), ethylenediamine (en), diethylenetriamine (dien), imidazole (im), sodium azide, and folic acid were purchased from Nacalai Tesque, and en and dien were distilled and converted to the HCl salts. 2,2′,6′,2″-Terpyridine (terpy) obtained from Sigma was recrystallized twice from petroleum ether, and the purity was checked by elemental analysis. Pterin-6-carboxylic acid (PC) was prepared from folic acid according to the literature[12] and purified through repeated precipitation from a solution containing PC·2Na and PC·1Na by adding aqueous HCl. PC obtained was dried under vacuum over P_4O_{10} and determined to be PC·$0.2H_2O$ by elemental analysis and pH titration. All reagents used were of highest grade available. Water was deionized, distilled, and further purified by Milli-Q Labo.

Synthesis of Ternary and Quaternary Complexes. The complexes isolated were analyzed for C, H, and N, and the number of water molecules was estimated from the analytical data and confirmed for $[Cu(bpy)(LM)](NO_3)\cdot H_2O$ by X-ray analysis.

$[Cu(bpy)(LM)](NO_3)\cdot H_2O$. Lumazine (LM) (0.16 g, 1 mmol) dissolved in 0.1 M NaOH (10 mL) was added to a MeOH solution (40 mL) containing $Cu(bpy)(NO_3)_2$ (0.34 g, 1 mmol) and heated at 60 °C for complete dissolution. The crystals which separated upon standing at room temperature were collected and recrystallized from EtOH: yield, ca. 30%. Anal. Calcd for $C_{16}H_{13}N_7O_6Cu$: C, 41.51; H, 2.83; N, 21.18. Found: C, 41.48; H, 2.54; N, 21.62.

$[Cu(bpy)(PC)(im)]\cdot 5H_2O$. Imidazole (im) (0.14 g, 2 mmol) dissolved in a small amount of dimethyl sulfoxide (DMSO) was added to a DMSO solution (150 mL) containing $[Cu(bpy)(PC)(H_2O)]\cdot 3H_2O$ (0.9 g, 2 mmol). After 10 days the precipitate was filtered and washed with MeOH and water: yield, 58%. Recrystallization from MeOH gave analytically pure crystals. Anal. Calcd for $C_{20}H_{25}N_9O_8Cu$: C, 41.20; H, 4.32; N, 21.62. Found: C, 41.42; H, 3.82; N, 21.73.

$[Cu(bpy)(PC)(NH_3)]\cdot 2.5H_2O$. An aqueous solution containing $[Cu(bpy)(PC)(H_2O)]\cdot 3H_2O$ (0.43 g, 1 mmol) and NH_3 (10-fold excess) was kept in an ice bath and concentrated under vacuum. The green crystals which separated on standing in the refrigerator were collected and washed with MeOH and water: yield, ca. 10%. Anal. Calcd for $C_{17}H_{19}N_8O_{5.5}Cu$: C, 41.93; H, 3.93; N, 23.01. Found: C, 41.97; H, 3.85; N, 22.80.

Table I. Crystal Data and Refinement Details for $[Cu(bpy)(LM)](NO_3)\cdot H_2O$

formula	$CuC_{16}O_6N_7H_{13}$
molecular wt	462.86
lattice type	orthorhombic
space group	$Pna2_1$
crystal size (mm)	0.02 × 0.02 × 0.05
a (Å)	11.437 (3)
b (Å)	23.849 (4)
c (Å)	6.680 (1)
V (Å^3)	1822.0 (9)
Z	4
$F(000)$	1880
D_{calcd} (g cm^{-3})	1.687
λ (Å)	1.54178
μ (cm^{-1})	42.64
2θ range (deg)	$2 < 2\theta < 120$
scan width (deg)	$0.8 + 0.5 \tan \theta$
scan type	ω–2θ
scan rate in ω (deg min^{-1})	4
total data collected	1665
unique data with $\lvert F_o\rvert > 3\sigma(F_o)$	1465
R	0.056
R_w	0.077

$K[Cu_2(bpy)_2(PC)_2(N_3)]\cdot 7H_2O$. To an aqueous solution (40 mL) of $[Cu(bpy)(PC)(H_2O)]\cdot 3H_2O$ (0.09 g, 0.2 mmol) was added NaN_3 (0.013 g, 0.2 mmol) dissolved in a small amount of water, and the mixture was heated to 50–60 °C. KNO_3 (1 M, 3 mL) was added to the hot solution, which was filtered and kept at room temperature. The crystals which separated upon standing were collected and dried: yield, 19%. Anal. Calcd for $C_{34}H_{36}N_{17}O_{13}Cu_2K$: C, 38.34; H, 3.43; N, 22.53. Found: C, 38.74; H, 3.09; N, 21.97.

Spectral Measurements. Absorption spectra were recorded at 25 °C in the range 400–870 nm with a Shimadzu UV-3100PC and a Hitachi 330 recording spectrophotometer. The samples of quaternary systems Cu^{II}–bpy–PC–X (X = im, NH_3, or N_3^-) contained 2 mM Cu(bpy)(PC) and 0–10 mM X. The pH values were adjusted with aqueous NaOH or HCl. ESR spectra were measured for 2 mM solutions of Cu^{II}–bpy–PC–X at 77 K and 25 °C with a JEOL JES-RE1X ESR spectrometer. IR spectra (KBr disk) were measured with a Perkin-Elmer 1600 FT-IR spectrometer.

pH Titrations. pH titrations were carried out at 25 °C and $I = 0.1$ M (KNO_3) for solutions containing $Cu(NO_3)_2$, L (L = bpy, phen, en, terpy, dien), PC, and/or im and an appropriate amount of KOH, or HNO_3. For titrations of the ternary and quaternary systems, Cu(L) and Cu(L)(PC) complexes isolated, respectively, were used. For titrations of the PC, Cu^{II}–PC, Cu^{II}–L–PC, Cu^{II}–PC–im, and Cu^{II}–bpy–PC–im systems, PC was first dissolved in a certain amount of aqueous KOH and titrated with 0.1 M HNO_3, because the solubility of PC in water is low at neutral pH. pH values were measured with an Orion Research 901A and a Beckman pHI 71 pH meter each equipped with a Beckman 39314 glass electrode and a Beckman 39419 double junction reference electrode. The pH meter was calibrated with NBS standard buffer solutions (4.008, 7.143, 9.180 at 25 °C). The titration data were collected and treated with the nonlinear least-squares computer program SUPERQUAD.[13] The overall stability constant β_{pqrst} for species $Cu_p(L)_q(PC)_r(im)_sH_t$ defined by eq 1 (charges are omitted for simplicity) were calculated by a FACOM M-680 computer at Nagoya University Computation Center

$$pCu + q(L) + r(PC) + s(im) + tH \xrightleftharpoons{\beta_{pqrst}} Cu_p(L)_q(PC)_r(im)_sH_t$$

$$\beta_{pqrst} = \frac{[Cu_p(L)_q(PC)_r(im)_sH_t]}{[Cu]^p[L]^q[PC]^r[im]^s[H]^t} \qquad (1)$$

where p, q, r, s, and t denote the numbers of moles of Cu(II), L (L = bpy, phen, en, terpy, dien), PC, im, and proton (H), respectively. Calculation of hydrogen ion concentrations from pH values was made by using $10^{-pH}/[H^+] = 0.855$ and $pK_w' = 13.96$.[14]

X-ray Structure Determination of $[Cu(bpy)(LM)](NO_3)\cdot H_2O$. A crystal with dimensions of 0.02 × 0.02 × 0.05 mm was used for collection of intensity data. Diffraction data were collected at 295 K with a Rigaku

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Table II. Bond Lengths (Å) and Angles (deg) for $[Cu(bpy)(LM)](NO_3)\cdot H_2O$

Cu–O(4)	2.316 (9)	Cu–N(1)	1.989 (9)
Cu–N(5)	1.974 (9)	Cu–N(1b)	1.963 (10)
Cu–N(2b)	2.016 (10)	O(2)–C(2)	1.197 (16)
O(4)–C(4)	1.226 (14)	N(1)–C(2)	1.342 (16)
N(1)–C(8a)	1.356 (14)	N(3)–C(2)	1.440 (16)
N(3)–C(4)	1.327 (16)	N(5)–C(4a)	1.356 (16)
N(5)–C(6)	1.350 (17)	N(8)–C(7)	1.386 (18)
N(8)–C(8a)	1.357 (16)	C(4)–C(4a)	1.487 (20)
C(4a)–C(8a)	1.391 (15)	C(6)–C(7)	1.340 (19)
N(1b)–C(1b)	1.363 (19)	N(1b)–C(5b)	1.362 (15)
N(2b)–C(6b)	1.359 (16)	N(2b)–C(10b)	1.333 (20)
C(1b)–C(2b)	1.363 (20)	C(2b)–C(3b)	1.337 (21)
C(3b)–C(4b)	1.389 (25)	C(4b)–C(5b)	1.383 (19)
C(5b)–C(6b)	1.493 (19)	C(6b)–C(7b)	1.371 (18)
C(7b)–C(8b)	1.375 (24)	C(8b)–C(9b)	1.413 (22)
C(9b)–C(10b)	1.346 (21)	O(1n)–N(1n)	1.182 (19)
O(2n)–N(1n)	1.178 (22)	O(3n)–N(1n)	1.115 (23)
O(4)–Cu–N(1)	118.1 (3)	O(4)–Cu–N(5)	78.6 (4)
O(4)–Cu–N(1b)	89.1 (4)	O(4)–Cu–N(2b)	93.8 (4)
N(1)–Cu–N(5)	90.5 (4)	N(1)–Cu–N(1b)	95.7 (4)
N(1)–Cu–N(2b)	148.0 (4)	N(5)–Cu–N(1b)	167.6 (4)
N(5)–Cu–N(2b)	98.9 (4)	N(1b)–Cu–N(2b)	81.5 (4)
Cu–O(4)–C(4)	106.5 (8)	Cu–N(1)–C(2)	118.9 (8)
Cu–N(1)–C(8a)	120.3 (7)	C(2)–N(1)–C(8a)	120.8 (9)
C(2)–N(3)–C(4)	125.4 (10)	Cu–N(5)–C(4a)	116.0 (9)
Cu–N(5)–C(6)	129.0 (8)	C(4a)–N(5)–C(6)	114.7 (10)
C(7)–N(8)–C(8a)	112.0 (10)	O(2)–C(2)–N(1)	122.5 (12)
O(2)–C(2)–N(3)	119.1 (12)	N(1)–C(2)–N(3)	118.4 (11)
O(4)–C(4)–N(3)	124.3 (12)	O(4)–C(4)–C(4a)	121.6 (11)
N(3)–C(4)–C(4a)	113.9 (10)	N(5)–C(4a)–C(4)	115.9 (9)
N(5)–C(4a)–C(8a)	124.0 (12)	C(4)–C(4a)–C(8a)	120.0 (11)
N(5)–C(6)–C(7)	120.8 (12)	N(8)–C(7)–C(6)	126.7 (13)
N(1)–C(8a)–N(8)	116.7 (9)	N(1)–C(8a)–C(4a)	121.5 (11)
N(8)–C(8a)–C(4a)	121.5 (10)	Cu–N(1b)–C(1b)	125.9 (8)
Cu–N(1b)–C(5b)	116.3 (9)	C(1b)–N(1b)–C(5b)	117.4 (10)
Cu–N(2b)–C(6b)	114.3 (9)	Cu–N(2b)–C(10b)	127.7 (9)
C(6b)–N(2b)–C(10b)	117.6 (11)	N(1b)–C(1b)–C(2b)	122.4 (12)
C(1b)–C(2b)–C(3b)	118.6 (15)	C(2b)–C(3b)–C(4b)	122.4 (14)
C(3b)–C(4b)–C(5b)	116.4 (13)	N(1b)–C(5b)–C(4b)	122.6 (12)
N(1b)–C(5b)–C(6b)	113.6 (11)	C(4b)–C(5b)–C(6b)	123.8 (12)
N(2b)–C(6b)–C(5b)	114.3 (11)	N(2b)–C(6b)–C(7b)	121.1 (12)
C(5b)–C(6b)–C(7b)	124.6 (12)	C(6b)–C(7b)–C(8b)	120.5 (13)
C(7b)–C(8b)–C(9b)	117.5 (14)	C(8b)–C(9b)–C(10b)	118.6 (15)
N(2b)–C(10b)–C(9b)	124.1 (13)	O(1n)–N(1n)–O(2n)	109.9 (17)
O(1n)–N(1n)–O(3n)	121.2 (19)	O(2n)–N(1n)–O(3n)	128.8 (18)

AFC-5R four-circle diffractometer using graphite-monochromated Cu Kα radiation ($\lambda = 1.54178$ Å). Crystal data and details of the parameters associated with data collection are given in Table I. The unit cell parameters were derived from least-squares refinement of 25 well-centered reflections ($22° < \theta < 27°$). Data collection showed systematic absence ($h + l = 2n + 1$ for $0kl$ and $h = 2n + 1$ for $h0l$) which led to two possible orthorhombic space groups $Pna2_1$ or $Pnam$. The intensities of three standard reflections measured every 100 reflections showed no significant variations. Intensity data were collected by the ω–2θ scan technique in the range $2° < 2\theta < 120°$. Reflection data were corrected for Lorentz and polarization effects. Absorption correction was not applied, because both the absorption coefficient and crystal size were small.

The structure was solved for two possible space groups by the heavy-atom method and refined anisotropically for non-hydrogen atoms by full-matrix least-squares calculations, which showed that the space group $Pna2_1$ was the most probable one. The electron density associated with H atoms could be detected at the expected locations in the difference Fourier map, and H atoms were refined isotropically. The final R and R_w values were 0.056 and 0.077, respectively. The weighting scheme $w^{-1} = [\sigma^2(F_0) + (0.02F_0)^2]$ was employed for the crystal. The final difference Fourier maps showed no residual peaks >0.6 eÅ^{-3} close to the Cu atom. Atomic scattering factors and anomalous dispersion terms were taken from the International Tables for X-ray Crystallography.[15] All calculations were carried out on a HITAC M-680H computer at the Computer Center of the Institute for Molecular Science by using the program system UNICS III.[16]

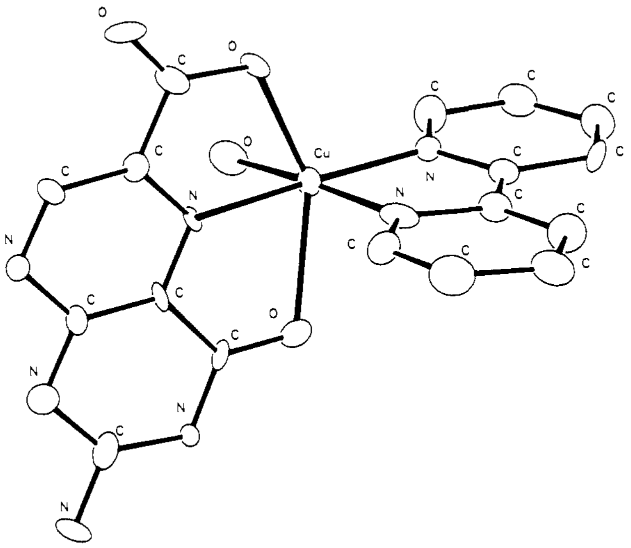


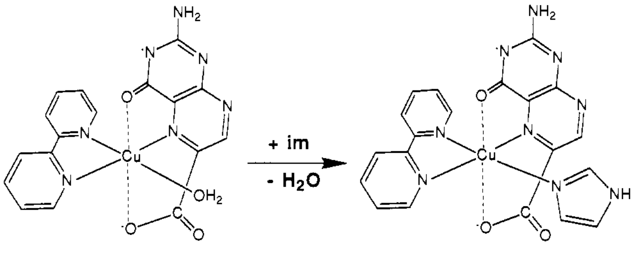
Figure 1. Molecular structure of $[Cu(bpy)(PC)(H_2O)]$.[10] Atoms are represented by thermal ellipsoids at 50% probability level.

Table III. ESR Parameters for Cu^{II}–bpy–PC–X Systems at 77 K[a]

X	$g_\parallel$	$g_\perp$	$\lvert A_\parallel\rvert$ (mT)	$\lvert A_\perp\rvert$ (mT)
H_2O	2.27	2.07	17.1	1.42
NH_3	2.24	2.06	18.5	1.42
im	2.25	2.06	17.9	1.56
N_3^-	2.24	2.06	17.3	1.25

[a] Conditions for spectral measurements: frequency, 9.234 GHz; field, 305 ± 50 mT; modulation, 0.32 mT; power, 1 mW; response, 0.1 s.

Scheme I



The final atomic parameters for non-hydrogen atoms are given in Table SII, and the selected bond lengths and angles are listed in Table II.

Results

Spectral Changes Due to Quaternary Complex Formation and Isolation of Complexes. On addition of im to the ternary system Cu^{II}-bpy–PC the ESR spectrum changed to give nine nitrogen superhyperfine splittings in the $g_\parallel$ and $g_\perp$ regions, indicating that Cu(II) had four coordinated nitrogens corresponding to the quaternary complex Cu(bpy)(PC)(im). The im molecule most probably replaces the equatorial water molecule in $Cu(bpy)(PC)(H_2O)$ (Figure 1).[9] The ESR parameters for 1:1:1:1 Cu^{II}–bpy–PC–X (X = NH_3, im, N_3^-; pH 7–9) measured at 77 K (Table III) show that addition of X to $Cu(bpy)(PC)(H_2O)$ results in shifts to higher fields and larger $\lvert A_\parallel\rvert$ values indicative of coordination of an additional nitrogen atom (Scheme I). The absorption spectra at pH 7–9 in the d–d region exhibited spectral changes due to addition of X; Cu(bpy)(PC)(X) at various [X]/[Cu(bpy)(PC)] ratios with X = im at pH 7 and NH_3 at pH 9 gave the spectra showing isosbestic points, the peak being shifted to shorter wavelengths (620–650 nm). The spectra for X = N_3^- showed a large intensity increase with a slight blue shift but no isosbestic points.

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(16) Sakurai, T.; Kobayashi, K. *Rikagaku Kenkyusho Houkoku* **1979**, *55*, 69.

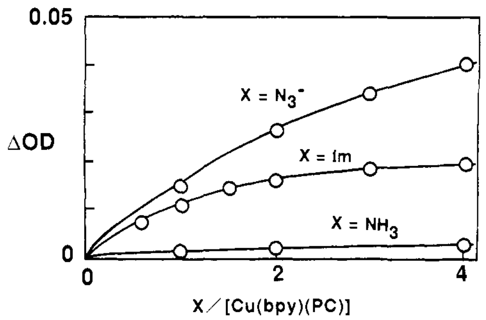

Figure 2. Differences (ΔOD) between the absorbances of Cu(bpy)(PC)(X) and Cu(bpy)(PC) plotted against [X]/[Cu(bpy)(PC)]. X, N_3^- (630 nm, pH 9.0); im (590 nm, pH 7.0); NH_3 (600 nm, pH 9.0).

These observations indicate that quaternary complexes Cu(bpy)(PC)(X) are formed at pH 7–9 and that for the systems with X = im and NH_3 the equilibrium for the quaternary complex formation (Scheme I) can be approximated mainly by the following step under the conditions employed:

$$\mathrm{Cu(bpy)(PC)} + \mathrm{X} \overset{K}{\rightleftharpoons} \mathrm{Cu(bpy)(PC)(X)} \qquad (2)$$

where X refers to im or NH_3 and K denotes the stepwise stability constant. Plots of the absorption increase against [X]/[Cu(bpy)(PC)] shown in Figure 2 suggest that im and NH_3 form the corresponding quaternary complex with considerable stability, whereas N_3^- forms a less stable one.

Because Cu(bpy)(PC) is less soluble and readily crystallizes as compared with Cu(bpy)(PC)(X), attempts to isolate quaternary complexes met some difficulties. The im-containing complex Cu(bpy)(PC)(im) was isolated from a DMSO solution of Cu(bpy)(PC) and im and recrystallized from methanol. Cu(bpy)(PC)(NH_3) was also isolated from aqueous solution containing Cu(bpy)(PC) and excessive NH_3. The products involving N_3^- were inferred to be K[$Cu_2(bpy)_2(PC)_2(N_3)$] from elemental analysis; the IR spectrum showed a peak at 2049 cm^{-1} due to N_3^- which is shifted from that of NaN_3 (2037 cm^{-1}) and the bands in the region 1600–700 cm^{-1} were similar to those for Cu(bpy)(PC)(H_2O) except that the peaks were shifted from each other. According to Karlin et al.[17] N_3^- exhibits a peak at around 2090–2050 and 2040–2020 cm^{-1} when coordinated in the end-on and the end-to-end manner, respectively. The peak for the present complex appears to be closer to the range for the end-on type coordination, but further investigations are required for structure determination. However, these results support that the quaternary complexes are formed in solution to a considerable extent from the ternary species Cu(bpy)(PC) which exists as a stable component for the quaternary complex formation.

Solution Equilibria in Ternary and Quaternary Systems. In order to analyze the solution equilibria in the ternary and quaternary systems, Cu^{II}–L–PC and Cu^{II}–L–PC–im, respectively, with L = bpy, phen, terpy, en, or dien, we carried out pH titrations of the relevant binary proton–ligand and copper(II)-ligand systems and the above ternary and quaternary systems. Because PC is sparingly soluble in water, the systems with PC were usually dissolved in solutions containing an excessive amount of KOH and titrated with 0.1 M HNO_3. From the titration curves for Cu^{II}–L–PC and related binary systems, the stability constants log β_{pqrst} (eq 1) for the ternary complexes have been calculated, and from the curve for Cu^{II}–bpy–PC–im the log β_{11110} value for the quaternary complex has been successfully determined (Table IV).

In line with the result of Sjöberg,[18] species Cu(im)$_n$ (n = 1–4), Cu(im)(OH), Cu(im)(OH)$_2$, and Cu(im)$_2$(OH) were detected by computer simulation of the titration curves for 1:1–1:7 Cu^{II}–im systems, but Cu(im)$_5$ and Cu(im)$_6$ were rejected. The stability constants for Cu(bpy)(OH)$_n$ (n = 1 and 2) agreed well with the reported values.[19] PC was found to give Cu(PC) and Cu(PC)(OH) but not Cu(PC)$_2$. The stability constants, log β_{11100}, for the ternary systems Cu^{II}–L–PC increase in the order of L, bpy < terpy ~ phen < en < dien, which coincides with the stabilities of the binary CuL complexes (log β_{11000}) (Table IV). The log β_{11101} for Cu^{II}–L–LM reported in an earlier paper[9] also showed the same trend. Although Cu(L)(LM) is thermodynamically favored when L is bpy or phen, the present results imply that PC has affinities for Cu(II) complexes with both π-accepting and σ-donating ligands. It is evident from the calculated species distribution curves for 1 mM solutions such as typically shown for Cu(bpy)(PC) in Figure 3 that the ternary species predominates in all the cases in neutral to weakly alkaline regions with a distribution peak at pH 6–8 (ca. 60% at pH 7.2 for Cu(bpy)(PC)), whereas the hydroxo species Cu(bpy)(OH)$_n$ are predominant at pH > 8. The log β_{11110} value for the quaternary system Cu^{II}–bpy–PC–im has been calculated to be 17.57, the difference log β_{11110} – log β_{11100} = 3.74 being larger than expected for a ligand bound to the fourth coordination site. Cu(bpy)(PC)(im) is the most abundant species at pH 7–9 (ca. 50% at pH 7.6 for 1 mM solution), and outside this pH range Cu(bpy)(OH)$_n$ (n = 1, 2) (pH > 9) and Cu(bpy)(PC) (pH < 7) are the main species (Figure 4). This confirms the quaternary complex formation from Cu(bpy)(PC) and im as inferred from the absorption and ESR spectral measurements.

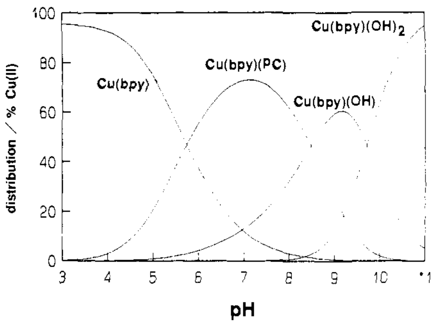

Figure 3. Calculated species distributions for $Cu_p(bpy)_q(PC)_rH_t$. [Cu(II)] = [bpy] = [PC] = 1 mM.

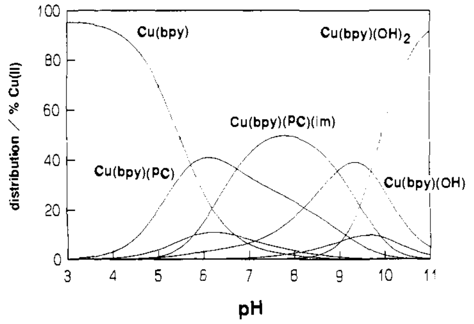

Figure 4. Calculated species distributions for $Cu_p(bpy)_q(PC)_r(im)_sH_t$. [Cu(II)] = [bpy] = [PC] = [im] = 1 mM.

Molecular Structure of [Cu(bpy)(LM)](NO_3)·H_2O. Figure 5 shows a perspective view of [Cu(bpy)(LM)](NO_3)·H_2O with the atomic labeling scheme. The coordination environment around the Cu atom is considered to be a slightly distorted square-pyramid with the two nitrogen atoms of bpy, the N(5) atom of LM, and the N(1) atom of the nearest neighboring LM in the equatorial plane (Cu–N(1B) = 1.963 (10), Cu–N(2B) = 2.016 (10), Cu–N(1) = 1.989 (9), and Cu–N(5) = 1.974 (9) Å) and with one carbonyl oxygen of LM at the apical position (Cu–O(4) = 2.316 (9) Å). The lumazine ring is nearly perpendicular to the Cu(II) coordination plane, which is similar to the orientation found in Cu(bpy)(PC). The counter ion nitrate and the water molecule

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[17] (a) Karlin, K. D.; Cohen, B. I.; Hayes, J. C.; Farooq, A.; Zubieta, J. *Inorg. Chem.* **1987**, *26*, 147–153. (b) Pate, J. E.; Ross, P. K.; Thamann, T. J.; Reed, C. A.; Karlin, K. D.; Sorrell, T. N.; Solomon, E. I. *J. Am. Chem. Soc.* **1989**, *111*, 5198–5209.

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Table IV. Stability Constants log β_{pqrst} for $Cu_p(L)_q(PC)_r(im)_sH_t$ Systems at 25 °C and $I = 0.1$ (KNO_3)

system	species pqrst	log β_{pqrst}[a]
Cu-bpy-PC-im	11110	17.57 (1)
Cu-bpy-PC	11100	13.83 (1)
Cu-bpy-im	11010	12.767 (2)
	11020	15.686 (2)
	1101-1	4.607 (8)
Cu-PC-im	10110	8.96 (1)
	10120	12.43 (1)
	10130	15.13 (3)
Cu-bpy	11000	9.0 (2)
	12000	14.724 (6)
	1100-1	2.04 (2)
	2200-2	-3.0 (2)
	1100-2	-7.68 (5)
Cu-PC	10100	4.947 (6)
	1010-1	-2.00 (1)
Cu-im	10010	4.223 (2)
	10020	7.675 (3)
	10030	10.484 (5)
	10040	12.44 (1)
	1001-1	-3.27 (2)
	1001-2	-11.29 (2)
	1002-1	-0.23 (1)
Cu-OH	1000-1	-7.223 (5)
	3000-4	-21.05 (3)
bpy	01001	4.394 (1)
PC	00101	7.272 (4)
	00102	10.202 (9)
im	00011	6.994 (1)
Cu-phen-PC	11100	14.286 (3)
Cu-en-PC	11100	15.577 (4)
Cu-terpy-PC	11100	14.326 (4)
Cu-dien-PC	11100	20.810 (7)
Cu-phen	11000	9.25[b]
	12000	16.00[b]
	1100-1	2.07 (1)
	1100-2	-5.21 (1)
phen	01001	4.95[b]
Cu-en	11000	10.523[c]
	12000	19.503[c]
en	01001	9.976[c]
	01002	17.148[c]
Cu-terpy	11000	9.068 (9)
	12000	17.09 (1)
	1100-1	0.879 (1)
	2200-1	11.54 (9)
terpy	01001	4.729 (6)
	01002	8.341 (6)
	01003	10.08 (2)
Cu-dien	11001	18.26 (5)
	11000	15.871 (2)
	1100-1	6.697 (0)
	2200-1	24.07 (2)
dien	01001	9.854 (3)
	01002	18.912 (2)
	01003	23.219 (4)

[a] Values in parentheses denote estimated standard deviations. [b] Fischer, B. E.; Sigel, H. *J. Am. Chem. Soc.* **1980**, *102*, 2998–3008. [c] Brookes, G.; Pettit, L. D. *J. Chem. Soc., Dalton Trans.* **1977**, 1918–1924.

do not participate in coordination. The basal plane formed by four coordinated nitrogen atoms are planar to within 0.15 Å, and the copper atom deviates by 0.10 Å from this plane toward the apical carbonyl oxygen.

The LM ligand is coordinated to a Cu(II) ion through O(4) and N(5) with concomitant deprotonation from the N(3) position and to the other Cu(II) ion through N(1), and thus the complex forms an infinite chain in the unit cell as shown in Figure 6. The C(4)–O(4) carbonyl bond, 1.226 (14) Å, retains a C=O double bond character (1.23–1.26 Å),[20] although the oxygen atom is

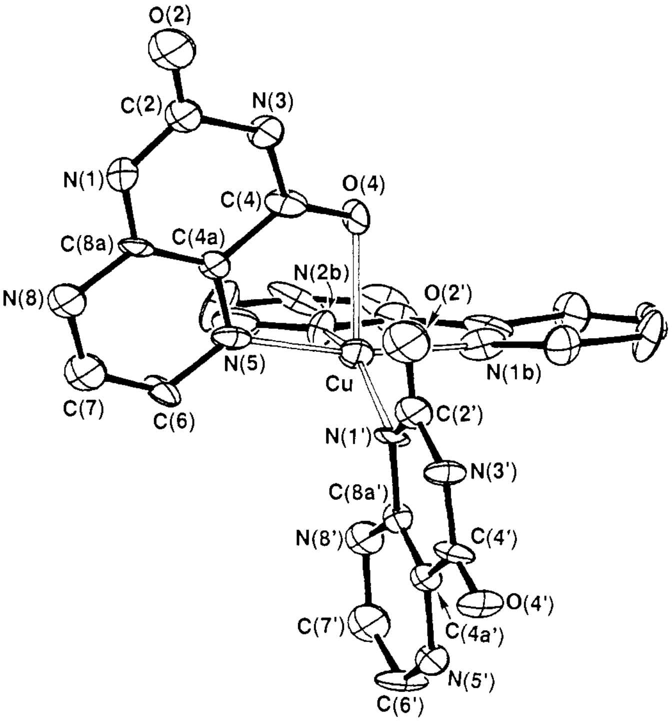

Figure 5. Molecular structure of $[Cu(bpy)(LM)]^+$. Atoms are represented by thermal ellipsoids at 50% probability level.

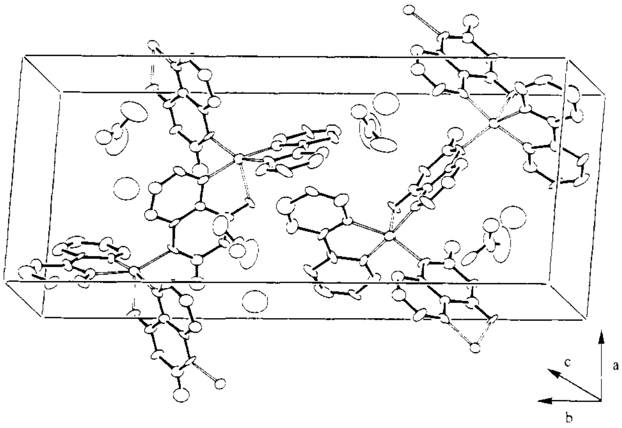

Figure 6. Crystal structure of $[Cu(bpy)(LM)](NO_3)\cdot H_2O$.

weakly coordinated to Cu(II). The C(2)–N(3) bond (1.440 (16) Å), on the other hand, is elongated in comparison with that of uncoordinated LM.[21] The solid state structure has a Cu^{II}–N(1) bond in addition to the bonds expected for the solution structure based on spectroscopic data, but we may infer that the monodentate Cu^{II}–N(1) bonding does not necessarily exist in dilute aqueous solution. In this connection, the binary complexes of neutral and deprotonated LM, $Cu(LM)_2$, $Cu(LM)_2X_2$ (X = Cl, Br), etc., have been isolated previously and concluded to have a 6O chromophore in $Cu(LM)_2X_2$ and a 5 N chromophore in $Cu(LM)_2$.[22]

Discussion

Formation of Quaternary Complexes in Solution. Considering that Cu(II) assumes a tetragonal or an axially distorted octahedral coordination structure, Cu(bpy)(PC) with PC occupying an equatorial and two axial positions (Figure 1) leaves only one coordination site available for the third ligand. Because bpy accepts π-back donation from Cu(II) through dπ–pπ interac-

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tions[23,24] and the pterin ring of PC is electron deficient, the Cu(II) ion of Cu(bpy)(PC) may retain the affinity for additional donor groups. In accordance with this, formation of the quaternary complexes from Cu(bpy)(PC) and X has now been established by isolation of the complexes as crystals and by spectrosopic and potentiometric studies. Coordination of three different ligands to a metal ion to form a stable quaternary complex depends on electronic, structural, and statistical factors,[24] because they share the coordination sites and the space around the central metal ion and should preferably be an electronically favorable combination. PC in [Cu(bpy)(PC)(H_2O)] is coordinated perpendicular to the Cu(II) coordination plane involving bpy, and, according to the absorption spectra in the d–d region which indicated that the equilibrium at neutral pH can be approximated by two dominant complex species Cu(bpy)(PC) and Cu(bpy)(PC)(im), combination of bpy and PC around the central Cu(II) ion is favored for electronic and structural reasons and probably makes Cu(bpy)(PC) thermodynamically stabilized (vide infra). ESR spectra already demonstrated that the water molecule is easily replaced by ligands such as im and NH_3 most probably without the disruption of the Cu(bpy)(PC) structure.[10]

Stabilization of Pterin-Containing Ternary and Quaternary Complexes. The titration curves for the Cu^{II}–PC system could be simulated by considering Cu(PC) and Cu(PC)(OH), and the simulation with $Cu(PC)_2$ was not successful. This indicates that PC requires two axial positions for effective binding with Cu(II) with its ring perpendicular to the coordination plane and without the axial sites no longer binds Cu(II). The $\Delta\log K$ values for formation of the ternary complexes Cu(L)(PC) defined by eq 3[24]

$$\mathrm{Cu(L)} + \mathrm{Cu(PC)} \overset{10^{\Delta\log K}}{\rightleftharpoons} \mathrm{Cu(L)(PC)} + \mathrm{Cu} \qquad (3)$$

$$\Delta\log K = \log \beta_{11100} - (\log \beta_{11000} + \log \beta_{10100})$$

are in the range –0.12~0.11 for L = bpy, phen, en, and dien and larger for L = terpy (0.31), which indicates that PC binds with Cu(terpy) and Cu(dien) having only one equatorial position available as strongly as with Cu(bpy), Cu(phen), and Cu(en) having two such positions. We concluded from these results that PC in solution prefers the Cu(II) binding in the manner revealed in the solid state and thus favorably binds with Cu(L). The smaller $\Delta\log K$ value for Cu(dien) is then interpreted as due to strong σ-donation by dien forming a very stable complex ($\log \beta_{11000} = 15.87$) as compared with 10.52 for Cu(en) and 9.07 for Cu(terpy). In this connection, the stabilization of Cu(L)(LM) (species 11101) as measured by the $\Delta\log \beta$ and log X values has been revealed to be larger for L = bpy and phen than for L = en. By assuming that the coordinating ability of the N(5) atom of PC is similar to that of pyridine ($pK_a = 5.26$; $\log \beta_{10100} = 2.49$)[25] and that the pK_a of N(5) is 2.0, we obtain the expected stability constant for Cu(PC) as 0.95 (= 2.49 × 2.0/5.26), and the constant for Cu(L)(PC) (L = bpy, phen) (eq 4) is expected to be 0.17 (= 0.95 – 2 × 0.39) according to Tanaka[26] by using his correction factor for a coordinated aromatic nitrogen (–0.39). The observed value for formation of Cu(PC) ($\log \beta_{10100} = 4.95$) and the $\log \beta_{11100}/\beta_{11000}$ values for eq 4

$$\mathrm{Cu(L)} + \mathrm{PC} \overset{\beta_{11100}/\beta_{11000}}{\rightleftharpoons} \mathrm{Cu(L)(PC)} \qquad (4)$$

which are calculated to be 4.8–5.3 (Table IV) are much larger than the above expected value. This extra stabilization further supports the view that PC effectively coordinates to Cu(L) as a terdentate ligand whose two axial Cu–O bondings probably make a substantial contribution to ternary complex stability.

The quaternary complex Cu(bpy)(PC)(im) again exhibits stabilization. The stepwise constant log K for complex formation between Cu(bpy)(PC) and X = im (eq 2) is expressed as follows:

$$\log K = \log \beta_{11110} - \log \beta_{11100} \qquad (5)$$

The log K value has been calculated to be 3.74 at 25 °C and I = 0.1 M(KNO_3), which is 0.8–1 log unit larger than the values for the coordination reactions of im as the fourth donor in the Cu(II) plane (Table IV):

$$\mathrm{Cu(bpy)(im)} + \mathrm{im} \rightleftharpoons \mathrm{Cu(bpy)(im)_2} \qquad (\log K = 2.92)$$

$$\mathrm{Cu(PC)(im)_2} + \mathrm{im} \rightleftharpoons \mathrm{Cu(PC)(im)_3} \qquad (\log K = 2.70)$$

The value of 3.74 is comparable with that for the equilibrium

$$\mathrm{Cu(bpy)} + \mathrm{im} \rightleftharpoons \mathrm{Cu(bpy)(im)}$$

where $\log \beta_{11010} - \log \beta_{11000} = 3.77$.

The binding of im to Cu(II) probably takes place in such a way that the angle between the imidazole ring and the copper coordination plane is close to zero[27] as in $Cu(im)_2(acetate)_2$,[28] where the two rings in the trans positions are nearly coplanar with the coordination plane. Since the stability difference between the rotational states of the Cu–N(im) bond is not known, the stability increase of 1 log unit could be due to absence of steric hindrance, but it may also be that the increase is canceled to a certain extent by the perpendicular Cu–im binding. The perpendicular binding of PC in Cu(bpy)(PC) makes room for incoming im or, in other words, does not interfere with the Cu–im binding. On the basis of these considerations we may conclude that coordinated PC does not interfere with coordination of im both sterically and electronically or even promote it if we take into account the statistical factor of –0.30 due to decrease in the number of coordination sites available for im.[26]

This conclusion is further supported by the apparent stability constants log K' for the step defined by eq 2. From the plots of absorption spectral magnitudes for Cu(bpy)(PC)(X) against X (Figure 2), we obtain the log K' values 3.6 for im (pH 7.0), 3.7 for NH_3 (pH 9.0), and 2.5 for N_3^- (pH 9.0). The value for im is in good agreement with log K obtained potentiometrically (Table IV), and that for NH_3 shows that the step for Cu(bpy)(PC)(NH_3) is more favored than expected on statistical grounds for the fourth NH_3 to coordinate to $Cu(NH_3)_3$ ($K_4/K_1 = 1/16$; $\log K_1 = 4.2$;[25] $\log K_4 = 4.2 - 1.20 = 3.0$). Considering that Cu(bpy)(PC) is a stable ternary species that is capable of accepting a further ligand and that the pterin ring has been reported to interact with the Cu(II) site of PAH from *Chromobacterium violaceum*[8c] with two coordinated imidazoles,[8b] Cu(bpy)(PC) may serve as a model for the Cu site and Cu(bpy)(PC)(X) as a model for the PAH–ptern cofactor–substrate complex possibly formed as an intermediate in the enzymatic reaction.

Coordination Modes of Pteridine Compounds. Structural features and orientations of the pteridine ring in the ternary complexes are provided by X-ray structural analysis of Cu(bpy)(LM), Cu(bpy)(PC), and two other pteridine-containing ternary Cu(II) complexes, $Cu(ethp)_2(phen)$ and Cu(tppb)(pterin) (ethp = 2-(ethylthio)-4-oxopteridine; tppb = tris(3-phenylpyrazolyl)hydroborate) reported recently by Burgmayer et al.[29,30] The molybdenum complex[31] of xanthopterin and the cobalt complex[32] of 2-(ethylthio)-4-hydroxypteridine have also been reported by Burgmayer and Stiefel. For comparison the structural data

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[30] After submission of the manuscript, Karlin et al. reported the structure of the Cu(II) complex of a new pterin ligand with the pterin N(5) atom coordinating from an axial position in a distorted square-pyramidal geometry (Nasir, M. S.; Karlin, K. D.; Chen, Q.; Zubieta, J. *J. Am. Chem. Soc.* **1992**, *114*, 2264–2265).

[31] Burgmayer, S. J. N.; Stiefel, E. I. *J. Am. Chem. Soc.* **1986**, *108*, 8310–8311.

[32] Burgmayer, S. J. N.; Stiefel, E. I. *Inorg. Chem.* **1988**, *27*, 4059–4065.

Table V. Comparison of the Bond Lengths (Å) of the Cu(II)–Pteridine Moiety and Orientations of the Pteridine Ring Relative to the Equatorial Plane in Ternary Copper(II) Complexes

structure	Cu(bpy)(PC)(H$_2$O)[b]	Cu(bpy)(LM)[c]	Cu(tppb)(pterin)[d]	Cu(ethp)$_2$(phen)[d]
coordination structure	distorted octahedral	square-pyramidal	square-pyramidal	distorted octahedral
pteridine ring orientation	perpendicular	perpendicular	planar	perpendicular
bond length[a]	Cu(bpy)(PC)(H$_2$O)[b]	Cu(bpy)(LM)[c]	Cu(tppb)(pterin)[d]	Cu(ethp)$_2$(phen)[d]
Cu–N(1)	–	1.989 (9)	–	–
Cu–N(5)	2.013 (3)	1.974 (9)	2.033 (3)	2.017 (4), 2.061 (3)
Cu–O(4)	2.499 (3)	2.316 (9)	1.968 (2)	2.303 (4), 2.305 (4)
C(4)–O(4)	1.238 (5)	1.226 (14)	1.282 (4)	1.250 (5), 1.246 (5)
N(1)–C(2)	1.361 (6)	1.342 (16)	1.333 (4)	1.325 (7), 1.318 (7)
C(2)–N(3)	1.359 (5)	1.440 (16)	1.378 (5)	1.335 (6), 1.353 (6)
N(3)–C(4)	1.339 (5)	1.327 (16)	1.318 (4)	1.349 (6), 1.353 (6)
C(4)–C(4a)	1.471 (6)	1.487 (20)	1.441 (4)	1.449 (7), 1.447 (7)
C(4a)–C(8a)	1.400 (5)	1.391 (15)	1.390 (5)	1.390 (6), 1.404 (6)
C(8a)–N(1)	1.341 (5)	1.356 (14)	1.337 (5)	1.351 (7), 1.353 (7)

[a] The numbering scheme shown in Figure 5 is used. [b] Reference 9. [c] This work. [d] Reference 27. tppb = tris(3-phenylpyrazolyl)hydroborate; ethp = 2-(ethylthio)-4-oxopteridine.

relevant to the copper(II)–pteridine moiety are summarized in Table V, from which we see that in the complexes with bpy or phen the pteridine ring coordinates to Cu(II) through N(5) at an equatorial position and a weak axial Cu–O(4) bond (2.30–2.50 Å). Only in the case of Cu(tppb)(pterin) does it bind with Cu(II) through the O(4) and N(5) atoms in the equatorial plane with the Cu(II)–O(4) length of 1.968 Å and an increased single bond character of the C(4)–O(4) bond. This equatorial bonding may be due to the structural requirements imposed by tppb coordination and the stacking between the pterin and phenyl rings. Probably due to the Cu(II) binding through N(1), a remarkable difference is observed in the C(2)–N(3) bond length in Cu(bpy)(LM) (1.440 Å), indicating that the bond has a single bond character with the negative charge probably localized on the C(2)–N(3) π^* bond orbital.

In the solid state both LM and ethp, which are devoid of the 6-substituent, coordinate to Cu(II) with their rings perpendicular to the coordination plane as has been found for PC in Cu(bpy)(PC)(H$_2$O) where PC cannot occupy two equatorial positions for steric reasons. This could mean that the pteridine ring has a tendency to coordinate to Cu(II) by using an equatorial and an axial position. However, the d–d absorption and ESR spectral data for Cu(bpy)(LM) in aqueous solution (pH 9.0) indicated that the Cu(II) chromophore has a 3N1O coordination, implying that LM occupies two equatorial positions. Orientation of Cu(II)-coordinated pteridine rings is therefore probably determined mainly by the steric requirements of the Cu(II) environment, but the stability constants for the ternary systems suggest that the two axial coordinations by the oxygen atoms of PC make additional contributions to $\mathrm{Cu^{II}}$–PC bonding.

Concluding Remarks

The PC-containing ternary complex Cu(bpy)(PC) has been revealed to have a unique tendency to form quaternary complexes with considerable stability. This is evident from the log K value of 3.74 for Cu(bpy)(PC)(im) (eqs 2 and 5), which is comparable with the value of 3.77 for formation of Cu(bpy)(im) from Cu(bpy) and im. Because PC in Cu(bpy)(PC) occupies only one equatorial position with its ring perpendicular to the Cu(II) plane, it presents no serious steric hindrance to the incoming ligand X and may not greatly decrease the electronegativity of Cu(II) with bound bpy owing to its electron-deficient nature, thus favoring quaternary complex formation.

According to Benkovic et al.,[8c] the pterin ring directly interacts with the copper site of PAH which has been reported to have two coordinated imidazole rings.[8b] Formation of Cu(bpy)(PC)(X) suggests that a PAH–pterin cofactor–substrate complex may be formed as an intermediate in the process of the enzyme activity. Interestingly pterin derivatives, which have a substituent at the C(6) position such as in biopterin, 6-methylpterin, and 6,7-dimethylpterin, are efficient in hydroxylation of phenylalanine by PAH requiring 1 mol of their tetrahydro form for conversion of 1 mol of phenylalanine to tyrosine,[33] whereas pterin and 7-methylpterin, neither of which have a 6-substituent, are less efficient and 3 mol of the tetrahydro form are required for the same reaction.[33,34] This may mean that the C(6)-substituent is necessary at least in part for the cofactor activity to make the perpendicular orientation and the substrate binding possible.

The present results indicate that the copper site of PAH may bind a pterin cofactor and still offer an additional binding site for the substrate, i.e., phenylalanine, or a dioxygen molecule to form a quaternary complex that would be favorable for site-specific hydroxylation of the benzene ring for conversion to *p*-tyrosine and has been proposed from biochemical studies.[11]

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Supplementary Material Available: Tables for compositions of the samples for pH titrations, atomic coordinates and isotropic thermal parameters, positional parameters for hydrogen atoms, and anisotropic temperature factors for non-hydrogen atoms (5 pages); table of observed and calculated structure factors (6 pages). Ordering information is given on any current masthead page.

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