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# Synthetic and Structural Studies on Copper(II) Complexes Containing Coenzyme PQQ and Terpyridine

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The mononuclear and dinuclear ternary Cu(II) complexes of coenzyme pyrroloquinoline quinone (PQQ) and 2,2′:6′,2″-terpyridine (terpy) have been isolated from aqueous solutions containing PQQ and [Cu(terpy)]$^{2+}$ complex at pH 3 and 7, respectively, regardless of the molar ratios of the starting materials. The structure of the dinuclear Cu(II) complex has been determined by X-ray diffraction method. Crystallographic data are as follows: [$Cu_2$(PQQ)(terpy)$_2$]·12$H_2O$·$CH_3CN$, triclinic, $P\bar{1}$, $a$ = 22.466(3) Å, $b$ = 10.862(5) Å, $c$ = 10.890(2) Å, $\alpha$ = 94.93(3)°, $\beta$ = 97.36(2)°, $\gamma$ = 90.31(2)°, and $Z$ = 2. The molecular structure consists of two six-coordinate Cu(II) ions bridged by PQQ with two equatorially coordinating terpy ligands. Each of the Cu(II) ions occupies the pyridine and the pyrrole moieties of PQQ, which has the tetraanionic form with three carboxylates and the deprotonated pyrrole group. On account of the binding of PQQ to Cu(II), the ring system is twisted. On the other hand, PQQ in the mononuclear complex ([Cu(PQQ)(terpy)]) obtained from acidic solution seems to be predominantly coordinated to Cu(II) with the pyridine moiety, as judged from the pH values of the complex syntheses and a comparison of the diffuse reflectance spectra of two ternary Cu(II) complexes. In neutral solution, the second [Cu(terpy)]$^{2+}$ complex would attach the pyrrole moiety of PQQ in the mononuclear complex, which results in the formation of the dinuclear Cu(II) complex containing PQQ with the deprotonated pyrrole moiety.

## Introduction

Since PQQ (4,5-dihydro-4,5-dioxo-1*H*-pyrrolo[2,3-*f*]quinoline-2,7,9-tricarboxylic acid) was first recognized as a coenzyme for alcohol dehydrogenases in methylotrophic bacteria,[1] it has been remarked to be also a coenzyme in numerous oxidoreductases.[2] In 1984, two research groups independently reported that copper-containing amine oxidases contain covalently bound PQQ, and accordingly the structural and the functional interactions between Cu and PQQ at the active site were of interest.[3,4] However, the most plausible candidate of an organic cofactor in the amine oxidases was demonstrated to be topa quinone (6-hydroxydopa quinone) residue, as mentioned in many recent papers.[2,5-11]

Ca(II) and Sr(II) ions are essential for the reconstitution of alcohol dehydrogenase from the apoenzyme plus PQQ.[12,13] Some transition metal ions are also required for the reconstitutions of membrane glycerol dehydrogenase (Co(II) and Ni(II))[14] and membrane-bound aldehyde dehydrogenase (Mn(II)).[15] Moreover, nitrile hydratase has been described to contain PQQ and Fe(III) in the active center.[16] Consequently, it is important to know about the interaction between PQQ and metal ions.

Several studies on the metal complexes of PQQ and its analogues have been reported so far.[17-22] The PQQ molecule possesses three binding sites as a multidentate ligand (Figure 1). Our previous short communications presented the preparation and characterization of PQQ and 1-methyl PQQ Cu(II) complexes containing 2,2′-bipyridine (bpy) or terpy; the bindings of PQQ and its derivative to Cu(II) probably take place at their pyridine moieties (site 1 in Figure 1).[19-21] Moreover, the binding site of PQQ with *cis*-[Ru(bpy)$_2$$Cl_2$] has been demonstrated to be the *o*-quinone moiety (site 2).[22] However, there has been no report on the crystal structure of a transition metal complex containing PQQ.

Here we describe the synthetic and spectroscopic investigations of the PQQ-containing mononuclear and dinuclear Cu(II) complexes (complexes **1** and **2**, respectively) together with the X-ray crystal analysis of complex **2**.

## Experimental Section

PQQ was purchased from Ube Industries (Tokyo, Japan). [Cu(terpy)]($NO_3$)$_2$·$H_2O$ was synthesized from Cu(II) nitrate according to a method described in the previous paper.[23] Other commercial chemicals were used without further purification. The diffuse reflectance spectra were recorded on a Hitachi U-3400 spectrophotometer.

**Preparation of Complex 1: [Cu(PQQ)(terpy)].**[19] Complex **1** was obtained as follows. PQQ (10 mg, 30 $\mu$mol) was dissolved in about 2 mL of 0.1 M KOH solution, and then the pH value of the solution was adjusted

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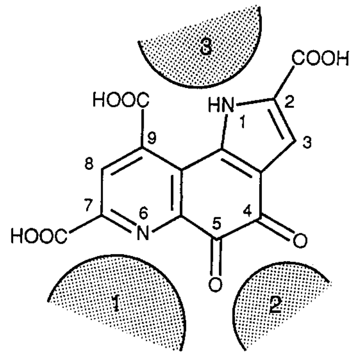

**Figure 1.** Possible coordination sites of PQQ.

to about 7 by the addition of 0.1 M HCl solution. After the solution was added to an aqueous solution (2 mL) of [Cu(terpy)](NO$_3$)$_2$·H$_2$O (26 mg, 60 μmol), the pH value of the mixture was adjusted to about 3 by the addition of 0.1 M HCl solution. The reddish brown powder was collected by filtration, washed with water, and then dried in vacuo overnight. Anal. Calcd for $C_{29}H_{15}N_5O_8Cu·2.5H_2O$: C, 51.98; H, 3.01; N, 10.45. Found: C, 52.04; H, 3.06; N, 10.51.

**Preparation of Complex 2: [Cu$_2$(PQQ)(terpy)$_2$].** PQQ (10 mg, 30 μmol) was dissolved in about 2 mL of 0.1 M KOH solution. [Cu(terpy)](NO$_3$)$_2$·H$_2$O (26 mg, 60 μmol) in 2 mL of water was added to the solution. The pH value of the mixture was adjusted to 7.0 by the addition of 0.1 M HCl solution. The resulting yellowish green solution was allowed to stand at room temperature. After a few days, microcrystals appeared, which were collected by filtration and washed with water. The dark-green microcrystals were powdered and then dried in vacuo overnight. Anal. Calcd for $C_{44}H_{24}N_8O_8Cu_2·7H_2O$: C, 50.52; H, 3.66; N, 10.71. Found: C, 50.26; H, 3.66; N, 10.67.

Complex **2** could also be obtained as follows. Complex **1** (40 mg, 60 μmol) was dissolved in a minimum quantity of 0.1 M KOH solution, and then the pH value of the solution was adjusted to 7.0 by the addition of 0.1 M HCl solution. The reddish brown solution was allowed to stand at room temperature. After a few days, the resulting dark-green microcrystals were collected by filtration and washed with water. The microcrystals were dried in vacuo overnight. Anal. Calcd for $C_{44}H_{24}N_8O_8Cu_2·6H_2O$: C, 51.41; H, 3.53; N, 10.90. Found: C, 51.40; H, 3.41; N, 10.86.

**Crystallographic Structure Determination on [Cu$_2$(PQQ)(terpy)$_2$]·12H$_2$O·CH$_3$CN.** The crystals suitable for X-ray diffraction were obtained as follows. Complex **1** (4 mg, 6 μmol) was dissolved in about 3 mL of 0.1 M KOH solution. After the pH value of the solution was adjusted to 7.0 by the addition of 0.1 M HCl solution, a small amount of acetonitrile was added to it. The mixture was allowed to stand at room temperature. After a few weeks, dark-green crystals were obtained as needles. A needle-shaped crystal was sealed in a glass capillary to retard loss of water and acetonitrile of crystallization. Diffraction data were collected using a Rigaku AFC-5R automated four-circle diffractometer with Ni-filtered Cu Kα radiation. The unit cell constants were refined from 12 centered reflections ($13° < 2\theta < 21°$). Intensity data in the range $2\theta < 120°$ were collected by the $\omega$–$2\theta$ scan technique; $\omega$-scan width $(1.30 + 0.15 \tan\theta)°$, scan speed (8.0° min$^{-1}$). The intensities of 7807 unique reflections were measured in the range $0 \leq 2\theta \leq 125°$ (*hkl* ranges: −25 to 25; −12 to 12; 0 to 12), of which 2137 had $I > 3\sigma(I)$ and were used. Intensities of three reflections, chosen as standards and measured every 100 reflections, showed no significant variations (<2%). Data were corrected for Lorentz and polarization effects. An empirical absorption correction based on a series of $\psi$-scans was applied to the data;[24,25] maximum and minimum correction factors were 1.001 and 0.804, respectively. The crystallographic data are summarized in Table 1.

**Table 1.** Crystallographic Data for [Cu$_2$(PQQ)(terpy)$_2$]·12H$_2$O·CH$_3$CN (**2**)

| | |
|---|---|
| formula | $C_{46}H_{51}N_9O_{20}Cu_2$ |
| fw | 1177.05 |
| color and habit | dark-green needles |
| cryst syst | triclinic |
| space group | $P\bar{1}$ |
| $a$, Å | 22.466(3) |
| $b$, Å | 10.862(5) |
| $c$, Å | 10.890(2) |
| $\alpha$, deg | 94.93(3) |
| $\beta$, deg | 97.36(2) |
| $\gamma$, deg | 90.31(2) |
| $V$, Å$^3$ | 2626(1) |
| $Z$ | 2 |
| cryst dimens, nm | 0.10 × 0.10 × 0.31 |
| $D_{calcd}$, g cm$^{-3}$ | 1.489 |
| $\mu$(Cu Kα), cm$^{-1}$ | 16.86 |
| $F$(000) | 1216 |
| scan method | $\omega$–$2\theta$ |
| $2\theta$ range, deg | 0–120 |
| no. of unique data collcd | 7807 |
| no. of data used in refinement | 2137 ($\|F_o\| > 3\sigma\|F_o\|$) |
| $R^a$ | 0.078 |
| $R_w{}^b$ | 0.062 |

$^a R = \sum||F_o| - |F_c||/\sum|F_o|$. $^b R_w = [\sum w(|F_o| - |F_c|)^2/\sum w(F_o)^2]^{1/2}$.

The structure was solved by the direct method (MULTAN 78)[26] and refined by a block-diagonal least squares method using the program HBLS-V.[27] Anisotropic temperature factors were used for non-hydrogen atoms. The positions of the hydrogen atoms, except on water and acetonitrile, were calculated based on the molecular geometry. These hydrogen atoms with isotropic temperature factors were included in further refinement. The weighting scheme used was $w = 1 / \sigma^2(F_o)$. The final $R$ and $R_w$ values were 0.078 and 0.062 for 2137 reflections ($|F_o| > 3\ \sigma|F_o|$), respectively. Atomic scattering factors are from ref 28. All the computations were carried out on an ACOS 2020 computer at the Computation Center, Osaka University, and on an ACOS S-850 computer at the Crystallographic Research Center, Institute for Protein Research, Osaka University.

The atomic positional parameters and equivalent isotropic temperature factors for the non-hydrogen atoms are listed in Table 2. The complete crystallographic summary and lists of the atomic coordinates of the hydrogen atoms, the non-hydrogen anisotropic thermal parameters, and the full listings of bond lengths and bond angles are available as supplementary material.

**EXAFS Analysis.** EXAFS spectra were measured at BL-6B of the Photon Factory in the National Laboratory for High Energy Physics (KEK-PF). The measurements were carried out with a beam current of about 250 mA and a storage-ring energy of 2.5 GeV. The data were collected with a Si(111) double crystal monochromator at room temperature in the transmission mode on a pellet sample diluted with polyethylene to reduce thickness effects. Nitrogen-filled ionization chambers ($I_0$ and $I$) were used. The energy was defined by assigning the first inflection point of the Cu foil spectrum to 8980.3 eV. The analysis of EXAFS was carried out by subtracting a smooth varying part estimated from a cubic spline and normalizing the spectrum to the absorption edge. Extracted EXAFS modulation was analyzed on the basis of plane-wave single-scattering theory.[29] The backscattering amplitude $F(\mathbf{k})$ and the phase shift $\phi(\mathbf{k})$ functions employed were the theoretical curves tabulated by Teo and Lee.[30,31] The fixed reducing factors $S$ obtained from the analysis of model compounds were used. The coordination number $N$, the Debye-Waller factor $\sigma$, the interatomic distance $r$, and the difference in threshold energy $\Delta E_0$ are determined in the nonlinear least-squares refined curve fitting procedure. The extraction of the EXAFS, the Fourier transformation, and the curve fitting were processed by using the systematic programs EXAFS 1.[32] All calculations were performed on a HITAC M-682H computer at the Computer Center of the University of Tokyo.

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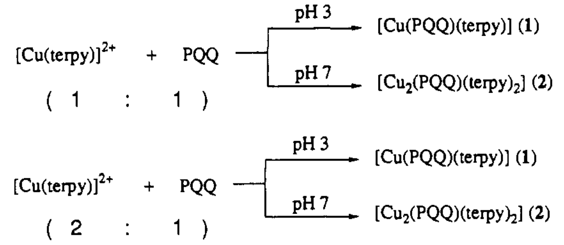
**Table 2.** Atomic Coordinates and Equivalent Isotropic Thermal Parameters (Å$^2$) for Non-Hydrogen Atoms in $[Cu_2(PQQ)(terpy)_2]\cdot12H_2O\cdot CH_3CN$ (**2**)[a]

| atom | *x*/*a* | *y*/*b* | *z*/*c* | $B_{eq}{}^b$ | atom | *x*/*a* | *y*/*b* | *z*/*c* | $B_{eq}{}^b$ |
|---|---|---|---|---|---|---|---|---|---|
| Cu(1) | 0.4014 (2) | 0.0683 (3) | 0.2168 (3) | 2.64 | C(8t) | 0.5972 (8) | −0.085 (2) | 0.273 (2) | 4.39 |
| Cu(2) | 0.1197 (2) | 0.5095 (3) | 0.3074 (3) | 2.52 | C(9t) | 0.5827 (8) | 0.005 (2) | 0.189 (2) | 3.15 |
| O(4p) | 0.3933 96) | 0.412 (2) | 0.573 (2) | 4.74 | C(10t) | 0.5233 (8) | 0.055 (2) | 0.176 (2) | 2.94 |
| O(5p) | 0.4090 (5) | 0.233 (2) | 0.390 (2) | 3.37 | C(11t) | 0.5010 (8) | 0.147 (2) | 0.095 (2) | 2.65 |
| O(15p) | 0.1939 (6) | 0.749 (2) | 0.610 (2) | 5.05 | C(12t) | 0.5361 (9) | 0.209 (2) | 0.024 (2) | 3.66 |
| O(16p) | 0.1256 (6) | 0.674 (2) | 0.448 (2) | 4.56 | C(13t) | 0.508 (1) | 0.296 (3) | −0.050 (2) | 5.43 |
| O(18p) | 0.2626 (7) | −0.022 (2) | −0.067 (2) | 6.21 | C(14t) | 0.4482 (9) | 0.326 (2) | −0.041 (2) | 5.02 |
| O(19p) | 0.3454 (5) | −0.044 (1) | 0.062 (2) | 2.57 | C(15t) | 0.4165 (7) | 0.265 (2) | 0.030 (2) | 2.46 |
| O(21p) | 0.1851 (6) | 0.458 (2) | 0.030 (2) | 4.30 | C(21t) | 0.1472 (9) | 0.721 (2) | 0.144 (2) | 4.65 |
| O(22p) | 0.1226 (5) | 0.367 (2) | 0.133(2) | 3.56 | C(22t) | 0.127 (1) | 0.796 (3) | 0.046 (2) | 5.83 |
| N(1p) | 0.2081 (6) | 0.514 (2) | 0.363 (2) | 1.65 | C(23t) | 0.071 (1) | 0.796 (3) | −0.010 (2) | 5.77 |
| N(6p) | 0.3214 (7) | 0.155 (2) | 0.203 (2) | 3.21 | C(24t) | 0.0283 (9) | 0.725 (2) | 0.039 (2) | 4.73 |
| N(1t) | 0.3910 (7) | −0.050 (2) | 0.343 (2) | 3.98 | C(25t) | 0.0476 (9) | 0.646 (2) | 0.133 (2) | 4.06 |
| N(2t) | 0.4836 (6) | 0.006 (2) | 0.239 (2) | 1.95 | C(26t) | 0.0048 (9) | 0.566 (2) | 0.185 (2) | 4.02 |
| N(3t) | 0.4432 (7) | 0.179(2) | 0.106 (2) | 3.61 | C(27t) | −0.0568 (9) | 0.554 (2) | 0.144 (2) | 3.97 |
| N(4t) | 0.1068 (6) | 0.642 (2) | 0.174 (2) | 3.08 | C(28t) | −0.091 (1) | 0.474 (3) | 0.207 (3) | 6.37 |
| N(5t) | 0.0312 (6) | 0.495 (2) | 0.269 (2) | 2.99 | C(29t) | −0.0611 (9) | 0.410 (2) | 0.303 (2) | 4.76 |
| N(6t) | 0.1008 (6) | 0.385 (2) | 0.425 (2) | 3.08 | C(30t) | 0.0021 (8) | 0.421 (2) | 0.332 (2) | 2.85 |
| C(2p) | 0.2227 (9) | 0.590 (2) | 0.475 (2) | 3.47 | C(31t) | 0.0420 (8) | 0.358 (2) | 0.421 (2) | 3.05 |
| C(3p) | 0.2805 (8) | 0.563 (2) | 0.532 (2) | 3.26 | C(32t) | 0.0168 (9) | 0.282 (2) | 0.503 (2) | 3.46 |
| C(4p) | 0.353 (1) | 0.395 (2) | 0.481 (2) | 4.78 | C(33t) | 0.0588 (8) | 0.225 (2) | 0.588 (2) | 3.64 |
| C(5p) | 0.3609 (8) | 0.287 (2) | 0.383 (2) | 3.07 | C(34t) | 0.1200 (9) | 0.258 (2) | 0.591 (2) | 4.94 |
| C(7p) | 0.2813 (7) | 0.120 (2) | 0.106 (2) | 2.37 | C(35t) | 0.1369 (8) | 0.330 (2) | 0.508 (2) | 3.09 |
| C(8p) | 0.2342 (8) | 0.193 (2) | 0.073 (2) | 1.63 | O(1w) | 0.0982 (7) | 0.120 (2) | 0.138 (2) | 6.54 |
| C(9p) | 0.2242 (8) | 0.297 (2) | 0.148 (2) | 2.43 | O(2w) | 0.0101 (8) | 0.024 (2) | 0.265 (2) | 8.75 |
| C(10p) | 0.2577 (8) | 0.435 (2) | 0.349 (2) | 2.85 | O(3w) | 0.1915 (7) | 0.026 (2) | 0.324 (2) | 7.48 |
| C(11p) | 0.2969 (8) | 0.462 (2) | 0.456 (2) | 2.79 | O(4w) | 0.0990 (8) | 0.925 (2) | 0.436 (2) | 8.99 |
| C(12p) | 0.3125 (7) | 0.257 (2) | 0.277 (2) | 2.46 | O(5w) | 0.2767 (6) | 0.161 (2) | 0.508 (2) | 5.95 |
| C(13p) | 0.2634 (8) | 0.329 (2) | 0.258 (2) | 2.37 | O(6w) | 0.4671 (8) | 0.579 (2) | 0.727 (2) | 9.96 |
| C(14p) | 0.1788 (8) | 0.683 (2) | 0.514 (2) | 2.76 | O(7w) | 0.2593 (7) | 0.174 (2) | 0.756 (2) | 8.20 |
| C(17p) | 0.2965 (8) | 0.006 (2) | 0.026 (2) | 3.20 | O(8w) | 0.2929 (8) | 0.770 (2) | 0.789 (2) | 8.04 |
| C(20p) | 0.1726 (7) | 0.380 (2) | 0.098 (2) | 1.86 | O(9w) | 0.3599 (8) | 0.581 (2) | 0.863 (2) | 8.48 |
| C(1t) | 0.3418 (8) | −0.070 (2) | 0.399 (2) | 2.43 | O(10w) | 0.0911 (7) | 0.084 (2) | 0.877 (2) | 6.60 |
| C(2t) | 0.3385 (9) | −0.151 (2) | 0.488 (2) | 4.57 | O(11w) | 0.2825 (7) | 0.394 (2) | 0.896 (2) | 9.66 |
| C(3t) | 0.389 (1) | −0.211 (2) | 0.528 (2) | 4.17 | O(12w) | 0.142 (2) | 0.947 (3) | 0.706 (3) | 22.21 |
| C(4t) | 0.443 (1) | −0.201 (2) | 0.476 (2) | 4.36 | N(1a) | 0.401 (1) | 0.515 (2) | 0.234 (2) | 8.68 |
| C(5t) | 0.4425 (8) | −0.121 (2) | 0.386 (2) | 3.44 | C(1a) | 0.368 (1) | 0.581 (2) | 0.201 (3) | 5.73 |
| C(6t) | 0.4936 (8) | −0.080 (2) | 0.324 (2) | 3.01 | C(2a) | 0.315 (1) | 0.654 (2) | 0.160 (3) | 5.47 |
| C(7t) | 0.554 (2) | −0.131 (2) | 0.343 (2) | 5.87 | | | | | |

[a] The letters p, w, and a represent the PQQ, water, and acetonitrile molecules, respectively. [b] $B_{eq} = {}^4/_3\sum_i\sum_j B_{ij}a_ia_j$.

**Scheme 1**



## Results and Discussion

**Syntheses of Ternary Cu(II) Complexes Containing PQQ.** The preparations of the ternary Cu(II) complexes containing PQQ are shown in Scheme 1. The mononuclear and dinuclear Cu(II) complexes were obtained at pH 3 and 7, respectively, regardless of the molar ratio of PQQ to $[Cu(terpy)]^{2+}$. This finding means that the binding of the second $[Cu(terpy)]^{2+}$ complex to PQQ is affected by the change of the pH value. The acid dissociation constants (p$K_a$) of the pyridine (N-6 in Figure 1) and the *o*-quinone (C-4 and C-5) moieties are less than 1.0; the p$K_a$ values of two carboxyl groups (C-7 and C-9) are 1.6 2.2.[33] On the other hand, the p$K_a$ values of the carboxyl group (C-2) and the N–H group (N-1) of the pyrrole moiety are 3.3 and 10.3, respectively.[33,34] At pH 3, therefore, the two carboxyl groups (C-7 and C-9) of the pyridine ring are almost dissociated but the carboxyl group (C-2) and the N–H group (N-1) of the pyrrole moiety are mostly protonated. At pH 7, all carboxyl groups are deprotonated. Taking into account these p$K_a$ values and the pH values of the complex syntheses, the predominant binding site of PQQ seems to be the pyridine moiety (site 1). Consequently, complexes **1** and **2** were probably produced under acidic and neutral pH conditions, respectively.

**Crystal Structure of $[Cu_2(PQQ)(terpy)_2]\cdot12H_2O\cdot CH_3CN$.** A view showing the features of complex **2** is given in Figure 2. The selected bond lengths and bond angles are presented in Table 3. Poor crystal quality requires that some caution must be exercised in interpreting the crystallographic results.[35] In particular, bond distances and angles should be discussed within the estimated standard deviations. The molecular structure of complex **2** consists of two six-coordinate Cu atoms bridged by PQQ with two equatorially coordinating terpy ligands in the terminal positions. Cu(1) and Cu(2) occupy site 1 and site 3 of PQQ (Figure 1), respectively. PQQ acts as a tridentate ligand, being perpendicular to two terpy rings which are almost parallel to each other, as shown in Figure 3. The dihedral angles between the PQQ ring and the terpy 1 or terpy 2 ring are estimated to be 82.4 and 92.6°, respectively. The dihedral angle between two terpy rings is estimated to be 24.3°.

The coordination spheres of two Cu(II) ions are nearly identical, as shown in Figure 4. Each Cu(II) ion is basically in a distorted

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(33) Kano, K.; Mori, K.; Uno, B.; Kubota, T.; Ikeda, T.; Senda, M. *Bioelectrochem. Bioenerg.* **1990**, *24*, 193.

(34) Kano, K.; Mori, K.; Uno, B.; Kubota, T.; Ikeda, T.; Senda, M. *Bioelectrochem. Bioenerg.* **1990**, *23*, 227.

(35) The crystal was used for data collection in spite of its poor quality, because single crystals were extremely difficult to obtain and treat, and repeated attempts by many methods over a year's time failed to produce crystals having better quality.

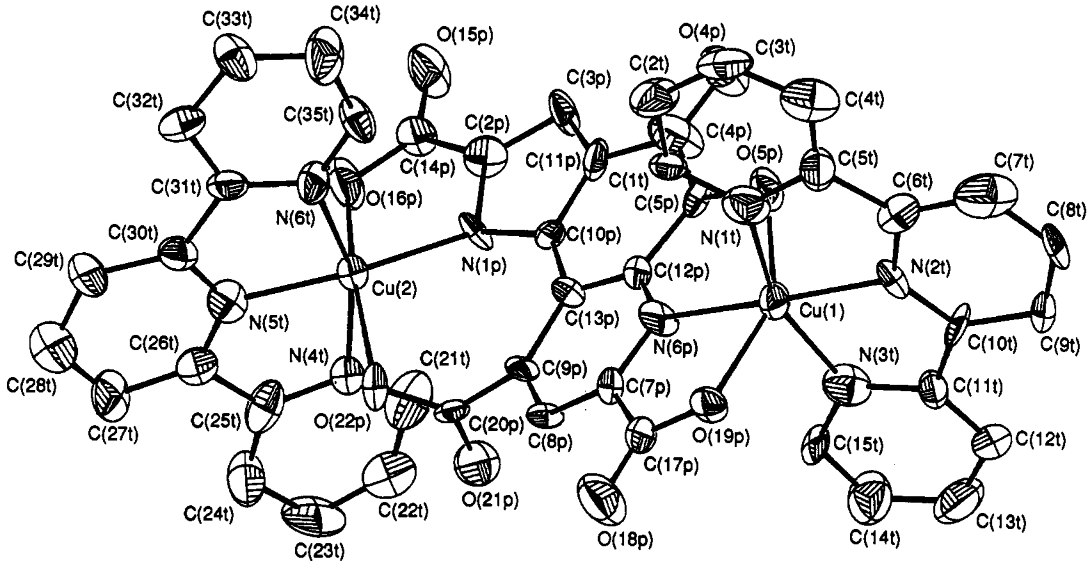

**Figure 2.** Molecular structure of $[Cu_2(PQQ)(terpy)_2]\cdot 12H_2O\cdot CH_3CN$ (**2**) showing the 50% probability thermal ellipsoids and atom-labeling scheme. Water and acetonitrile molecules and hydrogen atoms are omitted.

**Table 3.** Selected Bond Lengths (Å) and Bond Angles (deg) for $[Cu_2(PQQ)(terpy)_2]\cdot 12H_2O\cdot CH_3CN$ (**2**)[a]

| | | | |
|---|---|---|---|
| | Bond Lengths | | |
| Cu(1)–O(5p) | 2.47 (1) | Cu(1)–O(19p) | 2.23 (1) |
| Cu(1)–N(6p) | 2.03 (2) | Cu(1)–N(1t) | 2.00 (2) |
| Cu(1)–N(2t) | 1.96 (1) | Cu(1)–N(3t) | 2.08 (2) |
| Cu(2)–O(16p) | 2.24 (1) | Cu(2)–O(22p) | 2.36 (1) |
| Cu(2)–N(1p) | 2.00 (1) | Cu(2)–N(4t) | 2.12 (2) |
| Cu(2)–N(5t) | 1.98 (2) | Cu(2)–N(6t) | 2.02 (2) |
| O(4p)–C(4p) | 1.25 (2) | O(5p)–C(5p) | 1.23 (2) |
| O(15p)–C(14p) | 1.22 (2) | O(16p)–C(14p) | 1.31 (2) |
| O(18p)–C(17p) | 1.20 (2) | O(19p)–C(17p) | 1.27 (2) |
| O(21p)–C(20p) | 1.23 (2) | O(22p)–C(20p) | 1.24 (2) |
| N(1p)–C(2p) | 1.41 (2) | N(1p)–C(10p) | 1.43 (2) |
| N(6p)–C(7p) | 1.33 (2) | N(6p)–C(12p) | 1.35 (2) |
| C(2p)–C(3p) | 1.41 (3) | C(2p)–C(14p) | 1.49 (3) |
| C(3p)–C(11p) | 1.40 (3) | C(4p)–C(5p) | 1.54 (3) |
| C(4p)–C(11p) | 1.47 (3) | C(5p)–C(12p) | 1.49 (3) |
| C(7p)–C(8p) | 1.36 (2) | C(7p)–C(17p) | 1.52 (3) |
| C(8p)–C(9p) | 1.37 (2) | C(9p)–C(13p) | 1.41 (3) |
| C(9p)–C(20p) | 1.54 (2) | C(10p)–C(11p) | 1.38 (3) |
| C(10p)–C(13p) | 1.47 (3) | C(12p)–C(13p) | 1.36 (3) |
| | Bond Angles | | |
| O(5p)–Cu(1)–O(19p) | 149.5 (4) | O(5p)–Cu(1)–N(6p) | 73.5 (5) |
| O(5p)–Cu(1)–N(1t) | 86.9 (5) | O(5p)–Cu(1)–N(2t) | 99.9 (5) |
| O(5p)–Cu(1)–N(3t) | 92.0 (5) | O(19p)–Cu(1)–N(6p) | 76.5 (5) |
| O(19p)–Cu(1)–N(1t) | 94.1 (5) | O(19p)–Cu(1)–N(2t) | 110.4 (5) |
| O(19p)–Cu(1)–N(3t) | 96.9 (5) | N(6p)–Cu(1)–N(1t) | 101.8 (6) |
| N(6p)–Cu(1)–N(2t) | 172.3 (6) | N(6p)–Cu(1)–N(3t) | 96.9 (6) |
| N(1t)–Cu(1)–N(2t) | 81.6 (6) | N(1t)–Cu(1)–N(3t) | 160.1 (6) |
| N(2t)–Cu(1)–N(3t) | 79.1 (6) | O(16p)–Cu(2)–O(22p) | 167.6 (5) |
| O(16p)–Cu(2)–N(1p) | 80.1 (5) | O(16p)–Cu(2)–N(4t) | 85.0 (5) |
| O(16p)–Cu(2)–N(5t) | 98.8 (6) | O(16p)–Cu(2)–N(6t) | 96.0 (6) |
| O(22p)–Cu(2)–N(1p) | 96.3 (5) | O(22p)–Cu(2)–N(4t) | 84.4 (5) |
| O(22p)–Cu(2)–N(5t) | 86.0 (5) | O(22p)–Cu(2)–N(6t) | 96.2 (5) |
| N(1p)–Cu(2)–N(4t) | 104.7 (6) | N(1p)–Cu(2)–N(5t) | 173.6 (6) |
| N(1p)–Cu(2)–N(6t) | 94.9 (6) | N(4t)–Cu(2)–N(5t) | 81.5 (6) |
| N(4t)–Cu(2)–N(6t) | 160.2 (6) | N(5t)–Cu(2)–N(6t) | 78.9 (6) |

[a] The letters p, w, and a represent the PQQ, water, and acetonitrile molecules, respectively.

octahedral geometry with three nitrogens of terpy and one nitrogen of PQQ in the plane and with two oxygens of PQQ in the remote axial positions. The two terpy ligands reveal the similar bond lengths and angles each other. The nitrogen atoms [N(2t) and N(5t)] of the central pyridine rings are bonded to the Cu(II) ions at significantly shorter distances (Cu(1)–N(2t) = 1.96(1) Å and Cu(2)–N(5t) = 1.98(1) Å) than those of the other Cu–N(t) bonds (2.00(2)–2.12(2) Å), as a result of the constrained complex formation. The constraint due to the chelation of the terpy ligands also appears in the N(1t)–Cu(1)–N(3t) and N(4t)–Cu(2)–N(6t) angles of 160.1(6) and 160.2(6)°, respectively. These bond lengths and angles are approximately equal to those found in the structures of other terpy-containing Cu(II) complexes.[36,37] Differences between the coordination geometries around two Cu(II) ions are clear in the O–Cu–O angles (O(5p)–Cu(1)–O(19p) = 149.5(4)° and O(16p)–Cu(2)–O(22p) = 167.6(5)°); that is, the smaller bite angle of the Cu(1) site than that of the Cu(2) site results in larger deviations from octahedral geometry. The chelate ring comprised of the Cu(2), N(1p), C(10p), C(13p), C(9p), C(20p), and O(22p) atoms is the only seven-membered ring in complex **2** (Figure 3a), so that the O(22p)–Cu(2)–N(1p) angle of 96.3(5)° is larger than 90° and C(20p) deviates by 0.73 Å from the plane composed of the other six atoms (Figure 3(b)). The Cu(1)–O(5p) distance of 2.47(1) Å is longer than the other three

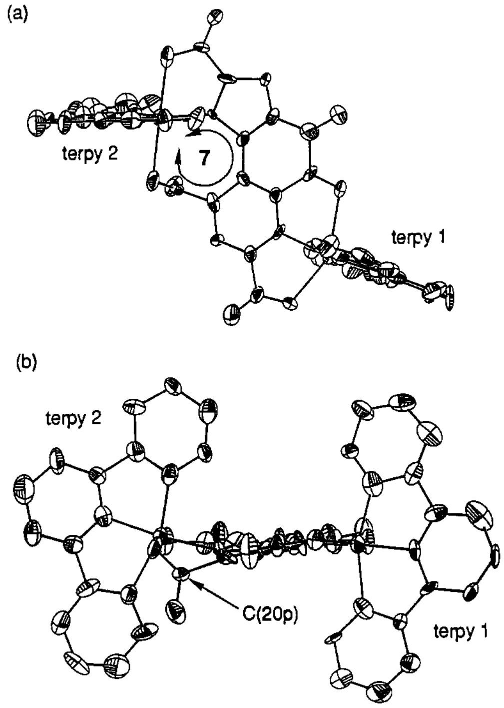

**Figure 3.** Complex **2** projected perpendicular (a) and parallel (b) to the PQQ molecule.

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(36) Anderson, O. P.; Packard, A. B.; Wicholas, M. *Inorg. Chem.* **1976**, *15*, 1613.

(37) Bresciani-Pahor, N.; Nardin, G.; Bonomo R. P.; Rizzarelli, E. *J. Chem. Soc., Dalton Trans.* **1984**, 2625.

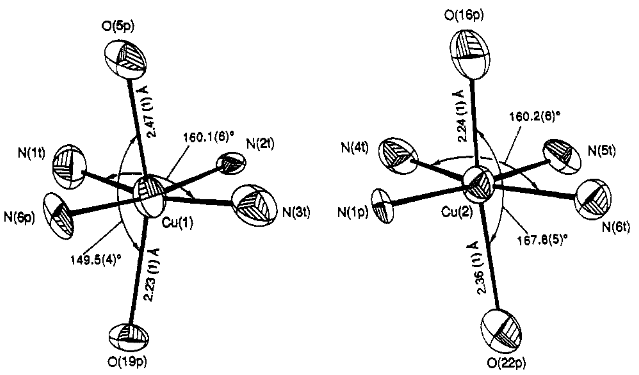

**Figure 4.** Coordination spheres of two Cu(II) ions in complex **2**. The letters p and t in parentheses refer to PQQ and terpyridine, respectively.

Cu–O bond distances in complex **2**. This is because O(5p) is carbonyl oxygen and the other three oxygens (O(16p), O(19p), and O(22p)) are carboxylate oxygens. Since the axial coordination of O(5p) is weak, Cu(1) deviates by 0.11 Å from the coordination plane (N(6p), N(1t), N(2t), and N(3t)) toward the O(19p) atom. On the other hand, Cu(2) atom lies on the plane consisting of the N(4t), N(5t), N(6t), and N(1p) atoms within experimental error.

Although PQQ has the tetraanionic form in complex **2**, the bond lengths and angles in the PQQ ligand are almost similar to those observed in the structures of the disodium $PQQ^{2-}$ salt,[38] the tripotassium $PQQ^{3-}$ salt,[39] and the 5-(2-oxopropyl)[1,40] and the 5-(2,4-dinitrophenylhydrazine)[41] adducts of PQQ. However, the longer N–C lengths in the pyrrole ring (1.41(2) and 1.43(2) Å for N(1p)–C(2p) and N(1p)–C(10p), respectively) than the corresponding N–C lengths (1.383(7) and 1.362(7) Å) of the free PQQ molecule[39] are caused by the deprotonation of the N(1p) group. A noticeable structural feature of the PQQ ligand is the twisting of the aromatic ring system. In the structures of PQQ and its adducts already reported, only the 5-(2-oxopropyl) adduct has the twisted ring system to accommodate the fully substituted $sp^3$ C-5 atom.[1,40] In spite of the absence of $sp^3$ carbon atom in the aromatic ring system of complex **2**, the ring system of PQQ is still twisted, which is due to the binding of Cu(2) to site 3 of PQQ. The large seven-membered chelate ring causes the distortion of the PQQ molecule. The dihedral angle between the pyridine and the pyrrole rings is 9.1°.

The lack of the intramolecular hydrogen bond between nitrogen of the pyrrole moiety (N(1p)) and oxygen of C-9 carboxylate (O(21p) or O(22p)) is also due to the coordination of Cu(2) to site 3 of PQQ, although such a hydrogen bond is observed in all structures of PQQ and its adducts already reported.[1,38–41] Therefore, the oxygen of the C-9 carboxylate is no longer in the plane of the aromatic ring system of PQQ.

Finally, Figure 5 shows the crystal packing of complex **2**. One of the most remarkable structural features observed in the crystal unit is the hydrogen bonding network. The oxygen atoms of PQQ and all solvent of crystallization are involved in the hydrogen bonding network. Possible hydrogen bonds and their distances are listed in Table 4. The extensive layer of the many solvent molecules in the crystal is formed through the hydrogen bonds. The hydrogen bonding network may play an essential role in stabilizing this crystal structure.

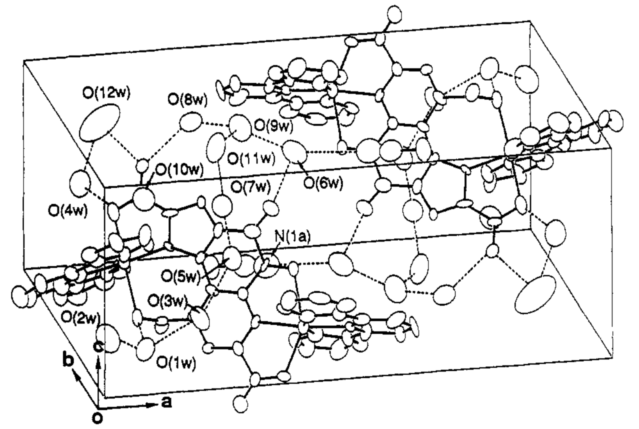

**Figure 5.** Crystal structure of complex **2**. The letters w and a in parentheses refer to water and acetonitrile, respectively. The dotted lines represent possible hydrogen bonds.

**Table 4.** Possible Hydrogen Bond Distances (Å)[a,b]

| | | | |
|---|---|---|---|
| O(4p)···O(6w) | 2.75 (2) | O(15p)···O(8w) | 2.76 (2) |
| O(15p)···O(12w) | 2.65 (3) | O(16p)···O(4w) | 2.80 (2) |
| O(22p)···O(1w) | 2.74 (2) | O(1w)···O(2w) | 2.80 (2) |
| O(1w)···O(3w) | 2.97 (2) | O(3w)···O(5w) | 2.89 (2) |
| O(4w)···O(12w) | 2.97 (4) | O(5w)···O(7w) | 2.76 (2) |
| O(6w)···O(9w) | 2.98 (3) | O(7w)···O(11w) | 2.74 (3) |
| O(8w)···O(9w) | 2.67 (2) | O(9w)···O(11w) | 2.74 (3) |
| O(4w)···O(2w)$^{i}$ | 2.83 (3) | O(4w)···O(3w)$^{i}$ | 2.80 (2) |
| O(12w)···O(10w)$^{i}$ | 2.66 (4) | O(7w)···O(18p)$^{ii}$ | 3.00 (2) |
| O(10w)···O(1w)$^{ii}$ | 2.82 (2) | O(11w)···O(21p)$^{ii}$ | 2.84 (2) |
| O(18p)···O(8w)$^{iii}$ | 2.77 (2) | O(6w)···N(1a)$^{iv}$ | 3.13 (3) |
| O(2w)···O(10w)$^{v}$ | 2.77 (2) | | |

[a] The letters p, w, and a represent the PQQ, water, and acetonitrile molecules, respectively. [b] Symmetry codes: (i) $x, 1 + y, z$; (ii) $x, y, 1 + z$; (iii) $x, -1 + y, -1 + z$; (iv) $1 - x, 1 - y, 1 - z$; (v) $-x, -y, 1 - z$.

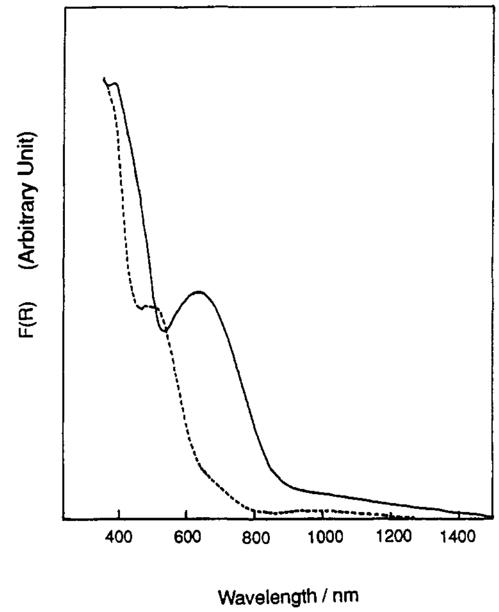

**Figure 6.** Diffuse reflectance spectra of complexes **1** (broken line) and **2** (solid line).

**Spectroscopic Investigations of Complexes 1 and 2 and Proposed Structure of Complex 1.** According to the previous NMR and EPR spectroscopies, we reported that PQQ in complex **1** might be coordinated to Cu(II) with N-6 and C-7 carboxylate of the pyridine moiety.[19] Additionally a comparison between the diffuse reflectance spectra of complexes **1** and **2** reveals a marked difference, as presented in Figure 6. The 480-nm absorption band of free PQQ in a solution (pH >9) exhibits the red shift and the decreasing of the intensity in order to deprotonate at the pyrrole moiety.[34] The diffuse reflectance spectrum of complex **2** has no absorption maximum around 480 nm, which suggests

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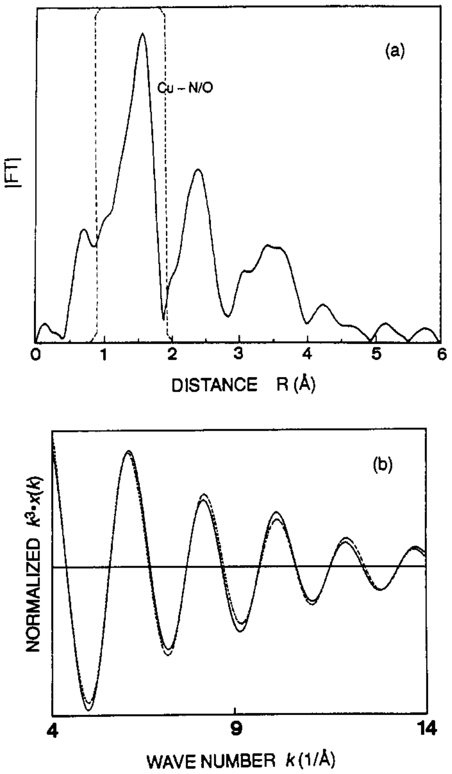

**Figure 7.** Fourier transform of the copper EXAFS spectrum of [Cu(PQQ)(terpy)] (**1**) (a) and Fourier-filtered Cu K-edge EXAFS spectrum from atoms in the first coordination sphere (back-transform window = 0.8–2.0 Å) of Cu (solid line) (b). The dashed lines in parts a and b represent the filter window used to extract the first coordination sphere EXAFS and the best curve-fitting simulation from Table 5, respectively.

the deprotonation of the pyrrole moiety by the binding of N-1 to Cu(II). On the other hand, complex **1** shows an intense band at 490 nm, and this can be explained by taking into account the protonation of the pyrrole moiety of PQQ. That is to say, PQQ in complex **1** seems to be predominantly coordinated to Cu(II) with the pyridine moiety (site 1).

EXAFS curve-fitting results for the first coordination sphere of complex **1** are shown in Figure 7, and the resulting structural details are given in Table 5. These results have supported that Cu(II) has six coordinating atoms of nitrogen and/or oxygen and the average Cu–N (or O) length is 2.03 Å.

**Table 5.** Curve-Fitting Result for the First Coordination Sphere of [Cu(PQQ)(terpy)] (**1**)

| complex | shell | scatterer | $N^a$ | $r^b$/Å | $\Delta E^c$/eV | $\sigma^d$ | $R^e$ |
|---|---|---|---|---|---|---|---|
| [Cu(PQQ)(terpy)] | 1st | N(O) | 5.9 | 2.00 | 11.57 | 0.07 | 0.022 |

[a] Number of scatterers per copper. [b] Copper-scatterer distance. [c] Change in the threshold energy. [d] Debye-Waller-like factor. [e] Reliability factor for curve fitting: $R = \{\sum[(x\cdot k^3)_{obsd}-(x\cdot k^3)_{calc}]^2/\sum[(x\cdot k^3)_{obsd}]^2\}^{1/2}$.

All experimental results (NMR[19], EPR[19], EXAFS, diffuse reflectance spectroscopy, and the pH dependence of the complex formation) substantiate that Cu(II) in complex **1** is attached to the pyridine moiety of PQQ and has a binding mode quite similar to that of Cu(1) in complex **2**.

## Conclusions

The PQQ molecule can be coordinated to Cu(II) by the pyridine moiety (site 1 in Figure 1) and the pyrrole moiety (site 3), but would not bind to Cu(II) through a quinone moiety (site 2). In particular, site 1 seems to be predominantly occupied by the Cu(II) ion. On the other hand, the complex of PQQ with *cis*-[Ru(bpy)$_2$Cl$_2$] has been described as the O,O-coordination of the quinone moiety (site 2).[22] The complexation modes of PQQ are of interest in connection with other transition metals and the comparison with the complex formations of aromatic coenzymes such as flavin and pterin. It is known that these coenzymes which have plural chelating sites are meridionally coordinated to Cu(II) like PQQ.[42,43]

**Acknowledgment.** We are very grateful to Dr. Takeshi Yamamura (Faculty of Science, Science University of Tokyo) for sharing his beam time for EXAFS measurement. We thank Dr. Shinobu Itoh (Faculty of Engineering, Osaka University) for his helpful discussions. This work was supported by a Grant-in-Aid from the Ministry of Education, Science, and Culture, Japan, for Research in Priority Areas (bioinorganic chemistry) (Grant 03241101).

**Supplementary Material Available:** Tables giving the complete crystallographic data summary for complex **2**, (Table S-1), atomic coordinates of the hydrogen atoms (Table S-2), the non-hydrogen anisotropic thermal parameters (Table S-3), and the complete bond lengths and bond angles (Table S-4 and Table S-5) and plots of the Cu K-edge XANES spectrum (Figure S-1) and k$^3$-weighted experimental EXAFS (k$^3\cdot x$(k)) of complex **1** (Figure S-2) (9 pages). Ordering information is given on any current masthead page.

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