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Inorganic Chemistry

Communications

Preparation and Characterization of Ternary Copper(II) Complexes Containing Coenzyme PQQ and Bipyridine or Terpyridine

Sir:

Copper-requiring amine oxidases are distributed widely in plants, mammals, and microorganisms. They contain nonblue copper and an organic cofactor that is covalently bound to the proteins; a subunit:copper:cofactor stoichiometry has been established to be 2:2:1 for most amine oxidases.[1,2] The cupric site indicated a tetragonal geometry with three imidazole-like nitrogens and one oxygen in the equatorial plane as ligand atoms,[3] whereas the cofactor responsible for a yellowish pink color of amine oxidases has not been conclusively identified. In 1984, 4,5-dihydro-4,5-dioxo-1*H*-pyrrolo[2,3-*f*]quinoline-2,7,9-tricarboxylic acid (PQQ, methoxatin), which is known to be the cofactor for certain bacterial dehydrogenases,[4] was first described to be present in copper-requiring amine oxidases as well.[5] Recent resonance Raman studies on 2,4-dinitrophenylhydrazone derivatives of plasma amine oxidases demonstrated that PQQ or a compound that closely resembles PQQ is the organic cofactor.[6] Further, it was suggested that the carbonyl cofactor of pea diamine oxidase,[7] lysyl oxidase,[8] or porcine kidney diamine oxidase[9] is also PQQ or its derivative.

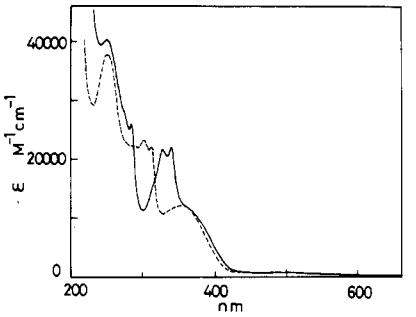

Figure 1. Absorption spectra of complex **1** (broken line) at pH 5.4 and complex **2** (solid line) at pH 6.1.

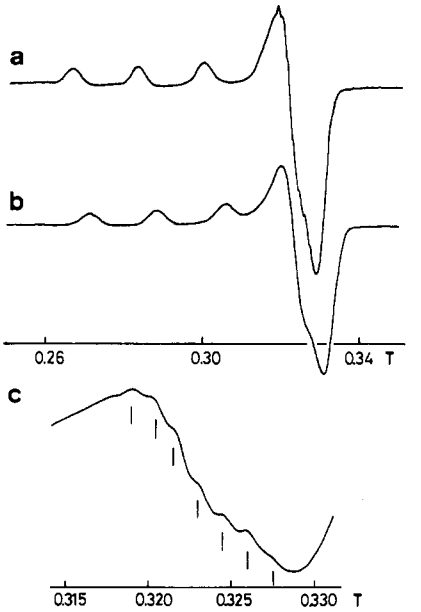

Figure 2. EPR spectra of complex **1** (a and c) and complex **2** (b) at 77 K: complex **1**, pH 5.4; complex **2**, pH 6.1.

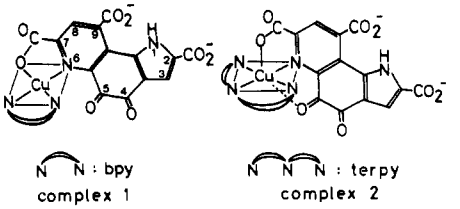

Figure 3. Proposed structures of complexes **1** and **2**.

In this communication, we report the preparation and characterization of ternary Cu(II) complexes containing PQQ in order

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to shed light on the structural and the functional relationships between nonblue copper and the organic cofactor in amine oxidases. Since the coordination of three aromatic nitrogens to the copper in the enzymes was proposed,[3] a Cu(II) complex of bipyridine (bpy) or terpyridine (terpy) was employed as a model for the active site of the enzymes. The complexation of Cu(II) with PQQ[10] or 9-decarboxylPQQ[11] or Cd(II) with 9-decarboxylPQQ[11] has been reported as to the coordination chemistry of PQQ and its derivatives so far.

The ternary Cu(II) complex of PQQ and bpy or terpy was isolated from an aqueous solution (about 10 mL) of PQQ (0.025 mmol) and $Cu(bpy)(NO_3)_2$ or $Cu(terpy)Cl_2$ (0.026 mmol) at about pH 3 as a reddish brown powder: $Cu(PQQ^{2-})(bpy)\cdot 2.5H_2O$ (complex **1**) and $Cu(PQQ^{2-})(terpy)\cdot 2.5H_2O$ (complex **2**) (PQQ^{2-} = PQQ where two carboxy groups are possibly deprotonated).[12] The electronic absorption spectrum of complex **1** at pH 5.4 exhibits three peaks assigned to the PQQ ligand at 249 (37 900), 353 (12 300), and 497 nm (760 M^{-1} cm^{-1}), as shown in Figure 1. Free PQQ shows absorption maxima at 249 (21 900), 330 (10 000), and 481 nm (600 M^{-1} cm^{-1}) in aqueous solution (pH 6). Two of the three peaks of PQQ in complex **2** appear at 250 (40 100) and 496 nm (610) (pH 6.1), but the absorption band near 350 nm is superimposed on the peaks of terpy (Figure 1). The red shifts of two peaks (near 350 and 500 nm) of PQQ in complexes **1** and **2**, compared with the corresponding bands of free PQQ, suggest the binding of the PQQ molecule to copper. A 20-nm red shift of the 330-nm band of PQQ was also observed by the addition of copper(II) ion to an aqueous solution of PQQ at pH 4.0.[10] These red shifts are probably due to the electronic change of PQQ bound to copper and/or an increase of the species hydrated at the C(5) position (C(5)=O) in the PQQ ligand; the NMR study of free PQQ in D_2O at pD 7.0 and 24 °C revealed an equilibrium between PQQ (ca. 60%) and C(5)-hydrated PQQ (ca. 40%), which exhibits absorption band near 350 nm.[13] In dimethyl sulfoxide (DMSO) the 338-nm band of PQQ hardly shifts in complex **1**, where the complexation of PQQ was observed by EPR.[14] Therefore, the hydration of the carbonyl group in the PQQ ligand is more likely than the electronic change of PQQ by the coordination. The EPR signals of complexes **1** and **2** reveal tetragonal Cu(II) ions (Figure 2a,b), displaying the parameters of $g_\parallel = 2.28$, $g_\perp = 2.07$, and $A_\parallel = 165$ G at pH 5.4 and $g_\parallel = 2.25$, $g_\perp = 2.07$, and $A_\parallel = 173$ G at pH 6.1, respectively. Additionally, seven superhyperfine lines ($A_N = 15$ G) due to nitrogens bound to Cu(II) ion were observed in the $g_\perp$ region of the signal of complex **1** (Figure 2c).[15] This finding suggests that there are three nitrogens (two nitrogens of bpy and one nitrogen of PQQ) in the equatorial plane of copper. The ^1H NMR signals of H(3) and H(8) of PQQ appear near 7.1 and 8.2 ppm, respectively, in D_2O at pD 6.8. The addition of the $Cu(bpy)^{2+}$ or $Cu(terpy)^{2+}$ complex to PQQ (PQQ:Cu(II) complex = 1:0.05) causes the preferential broadening of the H(8) signal. Thus, the NMR data of PQQ in the presence of the Cu complexes support that the binding of PQQ to copper takes place at the pyridine moiety of PQQ. Moreover, EPR parameters[16] of the ternary Cu complexes of bpy or terpy and the *N(1)*-methyl derivative[17] of PQQ were quite similar to those of complexes **1** and **2**, respectively. This result also indicates that the N(1) group of PQQ does not bind to copper. In conclusion, PQQ in complex **1** or **2** might be coordinated to copper by nitrogen 6 and carboxylate 7 (Figure 3),[18] as already recognized in the complex formation of 9-decarboxyPQQ.[11] The studies on amine oxidases by XAS and ENDOR spectroscopies could not exclude that at least one of the three imidazole-like nitrogens bound to copper is an aromatic nitrogen of the organic cofactor.[3]

The absorbance of complex **1** at 353 nm or complex **2** at 370 nm did not change in the range of pH 4–7 but decreased gradually above pH 7. The solution changed from orange-pink to yellow. The 353-nm band of complex **1** disappeared at pH 8.3, and shoulder bands were observed at around 340 and 375 nm. The electronic absorption spectra of complexes **1** and **2** were changed reversibly by alterations in pH. These spectral changes might be attributable to the hydration of the *o*-quinone moiety (C(4)=O and C(5)=O) in the PQQ ligand. The hydration of the quinone moiety in free PQQ is usually found above pH 10.[4g,19] Consequently, the electron-withdrawing effect of copper by the coordination of PQQ contributes to the pH decrease of the hydration.

In general PQQ catalyzes the nonenzymatic oxidations of amines in the presence of cationic detergents.[20] The oxidation of benzylamine (1 mM) with complex **1** or **2** (0.1 mM) was carried out in an aqueous solution without detergents at room temperature. After 24 h, benzaldehyde was determined by HPLC. In comparison with the control reaction with free PQQ at pH 6–7, the oxidation was 50% inhibited by complex **1**, while complex **2** promoted the reaction by a factor of about 15. PQQ and bpy in complex 1 are equatorially coordinated to copper, and the aromatic ring of bpy is extremely close to the carbonyl group (C(5)=O) of PQQ, so that this structure could prevent access of the bulky substrate to the carbonyl group[4a,g] which is considered to be the active site of PQQ. In complex **2**, nitrogen 6 and carboxylate 7 of PQQ are supposed to bind to copper in the equatorial and the axial directions, respectively. Since two aromatic planes of terpy and PQQ are perpendicular to each other, benzylamine is easily accessible to the *o*-quinone moiety of PQQ, which is activated by the electron-withdrawing effect of copper.

NMR relaxation measurements of ^{19}F nuclei suggested that copper in plasma amine oxidase treated with (trifluoromethyl)-phenylhydrazine derivatives lies in the aromatic ring of the inhibitors at distances of 0.11–0.16 nm from the fluorines.[21] Although the present model complexes afforded some useful information of the interaction between PQQ and copper, the distance between copper and the *o*-quinone moiety of PQQ is shorter than the above value estimated by the NMR study. The preparation of a model complex in which Cu(II) ion binds to nitrogen 1 or carboxylate 2 is in progress.

Registry No. **1**, 112440-12-7; **2**, 112440-13-8; PQQ, 72909-34-3; $Cu(bpy)(NO_3)_2$, 14871-95-5; $Cu(terpy)Cl_2$, 14973-00-3; Cu(*N(1)*-MePQQ)(bpy), 112459-47-9; Cu(*N(1)*-MePQQ)(terpy), 112440-14-9; triamine oxidase, 9059-11-4; benzylamine, 100-46-9.

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(12) Elemental analyses of complex **1** and complex **2** are as follows. Anal. Calcd for complex **1**, $CuC_{24}H_{17}N_4O_{10.5}$: C, 48.61; H, 2.90; N, 9.45; Cu, 10.7. Found: C, 48.43; H, 2.88; N, 9.43; Cu, 10.8. Calcd for complex **2**, $CuC_{29}H_{20}N_5O_{10.5}$: C, 51.98; H, 3.01; N, 10.45; Cu, 9.48. Found: C, 52.03; H, 2.91; N, 10.47; Cu, 9.51.
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(15) The seven superhyperfine lines were also observed in complex **1** containing the ^{63}Cu ion.
(16) EPR parameters at 77 K: Cu(*N*(1)-MePQQ)(bpy) complex at pH 5, $g_\parallel = 2.28$, $g_\perp = 2.07$, and $A_\parallel = 166$ G; Cu(*N*(1)-MePQQ)(terpy) complex at pH 5, $g_\parallel = 2.25$, $g_\perp = 2.07$, and $A_\parallel = 172$ G.
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