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Characterization of Ternary Copper(II) Complexes Containing Reduced PQQ ($PQQH_2$) and Bipyridine or Terpyridine

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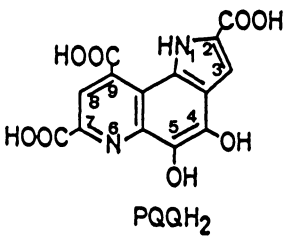
The ternary copper(II) complex containing reduced coenzyme PQQ ($PQQH_2$) and bipyridine or terpyridine was prepared and characterized by electronic absorption and electron paramagnetic resonance (EPR) spectroscopy. The structures of these complexes are quite similar to those of the corresponding ternary complexes containing PQQ (oxidized form), in which the binding of PQQ to Cu(II) through N(6) and COO^-(7) groups has been proposed.

Copper-requiring amine oxidase widespread in plants, microorganisms, and mammals catalyzes the oxidative deamination of amines by accepting two electrons from amines and transferring them to dioxygen. In 1984, two research groups independently suggested that PQQ (4,5-dihydro-4,5-dioxo-1H-pyrrolo[2,3-f]quinoline-2,7,9-tricarboxylic acid) functions as a prosthetic group together with copper ion in copper-requiring amine oxidase (bovine serum[1] or bovine plasma[2] amine oxidase, porcine kidney diamine oxidase,[2] and Aspergillus niger amine oxidase[2]). Thereafter the cofactors of pea diamine oxidase[3] and lysyl oxidase[4] as well as bovine plasma amine oxidase[5,6] and porcine kidney diamine oxidase[7] were also demonstrated to be PQQ or its derivative. The nonblue copper site in bovine amine oxidase indicated a tetragonal geometry with three imidazole-like nitrogen ligands and one oxygen ligand in the equatorial plane,[8,9] but the structural and the functional relationships between copper ion and the organic cofactor are not clear as yet. We prepared the ternary Cu(II) complex containing PQQ and bipyridine (bpy) or terpyridine (terp) as a model for the active site of amine oxidase.[10] This paper deals with the characterization of the ternary Cu(II) complex containing the reduced form of PQQ ($PQQH_2$) and bpy or terp on the basis of electronic absorption and EPR spectra. In addition, we represent the oxidations of these complexes by dioxygen.



$PQQH_2$

The $PQQH_2$ was obtained by the reduction of PQQ commercially available (Ube Industries, LTD.) with $Na_2S_2O_4$ in an aqueous solution and recrystallized from DMSO-acetonitrile.[11] The ternary Cu(II) complex of $PQQH_2$ and bpy or terp was

prepared by addition of a small amount of DMSO solution containing $PQQH_2$ to an aqueous solution (pH 5.5) of $[Cu(bpy)](NO_3)_2 \cdot 3H_2O$ or $CuCl_2(terp) \cdot 2H_2O$ under Ar atmosphere ($PQQH_2$/Cu complex = 1). Figure 1 exhibits the electronic absorption spectra of $Cu(PQQH_2)(bpy)$, $Cu(PQQH_2)(terp)$, and free $PQQH_2$ at pH 5.5 under anaerobic conditions. The 312-nm band of free $PQQH_2$ hardly shifts in the presence of $Cu(bpy)^{2+}$ or $Cu(terp)^{2+}$, although the absorption coefficients of the ternary complexes are larger than that of free PQQ: the absorption band of the $PQQH_2$ ligand superimposes the peaks of the bpy or terp ligand. However, about 20-nm blue shifts of the 400-nm band of $PQQH_2$ were observed in the complexes, suggesting the coordination of $PQQH_2$ to copper ion. $Cu(PQQH_2)(bpy)$ and $Cu(PQQH_2)(terp)$ were immediately oxidized to give rise to Cu(PQQ)(bpy) and Cu(PQQ)(terp), respectively, by dioxygen.

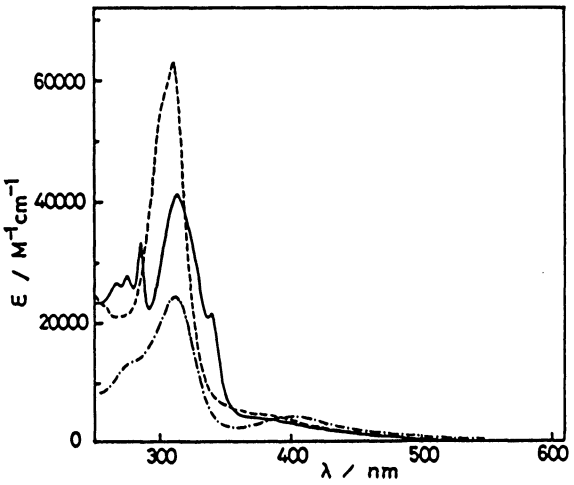


Fig. 1. Electronic absorption spectra of $Cu(PQQH_2)(bpy)$ (------), $Cu(PQQH_2)$-(terp) (———), and $PQQH_2$ (—·—·—) in aqueous solutions (pH 5.5) containing 0.6% DMSO under anaerobic conditions.

The EPR signals of $Cu(PQQH_2)(bpy)$ and $Cu(PQQH_2)(terp)$ under anaerobic conditions reveal tetragonal Cu(II) ions (Figs. 2(a) and 3(a)), displaying the spin Hamiltonian parameters of $g_{//}$ 2.28, $g_{\perp}$ 2.07, and $A_{//}$ 164 G and $g_{//}$ 2.25, $g_{\perp}$ 2.06, and $A_{//}$ 168 G, respectively. The former is quite similar to the signal ($g_{//}$ 2.28, $g_{\perp}$ 2.07, and $A_{//}$ 165 G) of Cu(PQQ)(bpy), as represented in Fig. 2. The latter also resembles the spectrum ($g_{//}$ 2.25, $g_{\perp}$ 2.06, and $A_{//}$ 173 G) of Cu(PQQ)(terp) in Fig. 3(b). These findings indicate the

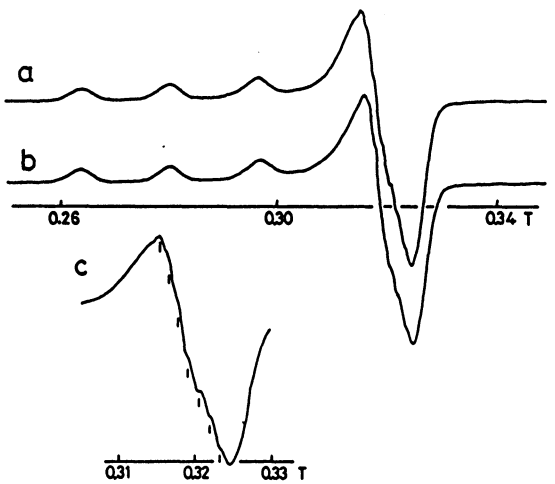


Fig. 2. EPR spectra of (a and c) $Cu(PQQH_2)(bpy)$ under Ar atmosphere and (b) Cu(PQQ)(bpy) at 77 K. solvent: 75% H_2O/25% DMSO (pH 5.5).

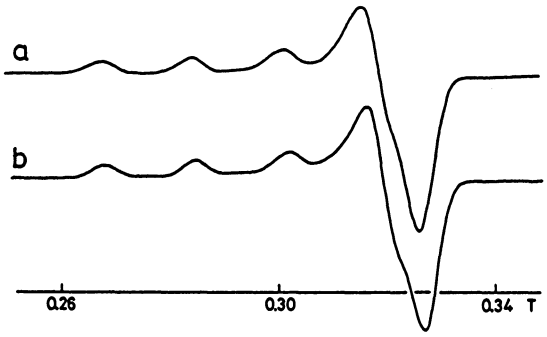


Fig. 3. EPR spectra of (a) $Cu(PQQH_2)$-(terp) under Ar atmosphere and (b) Cu-(PQQ)(terp) at 77 K. solvent: 75% H_2O/25% DMSO (pH 5.5).

similarity of the coordination geometry and the donor set of Cu(PQQH$_2$)(bpy) or Cu(PQQH$_2$)(terp) to those of Cu(PQQ)(bpy) or Cu(PQQ)(terp), respectively. It has been supposed that PQQ in Cu(PQQ)(bpy) having square planar Cu(II) are equatorially coordinated to copper through N(6) and COO$^-$(7) groups.[10] In Fig. 2(c) seven superhyperfine lines (A_N 15 G) due to nitrogens bound to Cu(II) ion, which was also observed in the $g_\perp$ region of the signal of Cu(PQQ)(bpy), demonstrate that three nitrogens (two nitrogens of bpy and one nitrogen of PQQH$_2$) locate around copper. The N(6) and COO$^-$(7) groups of PQQ in Cu(PQQ)(terp) were supposed to bind in the equatorial and the axial directions of Cu(terp)$^{2+}$ having a square planar geometry, respectively.[12] Moreover, there might be two species containing PQQ and hydrated-PQQ at the C(5)-carbonyl group in aqueous solutions of Cu(PQQ)(bpy) and Cu(PQQ)(terp).[10]

Jongejan et al. have reported that anaerobic titration of PQQH$_2$ with Cu^{2+} ion shows a complicated behavior; the addition of equimolar Cu^{2+} leads to the almost total collapse of the spectrum of PQQH$_2$ and the formation of a precipitate, but upon further addition of Cu^{2+}, (partial) formation of PQQ and hydrated-PQQ Cu(II) complexes occurs.[13] The formation of PQQ was concluded to be due to a rapid redox reaction. However, the complex formation between PQQH$_2$ and Cu(bpy)$^{2+}$ or Cu(terp)$^{2+}$ was not attended with such a redox reaction. The standard electrode potentials of Cu^{2+}, Cu(bpy)$^{2+}$, and Cu(terp)$^{2+}$ in aqueous solutions (I=0.1 (KNO_3)) at pH 5.5 were determined to be +237, +92, and +19 mV (NHE),[14] respectively, by a cyclic voltammetry. The value of free PQQ was found to be +150 mV (NHE) at pH 5.6,[15] which is higher than those of Cu(bpy)$^{2+}$ and Cu(terp)$^{2+}$ and is lower than that of Cu^{2+}. Therefore, these standard electrode potentials clearly support that PQQ is reduced in an aqueous solution containing Cu^{2+} under anaerobic conditions, but Cu(bpy)$^{2+}$ and Cu(terp)$^{2+}$ don't produce PQQ from PQQH$_2$. These electrochemical features of Cu(bpy)$^{2+}$ and Cu(terp)$^{2+}$ coincide with the fact that copper in amine oxidase doesn't show a change of oxidation state even under the anaerobic reduction of the organic cofactor with a substrate.[16-19] The oxidation rates of PQQH$_2$ in the presence or absence of the Cu(II) complexes are shown in Table 1. The oxidation of PQQH$_2$ with dioxygen was carried out by adding 0.01 ml of an anaerobic DMSO solution of PQQH$_2$ to an air saturated aqueous solution (3 ml, pH 5.6) containing the equimolar copper complex. The reaction was followed by observing the disappearance of the 310-nm band of PQQH$_2$. Both the Cu(II) complexes promote the oxidation of PQQH$_2$. An especially high activity of Cu(bpy)$^{2+}$ might be attributable to the structural unstabilization of Cu(PQQH$_2$)(bpy) by the repulsion between PQQH$_2$ and bpy coordinated to equatorial plane of Cu(II). Such a repulsion doesn't occur in Cu(PQQH$_2$)(terp) where two aromatic planes of terp and PQQ are perpendicular to each other.[10] On the other hand, the oxidative deamination of benzylamine in the presence of Cu(PQQ)(bpy) was 50% inhibited relative to the catalytic activity of free PQQ,

Table 1. Oxidation of PQQH$_2$ in an aqueous solution (pH 5.6) containing DMSO (0.33%)

Catalyst	Initial rate $V_0 \times 10^6$ M/s	Relative activity
Cu(bpy)$^{2+}$	$\geq$2	$\geq$50
Cu(terp)$^{2+}$	0.12	2.8
none	0.043	1

PQQH$_2$, Cu complex: 2.0×10^{-5} mol dm^{-3}, O_2: 5.3×10^{-5} mol dm^{-3}, I=0.1 (KCl), 25 °C

and Cu(PQQ)(terp) promoted the reaction by a factor of about 15. Since this reaction is considered to take place via the oxidation of the copper complex of $PQQH_2$ produced by the reaction of PQQ and benzylamine,[20,21] the lower activity of the oxidation of $PQQH_2$ with $Cu(terp)^{2+}$ than with $Cu(bpy)^{2+}$ (Table 1) clearly indicates that the oxidation step of $PQQH_2$ is not a rate-determining step in the oxidative deamination of benzylamine.

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