Mononuclear copper(II)–hydroperoxo complex derived from reaction of copper(I) complex with dioxygen as a model of DβM and PHM†‡

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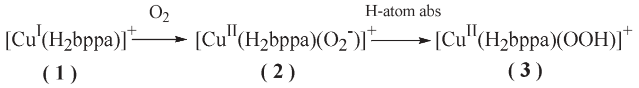
A mononuclear copper(II)–hydroperoxo species has been generated by the reaction of Cu(I)–H_2BPPA complex with dioxygen, which illustrates the enzymatic reaction process of the Cu_B site in the DβM and PHM.

Dopamine β-monooxygenase (DβM)[1] and peptidylglycine α-hydroxylating monooxygenase (PHM)[2] are copper containing enzymes which catalyze the conversion of dopamine to norepinephrine and the stereospecific hydroxylation of the glycine α-carbon of peptygylglycine substrates, respectively. These enzymes contain two copper ions as the active sites which are well separated. An oxidative H-atom abstraction reaction has been thought to occur at the Cu_B site.[3] The reaction mechanism of these enzymes has widely been proposed to proceed *via* mononuclear Cu–O_2 species.[4] The copper(II)–hydroperoxo species is one of the postulated active intermediates which has vigorously been synthesized and investigated as a model complex.[5,6] Recently, Solomon *et al.* have proposed that the copper(II)–superoxo species is the more readily reactive intermediate on the basis of their calculation analysis,[7] which supports a mechanism for the reaction whereby it is initiated by H-atom abstraction from the substrate by the copper(II)–superoxo species, which is generated before the formation of the copper(II)–hydroperoxo species.

In earlier work, Karlin *et al.* reported that the mononuclear copper(II)–superoxo species is generated by the reaction of the $[Cu^I(tmpa)]^+$ complex with O_2 at low temperatures.[8] Recently, there have been several reports on the preparation of quasi-stable copper(II)–superoxo complexes using a copper(I) complex with a series of tren-modification ligands.[9] Schindler *et al.* have also reported the generation of a stable copper(II)–superoxo complex from the reaction of $[Cu^I(TMG_3tren)]^+$ with O_2, which has been characterized by resonance Raman and X-ray crystallography.[10]

At this stage, it is important to understand the biological significance of the mononuclear copper(II)–superoxo species which has been proposed as a reaction intermediate in DβM and PHM,[7] because the H-atom abstraction process is considered as a key step in the generation of a copper(II)–hydroperoxo species in the enzymatic reaction process. In this paper, we report the first preparation of a well characterized mononuclear copper(II)–hydroperoxo species that is generated through the reaction of a copper(I) complex with dioxygen.



$$\underset{(1)}{[Cu^I(H_2bppa)]^+} \xrightarrow{O_2} \underset{(2)}{[Cu^{II}(H_2bppa)(O_2^{-})]^+} \xrightarrow{\text{H-atom abs}} \underset{(3)}{[Cu^{II}(H_2bppa)(OOH)]^+}$$

Scheme 1 The copper(II)–hydroperoxo generation process in this system.

To study the H-atom abstraction reaction, we took advantage of the ligand H_2bppa which stabilizes the copper(II)–hydroperoxo species.[5] Using the copper(I) complex of this ligand made the observation of the copper(II)–hydroperoxo species (Scheme 1).

The starting material $[Cu^I(H_2bppa)]^+$ (**1**) was prepared in acetone solution, and was obtained as a red crystal suitable for X-ray crystallography (Fig. 1).§ The molecular structure of the cation part of **1** revealed that the Cu^I ion is five-coordinated with the three pyridyl nitrogens, amine nitrogen and amide oxygen of one pivaloyl amide group. The calculated τ-value around the Cu^I ion (=0.53) showed that **1** is intermediate between a trigonal-bipyramidal and a square-pyramidal structure.[11] Selected bond lengths around the Cu^I ion [Cu–N(1) = 2.245 Å, Cu–N(2) = 2.148 Å, Cu–N(4) = 2.008 Å, Cu–N(6) = 2.062 Å, Cu–O(1) = 2.176 Å] were all elongated as compared with $[Cu^{II}(H_2bppa)]^{2+}$ reported hitherto.[12]

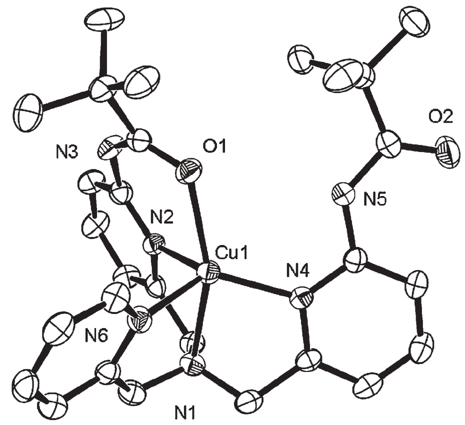


Fig. 1 ORTEP drawing of $[Cu^I(H_2bppa)]^+$ (**1**) with the atom labelling scheme. The thermal ellipsoids are at the 50% probability level, and the hydrogen atoms are omitted for clarity.

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‡ Abbreviations. H_2bppa = bis(6-pivalamido-2-pyridylmethyl)(2-pyridylmethyl)amine; Tmpa = tris(2-pyridylmethyl)amine; tren = tris(2-aminoethyl)amine; TMG_3-tren = tris(tetramethylguanidino)tren.

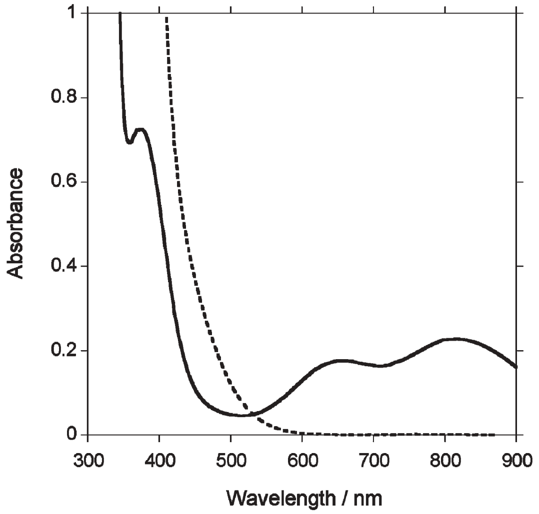


Fig. 2 UV–vis spectra of **1** (1 mM) (dotted line) and after bubbling O_2 to **1** (solid line) in acetone at -80 °C.

The reaction of **1** with dioxygen was performed in acetone and MeOH solutions, and was monitored using electronic absorption, ESR, ESI-mass and resonance Raman spectroscopies. Bubbling of dioxygen into the solution of **1** (1 mM) at -80 °C resulted in an apparent color change from light yellow to bluish green. The absorption spectral change in the reaction of **1** with dioxygen in acetone solution is shown in Fig. 2. This bluish green species showed an intense band at 375 nm (ε = 700 M^{-1} cm^{-1}) and two weak bands at 628 nm (ε = 190 M^{-1} cm^{-1}) and 819 nm (ε = 220 M^{-1} cm^{-1}), the former of which was assigned as the LMCT band for a series of Cu/O_2 adducts and the latter two bands of which were characteristic of d–d transition bands of the Cu(II) ion with a trigonal bipyramid structure.

The ESR spectrum of this bluish green solution showed that the reaction solution contained two or more kinds of Cu(II) species. (Fig. S1) This result was also supported from the ESI-mass spectrum measured immediately after the reaction of **1** and O_2 in acetone at -80 °C (Fig. S2 top). The ESI-mass spectrum showed two isotope clusters at *m/z* 550.3 and 584.3. The feature at *m/z* 550.3, which was obtained as a parent peak, was assigned to the $[Cu^{II}(Hbppa)]^+$ (**4**) species which was oxidized and deprotonated from **1**. Another feature at *m/z* 584.3 was assigned to $[Cu^{II}(H_2bppa)(OOH)]^+$ (**3**),[5] which was also confirmed from the isotope shift of the feature at *m/z* 588.3 when $^{18}O_2$ was used (Fig. S2 bottom). The resonance Raman spectra of acetone and MeOH solutions of this bluish green species measured at -80 °C (406.7 nm laser excitation) suggested the generation of a copper–dioxygen adduct (Table 1, Fig. S3 and S4). Strong resonance enhanced Raman peaks were observed at 861 cm^{-1} and 864 cm^{-1} in acetone and MeOH, respectively. These features shifted to 814 cm^{-1} ($\Delta\nu$ = 47 cm^{-1}) and 817 cm^{-1} ($\Delta\nu$ = 47 cm^{-1}), respectively, when ^{18}O-labeled dioxygen was used. The frequencies and ^{18}O-isotope shifts of these bands are characteristic of a peroxo O–O stretching vibration. The Cu–O stretching vibrations were also observed at 481 cm^{-1} and 471 cm^{-1} ($\Delta\nu$ = 10 cm^{-1}, when $^{18}O_2$ was used) in MeOH solution. These Raman bands were in good agreement with those of previously characterized $[Cu^{II}(H_2bppa)(OOH)]^+$ complex.[5]

Table 1 rRaman observation of the O–O stretching mode in cm^{-1}

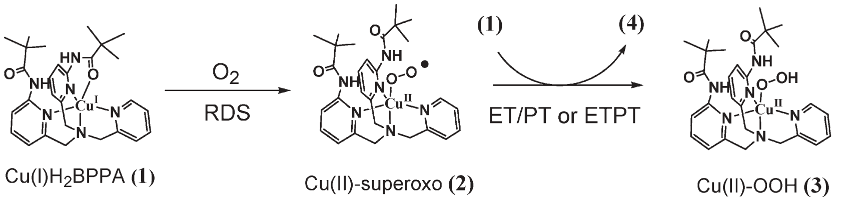
	$[Cu^I(H_2bppa)]^+ + O_2$		$[Cu^{II}(Hbppa)]^+ + H_2O_2$	
Reaction	$^{16}O–^{16}O$	$^{18}O–^{18}O$	$^{16}O–^{16}O$	$^{18}O–^{18}O$
Acetone	861	814	860	815
MeOH	864	817	863	817

The total consideration of these spectroscopic observations suggests that two kinds of copper(II) complexes, **3** and **4**, are included as the reaction products. It is very interesting that the mononuclear copper(II)–hydroperoxo species has been generated from the reaction of copper(I) complex with dioxygen.

Unfortunately, we were unsuccessful in the direct observation of mononuclear copper(II)–superoxo species in this system. This is because the reaction of **1** with dioxygen is very slow. The redox potential value of $[Cu^{II}(H_2bppa)]^{2+}$ in acetone solution (ΔE (Cu^I/Cu^{II}) = 0.64 V, *vs.* SHE) indicates that inter-molecular electron-transfer doesn't occur in the dioxygen reduction process (ΔE (O_2/O_2^-) = -0.61 V in acetone, *vs.* SHE).[13] These facts indicate that a direct contact of the copper(I) ion with dioxygen is required in the dioxygen reduction process.[14] In this step, the coordination of amido oxygen to the O_2 binding site and the bulky *tert*-butyl substituents prevent the reaction between the Cu(I) complex and dioxygen. Considering that the following H-atom transfer reaction is very fast, it may be difficult to directly observe the copper(II)–superoxo intermediate.

Since a metal–superoxo species is always proposed to be formed in the initial dioxygen binding step to the reduced metal center,[14] we tried another approach to observe the copper(II)–superoxo species which is always generated in the first step of the reaction of the mononuclear Cu(I) complex with dioxygen. The reaction of **1** with dioxygen was performed in both of acetone and MeOH solutions containing a large excess of 5,5-dimethyl-1-pyrroline *N*-oxide (DMPO, 40 eq.) which is often used as a spin trap reagent in ESR experiments. Bubbling dioxygen into the MeOH solution of **1** at -80 °C resulted in a drastic color change from light yellow to reddish purple. The absorption spectra of this reddish purple species showed intense bands at 488 nm (ε = 1080 M^{-1} cm^{-1}) and 561 nm (ε = 650 M^{-1} cm^{-1}) (Fig. S5). This resultant spectroscopic features were greatly different from that before addition of DMPO. The sample solution prepared under the same experimental conditions was ESR silent. The ESI-mass spectrum gave a parent peak at *m/z* = 696.4 corresponding to $[Cu^{II}(H_2bppa)(O_2^-)(DMPO)]^+$, which shifted to *m/z* = 700.4 when $^{18}O_2$ was used (Fig. S6), indicating that the copper(II)–superoxo precursor **2** generated in the first step of the reaction has been trapped by DMPO.[15]

These results clearly demonstrate that the copper(II)–hydroperoxo complex **3** has been generated *via* copper(II)–superoxo species **2**. In this process, an H-atom abstraction reaction by the copper(II)–superoxo species **2** will be required. The ESI-mass experiment indicated the generation of deprotonated copper(II) complex **4**, and a simulation of the UV–vis experiment suggested the ratio of generated species **3** and **4** to be about 1 : 1 (see supporting information, Fig. S7). It is reasonable to suppose that the first-generated copper(II)–superoxo species reacts with the unreacted copper(I) complex **1** as a substrate. A plausible reaction scheme may be presented as below (Scheme 2). (i) Mononuclear copper(II)–superoxo species **2** is generated by the reaction of **1** with



Scheme 2 A plausible reaction process.

dioxygen. (ii) The generated copper(II)–superoxo species **2** attacks with the unreacted **1** as a substrate to generate complex **3**. It is reasonable to consider that this reaction is accompanied by ET/PT or ETPT (ET = electron transfer, PT = proton transfer, ETPT = H-atom abstraction) process which is still unknown, although a solvent or water molecule might be related to this reaction process. We are now trying to obtain a detailed elucidation of this reaction process.

In the 1990's, Karlin *et al.* reported that the copper(II)–hydroperoxo species is generated from the reaction of the dinuclear–copper(I) complex with dioxygen.[16] In this reaction, the intermediate species was a binuclear copper(II) complex with a peroxo attached in an *end-on* fashion, and protonation is based on the added HBF_4 to generate a bridged hydroperoxo complex.

In this study, we succeeded in the observation of the copper(II)–hydroperoxo species **3** generated by the reaction of mononuclear copper(I) complex **1** with dioxygen. It is apparent that this reaction process took place on the mononuclear complex. This finding is a more reasonable reaction model for understanding the reactivity and reaction mechanism of copper(II)–superoxo species which has been proposed as one of the active intermediates in DβM and PHM.

Notes and references

§ Crystal data for **1**. $C_{29}H_{36}ClCuN_6O_{6.5}$, M_W 671.64, triclinic, $P\bar{1}$, a = 9.973(5), b = 12.38(3), c = 14.08(3) Å, α = 112.72(13), β = 91.375(9), γ = 98.20(4)°, V = 1581(5) Å^3, Z = 2, R = 0.0597, R_w = 0.1775. CCDC 612884. For crystallographic data in CIF or other electronic format see DOI: 10.1039/b609673e

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