Contribution of a Peroxide Adduct of Copper(II)-Peptide Complex to Modify the Secondary Structure of Albumin

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Copper(II)-peroxide Adduct, Modification of Protein, Copper(II)-peptide Complex

We have found that copper(II) compounds containing a peptide group in the chelate exhibit high activity for modification or degradation of albumin in the presence of hydrogen peroxide, whereas no activity was detected for the copper(II) compounds without an amide-group. It is suggested that presence of the amide-group in the ligand may play an important role in the formation of a peroxide adduct and in activation of the peroxide ion, leading to cleavage of the peptide bond of a neighboring protein. It is implied that conversion of normal cellular prion protein PrP^{C} into a disease-causing isoform, PrP^{Sc} is attributed to the activated peroxide ion coordinated to a copper(II) captured in the NH_2-terminal domain of the PrP^{C}.

In general, the neurons in human substantia nigra deteriorate during normal aging, and loss of these neurons is prominent in Parkinson's disease (Floor and Wetzel, 1998). These degenerative processes are hypothesized to involve oxidative stress (Stadtman, 1992; Sohal and Weindruch, 1996), and the oxidative stress hypothesis predicts that endogenous oxidants may cause greater cellular damage, which results in several neurodegenerations. In previous papers, we have proposed that among the species to induce oxidative stress, hydrogen peroxide is the most important one (Nishida and Ito, 1995). Very recently, Brown *et al.* (1997) have shown that the NH_2-terminal domain of PrP^{C} (the normal cellular prion protein) exhibits five to six sites that bind copper(II) presented as a glycine chelate, indicating that PrP^{C} can exist in a Cu-metalloprotein form *in vivo*. This finding suggests that formation of a copper(II) within the octapeptide may be closely related with the conversion of PrP^{C} into PrP^{Sc} (the abnormal disease-causing isoform) (Prusiner, 1997). Thus in this study the effect of a copper(II)-peroxide adduct on the modification of albumin was investigated, attempting to elucidate the possible contribution of a copper(II)-peroxide adduct to induce the modification of the NH_2-terminal region of PrP^{C}.

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Experimetal

Materials

The copper(II) compounds used in this study are of general form, Cu(L)ClY, where (L) are illustrated in Fig. 1. The structures of these compounds are similar to each other (Okuno *et al.*, 1997; Kobayashi *et al.*, 1996; Kobayashi *et al.*, 1998, in press), and as a representative one, the ORTEP drawing of $Cu(tfpy)Cl^{+}$ is illustrated in Fig. 2, which was determined in this study, where (tfpy) denotes N,N-bis(2-pyridylmethyl)-2-aminomethyltetrahydrofuran(see Fig. 1). Crystal data of $Cu(tfpy)ClClO_4 \cdot H_2O$: triclinic $P\bar{1}$, a = 10.673(6), b = 12.325(4), c = 8.999(4) Å, α = 96.78(4), β = 114.44(4), γ = 69.93(4)°, V = 1011.8(9) Å^3, Z = 2, D_x = 1.62 Mgm^{-3}; R = 0.093 for 3250 observed

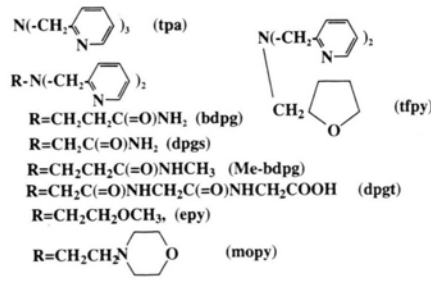


Fig. 1. Chemical structures of the ligands cited in this paper.

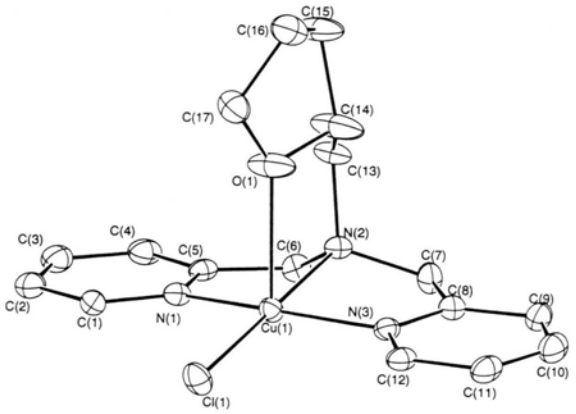


Fig. 2. ORTEP drawing of Cu(tfpy)Cl$^+$ cation. Cu(1)–Cl(1), 2.226(3); Cu(1)–N(2), 2.031(8); Cu(1)–N(1), 2.000(9); Cu(1)–N(3), 1.994(8); Cu(1)–O(1), 2.456(10) Å.

reflections (Fo $\geq 3\sigma$(Fo)). Supplementary data on atomic positions, bond lengths, bond angles, and Fo-Fc tables are not documented. The crystal structures of other compounds((L) = (bdpg) and (dpgs), (Okuno *et al.*, 1997); (mopy), (Kobayashi *et al.*, 1996); (dpgt) and (Me-bdpg) (Kobayashi *et al.*, 1998, in press) were already reported. Human carbonic anhydrase (HCA), albumin (bovine, BSA), and cytochrome c were purchased from Sigma (USA).

Degradation of albumin by copper(II)/H_2O_2 systems

To the reacton mixture containing a copper(II) compound(5 μl, 0.15–0.6 mM solution), Tris = tris (hydroxylmethyl)aminomethane)-HCl buffer (5 μl, 0.1 M pH = 7.8), hydrogen peroxide (5 μl, 6.25–100 mM solution), was added a protein solution (5 μl, 0.11 mM), and one hour after the mixing the solution was mixed with a solution containing 2-mercaptoethanol, and then electrophoresed (Rana and Meares, 1991); denatured samples were analyzed on 12.5% sodium dodecylsulfate-polyacrylamide gel electrophoresis. Products were visualized by Coomasie blue and the band intensities were quantified on an ATTO densitograph model AE-6920-M/W/V.

ESR spectral measurements

ESR spectra of the solutions containing copper(II) complex(1–10 mM in water/acetonitrile (1/1, v/v) solution), hydrogen peroxide(100 mM in water/acetonitrile (1/4, v/v) solution), PBN (α-phenyl-N-*t*-butylnitrone), one of the spin-trapping reagents for OH·radical (Janzen *et al.*, 1978) (100 mM in water/acetonitrile (1/4, v/v) solution) were measured with a JEOL ESR apparatus, model RE-2X using an X-band at 298 K.

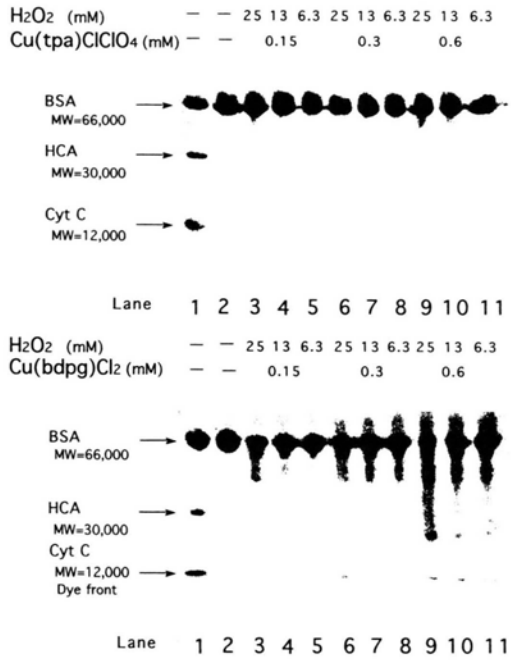


Fig. 3. Separation of the cleaved products by NaDodSO$_4$/acrylamide gel electrophoresis. BSA, HCA, Cyt c denote bovine serum albumin, human carbonic anhydrase, and cytochrome c, respectively. Lanes 1 and 2, proteins in the absence of copper(II) and hydrogen peroxide; lanes 3–11, albumin, hydrogen peroxide and copper(II) complex(in the upper (A), Cu(tpa)Cl$^+$, and the lower, (B), Cu(bdpg)Cl$^+$. The concentrations of hydrogen peroxide and copper(II) complex are written in the figure; concentrations of the copper(II) complex in the lanes 3–5, 6–8, and 9–11, are 0.15, 0.30, and 0.60 mM, respectively.

Results and Discussion

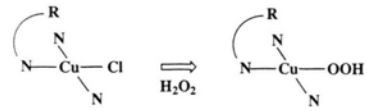
Degradation of albumin by copper(II)/H±$_2O_2$ systems

As a representative example, the electrophoresis done for the systems containing Cu(tpa)ClClO$_4$ and Cu(bdpg)ClClO$_4$ are illustrated in Fig. 3. We have confirmed that the presence of a copper(II) complex or hydrogen peroxide only does not induce the degradation of albumin under the experimental conditions used in this study. As shown in

Fig. 3, it is clear that the degradation of albumin is highly dependent on the copper(II) complex used, concentrations of a copper(II) complex and of hydrogen peroxide. It should be noted that the activity of the (tpa)-complex for degradation of albumin is much lower than that of the (bdpg)-complex. The activities of the compounds were estimated by measuring the band intensities (for example, see Table II), and the data showed that the activity for degradation is as follows: (bdpg) > (dpgs) > (Me-bdpg), (dpgt) >> (tfpy), (mopy), (epy) > (tpa). This indicates that the peptide group involved in the ligands, (bdpg), (dpgs) and (dpgt) is closely related with high activity observed for these copper(II) compounds.

Structure and reactivity of copper(II)-peroxide adduct

In our previous paper, we have investigated the activities of these copper(II) compounds for oxygenation of cyclohexane in the presence of hydrogen peroxide (Okuno *et al.*, 1997). Although ESR studies on these systems have demonstrated that in all the cases a peroxide adduct forms in the presence of hydrogen peroxide as illustrated below, the activity for oxygenation of cyclohexane is highly dependent on the R of the ligands; (bdpg) >> (dpgs) > (mopy), (epy), (tfpy), (tpa).



This order is essentially the same as observed for degradation of albumin as described above. The high activity of the (bdpg)-complex for oxygenation of cyclohexane has been attributed to the facile formation of a peroxide adduct, where the hydroperoxide ion binds to the oxygen atom of the amide-group through hydrogen bonding (see below), and in this conformation the peroxide ion is activated to react with cyclohexane directly, giving the oxygenated products (Okuno *et al.*, 1997). Formation of the peroxide adduct as proposed for the (bdpg)-complex is unfavorable for the (tpa), (tfpy), (epy), and (mopy)-complexes; in the cases of (tpa) and (mopy), the oxygen atom is absent, and in the (tfpy) and (epy) compounds, the longer copper(II)-oxygen atom of the tetrahydrofuran ring(2.456 Å) is unsuitable for formation of the peroxide adduct with hydrogen bonding as shown below. This situation is quite different from that observed for the iron(III) compound (Fe-O(tetrahydrofuran oxygen), 2.209 Å; Ito *et al.*, 1997; in

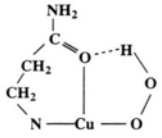
Table I. Selected bond lengths (Å) and angles (°) in the $Cu(tfpy)ClClO_4$ complex.

Bond lengths (Å)			
Cu(1)-Cl(1), 2.226(3); Cu(1)-N(2), 2.031(8); Cu(1)-N(1), 2.000(9); Cu(1)-N(3), 1.994(8); Cu(1)-O(1), 2.456(10) Å.			
Bond angles (°)			
Cl(1)-Cu(1)-N(2)	179.0(3)	Cl(1)-Cu(1)-N(1)	96.9(3)
Cl(1)-Cu(1)-N(3)	97.0(3)	Cl(1)-Cu(1)-O(1)	101.6(3)
N(2)-Cu(1)-N(1)	82.4(4)	N(2)-Cu(1)-N(3)	83.7(4)
N(2)-Cu(1)-O(1)	79.1(4)	N(1)-Cu(1)-N(3)	163.1(4)
N(1)-Cu(1)-O(1)	97.3(4)	N(3)-Cu(1)-O(1)	89.4(4)

Table II. Residual albumin (%) in the reaction mixture 1 hour after addition of hydrogen peroxide (see experimental section).

Complexes	$Cu(bdpg)ClClO_4$	$Cu(dpgs)ClClO_4$	$Cu(tpa)ClClO_4$
Cu(II) complex; none H_2O_2 none	100	100	100
Cu(II) complex; none H_2O_2(25 mM)	100	100	100
Cu(II) complex (0.3 mM) H_2O_2(1.6 mM)	100	100	100
Cu(II) complex (0.3 mM) H_2O_2(3.1 mM)	90	100	100
Cu(II) complex (0.3 mM) H_2O_2(6.3 mM)	62	83	100
Cu(II) complex (0.3 mM) H_2O_2(12.5 mM)	52	62	100
Cu(II) complex (0.3 mM) H_2O_2(25 mM)	30	46	100

the case of $Cu(bdpg)Cl^+$, Cu(II)-O(amide oxygen) is 2.287(2) Å, Okuno *et al.*, 1997).



Since no formation of PBN-OH was detected in the solution containing a copper(II) complex, hydrogen peroxide, and PBN in the ESR spectra, it seems quite likely that OH-radical formation does not occur in the solution, because PBN is one of the famous spin-trapping reagents for OH-radical (Janzen *et al.*, 1978). Thus, we would like to propose that the degradation of albumin is caused oxidatively by the copper(II)-peroxide adduct shown above, which exhibits a high electrophilic nature (Okuno *et al.*, 1997). This assumption may be supported by several facts, for example, some cytochrome P-450 enzymes exhibit high activity for oxidation of organic compounds containing carbonyl group and amide groups (L.-Robichaud *et al.*, 1995; Peng *et al.*, 1995; Vaz *et al.*, 1996), peptidylglycine α-hydroxylating monooxygenase contains a copper(II) ion (Prigge *et al.*, 1997), and the iron(III)-peroxide adduct exhibits a protease-like function (Rana and Meares, 1991).

Protein modifications in the biological systems

Present results suggest that the presence of the peptide-group near the copper(II) ion may play an important role in activating the peroxide ion, leading to modification or degradation of the neighboring peptide bonding. Thus, it seems most likely that the copper(II) ion coordinated by the peptide group in the octarepeat region in the PrP^C may contribute to the modification or degradation of the NH_2-terminal domain of PrP^C in the presence of hydrogen peroxide, and this may give a reasonable explanation for the formation of PrP^{Sc} reported in the previous papers (Prusiner, 1996, 1997).

It is known that the FALS (familial amyotrophic lateral sclerosis)-associated SOD1 (SOD = superoxide dismutase) mutations affect amino acid residues involved in enzymatic dimerization or β-barrel turns, rather than those corresponding to the active sites (Rabizadeh *et al.* 1995). Yim *et al.* (1993) have reported that in addition to its activity as a SOD, CuZnSOD catalyzes oxidation of substrates by hydrogen peroxide (Yim *et al.*, 1993; Pazos et al., 1996) Based on these facts and the present results, it seems reasonable to assume that in the FALS-associated mutations in a copper(II) peroxide adduct, which is an intermediate formed in the decomposition of superoxide ion, can oxidize the protein nearby, and that this may be a main origin for the increased openness of the three dimensional structure of the SOD in FALS. (Deng *et al.*, 1993).

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