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Ceruloplasmin Enhances DNA Damage Induced by Hydrogen Peroxide *In Vitro*

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Ceruloplasmin (Cp) was found to promote the oxidative damage to DNA, as evidenced by the formation of 8-hydroxy-2′-deoxyguanosine and strand breaks, when incubated with H_2O_2 *in vitro*. The capacity of Cp to enhance oxidative damage to DNA was inhibited by hydroxyl radical scavengers such as sodium azide and mannitol, a metal chelator, diethylenetriaminepentaacetic acid, and catalase. Although the oxidized protein resulted in an increase in the content of carbonyl groups, the ferroxidase activity and the proteolytic susceptibility were not significantly altered. The release of a portion of Cu from Cp was observed, and conformational alterations were indicated by the changes in fluorescence spectra. Based on these results, we suggest that damage to DNA is mediated in the H_2O_2/Cp system via the generation of $^{\bullet}OH$ by released Cu^{2+} and/or loosely bound Cu exposed from oxidatively damaged Cp through the conformational change. The release of Cu from Cp during oxidative stress could enhance the formation of reactive oxygen species and could also potentiate cellular damage.

Keywords: Ceruloplasmin, reactive oxygen species, DNA damage, conformational change

INTRODUCTION

Ceruloplasmin [Cp; ferroxidase; Fe(II): oxygen oxidoreductase; EC 1.16.3.1] is a 132-kDa multifunctional monomeric glycoprotein that contains greater than 95% of the Cu found in the plasma of vertebrate species.[1,2] Apoceruloplasmin is synthesized in the liver as a single polypeptide chain and secreted into plasma as a holoceruloplasmin associated with 6–7 atoms of Cu per molecule.[3] It has been suggested that Cp plays a role in protecting against reactive oxygen species-mediated cellular damage by acting as a ferroxidase, a scavenger of reactive oxygen species, and a peroxidase in the presence of reduced glutathione,[4–7] however, recent results indicate that Cp may also possess a damaging and oxidant activity.[8,9]

The superoxide anion radical O_2^- is generated in aerobic organisms, mostly as a result of normal metabolic processes and phagocytosis. O_2^- and its mutation product, H_2O_2, react with transition metal ions to produce the hydroxyl

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radical ($^{\bullet}OH$).[10] Transition metal ions such as Cu and Fe are extremely effective promoters of oxidation reactions and can cause the oxidative modification of lipids, proteins, and DNA.[11,12] During exposure to oxidative stress, many proteins with divalent cation binding sites will be likely susceptible to oxidative damage, and free transition metal ions could thus be released. The direct oxidation of Zn/Cd-metallothionein (MT) and Cu containing MT by H_2O_2 in a cell-free system has been shown to induce metal release.[13,14] It has also been reported that released as free Cu and/or Cu exposed at protein surfaces it could result in tissue damage in certain Cu, zinc superoxide dismutase (CuZn-SOD) mutant familial amyotrophic lateral sclerosis,[15–17] oxidatively inactivated CuZnSOD caused by a glycation, [18,19] and amyloid precursor protein in Alzheimer disease.[20] The catalytic action of Cu^{2+} from damaged Cu containing proteins in the generation of $^{\bullet}OH$ from H_2O_2 was suggested as a mechanism of deleterious effect.

In order to develop the *in vitro* model to elucidate the possible role of Cp as an oxidant via the release of Cu we investigated the enhancing effect of Cp on the DNA damage induced by H_2O_2. Free DNA is a convenient and sensitive substrate for assaying damage caused by the hydroxyl radicals. We report here that Cp undergoes damage induced by H_2O_2 with oxidation and conformational changes to release free Cu ion and/or expose loosely bound Cu ion. This Cu ion, together with H_2O_2, produces hydroxyl radicals which catalyze DNA damage, including the generation of strand breaks and the formation of 8-hydroxy-2′-deoxyguanosine (8-OH-dG).

MATERIALS AND METHODS

Materials

Calf thymus DNA, H_2O_2, mannitol, sodium azide, diethylenetriaminepentaacetic acid (DETAPAC), bathocuproine, o-dianisidine, o-phthaldehyde, apotransferrin, trypsin, chymotrypsin, pronase, nuclease P1, *Escherichia coli* alkaline phosphatase, and human Cp were obtained from Sigma (St. Louis, USA). In order to avoid the contamination of free Cu, Cp was treated with a chelating agent, Chelex-100 (Bio-Rad, Hercules, USA) for 4 h at room temperature, and Cp concentrations were determined using a BCA assay (Pierce, Rockford, USA) using bovine serum albumin as standard. pUC18 plasmid DNA was purified from *E. coli* cultures by using PROMEGA Magic Minipreps.

Generation of Strand Breaks in DNA

DNA single strand breaks were assayed by measuring the conversion of covalently closed circular double-stranded supercoiled DNA (form I) to open (relaxed) circular double-stranded DNA (form II). pUC18 plasmid DNA (200 ng) was incubated in 25 mM phosphate buffer, pH 7.5 under various conditions, as described in the figure legends, in a microfuge tube. The final volume was 10 μl. Incubations were at 37°C for 2 h. The reactions were terminated by the addition of 2 μl sixfold strength agarose gel loading solution (30% glycerol, 0.01% bromophenol blue). DNA samples were applied to 1% agarose gels in a TAE (40 mM Tris–acetate, pH 8.0/2 mM EDTA) buffer system, and electrophoresis was performed at 5 V/cm for 2 h at room temperature. Following electrophoresis, gels were stained with ethidium bromide and then destained for several hours. DNA bands were visualized by illuminating the gel with UV light and photographed.

Analysis of 8-OH-dG in DNA

Quantitation of the amount of 8-OH-dG in DNA was as described previously.[21] Calf thymus DNA (1 mg/ml) was incubated under various conditions, as described in the figure legends, in 100 μl of 25 mM phosphate buffer, pH 7.5, at 37°C for 2 h. After incubation, the DNA was separated by

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ethanol precipitation, dissolved in TE (10 mM Tris, pH 7.4/1 mM EDTA) buffer and digested to the nucleoside level with nuclease P1 and *E. coli* alkaline phosphatase. The resulting mixture was filtered and injected into an HPLC apparatus (Waters) equipped with both a UV detector and an electrochemical detector. The column was a Beckman Ultrasphere (0.46×25 cm) and the eluent was 10% aqueous methanol containing 50 mM phosphate buffer (pH 5.5). The flow rate was 1 ml/min. The molar ratio of 8-OH-dG to deoxyguanosine in each DNA sample was measured based on the peak height of authentic 8-OH-dG with the electrochemical detector and the UV absorbance at 254 nm for deoxyguanosine. All results presented are the means of at least duplicate, separate experiments yielded results with only minor variations from each other.

Oxidase Activity

Ceruloplasmic oxidase activity was measured using o-dianisidine as a substrate.[22] Ferroxidase activity of Cp was determined as described previously,[23] which measured the formation of red-colored transferrin–Fe^{3+} complex at 460 nm after the oxidation of iron in the presence of apotransferrin.

Determination of Protein Carbonyl Content

The carbonyl content of proteins was determined spectrophotometrically using the 2,4-dinitrophenylhydrazine (DNPH)-labeling procedure as described.[24] Both native and H_2O_2-treated Cp ($\sim$1 mg protein) were incubated with 0.4 ml 0.2% DNPH in 2 M HCl for 1 h. The protein hydrazone derivatives were sequentially extracted with 10% (w/v) trichloroacetic acid, treated with ethanol/ethyl acetate, 1 : 1 (v/v), and reextracted with 10% trichloroacetic acid. The resulting precipitate was dissolved in 6 M guanidine hydrochloride, and the difference spectrum of the sample treated with DNPH in HCl was examined versus a sample treated with HCl alone. Results are expressed as mol of DNPH incorporated per mol of protein calculated from an absorbtivity of 21.0 $mM^{-1} cm^{-1}$ at 360 nm for aliphatic hydrazones.

Copper Release

The concentration of Cu ion released from the H_2O_2-treated CuZnSOD was determined using bathocuproine disulfonic acid by the method described previously.[25] $CuSO_4$ solution was used as the standard. The concentration of free Cu was also determined by atomic absorption spectrophotometry. Protein samples were incubated with or without H_2O_2, and then subjected to ultrafiltration using a Millipore tube-5000, and then the concentration of Cu in the filtrate was determined.

Protease Susceptibility

Susceptibility to proteolysis was measured by the incubation of Cp samples (1.0 mg/ml) with 12.5 μg each of trypsin, chymotrypsin, or pronase in 250 μl of 25 mM HEPES (pH 8.0)/100 mM NaCl for 30–90 min at 37°C. After incubation, the aliquots were removed and subjected to a 10% trichloroacetic acid treatment. After centrifugation of precipitated proteins for 10 min at 14,000 rpm in an Eppendorf microfuge, the supernatant was first neutralized with a predetermined volume of 2 M potassium borate, pH 10. The amount of small peptides in the supernatant was then determined as described by Church *et al.*[26]

Fluorescence Measurements

Steady-state fluorescence measurements were performed on a Shimadzu RF-5301 PC spectrofluorophotometer with the sample compartment maintained at 22°C. A 150-W xenon source was used. The slit-width was fixed at 5 nm for excitation and emission. Unless otherwise stated, samples were excited at 280 nm, and the emission was monitored between 300 and 400 nm. Each recorded spectrum was an average of three

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separate scans and was corrected for background fluorescence of the relevant control. The intrinsic fluorescence of native Cp (4 μg of protein/ml) was routinely measured in 50 mM Tris–HCl, pH 7.4. Changes in protein fluorescence were monitored at the emission maximum of 333 nm. For quenching studies, the progressive addition of small aliquots of KI to the protein sample were made from quencher stock solutions (5 M) prepared in the same buffer as the protein sample; dilution never exceeded 10%, and in each experiment, the fluorescence intensity was corrected for the dilution factor. Quenching data were analyzed according to the standard Stern–Volmer relationship, $F_o/F = 1 + K_{sv}[Q]$, where F_o is the intensity of fluorescence at a given wavelength in the absence of quenching agent, F is the intensity of fluorescence at the same wavelength in the presence of a known concentration [Q] of quenching agent, and K_{sv} is the Stern–Volmer quenching constant obtained from the slope of a plot of F_o/F versus [Q].[27] To estimate the accessible fluorophore fraction f_a in $p47^{phox}$, modified Stern–Volmer plots were used, according to the relation,[28] $F_o/(F_o - F) = 1/f_a + 1/f_a K_{sv}[Q]$. The plot of $F_o/(F_o - F)$ versus $1/[Q]$ allows graphical determination of f_a.

Replicates

Unless otherwise indicated, each result described in this paper is representative of at least three separate determinations.

RESULTS

Generation of Strand Breaks and 8-OH-dG in DNA

When pUC18 DNA was incubated with 5 mM H_2O_2 itself yielded little, if any, strand breaks. However, Cp enhanced strand breaks in a dose-dependent manner, manifested as conversion to nicked plasmid (form II), as shown in Figure 1. Similar to the results obtained on strand breaks in plasmid DNA, a concentration-dependent increase in H_2O_2 induced 8-OH-dG formation in DNA was observed with 20–200 μg/ml Cp as shown in Figure 2. Cp alone did not cause any DNA damage. The modified base, 8-OH-dG, is considered to be one of the oxidative DNA products induced by oxygen radicals such as hydroxyl radicals and singlet oxygen, that can be easily measured by HPLC with electrochemical detection.[21] Therefore, it has been used as an

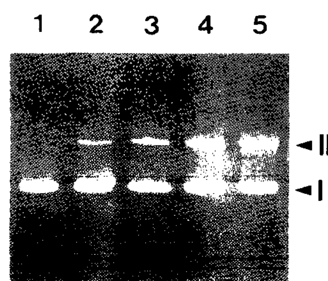

FIGURE 1 Concentration dependence of Cp enhancement on H_2O_2 induced strand breaks in pUC18 plasmid DNA. Lane 1, DNA + H_2O_2 (5 mM); lanes 2–5, DNA + H_2O_2 with 20, 50, 100, 200 μg/ml Cp, respectively.

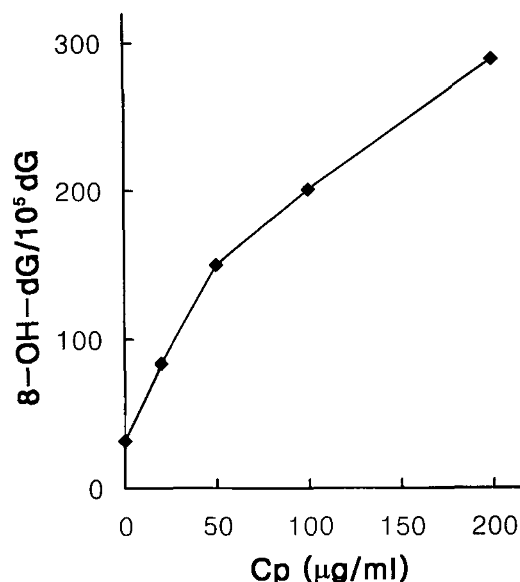

FIGURE 2 Concentration-dependent increase of 8-OH-dG in calf thymus DNA treated with 5 mM H_2O_2 and various concentrations of Cp.

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TABLE I Effect of inhibitors on the Cp/H_2O_2-mediated 8-OH-dG formation in calf thymus DNA

Addition	Inhibition of 8-OH-dG formation (%)
Azide (50 mM)	42
Mannitol (200 mM)	35
DETAPAC (1 mM)	100
EDTA (2 mM)	89
Catalase (100 µg/ml)	100

The amount of 8-OH-dG formed with 5 mM H_2O_2 and 100 µg/ml Cp was 198 8-OH-dG/10^5 dG (mean, $n = 3$).

indicator of oxidative DNA damage *in vivo* and *in vitro*. The enhancing effect of Cp on the formation of 8-OH-dG in DNA was inhibited by scavengers for •OH, such as sodium azide and mannitol, and DETAPAC, a chelating agent for Cu^{2+} in the same experimental setting as described above. Addition of catalase also inhibited 8-OH-dG formation in DNA (Table I).

Protein Oxidation

Previous *in vitro* studies have shown that protein oxidation modified by the metal-catalyzed oxidation system is accompanied by the conversion of proline, lysine, arginine, and histidine residues into carbonyl derivatives which can be detected by the formation of hydrazone conjugates upon treatment with 2,4-dinitrophenyl-hydrazine,[29] The level of carbonyl content was increased 2–3-fold with treatment of 5 mM H_2O_2 to Cp compared to untreated Cp (Figure 3).

Oxidase Activity

Cp oxidase activity was measured after the incubation of Cp with H_2O_2 to determine if the increase in Cp carbonyl formation resulted in a loss of functional activity. Reactive oxygen species can damage proteins, resulting in the formation of carbonyl derivatives with a concomitant loss of enzyme function. Despite the oxidative modification of Cp by H_2O_2, the oxidase activity was essentially unaltered. Cp ferroxidase activity was

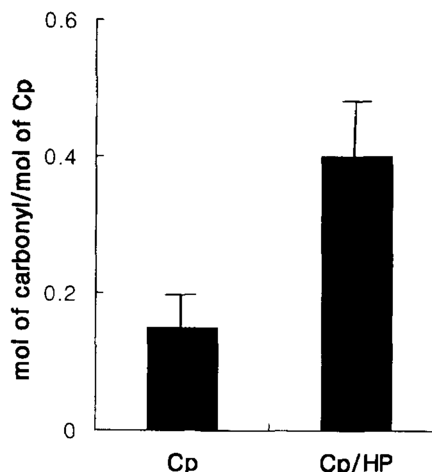

FIGURE 3 Protein carbonyl content of Cp exposed to H_2O_2 (Cp/HP). Protein carbonyls were measured in native and oxidized Cp by the method of Levine and co-workers[24] with the use of 2,4-dinitrophenyl hydrazine. Results are mean ± SD of three determinations.

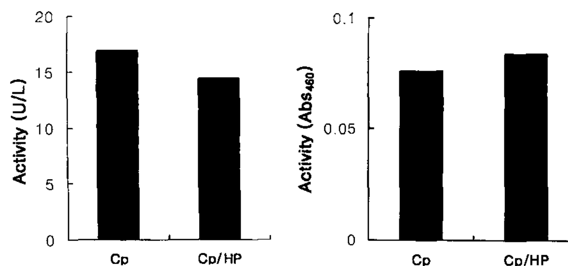

FIGURE 4 Effect of H_2O_2 on the activity of Cp. Oxidase activity toward o-dianisidine (left) and ferroxidase activity (right) of native Cp and H_2O_2-treated Cp (Cp/HP) were determined.

also quite resistant to damage by oxygen radicals (Figure 4).

Release of Copper Ion

Hydroxyl radicals can be formed from H_2O_2 in the presence of transition metal ions such as Cu and Fe through Fenton-type reactions. We determined the amount of Cu released from oxidized Cp using bathocuproine and atomic absorption spectrophotometry. H_2O_2 led to an increased amount of Cu^{2+} available with bathocuproine sulfate from

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Cp, which can participate in a Fenton-type reaction. It is responsible for the DNA damage. The concentration of Cu from Cp (100 μg/ml) is equivalent to 0.75 μM.[8] The results showed that 9.5–12.1% of weakly bound and/or free Cu was released by incubation of Cp with 5 mM H_2O_2 for 2 h at 37°C. These data confirm that the binding sites of Cu^{2+} in Cp were disintegrated by treatment with H_2O_2. This notion is also supported by the evidence that DETAPAC, which chelates transition metals, could markedly inhibit the formation of 8-OH-dG.

Protease Susceptibility

Protein damage by reactive oxygen species usually results in enhanced proteolytic susceptibility due to protein unfolding and increased accessibility of peptide bonds to proteases.[30] We have examined whether or not Cp becomes more susceptible to proteolytic digestion following H_2O_2 treatment. The results indicate that treated Cp does not show any noticeable change in its degradation by trypsin, chymotrypsin, or pronase (Figure 5).

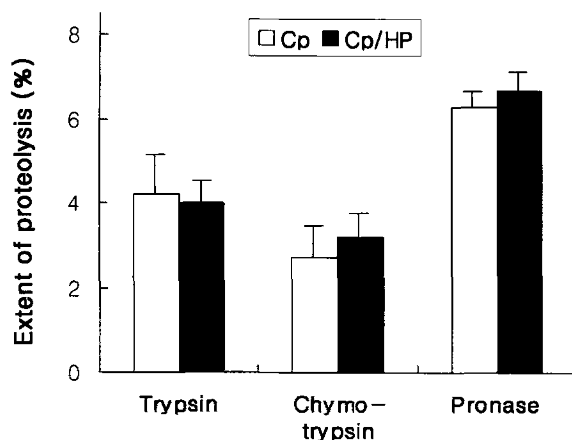

FIGURE 5 Proteolytic susceptibility of Cp and oxidized Cp. The extent of proteolysis observed when native and H_2O_2-treated Cp (Cp/HP) were digested with 12.5 μg each of proteases in 25 mM HEPES at pH 8.0 containing 100 mM NaCl for 30 min (pronase) or 90 min (trypsin, chymotrypsin) at 37°C, and then the extent of amine liberation was assayed and calculated using the method of Church *et al.*[26] The values are the mean ± range of two separate experiments.

Conformational Change

As can be seen in Figure 6, native Cp exhibits a fluorescence emission spectrum typical for Trp residues in proteins. Upon excitation of native Cp at 280 nm, an emission spectrum with a maximum at 333 nm was obtained. The fluorescence spectra of native and oxidized Cp, normalized to protein content, show that oxidized Cp displays a lowered quantum yield (73.2%) and a small red-shift (1 nm) of the emission spectra. This suggests that the Trp is supposedly in more hydrophobic environment. The lower quantum yield document perturbations in the environment of Trp which reflect conformational changes. A surface (I^-) quencher was employed to investigate the accessibility of the fluorophore. The Stern–Volmer constant (K_{sv}), derived from the slope of the linear portion of the Stern–Volmer plot at low KI, can be taken as a crude estimation of the accessibility of

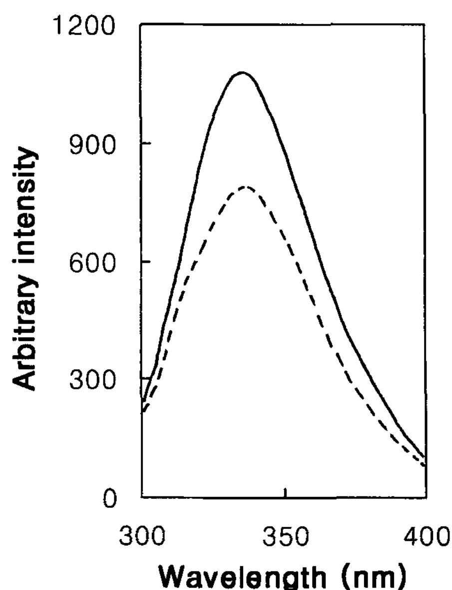

FIGURE 6 Fluorescence emission spectra of native Cp (solid line) and H_2O_2-treated Cp (dotted line) dissolved in 50 mM Tris–HCl, pH 7.4. The concentration of protein was 4 μg/ml. Spectra were obtained using an excitation wavelength of 280 nm and excitation and emission slits of 5 nm. All spectra were corrected for a protein blank emission. The fluorescence intensity is expressed in arbitrary units.

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TABLE II Quenching parameters for KI quenching of Cp fluorescence

Sample	K_{sv}[a] (M^{-1})	f_a[b]
Cp	1.42	0.69
Cp + H_2O_2	1.77	0.80

[a]The K_{sv} constant was graphically estimated as the initial slope from the Stern–Volmer plots. [b]The fractional number of accessible fluorophores (f_a) was extrapolated from the modified Stern–Volmer relationship[28] with concentrations of KI ranging from 0.05 to 0.5 M.

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Trp residues in protein.[27] As shown in Table II, it slightly increased by the Cp treated with H_2O_2, which confirms the increase of accessibility of Trp residues of Cp by oxidation with H_2O_2. It was also deduced from modified Stern–Volmer plots that the value of f_a, which represents the fraction of tryptophan residues accessible to the quencher, increased with the treatment of Cp with H_2O_2. Quenching efficiency is increased in the treated protein compared to the native Cp, and the fraction of accessibility is increased in the treated protein for KI, confirming that the environment of Trp, which acts as an indicative for local conformation changes, is modified.

DISCUSSION

The present *in vitro* study demonstrates the deleterious effect of Cp in the presence of H_2O_2 on DNA strand breaks and base damage as reported by the 8-OH-dG formation. Our data can be interpreted in the following way: $^{\bullet}OH$ generation arises from the H_2O_2 and the free Cu^{2+} liberated and/or Cu^{2+} exposed to the surface from the oxidatively damaged Cp. The following lines of evidence support the notion that Cp is a source of Cu^{2+} for the Fenton reaction in H_2O_2/Cp: (1) a Cu chelator, DETAPAC, abolished strand breaks and prevented the formation of 8-OH-dG in DNA; (2) free Cu^{2+} was released from the damaged Cp; and (3) exogenously added free Cu^{2+} instead of Cp augmented the generation of 8-OH-dG in DNA. The 8-OH-dG formation and strand breaks in DNA are known indicators of hydroxyl radical attack on DNA.[31,32] Furthermore, the protective effect of hydroxyl radical scavenger mannitol on the DNA damage and an increase of degradation of 2-deoxy-D-ribose, which reflects the generation of hydroxyl radicals, in the addition of Cp compared to H_2O_2 alone (data not shown), support the proposal that reaction of H_2O_2 with Cp produced $^{\bullet}OH$. It has been proposed that Cu^{2+} liberated from oxidatively damaged antioxidant enzyme CuZnSOD[33] and glycated CuZnSOD[18] catalyzes the formation of $^{\bullet}OH$ from H_2O_2 via a Fenton-like reaction, which resulted in the inactivation of α_1-protease inhibitor[33] and in the oxidative damage to DNA.[34,35] Antioxidant enzymes provide a substantial defense network against the accumulation of reactive oxygen species and Cu sequestration by binding proteins such as Cp and MT may afford protection from Cu-induced oxidant injury. Therefore, the damage brought about by oxidative stress is expected to be exacerbated if the antioxidant proteins themselves are damaged and to release a catalyst in Fenton-type reactions.

It has been shown that Cp induces low density lipoprotein (LDL) oxidation *in vitro*, and it has been suggested that the prooxidant activity depends on the presence of a single chelatable Cu atom.[8] Chelex-100 removes one of the seven Cu atoms that cause oxidation of LDL.[8] However, Chelex-100 treated Cp shows oxidant activity to damage DNA similar to that of untreated Cp in the presence of H_2O_2. Therefore, it can be assumed that the Cu atom specific for LDL oxidation does not play a critical role in the enhancing effect of Cp on the H_2O_2-mediated DNA damage. DNA damage induced by H_2O_2/Cp is either mediated by other specific Cu site(s) or by the global and nonspecific effect of various Cu sites.

Recently, it has been suggested that during aging, Cp is subjected to oxidative modifications, although the oxidase activity was not significantly altered. This is likely to be the source of

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conformational changes around the Cu sites leading to an intramolecular electron rearrangement among the various Cu sites.[36] Our results of H_2O_2-treated Cp shows a similar increase of oxidation and no significant alteration in oxidase activity and protease susceptibility. In addition, there are several lines of evidence obtained from the present study, indicating that Cp treated with H_2O_2 induces conformational changes, as reflected by a fluorescence change with three contributing factors: (1) a significant decrease of protein quantum yield; (2) a shift of emission maximum; and (3) an increase in the accessibility to a hydrophilic quencher, which is reflected by the observed increase in the quenching parameters, K_{sv} and f_a. The conformational integrity of the Cp molecule was destroyed, and thus Cu^{2+} became accessible from the outside, and $^{\bullet}OH$ generated could be liberated toward the outside of the Cp molecule, which resulted in the damage to DNA. H_2O_2 did not alter Cp oxidase activity significantly, indicating that either the conformational change is not gross, which can be also implicated from the nonsignificant change in protease susceptibility, or specific Cu atoms responsible for oxidase and oxidant activities may not be the same.

In conclusion, the present results indicate that the generation of DNA strand breaks and the formation of 8-OH-dG within DNA may be augmented by reactive oxygen species formed by the reaction of free Cu^{2+}, released and/or exposed from oxidatively damaged Cp and H_2O_2. The release of Cu from Cu-containing proteins, such as Cp, CuZnSOD, Cu containing MT, and amyloid precursor protein, may serve to potentiate cell damage by promoting Cu-dependent redox cycle when an excess amount of O_2^- and/or H_2O_2 is generated during oxidative stress.

Acknowledgment

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References

[1] L. Ryden and I. Bjork (1976) Reinvestigation of some physicochemical and chemical properties of human ceruloplasmin (ferroxidase). *Biochemistry*, **15**, 3411–3417.
[2] S.J. Orena, C.A. Goode and M.C. Linder (1986) Binding and uptake of copper from ceruloplasmin. *Biochemical and Biophysical Research Communications*, **139**, 822–829.
[3] N. Takahashi, T.L. Ortel and F.W. Putnum (1984) Single-chain structure of human ceruloplasmin: The complete amino acid sequence of the whole molecule. *Proceedings of the National Academy of Sciences of the United States of America*, **81**, 390–394.
[4] I.M. Goldstein, H.B. Kaplan, H.S. Edelson and G. Weissmann (1979) Ceruloplasmin. A scavenger of superoxide anion radicals. *Journal of Biological Chemistry*, **254**, 4040–4045.
[5] S. Yamashoji and G. Kajimoto (1983) Antioxidant effect of caeruloplasmin on microsomal lipid peroxidation. *Federation of European Biological Society Letters*, **152**, 168–170.
[6] V.M. Samokyszyn, D.M. Miller, D.W. Reif and S.D. Aust (1989) Inhibition of superoxide and ferritin-dependent lipid peroxidation by ceruloplasmin. *Journal of Biological Chemistry*, **264**, 21–26.
[7] I.G. Kim, S.Y. Park, K.C. Kim and J.J. Yum (1998) Thiol-linked peroxidase activity of human ceruloplasmin. *Federation of European Biological Society Letters*, **431**, 473–475.
[8] E. Ehrenwald, G.M. Chisolm and P.L. Fox (1994) Intact human ceruloplasmin oxidatively modifies low density lipoprotein. *Journal of Clinical Investigation*, **93**, 1493–4501.
[9] C.K. Mukhopadhyay, E. Ehrenwald and P.L. Fox (1996) Ceruloplasmin enhances smooth muscle cell- and endothelial cell-mediated low density lipoprotein oxidation by a superoxide-dependent mechanism. *Journal of Biological Chemistry*, **271**, 14 773–14 778.
[10] B. Chance, H. Sies and A. Boveris (1979) Hydroperoxide metabolism in mammalian organs. *Physiological Review*, **59**, 527–605.
[11] B. Halliwell and J.M.C. Gutteridge (1984) Oxygen toxicity, oxygen radicals, transition metals and disease. *Biochemical Journal*, **219**, 1–14.
[12] D. Steinberg (1995) Role of oxidized LDL and antioxidants in atherosclerosis. *Advances in Experimental and Medical Biology*, **369**, 39–48.
[13] I. Jimenez, M. Gotteland, A. Zarzuelo, R. Uauy and H. Speisky (1997) Loss of the metal binding properties of metallothionein induced by hydrogen peroxide and free radicals. *Toxicology*, **120**, 37–46.
[14] J.P. Fabisiak, V.A. Tyurin, Y.Y. Tyurina, G.G. Borisenko, A. Korotaeva, B.R. Pitt, J.S. Lazo and V.E. Kagan (1999) Redox regulation of copper–metallothionein. *Archives of Biochemistry and Biophysics*, **363**, 171–181.
[15] M. Wiedau-Pazos, J.J. Goto, S. Rabizadeh, E.B. Gralla, J.A. Roe, M.K. Lee, J.S. Valentine and D.E. Bredesen (1996) Altered reactivity of superoxide dismutase in familial amyotrophic lateral sclerosis. *Science*, **271**, 515–518.
[16] T. Siddique and H.X. Deng (1996) Genetics of amyotrophic lateral sclerosis. *Human Molecular Genetics*, **5**, 1465–1470.
[17] M.B. Yim, J.H. Kang, H.S. Yim, H.S. Kwak, P.B. Chock and E.R. Stadtman (1996) A gain-of-function of an amyotrophic lateral sclerosis-associated Cu,Zn-superoxide dismutase mutant: An enhancement of free radical formation due to a decrease in K_m for hydrogen peroxide.

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Proceedings of the National Academy of Sciences of the United States of America, **93**, 5709–5714.

[18] T. Ookawara, N. Kawamura, Y. Kitagawa and N. Taniguchi (1992) Site-specific and random fragmentation of Cu,Zn-superoxide dismutase by glycation reaction. Implication of reactive oxygen species. *Journal of Biological Chemistry*, **267**, 18 505–18 510.

[19] K.N. Islam, M. Takahashi, S. Higashiyama, T. Myint, N. Uozumi, Y. Kayanoki, H. Kaneto, H. Kosaka and N. Taniguchi (1995) Fragmentation of ceruloplasmin following non-enzymatic glycation reaction. *Journal of Biochemistry*, **118**, 1054–1060.

[20] G. Multhaup, A. Schlicksupp, L. Hesse, D. Beher, T. Rupper, C.L. Masters and K. Beyreuther (1996) The amyloid precursor protein of Alzheimer's disease in the reduction of copper(II) to copper. *Science*, **271**, 1406–1409.

[21] J.-W. Park and R.A. Floyd (1992) Lipid peroxidation products mediate the formation of 8-hydroxydeoxyguanosine in DNA. *Free Radical Biology and Medicine*, **12**, 245–250.

[22] K.H. Schosinsky, H.P. Lehmann and M.F. Beeler (1974) Measurement of ceruloplasmin from its oxidase activity in serum by use of o-dianisidine dihydrochloride. *Clinical Chemistry*, **20**, 1556–1563.

[23] P.G. Winyard, R.C. Hider, S. Brailsford, A.F. Drake, J. Lunec and D.R. Blake (1989) Effects of oxidative stress on some physiochemical properties of caeruloplasmin. *Biochemical Journal*, **258**, 435–445.

[24] R.L. Levine, D. Garland, C.N. Oliver, A. Amici, I. Climent, A.G. Lenz, B.W. Ahn, S. Shaltiel and E.R. Stadtman (1990) Determination of carbonyl content in oxidatively modified proteins. *Methods in Enzymology*, **186**, 464–478.

[25] S.L. Jewett, S. Cushing, F. Gillespie, D. Smith and S. Sparks (1989) Reaction of bovine-liver copper–zinc superoxide dismutase with hydrogen peroxide. Evidence for reaction with H_2O_2 and HO_2^- leading to loss of copper. *European Journal of Biochemistry*, **180**, 569–575.

[26] F.C. Church, D.H. Porter, G.L. Catignani and H.E. Swaisgood (1985) An o-phthalaldehyde spectrophotometric assay for proteinases. *Analytical Biochemistry*, **146**, 343–348.

[27] M.R. Eftink and C.A. Ghiron (1976) Exposure of tryptophanyl residues in proteins. Quantitative determination by fluorescence quenching studies. *Biochemistry*, **15**, 672–680.

[28] S.S. Lehrer (1971) Solute perturbation of protein fluorescence. The quenching of the tryptophyl fluorescence of model compounds and of lysozyme by iodide ion. *Biochemistry*, **10**, 3254–3262.

[29] A. Amici, R.L. Levine, L. Tsai and E.R. Stadtman (1989) Conversion of amino acid residues in proteins and amino acid homopolymers to carbonyl derivatives by metal-catalyzed oxidation reactions. *Journal of Biological Chemistry*, **264**, 3341–3346.

[30] E.R. Stadtman (1992) Protein oxidation and aging. *Science*, **257**, 1220–1224.

[31] O.I. Aruoma, B. Halliwell, E. Gajewski and M. Dizdaroglu (1991) Copper-ion-dependent damage to the bases in DNA in the presence of hydrogen peroxide. *Biochemical Journal*, **273**, 601–604.

[32] J.-W. Park and R.A. Floyd (1994) Generation of strand breaks and formation of 8-hydroxy-2′-deoxyguanosine in DNA by a thiol/Fe^{3+}/O_2-catalyzed oxidation system. *Archives of Biochemistry and Biophysics*, **312**, 285–291.

[33] K. Sato, T. Akaike, M. Kohno, M. Ando and H. Maeda (1992) Hydroxyl radical production by H_2O_2 plus Cu,Zn-superoxide dismutase reflects the activity of free copper released from the oxidatively damaged enzyme. *Journal of Biological Chemistry*, **267**, 25 371–25 377.

[34] J.-W. Park and R.A. Floyd (1997) Glutathione/Fe^{3+}/O_2-mediated DNA strand breaks and 8-hydroxydeoxyguanosine formation. Enhancement by copper, zinc superoxide dismutase. *Biochimica et Biophysica Acta*, **1336**, 263–268.

[35] H. Kaneto, J. Fujii, K. Suzuki, H. Kasai, R. Kawamori, T. Kamada and N. Taniguchi (1994) DNA cleavage induced by glycation of Cu,Zn-superoxide dismutase. *Biochemical Journal*, **304**, 219–225.

[36] G. Musci, M.C. Bonaccorsi di Patti, U. Fagiolo and L. Calabrese (1993) Age-related changes in human ceruloplasmin: Evidence for oxidative modifications. *Journal of Biological Chemistry*, **268**, 13 388–13 395.

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