Dithiothreitol inhibits the acceleration of DNA strand breaks by human serum albumin in a metal-catalyzed oxidation and γ-irradiation system

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ABSTRACT

Intact or Cys-34 modified human serum albumin (HAS) was significantly damaged and degraded in the metal-catalyzed oxidation (MCO) system containing dithiothreitol (DTT) as an electron donor and, while it lasted, this protein might have prohibited the •OH or H_2O_2 from attacking DNA. However, in the glutathione (GSH) and ascorbate (non-thiol) MCO system, HSA was not sacrificially degraded and accelerated rather than prevented DNA strand breaks. In contrast, human ceruloplasmin was not sacrificially degraded but significantly prevented DNA strand breaks in both GSH and DTT MCO system. In γ-irradiation system, only DTT attenuated the acceleration of DNA strand breaks by HSA. This protein did not remove more H_2O_2 in the presence of reduced GSH (thiol-linked peroxidase) than in the absence of GSH. Therefore it seems that in an MCO system, the antioxidant activity of HSA is dependent on the effectiveness of reducing equivalents to induce the exposure of a functional group scavenging •OH or H_2O_2 by reduction of disulfide bonds in HSA. In the presence of DTT, disulfide bonds in HSA were completely reduced to cysteinyl residues but not significantly converted into cysteines by ascorbate or GSH. On the other hand, human ceruloplasmin has a resistance to reduction. In conclusion, the antioxidant activity of HSA in the DTT MCO system is thought not to be due to peroxidase function by thiol specificity, but due to sacrificial antioxidant activity by the functional groups, such as hydrophobic compounds exposed to the protein surface by DTT.

INTRODUCTION

ROS and their derivatives have been thought to play a significant role in the pathogenesis of human diseases such as rheumatoid arthritis, atherosclerosis and cancer (1-2). To circumvent the damage caused by the ROS, multiple defense enzyme systems, collectively called antioxidant proteins, are present not only in intracellular fluids but also in extracellular fluids (3-5). Human blood plasma is more sensitive to ROS, which are generated from granulocytes and macrophages during phagocytosis and the reactions of oxygen with a variety of metabolic products derived from cellular metabolism or γ-irradiation (6). Therefore, proteins that are suspected to have antioxidant properties have been identified in extracellular fluids (7-9).

Thiol compounds, including tripeptide GSH, are important antioxidant members since they have been shown to scavenge ROS and other oxygen derivatives by enzymatic, as well as non-enzymatic, mechanisms (10). Thiol-linked peroxidase is a class of enzymes exerting peroxidase activity in support of thiol-reducing equivalents. In human blood plasma GSH peroxidase containing selenium has been identified. However, it decomposes hydrogen peroxide (H_2O_2) when used with GSH in high non-physiological concentration (5 mM) (11). Therefore, the extracellular thioredoxin or glutaredoxin have been shown to be efficient electron donors for selenium-dependent peroxidase rather than GSH (12). Recently it has been proposed that human ceruloplasmin plays a significant role in H_2O_2-removing peroxidase activity in the presence of DTT or reduced GSH (13, 14).

HSA was speculated to be a “sacrificial” antioxidant in blood plasma and extracellular fluid (15, 16). In contrast, a recent study has suggested that HSA functions as an extracelluar enzymatic antioxidant, and should be classified as a “GSH (thiol)-dependent peroxidase”, not as a “sacrificial” antioxidant (17). It has been shown that an MCO system, which is composed of trace amount of iron, O_2 and electron donors, induces strand breaks in plasmid and mammalian DNA as well as the inactivation of several enzymes (18, 19). The aim of the present study is to investigate the antioxidant role of HSA and the effect of DTT on the DNA strand breaks by •OH or H_2O_2 produced in an MCO and γ-irradiation system. We also provide the first demonstration that the antioxidant property of HSA is different from that of ceruloplasmin, which has been suspected of GSH (thiol)-linked peroxidase, in the MCO system containing thiol compounds.

MATERIALS AND METHODS

Materials

All reagents were of analytical grade. Ammonium sulfate, agarose, GSH, β-mercaptoethanol, DTT, trichloroacetic acid, 5,5’-dithiobis-(2-nitrobenzoic acid), ascorbic acid, ovalbumin, cysteine and HEPES were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Human ceruloplasmin was obtained from Calbiochem. DEAE-Sepharose, pUC and ϕX174 plasmid DNA were purchased from Pharmacia Fine Chemicals (Uppsala, Sweden). Cibacron Blue F3-GA-sepharose was supplied by Bio-Rad (Richmond, CA, U.S.A.) and ultrafiltration membrane was purchased from Amicon Co. (Beverly, MA, U.S.A.). KSCN, NaCl, $FeCl_3$ and

$FeSO_4 \cdot 7H_2O$ were obtained from Waco Chemical Co. (Japan).

Preparation of human serum albumin

HSA was prepared from fresh blood serum (Taejon National Blood Bank) according to the modified methods of (20, 21), and all the purification steps were performed at 4°C. Plasma proteins were precipitated by the addition of an equal volume of 60% ammonium sulfate. The supernatant was extensively dialyzed against a Cibacron Blue F3-GA-Sepharose equilibrium buffer (20 mM phosphate buffer, pH 7.4). After centrifugation, the supernatant was loaded into a Cibacron Blue F3-GA-Sepharose column. The column was washed with an equilibrium buffer and eluted with an elution buffer (20 mM phosphate buffer containing 1M NaCl). Eluted fractions were pooled, concentrated and washed with a DEAE-Sepharose equilibrium buffer (20 mM phosphate buffer, pH 7.4) in order to remove salts by the Amicon ultrafiltration membrane (molecular weight of membrane; 10,000 dalton). The concentrated sample was loaded into a DEAE-Sepharose column. The column was washed with an equilibrium buffer and eluted with a linear NaCl gradient (0 – 400 mM). The main peak shows a homogeneous HSA. The analysis of the protein was estimated by native and sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) (22). Protein estimations were carried out according to the dye-binding methods of (23), using ovalbumin as the standard.

Determination of H_2O_2-removing activity of HSA in the presence of GSH and cysteine

To determine the peroxidase activity of HSA, the reaction was started by the addition of 500 μM H_2O_2 into a 50 μl reaction mixture containing 500 μM GSH, 100 mM NaCl, 50 mM HEPES buffer at pH 7.0, and an appropriate amount of the sample. The reaction mixtures were then incubated at 37°C. After 40 min, trichloroacetic acid solution (0.8 ml, 12.5%, w/v) was added to the reaction mixture to stop the reaction, followed by the addition of 200 μl of 10 mM $FeSO_4$ and 100 μl of 2.5 N KSCN to develop a purple color. The remaining H_2O_2 was measured by monitoring the decrease of absorbance at 480nm (24).

Analysis of DNA breaks induced by MCO systems and γ-radiation

Before the experiment, Tris-EDTA in a commercial plasmid must be completely discarded because this affects the function of ceruloplasmin. To analyze DNA strand breaks by hydroxyl radicals (•OH), 0.3 μg of ϕX174 or pUC plasmid DNA was mixed with 3 μM $FeCl_3$, 10 mM reducing equivalents, 50 mM NaCl and purified HSA in a total volume of 20 μl of 50 mM HEPES (pH 7.0). The reaction mixtures were then incubated for 100 – 160 min at 37°C. In the Fenton reaction, 10 μM $FeSO_4$ and 500 μM H_2O_2 were used and the reaction mixture was incubated at 37°C. Plasmid (pUC) DNA (0.3 μg) in 50 mM HEPES buffer (pH 7.0) containing 100 mM NaCl was irradiated with a single exposure to a dose of 60 Gy at a dose rate of 1.0 Gy/min using a ^{60}Co irradiator. The separation of the different conformations of ϕX174 or pUC was performed by electrophoresis using 0.8% agarose gel. Electrophoresis was carried out in 80 mM Tris-acetate (8 mM) buffer, pH 8.0, containing 2 mM EDTA. The DNA band on agarose gel was stained with ethidium bromide (0.5 μg/ml). After gel electrophoresis, the fractions of the three different forms (open circular, linear form and supercoiled form) were estimated by densitometric analysis using a Bio-profile image analysis system (Vilber Lourmat, France).

Modification of free sulfhydryl group

Freshly prepared HSA (2 mg/ml) was incubated with 2 mM 5,5'-dithiobis-(2-nitrobenzoic acid) in 100 mM HEPES buffer, pH 7.0 for 60min at 37°C and the absorbance at 412nm was measured (25). Before being used for the experiment, excess reagent was removed using an Ultrafree-15 centrifugal membrane.

Results

Protective effect against DNA strand breaks by HSA and ceruloplasmin in an MCO and γ-irradiation system

HSA has a molecular weight of 66,000 dalton and accounts for up to 60% of the total plasma protein. It is one of the few plasma proteins that have no oligosaccharides. HSA contains a total of 17-disulfide linkages and one free cysteinyl residue. Therefore, in the absence of β-mercaptoethanol, the protein band migrated significantly faster on the sodium dodecyl sulfate-polyacrylamide gel (Fig. 1).

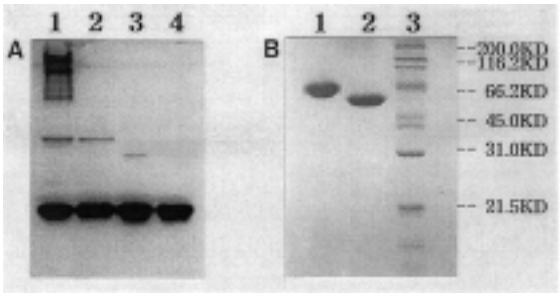

Figure 1. Native (A) and Sodium dodecyl sulfate (B) polyacrylamide gel electrophoresis analysis of HSA at different stages of purification (7.5% and 12.5% acrylamide, respectiviely). A: Lane 1, sample from blood plasma; lane 2, sample from supernatant precipitated by ammonium sulfate; lane 3, sample from Blue-Sepharose chromatography; lane 4, sample from DEAE-Sepharose chromatography. B: Lane 1, non-reducing sample; lane 2, sample adding β-mercaptoethanol; lane 3, standard markers.

We examined HSA for antioxidant activity by measuring the extent of the DNA strand breaks to convert to open circular and linear forms of supercoiled plasmid in an MCO reaction and γ-irradiation system. When GSH (thiol compound) and ascorbate (non-thiol compound) were used as the electron donors in the MCO system, the prevention of DNA strand breaks by HSA was not observed. On the other hand, it was shown that HSA might have the ability to prevent DNA strand breaks in the DTT MCO system (Fig. 2A). In the GSH MCO system, HSA accelerated, in a concentration-dependent manner, rather than prevented DNA strand breaks (Fig. 2B). Modification of free sulfhydryl residue (Cys-34) by 5,5'-dithiobis-(2-nitrobenzoic acid) also led to a prevention of DNA strand breaks in the DTT MCO system and DNA damage was also accelerated by modified HSA in the GSH MCO system (Fig. 2C). However, human ceruloplamsin effectively protected against DNA strand breaks in both GSH and DTT MCO [13] systems

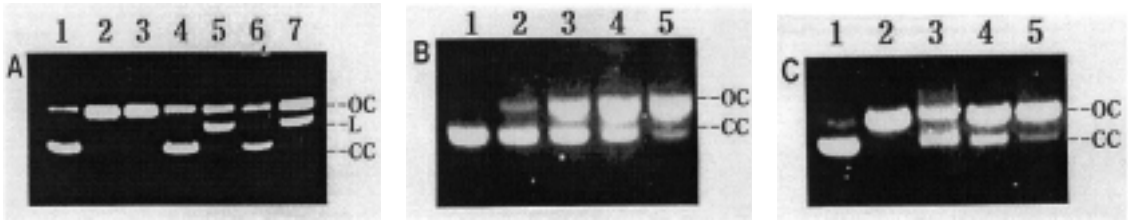

Figure 2. **A**: Antioxidant effect of HSA on DNA strand breaks induced by the MCO (10 mM reducing equivalents/3 μM $FeCl_3$/100 mM NaCl in 20 μl of reaction volume; incubation time; 100min) system. 0.3 μg of ϕX174 or pUC plasmid DNA was used. Lane 1, 0.3 μg of ϕX174; lane 2, 0.3 μg of ϕX174 in ascorbate MCO system; lane 3, 0.3 μg of ϕX174 in DTT MCO system; lane 4, 0.3 μg of ϕX174 in GSH MCO system; lane 5, 0.3 μg of ϕX174 in ascorbate MCO system + 200 μg HSA; lane 6, 0.3 μg of ϕX174 in DTT MCO system + 200 μg HSA; lane 7, 0.3 μg of ϕX174 in GSH MCO system + 200 μg HSA. **B**: Effect of HSA concentration on the DNA strand breaks in the GSH MCO system. Lane 1, 0.3 μg of pUC;

lane 2, 0.3 μg of pUC in GSH MCO system; lane 3, 0.3 μg of pUC in GSH MCO system reaction + 150 μg HSA; lane 4, 0.3 μg of pUC in GSH MCO system + 300 μg HSA; lane 5, 0.3 μg of pUC in GSH MCO system + 450 μg HSA. **C**: Antioxidant effect of Cys-34 modified HSA by DTNB. Lane 1, 0.3 μg of pUC; lane 2, 0.3 μg of pUC in DTT MCO system; lane 3, 0.3 μg of pUC in DTT MCO system reaction + 200 μg DTNB-modified HSA; lane 4, 0.3 μg of pUC in ascorbate MCO system; lane 5, 0.3 μg of pUC in ascorbate MCO system + 200 μg DTNB-modified HAS. CC: closed circular; OC: open circular; L: linear form.

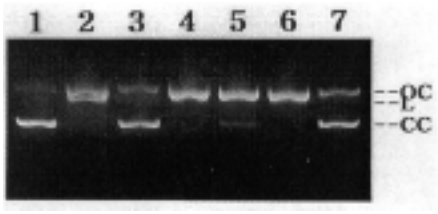

Figure 3. Antioxidant effect of human ceruloplasmin on DNA strand breaks induced by the MCO (10 mM reducing equivalents/3 μM $FeCl_3$/100 mM NaCl in 20 μl of reaction volume, incubation time; 160min) system. 0.3 μg of ϕX174 plasmid DNA was used. Lane 1, 0.3 μg of ϕX174; lane 2, 0.3 μg of ϕX174 in GSH MCO system; lane 3, 0.3 μg of ϕX174 in GSH MCO system + 10 μg ceruloplasmin; lane 4, 0.3 μg of ϕX174 in reduced ascorbate MCO system; lane 5, 0.3 μg of ϕX174 in ascorbate MCO system + 10 μg ceruloplasmin ; lane 6, 0.3 μg of ϕX174 in ascorbate(5 mM) + GSH(5 mM) MCO system; lane 7, 0.3 μg of ϕX174 in ascorbate (5 mM) + GSH(5 mM) MCO system + 10 μg ceruloplasmin. CC: closed circular; OC: open circular; L: linear form.

and did not significantly affect the prevention of molecular damage in the ascorbate MCO system (Fig 3). In the Fenton reaction system, HSA did not prevent DNA strand breaks. However, this protein did significantly protected against DNA damage when DTT was added to the reaction system (Fig. 4A). HSA did not show a preventive effect against DNA strand breaks by the addition of GSH or cysteine (Fig. 4B). In contrast to HSA, human ceruloplasmin effectively prevents DNA strand breaks by the addition of GSH in the Fenton reaction (Fig. 4C).

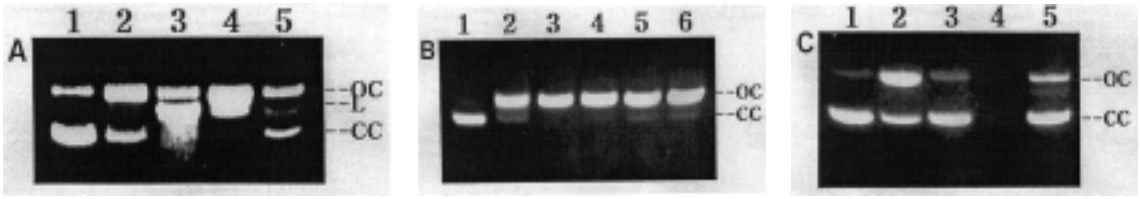

Fig. 4. **A**: Antioxidant effect of HSA on DNA strand breaks induced by the Fenton reaction (10 μM Fe^{2+}/500 μM H_2O_2/50 mM NaCl, incubation time: 60 min). Lane 1, 0.3 μg of ϕX174; lane 2, 0.3 μg of ϕX174 in Fenton system; lane 3, 0.3 μg of ϕX174 in Fenton system + 200 μg HSA; lane 4, 0.3 μg of ϕX174 in Fenton system + 1 mM DTT (incubation time: 10 min); lane 5, 0.3 μg of ϕX174 in Fenton system + 1 mM DTT + 200 μg HSA (incubation time: 10 min). **B**: Lane 1, 0.3 μg of pUC; lane 2, 0.3 μg of pUC in Fenton system; lane 3, 0.3 μg of pUC in Fenton system + 1 mM GSH (incubation time: 10 min); lane 4, 0.3 μg of pUC in Fenton system + 200 μg HSA + 1 mM GSH (incubation time: 10 min); lane 5, 0.3 μg of pUC in Fenton system + 1 mM cysteine; lane 6, 0.3 μg of pUC in Fenton system + 1 mM cysteine + 200 μg HSA. **C**: Lane 1, 0.3 μg of pUC; lane 2, 0.3 μg of pUC in Fenton system; lane 3, 0.3 μg of pUC in Fenton system + 10 μg ceruloplasmin; lane 4, 0.3 μg of pUC in Fenton system + 5 mM GSH; lane 5, 0.3 μg of pUC in Fenton system + 5 mM GSH + 10 μg ceruloplasmin. CC: closed circular; OC: open circular.

In the γ-irradiation system (60Gy) generating •OH from H_2O, HSA also accelerated the DNA damage and DTT additives attenuated the acceleration of DNA strand breaks by HSA. On the other hand, ascorbate and GSH did not affect the attenuation of DNA damage acceleration by HSA. (Fig. 5)

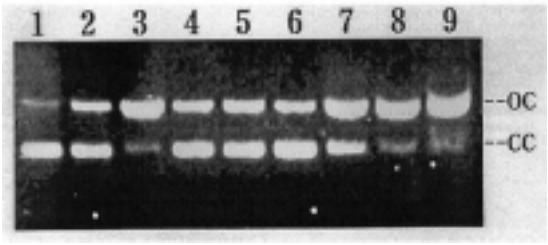

Figure 5. Effect of DTT, GSH and ascorbate on DNA strand breaks accelerated by HSA in γ-irradiation system. The reaction mixture (20 μl) contained 0.3 μg of pUC plasmid DNA and 100 mM NaCl in 50 mM HEPES. Lane 1, 0.3 μg of pUC; lane 2, 0.3 μg of pUC + 100 mM NaCl (60 Gy); lane 3, 0.3 μg of pUC + 100 mM NaCl + 300 μg HSA (60Gy); lane 4, 0.3 μg of pUC + 100 mM NaCl + 0.5 mM DTT (60Gy); lane 5, 0.3 μg of pUC + 100 mM NaCl + 0.5 mM GSH (60Gy); lane 6, 0.3 μg of pUC + 100 mM NaCl + 0.5 mM ascorbate (60Gy) ; lane 7, 0.3 μg of pUC + 100 mM NaCl + 0.5 mM DTT + 300 μg HSA (60Gy); lane 8, 0.3 μg of pUC + 100 mM NaCl + 0.5 mM GSH + 300 μg HSA (60Gy); lane 9, 0.3 μg of pUC + 100 mM NaCl + 0.5 mM ascorbate + 300 μg HSA (60Gy). CC: closed circular; OC: open circular; L: linear form.

H_2O_2-removing activity of HSA

The H_2O_2–removing peroxidase activity of HSA was also examined by measuring the decrease of H_2O_2 in the presence of thiol compounds (reduced GSH and cysteine). Although GSH or cysteine were absent in the reaction mixture, some quantity of H_2O_2 was eliminated by HSA (0.048 nmole H_2O_2/mg protein). However, the HSA of the reaction mixture, to which reduced GSH or cysteine was added, did not remove any more of the H_2O_2 than did the reaction mixture without GSH or cysteine (Fig. 6).

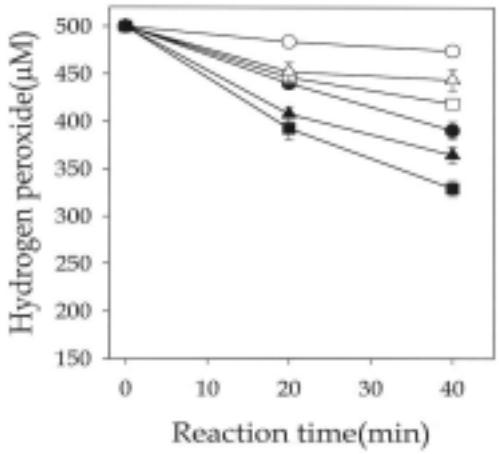

Figure 6. H_2O_2-removal activity of HSA supported by GSH and cysteine. Peroxidase reaction was carried out in a 50 μl reaction mixture according to the methods described under materials and methods. Open circle, 500 μM H_2O_2; closed circle, 100 μg HSA + 500 μM H_2O_2 + 100 mM NaCl; open square, 500 μM H_2O_2 + 500 μM GSH + 100 mM NaCl; closed square, 100 μg HSA + 500 μM H_2O_2 + 500 μM GSH + 100

mM NaCl; open triangle, 500 μM H_2O_2 + 500 μM cysteine + 100 mM NaCl; closed triangle, 100 μg HSA + 500 μM H_2O_2 + 500 μM cysteine + 100 mM NaCl. Data represent the means of triplicate analyses.

HSA and cerulopalsmin degradation of in an MCO system

In the DTT MCO system, HSA was severely degraded. On the other hand, they were not significantly degraded in the MCO system containing GSH or ascorbate as electron donors. However, human ceruloplasmin was not significantly degraded in both thiol (GSH and DTT) and nonthiol MCO system (Fig. 7). In the presence of DTT, HSA was completely reduced and so the electrophoretic mobility of the protein

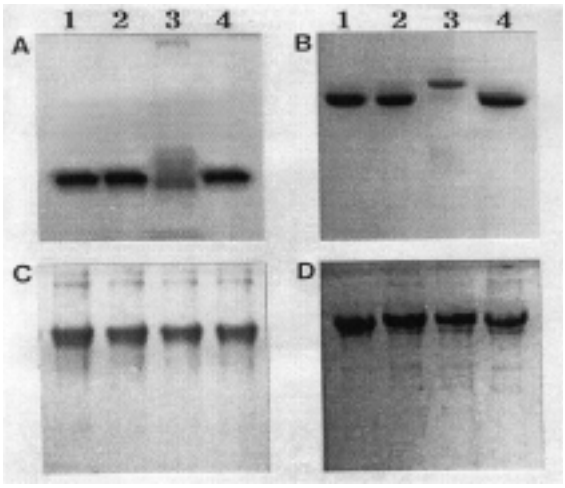

Figure 7. Native (A, C) and sodium dodecyl sulfate (B, D) polyacrylamide gel electrophoresis analysis of HSA (A, B) and ceruloplasmin (C, D) in a MCO system (7.5% and 12.5% acrylamide, respectiviely). Lane 1, HSA; lane 2, HSA in ascorbate MCO system; lane 3, HSA in DTT MCO system; lane 4, HSA in GSH MCO system. The reaction mixture contained 10 mM reducing equivalent, 3 μM $FeCl_3$, 100 mM NaCl and 200 μg HSA in 50 μl of reaction volume and was incubated for 60 min at 37°C.

on SDS-PAGE was changed in the case of β-mercaptoethanol. However, in the presence of ascorbate and GSH, disulfide of HSA was not significantly reduced and so the electrophoretic mobility of the protein on SDS-PAGE was not significantly changed. On the other hand, human ceruloplasmin that has been speculated to be thiol-linked peroxidase had a resistance to complete reductions of S-S bonds by DTT, as well as GSH and ascorbate.

DISCUSSION

The physiological role of HSA continues to be actively investigated since many diseases dramatically alter the level of this plasma protein (26-28). Besides the regulation of the osmotic pressure as principal function, it also binds and transports fatty acids, bile pigment and other lipophilic compounds in blood (29, 30). Recently it has been proposed that HSA inhibit the formation of apoceruloplasmin *in vitro* (31). HSA also functions as an antioxidant to protect against the damage of other important molecules, although this protein has no distinctive enzyme activity. In the presence of albumin, copper does not react with H_2O_2 to form •OH in solution. Although albumin-bound copper with H_2O_2 leads to formation of •OH, it site-specifically reacts further with albumin. Therefore albumin is able to prevent copper-stimulated lipid peroxidation and hemolysis of erythrocyte (32). It was previously mentioned that damage of albumin might be biologically insignificant since there is so much albumin in the serum and damaged albumin may be proteolytically degraded and quickly replaced *in vivo* (15). It also protects against α_1-antiprotease damage by a powerful oxidant, hypochlorous (HOCl)(33). These functions of albumin might be thought to be those of a sacrificial antioxidant (15, 16). In contrast, HSA has also recently been suspected of functioning as a GSH (thiol)-linked peroxidase. It was proposed that HSA has a GSH-linked peroxidase activity, so H_2O_2 generated as an intermediate in a thiol (DTT and GSH) MCO system is removed enzymatically and the inactivation of glutamine synthetase by •OH was prevented ($RSH + Fe^{3+} + O_2 \rightarrow H_2O_2 + Fe^{2+} \rightarrow Fe^{3+} + •OH + OH^-$). Therefore, it has been suggested that HSA can be a major antioxidant enzyme of extracellular fluids (17, 19).

In these studies, we demonstrate that HSA protected against DNA strand breaks in the MCO and γ-irradiation system containing DTT as the reducing equivalents. On the contrary, DNA strand breaks were

accelerated with the increase of HSA additive in the GSH or ascorbate metal-catalyzed oxidation and γ-irradiation system. The reason may be that protein peroxy radicals are very quickly formed by •OH or H_2O_2 generated in the MCO (or γ-irradiation) system and react with DNA to give strand breaks. (34). If HSA had GSH (thiol)-dependent peroxidase activity, DNA strand breaks would be prevented in the GSH MCO system because H_2O_2-removing activity of HSA caused activated oxygen species (•OH) not to be produced. It has been reported that Cys-34 of HSA plays an important role in phospholipid hydroperoxide peroxidase activity in the presence of cysteine (35). The fact that Cys-34 modified HSA also prevented DNA strand breaks only in the DTT MCO system, while aminothiol, cysteine or GSH, did not affect the prevention of DNA damage means that Cys-34 was not closely related with protectiveness of DNA damage by removing H_2O_2 in an MCO system.

It would appear from the above results that HSA might not have H_2O_2-removing peroxidase activity in the presence of GSH (thiol groups). In addition, HSA did not remove more H_2O_2 in the presence of reduced GSH or cysteine than in the absence of GSH or cysteine, and this protein did removed some amount of H_2O_2 in the absence of GSH or cysteine. This discrepancy between previous data (17) and the present data about the H_2O_2 removing properties may be a result of the use and non-use of the direct reaction of HSA with H_2O_2 as the control group. The H_2O_2 removing activity of HSA may not be enzymatic but stoichiometric, making HSA appear to be a sacrificial antioxidant.

HSA is easily damaged and degraded in the DTT MCO system and, while it lasts, HSA may prohibit the •OH or H_2O_2 generated by the MCO system, or the γ-irradiation from attacking DNA. If HSA has thiol-dependent peroxidase, the reversible formation of disulfide bonds prohibits the protein degradation. Sacrificial antioxidant activity of HSA may be dependent on the effectiveness of reducing equivalents (electron donors) to reduce S-S bonds in HSA. Since HSA contains many disulfide linkages, DTT, a possible strong reducing agent in HSA, may easily make the functional groups responsible for the sacrificial antioxidant activity, including hydrophobic groups and free sulfhydryl residues, exposed to the protein surface. The exposure of the hydrophobic groups in protein seems to be an important factor for further degradation by •OH or proteolysis. Therefore, it seems that •OH or H_2O_2 are effectively scavenged by these functional groups and protein degradation is accelerated before intact HSA forms protein peroxy radicals. In contrast, when DTT was exchanged with GSH or ascorbate in an MCO and γ-irradiation system, HSA accelerated rather than prevented DNA strand breaks in spite of far reduced formation of •OH or H_2O_2 (approximately 1/10 of DTT and ascorbate MCO system). It may be that because GSH is not a strong and effective reducing equivalent for the reduction of disulfide linkage of HSA, this protein is not significantly and sacrificially degraded by scavenging •OH or H_2O_2. Therefore, protein peroxy radicals reacting with DNA can be easily and quickly formed from the intact HSA. These results indicate that maintenance of DNA or enzyme activity in the DTT MCO system may be due to the reaction of reduced HSA with •OH (or H_2O_2) and so the attack on DNA or other important target molecules by •OH (or H_2O_2) may be prevented.

In this investigation, human ceruloplasmin, which has been suspected of thiol-linked peroxidase, was used as a control. Because human ceruloplasmin has a resistance to the reductions of disulfide linkages by DTT in this experimental condition, the electrophoretic mobility of the protein on SDS-PAGE was not changed. An important factor in the screening of thiol-linked peroxidase activity seems to be the resistance to full reductions of disulfide linkages by a thiol compound (DTT). Therefore, in the DTT MCO system, human ceruloplasmin was not sacrificially and significantly degraded.

We would like to propose that HSA forms protein peroxy radicals in an MCO and γ-irradiation system and DNA strand breaks can take place, presumably via protein peroxy radicals. DTT as a strong reducing agent of HSA can protects against molecular damage, including DNA strand breaks, in an MCO and γ-irradiation system. However, this prevention of DNA strand breaks may result from the stoichiometric reaction of reduced (unfolding) HSA with •OH or H_2O_2, not removing H_2O_2 enzymatically. Therefore, it may be that the antioxidant activity of HSA is not due to H_2O_2-removing peroxidase function by GSH (thiol) specificity, but due to its sacrificial property.

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