Reduction of phosphatidylcholine hydroperoxide by apolipoprotein A-I: purification of the hydroperoxide-reducing proteins from human blood plasma

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Abstract Plasma glutathione peroxidase (GSHPx) has been suggested to reduce submicromolar levels of free fatty acid hydroperoxides and phosphatidylcholine hydroperoxides (PC-OOH), and therefore these hydroperoxides are undetectable in human blood plasma. The capacity for the reduction should be about 2.5 μm as the level of glutathione in human plasma is about 5 μm. However, 2 h of aerobic incubation of 58 μm PC-OOH in human plasma at 37°C resulted in the formation of 36 μm phosphatidylcholine hydroxide (PC-OH). The presence of PC-OOH-reducing protein other than plasma GSHPx was suggested by the results. *a*) The same rates of PC-OOH decay and PC-OH formation were observed in both sera from rats with selenium-deficient and selenium-supplemented diet; *b*) the PC-OOH-reducing activity was observed only in the high molecular weight fraction but not in the low molecular weight fraction; and *c*) albumin did not work as a reducing substrate of plasma GSHPx. We have isolated two hydroperoxide-reducing protein fractions from human plasma by a sequential purification scheme, comprising an ammonium sulfate precipitation followed by sequential chromatography on anion exchange, hydrophobic interaction, and heparin columns. One of the proteins was identified as apolipoprotein A-I by N-terminal amino acid sequence analysis. Moreover, the hydroperoxide-reducing activity of one of the fractions was inhibited almost completely by the addition of anti-apolipoprotein A-I antibody. These findings demonstrate that apolipoprotein A-I in high density lipoprotein can reduce PC-OOH to PC-OH.—Mashima, R., Y. Yamamoto, and S. Yoshimura. **Reduction of phosphatidylcholine hydroperoxide by apolipoprotein A-I: purification of th hydroperoxide-reducing proteins from human blood plasma. *J. Lipid Res.* 1998.** 39: **1133–1140.**

Supplementary key words phosphatidylcholine hydroxide • selenium-deficient • selenium-supplemented • plasma glutathione peroxidase • high density lipoprotein • peroxidase

The mechanisms of protection against oxygen radicals have attracted extensive attention since oxygen radicals have been suggested to be a causative factor in aging and are implicated in degenerative diseases such as heart attack, diabetes, cancer, and others (1). Despite the presence of various antioxidants and antioxidant enzymes, human and rat plasmas contain nanomolar levels of cholesteryl ester hydroperoxide (CE-OOH) (2–4) and this is regarded as one of the direct pieces of evidence of oxygen radicals-mediated injury in vivo as lipid hydroperoxides are the primary oxidation products of lipids. However, we could not detect phosphatidylcholine hydroperoxide (PC-OOH) in human and rat plasmas (3, 5, 6) although in vitro oxidation of lipoprotein gives both PC-OOH and CE-OOH (4, 7–9). The instability of PC-OOH in plasma (7, 9) is likely to be the reason. We (10) and others (11, 12) have shown that plasma glutathione peroxidase (GSHPx) can reduce PC-OOH to its hydroxide (PC-OH) in the presence of glutathione. Moreover, we have shown that PC-OOH in high density lipoprotein (HDL) can be converted to CE-OOH by the action of lecithin:cholesterol acyltransferase (LCAT) (13).

Human and rat plasma GSHPx have been characterized as a tetrameric protein of identical subunits of 22.5 kDa molecular mass containing selenium (14–17). Therefore, the selenium content in diet can manipulate not only the enzymatic activity (18) but also protein levels of plasma GSHPx in blood plasma. GSHPx requires two molecules of glutathione for the reduction of one molecule of hydroperoxide (19). This implies that in vitro human plasma can reduce only about 2.5 μm hydroperoxides because the level of glutathione usually available in human plasma is 5 μm (20). However, we have noticed that human plasma has a capacity to reduce 20 μm PC-OOH (13) or more. To understand why human plasma can reduce such large

Abbreviations: apoA-I, apolipoprotein A-I; HDL, high density lipoprotein; GSHPx, glutathione peroxidase; PC-OOH, phosphatidylcholine hydroperoxide; PC-OH, phosphatidylcholine hydroxide; PLPC, 1-palmitoy1-2-linoleoylphosphatidylcholine; PLPC-OOH, 1-palmitoyl-2-linoleoylphosphatidylcholine hydroperoxide; PLPC-OH, 1-palmitoyl-2-linoleoylphosphatidylcholine hydroxide.

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amounts of PC-OOH, we confirmed that human plasma can reduce to a large degree the concentration of 36 μm PC-OOH. We also assessed the possibility that albumin may work as a reducing substrate for plasma GSHPx. We compared the rates of the reduction of PC-OOH in sera from rats with selenium-deficient and selenium-supplemented diets. Finally, we isolated the proteins that can reduce PC-OOH. Here we report the inability of albumin to act as a reducing substrate for plasma GSHPx, a lack of difference in the reduction of 50 μm PC-OOH between sera from selenium-deficient and selenium-supplemented rats, and the isolation of two protein fractions that can reduce PC-OOH. We have identified one of the proteins by amino acid sequence as apolipoprotein A-I (apoA-I).

MATERIALS AND METHODS

Reagents

Soybean phosphatidylcholine (PC), 1-palmitoyl-2-linoleoyl phosphatidylcholine (PLPC), rat serum albumin, *tert*-butyl hydroperoxide (BOOH), cumene hydroperoxide, and phenylmethylsulfonyl fluoride (PMSF) were obtained from Sigma (Tokyo). 2,2′-Azobis(2,4-dimethylvarelonitrile) (AMVN) and choline chloride were obtained from Wako Pure Chemical (Osaka). HiLoad Q Sepharose 26/10 HP, Phenyl Sepharose 6FF (low sub), HiTrap Heparin, Protein A Sepharose CL-4B, and PD-10 columns were from Pharmacia (Tokyo). Ultrafiltration membrane CENTRIFLO CF-25 was purchased from Amicon (Tokyo). Solvents and other reagents were of the highest grade commercially available. Mouse monoclonal antibody raised against human apoA-I was a kind gift from Japan Immunoresearch Laboratory (Takasaki).

PC-OOH was prepared by the aerobic oxidation of soybean PC (50 μmol) with AMVN (5 μmol) in 100 ml hexane at 37°C for 2 h. After exchanging solvent from hexane to methanol, PC-OOH was purified on a semipreparative octadecylsilyl column (25 cm × 10 mm i.d., Japan Spectroscopic, Tokyo) using methanol containing 0.02% triethylamine as the mobile phase at a flow rate of 4 ml/min. PLPC-OOH was prepared as described previously (13).

Plasma GSHPx was separated from rat serum as described previously (21). Fractions that decomposed cumene hydroperoxide were concentrated on a CF25 membrane and stored at −80°C. 2-Mercaptoethanol was present during all the purification steps to preserve the enzyme activity but was removed by gel filtration prior to activity determination because this thiol works as a reducing substrate of the enzyme (10). Enzyme activity was measured by the oxidation of NADPH in the presence of 0.23 mm cumene hydroperoxide, 0.25 mm GSH, 0.12 mm NADPH, and 1 unit/ml glutathione reductase at 37°C under aerobic conditions (21). One unit of the enzyme activity is defined as 1 μmol NADPH oxidized/min at 37°C.

Animals

Male Wistar rats were fed a selenium-deficient diet (less than 0.05 ppm) obtained from Oriental Yeast (Tokyo) or a conventional diet containing 1 ppm of sodium selenite for 12 weeks after weaning. The activity of plasma GSHPx in sera from these animals was measured as described above.

Incubation of PLPC-OOH in human plasma or rat serum

5 μl of 1.2 mm PLPC-OOH in methanol was mixed with 95 μl of human heparinized plasma obtained from a 26-year-old healthy male donor and incubated at 37°C under aerobic conditions. Incubation of PLPC-OOH in sera obtained from rats fed selenium-deficient or selenium-supplemented diets was carried out similarly. Aliquots (5 μl) of human plasma or rat serum were withdrawn during the course of incubation and stored at −80°C until analysis. Analyses of PLPC-OOH and PLPC-OH were carried out by a reported HPLC method (22) with slight modification. Briefly, aliquots (5 μl) of human plasma or rat serum were mixed with 4 volumes of methanol. After centrifugation at 12,000 *g* for 3 min, the supernatant (5 μl) was injected onto a CAPCELL PAK ODS column (5 μm, 4.6 × 250 mm; Shiseido, Tokyo) with UV detection at 234 nm. The mobile phase used was acetonitrile–methanol–water 100:99:1 (v/v/v) containing 10 mm choline chloride and the flow rate was 2 ml/min.

Albumin as a reducing substrate of plasma GSHPx

Sulfhydryl groups on albumin (albumin-SH) were measured by a reported method (23). Thirty μm BOOH was incubated in 50 mm Tris buffer (pH 7.6) containing 100 μm EDTA in the presence of 1.0 unit/ml rat plasma GSHPx and in the absence or presence of either 100 μm rat serum albumin-SH or glutathione at 37°C under aerobic conditions. The concentration of BOOH was determined by a hydroperoxide-specific chemiluminescence method (24) after separation on an octadecylsilyl column (CAPCELL PAK ODS; 5 μm, 4.6 × 250 mm; Shiseido, Tokyo) using acetonitrile–water 1/3 (v/v) as an eluant at a flow rate of 1 ml/min. A 70% aqueous solution of BOOH was used as a standard.

PC-OOH-reducing activity in high and low molecular weight materials in human plasma

High and low molecular weight materials were separated from human heparinized plasma by a PD-10 column using 10 mm phosphate-buffered saline containing 1 mm EDTA (PBS, pH 7.4) as the eluant. Five μl of a solution of 1.0 mm PLPC-OOH in methanol was mixed with 95 μl of either high molecular or low molecular weight fraction after diluting them with an equal volume of PBS. PLPC-OOH was also mixed with the combined solution of equal volume of high molecular weight fraction and low molecular weight fraction. The decay of PLPC-OOH and the formation of PLPC-OH were monitored as described above.

Purification of PC-OOH-reducing proteins from human blood plasma

All the purification steps were carried out at 4°C or on ice. The PC-OOH-reducing activity of protein fractions was assessed as follows. Five μl of a 100 μm solution of soybean PC-OOH in methanol was added to 100 μl of protein fractions and incubated at 37°C for 1 h under aerobic conditions. Then, 400 μl of methanol was added to the reaction solution to precipitate salts and proteins. After centrifugation at 12,000 *g* for 3 min, aliquots (100 μl) of the supernatant were injected onto HPLC equipped with a hydroperoxide-specific, chemiluminescence detection system (24). PC-OOH was separated on a silica gel column (LC-Si, 5 μm, 25 cm × 4.6 mm i.d., Supelco Japan, Tokyo) using methanol/40 mm monobasic sodium phosphate (9:1, v/v) as an eluant at a flow rate of 1 ml/min.

Ammonium sulfate precipitation. Heparinized human plasma (20 ml) obtained from a 26-year-old healthy male donor was mixed with the same volume of ice-cold buffer A (20 mm sodium phosphate (pH 8.0) containing 1 mm EDTA, 0.2 mm PMSF, and 0.1 m NaCl). Solid ammonium sulfate was then added slowly to the above solution to give a final concentration of 50% saturation. This protein solution was stirred on ice for 30 min, then kept on ice for a further 30 min. After centrifugation at 15,000 *g* for 10 min at 4°C, the supernatant was decanted, the precipitate was collected and resuspended in buffer A (20 ml), and the ammonium sulfate precipitation and the centrifugation were repeated.

Q Sepharose column chromatography. The precipitate was desalted by a PD-10 column gel filtration using buffer A and subsequently

loaded onto a HiLoad Q Sepharose 26/10 HP column (50 ml) equilibrated with buffer A. After washing thoroughly with buffer A (100 ml), proteins were fractionated by elution with a linear gradient of 0.1–0.4 M NaCl in buffer B (20 mM sodium phosphate (pH 8.0) containing 1 mM EDTA and 0.2 mM PMSF) using an FPLC system (Pharmacia) over a period of 50 min at a flow rate of 5 ml/min. Then, 1.1 M NaCl in buffer B was used as an eluant. Ten-ml fractions of the eluant were collected. The PC-OOH-reducing activity eluted around 0.2–0.3 M NaCl as a broad peak.

Phenyl Sepharose column chromatography. Fractions with PC-OOH-reducing activity were combined and mixed with solid ammonium sulfated to give a final concentration of 40% saturation. This turbid protein suspension was then applied onto a Phenyl Sepharose 6FF (low sub) column (1.6 × 5 cm) equilibrated with buffer B containing 40% ammonium sulfate. Proteins were eluted by a stepwise gradient with 40, 20, and 0% ammonium sulfate in buffer B at a flow rate of 1 ml/min. The PC-OOH-reducing proteins, which remained bound under these conditions, were eluted using 50% propylene glycol in buffer B, and exchanged into buffer A using a PD-10 column.

HiTrap Heparin chromatography. The resulting fraction with PC-OOH-reducing activity was purified further using a HiTrap Heparin column (1 ml). After loading and washing extensively with buffer A, proteins were eluted with a linear 20 min gradient of 0.1–2.1 M NaCl in buffer B at a flow rate of 1 ml/min using an FPLC system. One-ml fractions of the eluant were collected. One fraction with PC-OOH-reducing activity (fraction A) eluted without retention and was concentrated (640 μg protein/ml) using a CF-25 membrane. The other PC-OOH-reducing fraction (fraction B) was eluted with 0.5–1.0 M NaCl in buffer B.

Amino acid sequence determination

The purity of proteins in fraction A was assessed by a 10% SDS-polyacrylamide gel electrophoresis (PAGE) and the protein bands were transblotted onto polyvinylidene difluoride membrane (Bio-Rad). The blotted proteins were detected by staining with Coomassie Brilliant Blue R-250. The major band was excised and subjected to automated gas–liquid phase sequencer (model PPSQ-10, Shimadzu, Kyoto).

Immunoprecipitation

Five hundred μl of mouse anti-human apoA-I antibody (2 mg/ml), 200 μl (wet gel) of Protein A Sepharose CL-4B (Pharmacia), and 500 μl of 1.5 M glycine buffer (pH 8.9) containing 3 M NaCl were incubated for 1 h at room temperature to bind the antibody to the gel. After centrifugation at 12,000 *g* for 3 min, the gel was washed with PBS; and centrifugation and washing were repeated 3 times. Then, the antibody-fixed Protein A Sepharose gel (about 60 μl) was mixed with 60 μl of fraction A (26 μg protein/ml) in PBS, left to stand for 10 min at room temperature, and centrifuged at 12,000 *g* for 3 min. Fifty μl of the supernatant or PBS was mixed with 2.5 μl of a 100 μM PLPC-OOH in methanol and incubated for 1 h at 37°C under aerobic conditions. Then, 200 μl of methanol was added, the mixture was centrifuged at 12,000 *g* for 3 min, and 100 μl of the supernatant was injected onto an ODS column to measure the concentrations of PLPC-OOH and PLPC-OH. HPLC conditions were as described above.

RESULTS AND DISCUSSION

Lipid hydroperoxides are the primary oxidation product of lipids and are detoxified by reduction because they can initiate further oxidation of lipids, proteins, and other biological components in the presence of metal ions. In the course of our study on the fate of PC-OOH in human plasma, we demonstrated that plasma GSHPx can reduce PC-OOH in the presence of glutathione. The capacity for this reduction should be about 2.5 μM as plasma of healthy humans contains 5 μM glutathione (20) and two mole GSH are needed to reduce one mole PC-OOH. However, we found that human plasma can reduce more than the 20 μM level of PC-OOH (13). To confirm

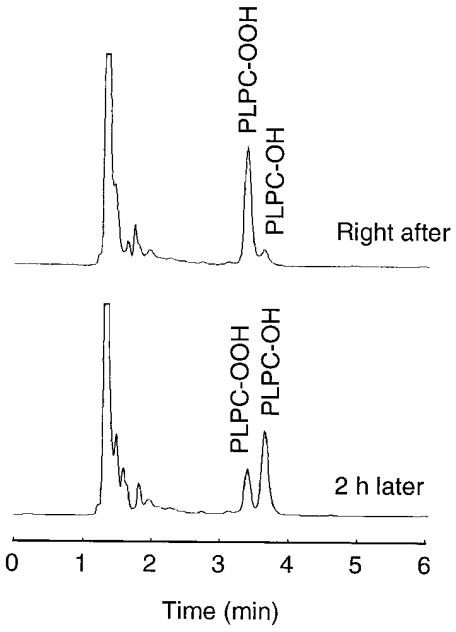

Fig. 1. Reduction of PLPC-OOH to PLPC-OH by human plasma isolated from a 26-year-old healthy male donor at 37°C under aerobic conditions. Two HPLC chromatograms obtained from 0 (right after the addition of 58 μM PLPC-OOH to the plasma) and 2 h incubation were compared. The HPLC conditions were described in Materials and Methods.

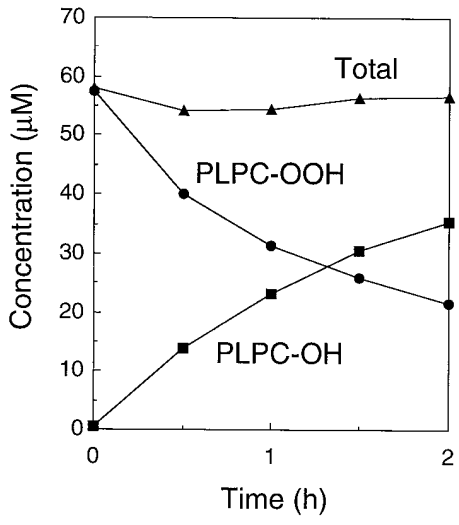

Fig. 2. Time course changes in the levels of PLPC-OOH (●), PLPC-OH (■), and PLPC-OOH + PLPC-OH (▲) during the incubation of 58 μM PLPC-OOH in human plasma at 37°C under aerobic conditions. Plasma was isolated from a 26-year-old healthy male donor. Values are the mean of two independent and reproducible analyses.

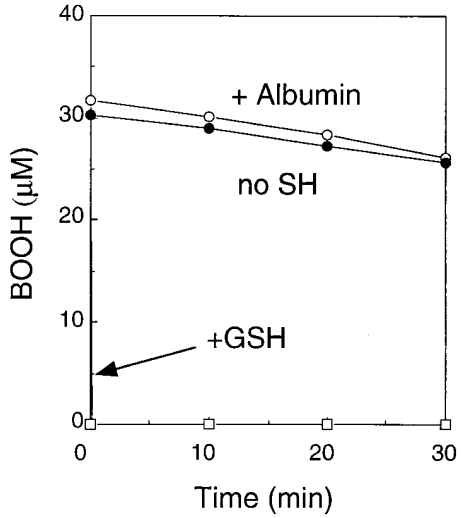

Fig. 3. The decay of *tert*-butyl hydroperoxide (BOOH) by rat plasma glutathione peroxidase (1.0 unit/ml) in the absence or the presence of 100 μm glutathione (GSH) or rat serum albumin at 37°C under aerobic conditions. Values are the mean of two independent and reproducible analyses.

this observation, we incubated 58 μM PLPC-OOH in human plasma for 2 h under aerobic conditions at 37°C. **Figure 1** shows the resulting decrease in PLPC-OOH and the formation of PLPC-OH, as measured by an HPLC method.

Figure 2 shows the time course of this reaction and that the total concentration of PLPC-OOH and PLPC-OH was almost constant, indicating that the two-electron reduction is the predominant reaction because one-electron reduction of PC-OOH gives alkoxyl radical (PC-O$^{\bullet}$) and this gives aldehydes in high yields by β-scission reaction (1). After 2 h incubation, the concentration of PLPC-OH was 36 μm. This observation may be explained if albumin, in addition to GSH, works as a reducing substrate of plasma GSHPx as albumin is present at about 600 μm in human plasma and has one free sulfhydryl group at position 34 from the N-terminal. However, we failed to demonstrate that rat serum albumin accelerated the reduction of BOOH in the presence of rat plasma GSHPx (**Fig. 3**). Human albumin freshly isolated from plasma using a Blue Sepharose column (25) also failed to function as a reducing substrate (data not shown).

To assess the involvement of plasma GSHPx in the reduction of large amounts of PC-OOH, we further compared the PC-OOH-reducing activity of sera from rats fed selenium-deficient or selenium-supplemented diets. The activity of the selenium-dependent plasma GSHPx in the sera was 0.07 and 9.92 units/ml, respectively, as measured by the decrease of NADPH in the presence of glutathione reductase, cumene hydroperoxide, and glutathione (21), indicating that rats fed a selenium-deficient diet were indeed deficient in selenium. Despite such a big difference in plasma GSHPx activity, there was a reduction in concentration of 50 μM PLPC-OOH by sera from both groups that proceeded at the same rate as shown in **Fig. 4**. After 2 h of incubation the concentration of PLPC-OH was about 40 μm (Fig. 4). The above data strongly suggest the presence in rat serum of the hydroperoxide-reducing activity other than plasma GSHPx.

We next separated human plasma into high and low molecular weight fractions by gel filtration using a PD-10 column and measured the PC-OOH-reducing activity in both fractions. We observed the activity only in the high molecular weight fraction (**Fig. 5**). Moreover, the addition of the low molecular weight fraction to the high molecular weight fraction instead of PBS did not accelerate the reduction of PC-OOH (Fig. 5), indicating that the PC-OOH-reducing activity does not require low molecular weight cofactors.

Purification of PC-OOH-reducing proteins

We therefore tried to isolate the PC-OOH-reducing proteins from human plasma by a combination of ammonium sulfate precipitation, and chromatography in HiLoad Q Sepharose, Phenyl Sepharose and HiTrap Heparin columns (**Table 1**). Ammonium sulfate (50%) precipitation increased the PC-OOH-reducing activity (pmol/h per mg) 3-fold. **Figure 6** shows the elution profiles of proteins, the

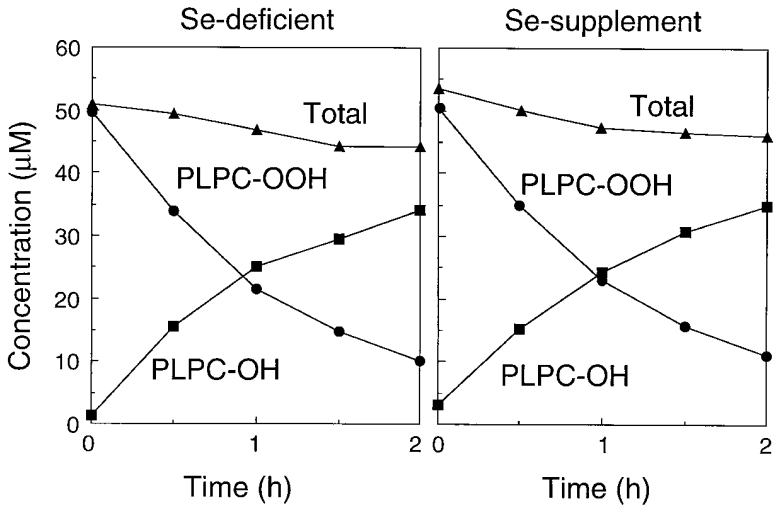

Fig. 4. Time course changes in the levels of PLPC-OOH (●), PLPC-OH (■), and PLPC-OOH + PLPC-OH (▲) during the aerobic incubation at 37°C of 50 μm PLPC-OOH in sera from rats fed a selenium-deficient or selenium-supplemented diet for 12 weeks; their plasma GSHPx activities were 0.07 and 9.92 units/ml, respectively. Values are the mean of two independent and reproducible analyses.

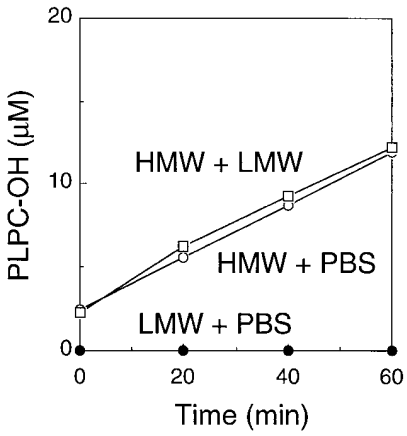

Fig. 5. Formation of PLPC-OH during the incubation of 50 μm PLPC-OOH in equal volume of the high and/or low molecular weight fractions from human plasma and 10 mm phosphate-buffered saline containing 1 mm EDTA (pH 7.4) at 37°C under aerobic conditions. Values are the mean of two independent and reproducible analyses.

PC-OOH-reducing activity, and plasma GSHPx by anion exchange chromatography using a HiLoad Q Sepharose column. The PC-OOH-reducing activity eluted as a broad peak while plasma GSHPx eluted as a single, sharp peak with maximal activities in fractions 28 and 31, respectively, indicating that the PC-OOH-reducing activity was different from plasma GSHPx.

Fractions 24 to 28 eluting from the Q Sepharose column were combined and applied to a Phenyl Sepharose column. Ammonium sulfate was added to the fractions to a final concentration of 40% as the PC-OOH-reducing activity did not bind to the hydrophobic Phenyl Sepharose gel in the absence of 40% of ammonium sulfate (data not shown). The PC-OOH-reducing activity was eluted as a single peak by a 50% propylene glycol in buffer B after a stepwise decrease of ammonium sulfate from 40 to 0 % in buffer B. This purification step decreased the amount of protein recovered from 73 to 3.2 mg (4.4%) while the specific activity increased from 828 to 5588 pmol/h per mg (Table 1).

The PC-OOH-reducing activity was separated into two fractions (fractions A and B) by a HiTrap Heparin column; the protein(s) in fraction A were not retained by the column whereas the protein(s) in fraction B eluted with 0.5–1.0 m NaCl. Fractions A and B both yielded 6% of the initial PC-OOH-reducing activity with an increase in specific activity of 55- and 143-fold, respectively (Table 1). However, these numbers should be judged with caution as the specific PC-OOH-reducing activity of fraction A was dependent on the protein concentration; i.e., the activity increased with increasing protein concentration of fraction A but the relationships were not linear (data not shown).

Fractions A and B were applied to SDS-PAGE to assess the purity of the proteins. It was found that fraction A contained a 28 kDa protein as the major protein with several minor proteins as shown in **Fig. 7**. Fraction B yielded a single band (data not shown).

To demonstrate the PC-OOH-reducing activity of fraction A, fraction A (64 μg protein/ml) was incubated with PLPC-OOH (7 μm) in PBS at 37°C under aerobic conditions. **Figure 8** shows the stoichiometric reduction of PLPC-OOH to PLPC-OH. As 0.8 μm PLPC-OOH was reduced in 1 h, the specific activity can be calculated as $0.8 \times 1000000/64 = 12500$ pmol/h per mg. This number is much higher than 1962 pmol/h per mg shown in Table 1 where the protein concentration of fraction A was 640 μg/ml. However, this is not surprising because of the reason described above.

The N-terminal amino acid sequence of the 28 kDa protein in fraction A was found to be XXPPQSPXDRVKD LATVY (X denotes an unidentified amino acid), matching that of apoA-I (26). The amino acid sequence of minor proteins was not determined because of their insufficient amounts. Characterization of the protein in fraction B is ongoing.

Figure 9A shows that PLPC-OOH (5 μm) was stable in PBS for 1 h. Although the addition of fraction A resulted in the formation of 0.38 μm PLPC-OH (Fig. 9B), the treatment of fraction A with anti-human apoA-I monoclonal antibody prior to the addition reduced the formation of PLPC-OH almost completely (Fig. 9C), indicating that the PC-OOH-reducing activity of fraction A is due to apoA-I.

Mechanism of the reduction

Apo A-I is the major protein of high density lipoprotein (HDL) and accelerates the transport of peripheral free cholesterol (reverse cholesterol transport) by activating

TABLE 1. Purification of PC-OOH-reducing activity from human blood plasma

Purification Step	Activity	Protein	Specific Activity	Purification	Yield
	pmol/h	*mg*	*pmol/h/mg*	*-fold*	*%*
Plasma	51338	1450	36	1	100
Ammonium sulfate cut	68425	733	93	3	133
HiLoad Q Sepharose	60113	73	828	23	117
Phenyl Sepharose	18039	3.2	5588	158	35
HiTrap heparin					
Passed through (fraction A)	3048	1.6	1962	55	6
Eluted with 0.5–1.0 m NaCL (fraction B)	2840	0.6	5072	1436	6

The PC-OOH-reducing activity at each step was determined using soy PC-OOH as a substrate as described in Materials and Methods.

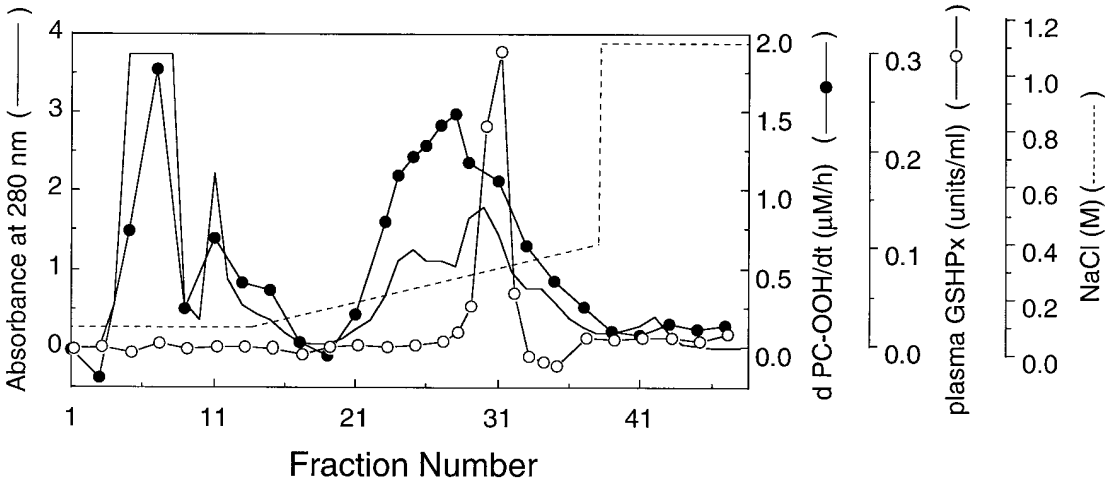

Fig. 6. Elution profiles of proteins (——; absorbance at 280 nm), plasma GSHPx (○), and PC-OOH-reducing proteins (●) from HiLoad Q Sepharose 26/10 HP column eluted with 20 mm sodium phosphate (pH 8.0) containing 1 mm EDTA and 0.2 mm PMSF at a flow rate of 5 ml/min. Concentration of NaCl was indicated (-----).

LCAT which catalyzes the esterification of free cholesterol to cholesteryl esters (27). ApoA-I is a 28 kDa protein of a single peptide with 243 amino acids and contains three methionine residues at the 86-, 112-, and 148-positions from the N-terminal (26). The oxidation of apoA-I by hydrogen peroxide converts methionine at the 112- and 148-positions to methionine sulfoxide but not methionine at the 86-position (28). Methionine residues at the 112- and 148-positions locate in the amphipathic region of α-helix of apoA-I molecule (26) where hydrogen peroxide is accessible. Accessibility of hydroperoxide should be very important for the reduction of hydroperoxide by methionine. In fact, albumin did not reduce BOOH (Fig. 3) and PC-OOH (data not shown) although it contains six methionine residues (29).

Recently, Garner et al. (30, 31) observed the reduction of CE-OOH to CE-OH by apoA-I with a concomitant formation of methionine sulfoxide. It is reasonable to assume that the reduction of PC-OOH to PC-OH by apo A-I proceeds by the same reaction mechanism. It has been observed that HDL can reduce CE-OOH (32) and PC-OOH (13) to their corresponding alcohols.

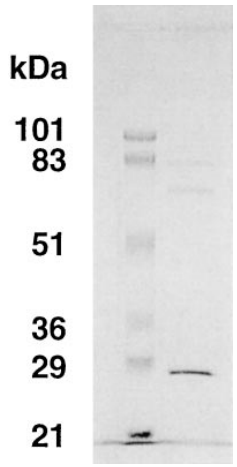

Fig. 7. 10% SDS-polyacrylamide gel electrophoresis (PAGE) of fraction A and molecular markers.

Role of apo A-I-dependent reduction of hydroperoxides

Under physiological conditions the levels of lipid hydroperoxides in human plasma are likely to be submicromolar as the detected levels of CE-OOH are 3 nm (2). Under these conditions, plasma GSHPx is likely to play the most important role in reducing lipid hydroperoxides except CE-OOH (10) as plasma contains micromolar GSH and plasma GSHPx reduces hydroperoxides very rapidly (20). However, the reduction of lipid hydroperoxides by apoA-I may play a significant role under pathophysiological condi-

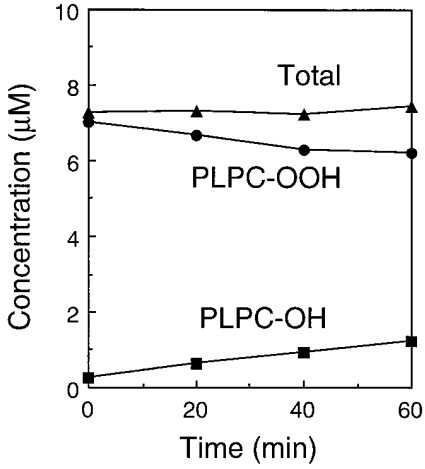

Fig. 8. Time course changes in the levels of PLPC-OOH (●), PLPC-OH (■), and PLPC-OOH + PLPC-OH (▲) during the aerobic incubation of 7 μm PLPC-OOH in 10 mm phosphate-buffered saline (pH 7.4) containing fraction A (64 μg protein/ml) and 1 mm EDTA at 37°C. Values are the mean of two independent and reproducible analyses.

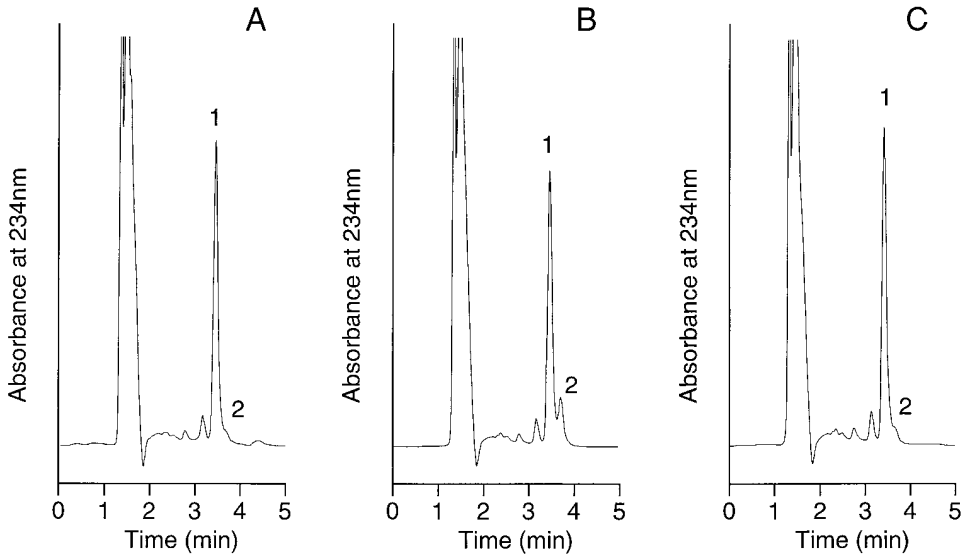

Fig. 9. HPLC chromatograms after 1 h aerobic incubation of 5 μm PLPC-OOH in (A) 10 mm phosphate-buffered saline containing 1 mm EDTA (PBS, pH 7.4), (B) PBS containing fraction A (13 μg protein/ml), and (C) PBS containing fraction A pretreated with anti-human apoA-I monoclonal antibody. Same results were obtained in triplicate experiments. Details were described in Materials and Methods. **1**, PLPC-OOH; **2**, PLPC-OH.

tions such as inflammation and plasma GSHPx deficiency (33). Concentration of apoA-I in human plasma is about 1.27 g/l (45 μm) (34). This implies that human plasma has a capacity of reducing at least 90 μm hydroperoxides if two methionine residues in apoA-I are active (28). If we could detect methionine sulfoxide-containing apoA-I in biological samples, it would be good evidence of apoA-I-dependent reduction of hydroperoxides; further investigation is being considered. The presence of methionine sulfoxide reductase in humans (35, 36) may also suggest a role of apoA-I-dependent reduction of hydroperoxides.

In summary, we have demonstrated that human plasma and rat serum contain PC-OOH-reducing activities. We have succeeded in isolating two purified fractions of PC-OOH-reducing activities and apoA-I has been identified in one of them as a major PC-OOH-reducing protein.

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