Role of Hemopexin in Protection of Low-Density Lipoprotein against Hemoglobin-Induced Oxidation

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ABSTRACT: Globin-free hemin and certain hemoproteins, predominantly hemoglobin, are active triggers of low-density lipoprotein (LDL) peroxidation, a contributing cause of atherosclerosis. The role of the plasma heme-binding protein, hemopexin, in protecting apolipoprotein B and LDL lipids from oxidation triggered by either hemin or hemoglobin in the presence of low amounts of H_2O_2, was investigated at physiological pH and temperature. Significantly, hemopexin prevented not only hemin-mediated modification of LDL but also LDL peroxidation induced by hemoglobin, both by met and oxy forms. Analysis of the data revealed that the rate of heme transfer from methemoglobin to hemopexin was highly dependent upon temperature: only minimal heme transfer occurred at 20 °C, whereas at the physiological temperature of 37 °C, heme transfer was rapid, within the lag phase of LDL oxidation, regardless of the presence or absence of H_2O_2. Heme did transfer to hemopexin from oxyhemoglobin as well, but only in the presence of H_2O_2. The proposed mechanism of the inhibition of oxyhemoglobin oxidative reactivity by hemopexin involves peroxidation of oxyhemoglobin (Fe^{II}) to ferrylhemoglobin (Fe^{IV}), followed by a comproportionation reaction ($Fe^{IV} + Fe^{II} \rightarrow 2Fe^{III}$), yielding methemoglobin (Fe^{III}) from which heme is readily transferred to hemopexin. Taken together, the data demonstrate that hemopexin can act as an extracellular antioxidant against hemoglobin-mediated damage in inflammatory states, which is especially important when haptoglobin is depleted or absent.

Several hemoproteins, including hemoglobin, as well as free hemin are capable of inducing oxidative damage in various biological systems (Everse et al., 1994; Hebbel & Eaton, 1986; Wieland et al., 1993). A recently reported novel target for heme-induced oxidations is the plasma low-density lipoprotein (LDL)[1] (Balla et al., 1991; Wieland et al., 1993; Paganga et al., 1992; Hogg et al., 1994; Savenkova et al., 1994). Oxidation of LDL is considered to be a causative factor of atherosclerosis (Steinberg et al., 1989; Rice-Evans & Brukdorfer, 1992; Esterbauer et al., 1992), and thus intensive research has been carried out to identify plasma components which either elicit LDL oxidation or can act as LDL antioxidants. The following heme compounds have been reported to act as strong oxidants of LDL: the heme enzymes myeloperoxidase and horseradish peroxidase (HRP), the oxygen transporters myoglobin and hemoglobin, and free hemin (Balla et al., 1991; Miller & Shaklai, 1994; Wieland et al., 1993; Paganga et al., 1992; Miller et al., 1996; Hogg et al., 1994; Savenkova et al., 1994). Hemoglobin was recently reported to be an extremely active oxidizer of LDL, even more so than the active peroxidase HRP (Paganga et al., 1992; Miller et al., 1996). Since red cells forming about 50% of the blood volume contain as much as 20 mM hemoglobin, even a minimal vascular hemolysis is sufficient to yield plasma hemoglobin at the micromolar concentration range that can affect LDL peroxidation (Miller et al., 1996).

The rapid removal of hemoglobin from the circulation by haptoglobin obviates this potential hazard. However, haptoglobin is readily depleted even by small amounts of hemoglobin, leading to free hemoglobin in the plasma (Muller-Eberhard et al., 1968). According to general understanding, hemin is then released from circulating hemoglobin and becomes available for hemopexin or albumin binding (Smith, 1990). Since albumin binds hemin much weaker than hemopexin (Hrkal et al., 1974; Beaven et al., 1974), it serves as a temporary hemin reservoir, while hemopexin functions as a transporter of globin-free hemin to the liver by receptor-mediated endocytosis (Smith & Morgan, 1985; Smith et al., 1991), thereby conserving heme iron (Muller-Eberhard, 1988). Although hemopexin recycles intact after endocytosis (Smith & Morgan, 1979), elevation of plasma-free hemin upon acute severe or chronic hemolysis or by intravenous hemin administration as a drug is accompanied by reduction of circulating hemopexin levels (Muller-Eberhard, 1988). On the other hand, hemopexin has been shown to be one of the acute phase plasma proteins whose mRNA levels are increased by cytokines, and the synthesis of hemopexin increases in response to inflammation (Muller-Eberhard, 1988; Greieninger et al., 1986). Thus, hemopexin levels in human plasma can vary in the range of 8−21 μM (Smith, 1990). The binding affinity of heme to hemopexin ($K_a \sim 10^{14}$ M^{-1}), although not reaching that to globin in oxyhemoglobin, is much higher than to any other vascular components and is comparable to that in methemoglobin (Hrkal et al., 1974; Beaven et al., 1974). Unlike the globins,

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[1] Abbreviations: LDL, low-density lipoprotein; apo B, apolipoprotein B-100; PBS, phosphate-buffered saline; SDS−PAGE, sodium dodecyl sulfate−polyacrylamide gel electrophoresis; TBARS, thiobarbituric acid reactive substances; PMSF, phenylmethanesulfonyl fluoride.

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which despite tight binding of heme retain peroxidative activity (Everse et al., 1994), hemopexin-bound heme is completely inactive as an oxidant (Vincent et al., 1988; Gutteridge & Smith, 1988). Indeed, globin-free hemin-induced peroxidation of either linolenic acid micelles, phospholipid liposomes, or LDL was abrogated by hemopexin (Gutteridge & Smith, 1988; Balla et al., 1991). Thus, by binding hemin hemopexin serves as a major protector of plasma components from oxidation triggered by free hemin dissociated from hemoglobin or myoglobin appearing in plasma following hemolysis or trauma.

The appearance of extracorpuscular hemoglobin in the circulation is a much more frequent event than that of globin-free hemin. Nevertheless, on the basis of the available information, one would expect that the hemopexin antioxidative activity will not affect hemoglobin-induced oxidation. In the present study, we compared the effects of hemopexin on LDL peroxidations promoted by globin-free hemin and hemoglobin. It was found that, under physiological conditions, hemopexin inhibited not only the reactivity of globin-free hemin but also hemoglobin-induced LDL peroxidation. These results demonstrate that hemopexin acts as a crucial antioxidant by providing a defense against hemoglobin-triggered oxidative damage.

EXPERIMENTAL PROCEDURES

Chemicals. Bovine hemin, phenylmethanesulfonyl fluoride (PMSF), and Chelex-100 were purchased from Sigma Chemical Co., St. Louis, MO. Hydrogen peroxide (H_2O_2) was from Merck, Darmstadt, Germany. DEAE-Sepharose CL-6B was from Pharmacia, Uppsala, Sweden. Chemicals for sodium dodecyl sulfate−polyacrylamide gel electrophoresis (SDS−PAGE) were purchased from Bio-Rad Laboratories, Richmond, CA.

Isolation of LDL, Hemoglobin, and *Hemopexin*. LDL was isolated from blood drawn into Na_4EDTA-containing tubes (final concentration 1 mg/mL). Plasma was isolated by centrifugation (3000*g* for 15 min) and supplemented with 15 μg/mL PMSF to prevent proteolysis. LDL ($d = 1.019-1.063$ g/mL) was isolated from the plasma by two sequential ultracentrifugations using a Beckman 70.1 Ti rotor at 40 000 rpm for 18 h (Schumaker & Puppione, 1986). To clear the LDL from KBr and EDTA added during preparation, the samples were extensively dialyzed (three changes within 24 h) against nitrogen-flushed, Chelex 100-treated phosphate (10 mM) buffered saline, pH 7.4 (PBS), at 4−8 °C. LDL preparations were stored under N_2 for up to 3 days at 2−4 °C.

Erythrocytes were washed three times with PBS and lysed by ice-cold water. The hemolysate was separated from erythrocyte membranes by centrifugation at 30000*g* for 30 min, and hemoglobin was purified from it by ion-exchange chromatography using DEAE-Sepharose CL-6B (Hebbel et al., (1988). This hemoglobin was verified spectrophotometrically to be oxyhemoglobin and was used to prepare methemoglobin by adding 1.1 mM $K_3Fe(CN)_6$ to 1.0 mM oxyhemoglobin, incubating for 30 min at room temperature, and dialyzing (Antonini & Brunori, 1971).

Hemopexin was isolated from rabbit or human plasma as reported (Morgan & Smith, 1984; Vretblad & Hjorth, 1977).

Measurement of Heme Transfer. Transfer of heme from hemoglobin to hemopexin was measured both spectrophotometrically and fluorometrically. Using extinction coefficients of $\epsilon_{404} = 1.78 \times 10^5$ M^{-1} cm^{-1} and $\epsilon_{416} = 1.04 \times 10^5$ M^{-1} cm^{-1} for methemoglobin and $\epsilon_{404} = 6.5 \times 10^4$ M^{-1} cm^{-1} and $\epsilon_{416} = 8.4 \times 10^4$ M^{-1} cm^{-1} for heme−hemopexin, a formula for the concentration of residual heme in a methemoglobin/hemopexin mixture was derived:

$$\text{methemoglobin } (\mu\text{M}) = 10.25A_{404} - 7.89A_{416}$$

This calculation is accurate as long as no light scattering from aggregated globin appears, but it is applicable only to the methemoglobin/hemopexin mixture since the absorbance maxima in the Soret region of oxyhemoglobin and heme hemopexin are very close, and it is difficult to distinguish between the visible spectra of heme−hemopexin and ferryl-hemoglobin (Paganga et al., 1992; Antonini & Brunori, 1971; Morgan, 1976). Therefore, heme transfer from the hemoglobin species to hemopexin was also measured fluorometrically, taking advantage of the fact that hemopexin has 7-fold higher intensity of fluorescence emission than equimolar globin at excitation/emission wavelengths of 285/345 nm, respectively. This finding is explained by the high content of tryptophan residues in hemopexin (Morgan, 1976; Muller-Eberhard, 1988). Thus, binding of heme causes drastic quenching of hemopexin fluorescence, while dissociation of heme from globin yields a relatively nonsignificant recovery of globin fluorescence. Titration of hemopexin by hemin and measurements of fluorescence intensity quenching were used to calibrate emission intensity with heme−hemopexin concentration.

Analytical Procedures. Absorbance was measured using a HP 8452A diode array spectrophotometer (Hewlett-Packard Co., Waldbronn, Germany) or by an Uvikon 930 spectrophotometer (Kontron Instruments, Zurich, Switzerland). Fluorescence measurements were performed on a SLM-Aminco 8000 spectrofluorometer (SLM Instruments, Inc., Urbana, IL). Concentration of LDL was determined by the method of Lowry et al. (1951) with 1% sodium dodecyl sulfate added to the assay buffer to facilitate dissolution of the lipoprotein. Concentrations of hemin and hemoglobin were expressed as heme equivalents, and concentrations of apohemopexin were determined using an extinction coefficient of 1.1×10^5 M^{-1} cm^{-1} at 280 nm (Morgan, 1976). The protein patterns were followed by SDS−PAGE with β-mercaptoethanol (Laemmli, 1970) in bilayer gel slabs of 6% (two-thirds of the length) and 12% (one-third) acrylamide. Gels were stained with Coomassie brilliant blue R-250. Formation of conjugated dienes was followed kinetically by the absorbance at 234 nm (Esterbauer et al., 1992). Thiobarbituric acid reactive substances (TBARS) were determined as described (Balla et al., 1991). All experiments were performed at least in triplicate, and the data are presented as the means of the individual values.

RESULTS AND DISCUSSION

Neutrophils are activated *in vivo* as part of host defense responses to trauma and hemolysis, which produce intravascular hemoglobin and heme. These cells generate reactive oxygen intermediates, including H_2O_2 in the respiratory burst. We therefore compared the effect of hemopexin on hemin- and hemoglobin-induced LDL oxidation in the presence of a low amount of H_2O_2 as a model for these pathophysiological conditions. As we have previously shown (Miller &

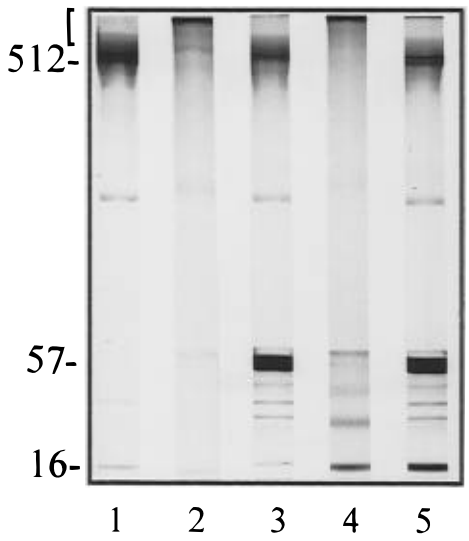

FIGURE 1: Inhibition of hemin- and hemoglobin-induced apo B cross-linking by hemopexin. LDL (600 μg/mL) was incubated at 37 °C for 3 h with the following compounds: (1) alone or in the presence of 14 μM H_2O_2; (2) 10 μM hemin + 14 μM H_2O_2; (3) 12 μM hemopexin + 10 μM hemin + 14 μM H_2O_2; (4) 10 μM oxyhemogobin + 14 μM H_2O_2; (5) 12 μM hemopexin + 10 μM oxyhemoglobin + 14 μM H_2O_2. The samples were then analyzed by SDS−PAGE. The numbers on the left-hand side designate the relative molecular mass ($\times 10^{-3}$): 16, globin; 57, hemopexin, 512, apo B monomer. The zone marked in the gel origin ([) contains covalent aggregates of apo B produced in the oxidative process.

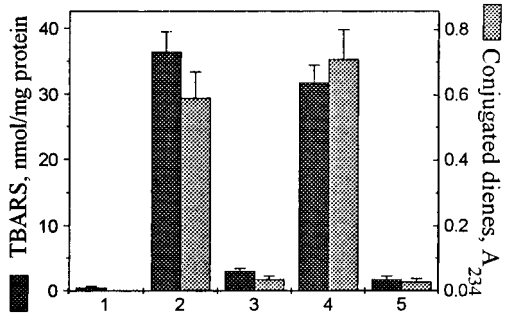

FIGURE 2: Inhibition of hemin- and hemoglobin-induced LDL lipid peroxidation by hemopexin. TBARS and conjugated dienes were determined in the same samples as in Figure 1. The dienes were measured relative to the control sample (1) using the A_{234} of 10-fold diluted samples. Shown are data following 3 h of incubation, which present end point levels reached for each oxidation product.

Shaklai, 1994; Miller et al., 1996), hemin, oxyhemoglobin, and methemoglobin induced peroxidative modification of apo B, resulting in its covalent cross-linking to high molecular weight aggregates that do not migrate in the gel, within 3 h of incubation at 37 °C (lanes 2 and 4 in Figure 1). As expected, hemopexin, due to its high affinity for hemin, prevented hemin-mediated cross-linking of apo B (Figure 1, lane 3). Surprisingly, hemopexin also inhibited the cross-linking mediated by hemoglobin as judged by the presence of apo B in its monomer position (Figure 1, lane 4). The extent of oxidation of LDL lipids by hemin and hemoglobin was also strongly reduced by hemopexin, as assessed by the formation of TBARS and conjugated dienes (Figure 2). These experiments performed with rabbit hemopexin were repeated with human hemopexin with nearly identical results (data not shown).

The above results confirm previous studies showing that hemopexin is an inhibitor of oxidations induced by free hemin (Balla et al., 1991; Gutteridge & Smith, 1988).

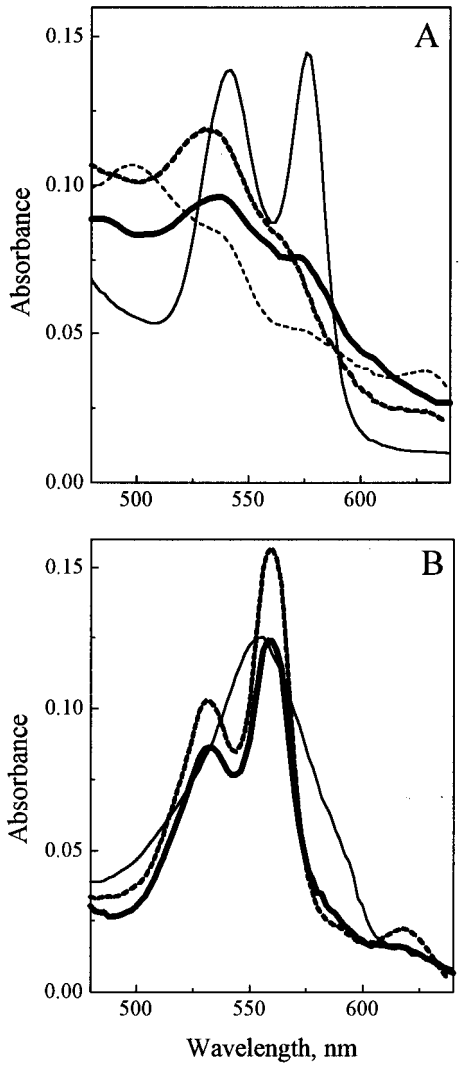

FIGURE 3: Spectrophotometric evaluation of heme transfer from hemoglobin to hemopexin. (A) Shown are visible spectra of the reaction mixture, which contained 600 μg/mL LDL, 12 μM hemopexin 10 μM oxyhemoglobin, and 14 μM H_2O_2, at time 0 (solid thin line) and following 3 h of incubation at 37 °C (solid heavy line), and visible spectra of the control, containing 12 μM hemopexin and 10 μM methemoglobin, at time 0 (dashed thin line) and after 30 min of incubation at 37 °C (dashed heavy line). (B) Spectra of the same samples as in (A) immediately after addition of solid sodium dithionite are shown. Note that both spectra coincided at time 0 (solid thin line).

However, no role of hemopexin in protecting against hemoglobin-mediated oxidations was yet suggested. To explore this activity, we sought to identify the species of heme generated in the reaction mixture by following the changes in the protein-bound state of heme, which are evident in the visible absorbance spectra. Furthermore, hemoglobin is a high-spin heme protein, while heme−hemopexin is a low-spin heme protein, and the two states can be distinguished by characteristic absorbance spectra (Morgan, 1976). Visible spectra of the reaction mixtures containing hemoglobin, hemopexin, LDL, and H_2O_2 were recorded at time 0 and after 3 h of incubation when the reaction was terminated (Figure 3). The final spectra obtained were similar whether oxy- or methemoglobin was used, but in both cases they differed from the time 0 spectra. To determine whether LDL influenced these spectral changes, the reactions were repeated in the absence of LDL, and the same spectral changes were observed (data not shown). When H_2O_2 was also excluded

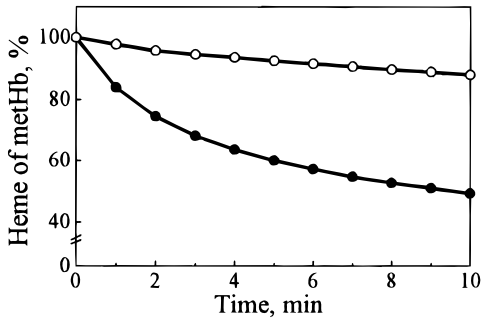

FIGURE 4: Temperature dependence of heme transfer from methemoglobin to hemopexin. The mixtures containing 1.2 μM hemopexin and 1.0 μM methemoglobin (metHb) were incubated at 20 °C (open circles) and at 37 °C (full circles), and the distribution of heme between globin and hemopexin was calculated from the absorbance spectra in the Soret region as described in Experimental Procedures.

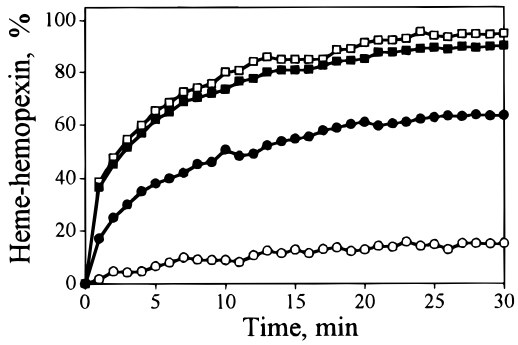

FIGURE 5: Dependence of the heme transfer rate from hemoglobin to hemopexin on the iron oxidative state. The intrinsic fluorescence of hemopexin was followed at 37 °C before and after addition of methemoglobin (open squares), methemoglobin/H_2O_2 (filled squares), oxyhemoglobin (open circles), and oxyhemoglobin/H_2O_2 (filled circles). Quenching of the hemopexin fluorescence intensity was used to calculate the percent of heme−hemopexin in the mixtures (see Experimental Procedures). Excitation and emission wavelengths were 285 and 345 nm, respectively. The mixtures contained 1.2 μM hemopexin, 1.0 μM hemoglobin, and 1.4 μM H_2O_2.

from the reactions (control mixtures), practically no changes occurred in the oxyhemoglobin/hemopexin mixture (not shown), but in the methemoglobin/hemopexin mixture the typical methemoglobin spectrum rapidly changed into a spectrum with a distinct absorbance maximum at 532 nm and a shoulder near 564 nm (Figure 3A, dashed lines). To assist analysis of the spectra shown in Figure 3A, the heme iron in all mixtures was reduced by dithionite, and the spectra were recorded again and shown in Figure 3B. Evidently, both time 0 spectra changed to typical deoxyhemoglobin spectra (solid thin line), implying that heme remained globin-bound. On the other hand, the mixtures incubated for 3 h exhibited spectra characteristic of heme(Fe^{II})−hemopexin (Morgan, 1976). Thus, within the experimental time scale used, heme was transferred from hemoglobin to hemopexin.

The globin−heme interaction is weaker in methemoglobin than in oxyhemoglobin (Banerjee, 1962). The transfer of hemin from methemoglobin to hemopexin was studied by Hrkal and co-workers (Hrkal et al., 1974), who found that equilibrium was attained within 96 h at 5 °C and 24 h at 20 °C, suggesting a slow transfer of hemin to hemopexin. Importantly, such a slow transfer cannot account for the aforementioned ability of hemopexin to inhibit hemoglobin-induced LDL peroxidation at 37 °C, which occurs within 3 h. To determine whether the difference in temperature accounts for the difference in heme transfer rate, the rates of heme transfer from methemoglobin to hemopexin at 20 and 37 °C were compared by using the differences in the Soret absorption between met hemoglobin and hemin−hemopexin (Antonini & Brunori, 1971; Morgan, 1976). Since at 37 °C light scattering from globin aggregates increases as the reaction proceeds and interferes with absorbance in the Soret region [a technical obstacle which may have led to the choice of low temperatures to investigate heme transfer (Hrkal et al., 1974)], only the initial transfer rates are presented in Figure 4. Clearly, heme transfer from methemoglobin to hemopexin is highly temperature dependent, and the initial rate at 37 °C was calculated to be 7-fold higher than at 20 °C. In fact, as judged by the time-dependent absorbance spectra in the methemoglobin/hemopexin mixture, heme is completely transferred from globin to hemopexin within 30 min at 37 °C (Figure 3).

These data demonstrate that hemopexin affords protection to LDL from methemoglobin-induced peroxidative damage at physiological temperature by efficient heme transfer. Nevertheless, the probability of heme transfer from oxyhemoglobin (due to its high affinity for heme) is extremely low even at 37 °C, whereas the data in Figures 1 and 2 show full protection by hemopexin against oxyhemoglobin-driven peroxidation as well. Therefore, the rates of heme transfer from met- and oxyhemoglobin to hemopexin in the presence and absence of H_2O_2 were compared. In this set of experiments, changes in the intrinsic fluorescence emission intensity of hemopexin were used to monitor heme binding. Hemoglobin tends to undergo temperature-dependent autoxidation (Hebbel et al., 1988), so that, for the kinetic measurements at 37 °C, isolated hemoglobin was replaced by fresh red blood cell lysate (containing oxyhemoglobin and a reductive system) to maintain the oxy form. As shown in Figure 5, in the presence of methemoglobin hemopexin became fully saturated with hemin within 30 min with an apparent transfer rate constant of 83 min^{-1}. Under the same experimental conditions, there was significantly less transfer of heme from oxyhemoglobin to hemopexin (15% as compared to 94% from the methemoglobin) with a transfer rate constant of 0.28 min^{-1}.

The pattern of heme transfer from methemoglobin to hemopexin was not changed by the presence of H_2O_2. Many studies have dealt with the reaction of methemoglobin with H_2O_2 (Giulivi & Davies, 1990; Paganga et al., 1992; Hogg et al., 1994), and it is generally accepted that the heme iron is oxidized to the ferryl state (Fe^{IV}). The fact that all the heme was transferred to hemopexin indicates either that the ferryl state was not formed or that any ferryl heme formed was transferred to hemopexin. Whatever the details of this reaction are, the important observation is that all the heme was neutralized in our reaction mixture by transfer to hemopexin within 30 min, a time shorter than the lag phase preceding LDL oxidation (Paganga et al., 1992; Hogg et al., 1994; Miller et al., 1996).

In the case of oxyhemoglobin/H_2O_2, the picture is somewhat different. Although normally very little heme is transferred to hemopexin from oxyhemoglobin, in the presence of H_2O_2 a large fraction of the heme was identified as heme−hemopexin within 30 min (Figure 5). The increased heme transfer can be explained as follows: in the presence of H_2O_2, oxyhemoglobin (Fe^{II}) oxidizes first to ferrylhemo-

globin (Fe^{IV}) (Paganga et al., 1992) and then to methemoglobin (Fe^{III}) by a comproportionation reaction (Giulivi & Davies, 1990):

$$\text{globin}-\text{heme}(Fe^{II}) + H_2O_2 \rightarrow \text{globin}-\text{heme}(Fe^{IV})=O + H_2O \quad (1)$$

$$\text{globin}-\text{heme}(Fe^{IV})=O + \text{globin}-\text{heme}(Fe^{II}) \rightarrow 2[\text{globin}-\text{heme}(Fe^{III})] + {}^1/_2O_2 \quad (2)$$

Thus, in the oxyhemoglobin/H_2O_2 mixture, methemoglobin and possibly ferrylhemoglobin are responsible for the heme which transfers to hemopexin. The lower transfer of heme from oxyhemoglobin/H_2O_2 as compared to methemoglobin/H_2O_2 is determined by the fraction of hemoglobin that remains in the oxy state under reaction conditions of minimal H_2O_2 excess. Because, at this stage, all the H_2O_2 was consumed, the reminder of the oxyhemoglobin is incapable of serving as an active LDL oxidizer any longer.

As stated, hemoglobin has a high peroxidase activity toward LDL, and the products are covalent aggregates of apo B (Miller et al., 1996; Figure 1). This reactivity resembles that of the other oxygen binding protein, myoglobin (Hanan & Shaklai, 1995), and appears to arise from the ability of globin to form heme-induced radicals which initiate a cascade of radicals in target proteins resulting in their cross-linking (Shaklai et al., 1987). According to this mechanism, following reactions 1 and 2 above, the two oxidative equivalents of a peroxide ROOH (H_2O_2 or LDL lipid hydroperoxides) activate methemoglobin to form a ferryl iron and a globin radical (·globin):

$$\text{globin}-\text{heme}(Fe^{III}) + \text{ROOH} \rightarrow \cdot\text{globin}-\text{heme}(Fe^{IV})=O + \text{ROH} \quad (3)$$

This active intermediate oxidizes LDL and forms covalently cross-linked apo B, $(\text{apo B}-\text{apo B})_n$:

$$\cdot\text{globin}-\text{heme}(Fe^{IV}) + \text{apo B} \rightarrow \text{globin}-\text{heme}(\text{FeIV}) + \cdot\text{apo B} \quad (4)$$

$$2n[\cdot\text{apo B}] \rightarrow (\text{apoB}-\text{apo B})_n \quad (5)$$

It should be mentioned that another, indirect pathway may take place: LDL lipids can first be oxidized by the apo B radical, and thus lipid oxidation products may be involved in the process of protein cross-linking (Jessup et al., 1992).

In the presence of hemopexin, a different route of reactions takes place. Following reactions 1 and 2, hemopexin removes heme from methemoglobin:

$$\text{globin}-\text{heme}(Fe^{III}) + \text{hemopexin} \rightarrow \text{globin} + \text{hemopexin}-\text{heme}(Fe^{III}) \quad (6)$$

Depriving hemoglobin of heme enables hemopexin to abrogate the system's peroxidative potential (Shaklai et al., 1987) and thereby prevents oxidation of LDL.

Taken together, the results of this study show that hemopexin, previously considered to prevent only hemin-mediated oxidation, is also a potent antioxidant toward hemoglobin. When there is sufficient hemolysis by one of the many ways in which red blood cells are damaged, hemoglobin appears in the plasma. This is often concurrent with inflammation, when activated leukocytes produce oxidants like H_2O_2. The presence of hemoglobin/H_2O_2 is hazardous for LDL and other plasma constituents, and under these conditions, LDL could be protected by hemopexin from hemoglobin-derived peroxidative reactivity. This is biologically consistent with the fact that hemopexin is one of the plasma acute phase proteins which are synthesized in higher levels following inflammation (Muller-Eberhard, 1988; Greieninger et al., 1986).

In contrast to albumin, hemopexin, by binding heme tightly and delivering it to the liver for catabolism, denies heme access to other vascular components, thus protecting them from structural and functional modifications that follow heme-induced peroxidations (Solar et al., 1987). Hemopexin damps the chemical reactivity of heme by tightly binding it in a bishistidyl, low-spin complex (Morgan et al., 1993), and recent studies implied that oxidants that are otherwise activated by heme become inactive in the presence of hemopexin due to reduced access to the hemopexin-bound heme (Timmins et al., 1995). Notably, hemopexin has no free sulfhydryl groups (Morgan, 1976) in contrast to the globins, which may also contribute to the unusual resistance of hemopexin to deleterious effects of heme.

Hemopexin is not only utilized to conserve body iron stores but also acts protectively in two major ways: as a pleotropic regulator of gene expression and as an extracellular antioxidant (Muller-Eberhard, 1988; Vincent et al., 1988; Alam & Smith, 1989). The sequence of events set in motion by exposure of cells to heme hemopexin has recently been shown to enable retinal pigment epithelial cells to survive heme-mediated oxidative stress (Hunt et al., 1996a). Here, it is shown that hemopexin also prevents hemoglobin-mediated oxidative damage in addition to its known role in protecting against the toxic effects of globin-free heme. Hemoglobin is a trigger of oxidative pathology of vascular components as well as other tissues including the neural retina (Ito et al., 1995) and the central nervous system (Sadrzadeh et al., 1987; Regan et al., 1993). Interestingly, it has recently been shown that hemopexin is synthesized not only in the liver parenchymal cells (Muller-Eberhard & Morgan, 1975) but also in cells of the peripheral nerves in response to injury, e.g., in the sciatic nerve and in ganglions of the neural retina and skeletal muscle cells (Swerts et al., 1992; Hunt et al., 1996b). Hemopexin was shown to be present within neurons of the human brain (Morris et al., 1993), and it was specifically expressed in brain of transgenic mice harboring the human hemopexin promoter sequence (Tolosano et al., 1996). The occurrence of hemopexin in organs which are bathed by plasma, such as the liver, as well as in barrier tissues, such as the brain, suggests that it may have evolved in vertebrates in part due to a need for protection against hemoglobin-mediated oxidative damage not only in the vascular compartment but throughout the body.

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