Comparison between copper-mediated and hypochlorite-mediated modifications of human low density lipoproteins evaluated by protein carbonyl formation

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Abstract The purpose of this study was to evaluate the mechanisms of apolipoprotein B (apoB) modification during oxidation of human low density lipoproteins (LDL) mediated either by copper or by hypochlorite (HOCl). The kinetics of protein carbonyl formation, the relationship of apoB carbonyl formation to lipid peroxidation, and the loss of apoB lysine residues were determined. During copper-mediated LDL oxidation, apoB carbonyls appeared to increase slowly, displayed saturation kinetics in response to increasing copper concentrations, and correlated with lipid peroxidation. During HOCl-mediated LDL oxidation, apoB carbonyls increased with increasing HOCl concentrations reaching plateau with time; however, lipid peroxidation was not observed. During copper-mediated, but not during HOCl-mediated LDL oxidation, LDL vitamin E was depleted. ApoB carbonyls formed more efficiently during copper-mediated LDL oxidation at low (<5 μM) copper concentrations compared with higher copper concentrations or during HOCl-mediated LDL oxidation. The differences in oxidation kinetics between copper- and HOCl-mediated LDL oxidation support the concept that the binding of copper to LDL is a site specific process, and suggest that HOCl modifies apoB amino acids randomly.—**Yan, L-J., J. K. Lodge, M. G. Traber, S. Matsugo, and L. Packer.** Comparison between copper-mediated and hypochlorite-mediated modifications of human low density lipoproteins evaluated by protein carbonyl formation. *J. Lipid Res.* 1997. **38:** 992–1001.

Supplementary key words apolipoprotein B • carbonyls • copper • HOCl • low density lipoproteins • thiobarbituric acid reactive substances • vitamin E

Oxidatively modified low density lipoproteins (LDL) are likely to be the main source of cholesterol that accumulates in arteriosclerotic plaques (1–3). Oxidized LDL are metabolized by macrophages and smooth muscle cells via the scavenger pathway that, unlike the native LDL receptor, is not down-regulated (4, 5). The evidence for the participation of LDL oxidation in atherosclerosis includes: oxidized LDL are present in atherosclerotic lesions (6); in vitro experiments show increased uptake of oxidized LDL by macrophages (4); and oxidized LDL cause injury to cultured cells (7). Oxidized LDL also alter growth factor and cytokine production (8, 9), induce monocyte recruitment and adhesion to endothelium (10, 11), and alter cell migration and growth (4, 12, 13).

Trace amounts of copper can induce LDL oxidation (14), resulting in highly reproducible oxidative modifications in vitro (15, 16). This process leads to oxidized LDL that shares many structural and functional properties with LDL oxidized by cells (17) or LDL extracted from arterial atherosclerotic plaques (18). Moreover, this process mimics the in vivo situation because copper ions are present in the arterial wall and plaque extracts have been shown to oxidize LDL in vitro (19). Recognition of oxidized LDL by macrophage scavenger receptors may result from derivatization of apolipoprotein (apoB) lysine residues (20) or apoB modification by lipid peroxidation breakdown products such as malondialdehyde and 4-hydroxynonenal (21).

Although copper-mediated LDL oxidation produces an LDL with physiologically relevant characteristics, the finding that human atherosclerotic plaque contains relatively large amounts of the antioxidants, α-tocopherol

Abbreviations: apoB, apolipoprotein B; DNPH, 2,4-dinitrophenylhydrazine; EDTA, ethylenediaminetetraacetic acid; HOCl, hypochlorite/hypochlorous acid; LDL, low density lipoprotein; SDS, sodium dodecyl sulfate; TBARS, thiobarbituric acid reactive substances.

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and ascorbate (22), that should prevent LDL oxidation, suggests that some other mechanisms may be involved in its oxidation in vivo. One such potential mechanism occurs during the neutrophil/monocyte respiratory burst. Upon stimulation, neutrophils/monocytes release reactive oxygen species, such as superoxide ($O_2^{\cdot -}$) and hydrogen peroxide (H_2O_2). Moreover, they release myeloperoxidase (EC 1.11.1.7, MPO), an enzyme that catalyzes the following reaction to yield the powerful oxidant, hypochlorous acid (HOCl) (23–26):

$$H_2O_2 + H^+ + Cl^- \rightarrow HOCl + H_2O$$

Both the reactive oxygen species and HOCl contribute to the bactericidal action of neutrophils/monocytes. However, the damaging effect of these products is not limited to bacteria, vulnerable too is the surrounding tissue or plasma lipoproteins. Oxidative modification of LDL by HOCl results in modification of the protein portion, while lipid peroxidation occurs to a very limited extent (27–29). Interestingly, both myeloperoxidase (30) and HOCl-modified proteins (31) have been detected in human atherosclerotic lesions.

Protein carbonyl formation is a biomarker of protein oxidation (32). Increased levels of protein carbonyls have been detected in several disease states such as rheumatoid arthritis (33), ischemia-reperfusion injury to heart muscle (34, 35) and skeletal muscle damage due to exhaustive exercise (36). We have developed a sensitive and a reliable method for apoB carbonyl quantitation and found that apoB carbonyl is also a good biomarker for the evaluation of LDL oxidation (37). While the literature abounds with studies on copper- or HOCl-mediated LDL oxidative modification, direct comparisons of the kinetics of apoB carbonyl formation during Cu^{2+}- and HOCl-mediated LDL oxidation have not been carried out. Although it is known that apoB can be modified by lipid peroxidation products during LDL oxidation (38, 39), the relationship of apoB carbonyl formation to LDL lipid peroxidation, as well as to the loss of those amino acids (such as lysine), which are readily attacked by lipid peroxidation products during oxidative modification, has not been investigated. Therefore, in the present study, we compared LDL oxidation by copper and HOCl to address these questions.

MATERIALS AND METHODS

Materials

Cupric sulfate, 2,4-dinitrophenylhydrazine (DNPH), sodium dodecyl sulfate (SDS), thiobarbituric acid, bovine serum albumin (BSA), and ethylenediamine tetraacetic acid (EDTA) were from Sigma Chemical Co. (St. Louis, MO). Malonaldehyde bis(dimethylacetal) was from Aldrich. Heptane, reagent alcohol, and NaOCl were from Fisher Scientific (Pittsburgh, PA).

Preparation of low density lipoproteins

LDL were isolated by sequential ultracentrifugation (40) from fresh plasma of normolipidemic donors obtained from the local blood bank. The density of whole plasma was adjusted to 1.24 g/ml by adding potassium bromide. The plasma was layered under saline (d 1.006), and centrifuged at 242,000 *g* in a Beckman VTi 50 vertical-rotor for 2 h at 10°C. The LDL, which appeared as a yellow band in the center of the tube, were removed using a needle and syringe. This layer was then ultracentrifuged at d 1.063 in a Beckman Ti 60 rotor at 260,000 *g* for 24 h at 10°C. LDL, a yellow band at the top of the tube, were collected and then dialyzed against sodium phosphate buffer (10 mM, pH 7.4) at 4°C for 24 h. LDL were used within 2 days of isolation.

Oxidative modification of low density lipoproteins

Modification by Cu^{2+}. LDL (0.5 mg protein/ml) were incubated for indicated periods at 37°C in 10 mM sodium phosphate buffer, pH 7.4, containing the indicated concentrations of $CuSO_4$. For time-dependent studies, aliquots were taken at indicated times. The oxidation reaction was stopped by the addition of EDTA (100 μM final concentration).

Modification by HOCl. The concentration of hypochlorite/hypochlorous acid present in the diluted commercial NaOCl solution was determined spectrophotometrically using published extinction coefficients ($\epsilon_{290} = 350\ M^{-1}\ cm^{-1}$) (41). Hypochlorous acid (pK_a = 7.5 (42)) is present as both OCl^- and HOCl at physiological pH. In this paper, HOCl is used to describe the mixture of OCl^- and HOCl. HOCl was diluted in 10 mM phosphate buffer (pH 7.4) and added to LDL (0.5 mg/ml) solution in phosphate buffer (10 mM, pH 7.4). The oxidation reactions were carried out in the air at 37°C, and oxidized LDL were analyzed immediately after the oxidation.

Protein carbonyl assay

ApoB carbonyls were measured spectrophotometrically with the use of the carbonyl specific reagent 2,4-dinitrophenylhydrazine (DNPH) as previously reported (37). Briefly, native LDL or oxidized LDL (0.5 mg protein/ml) were divided into two portions (1 ml each). To each portion either 0.2 ml 2 N HCl, or 10

mM DNPH in 2 N HCl was added. After incubation at room temperature for 1 h, half volume (0.6 ml) of denaturing buffer (0.15 M sodium phosphate buffer, pH 6.8, containing 3% SDS) was added. The samples were mixed vigorously followed by the sequential addition of one volume of ethanol and heptane (1.8 ml each). The mixture was centrifuged, and the precipitated protein was isolated from the interface. The protein was washed three times with 3 ml of ethyl acetate–ethanol 1:1 (vol/vol), and dissolved in 1 ml denaturing buffer. Each DNPH sample was scanned from 320 nm to 410 nm in the Perkin-Elmer Lambda 5 UV/VIS spectrophotometer against the corresponding HCl sample and the peak absorbance (around 360 nm) was used to quantitate the protein carbonyls (extinction coefficient 22,000 M^{-1} cm^{-1} (43)).

Other methods

TBARS assay was performed according to Schuh, Fairclough, and Haschemeyer (44) with slight modification (45). In a typical assay, trichloroacetic acid (10%, final concentration) was added to aliquots of lipoproteins (50 μg protein). One milliliter of 0.8% thiobarbituric acid was then added and the mixture was heated in a Fisher Brand dry bath at 100°C for 60 min. After centrifugation to clarify the solution, the peak absorbance at 532 nm was read against a buffer blank. TBARS were calculated from a standard curve, using malonaldehyde bis(dimethylacetal) as the standard (46).

Unmodified amino groups (mainly lysine residues) were quantified using fluorescamine according to Böhlen et al. (47). In a typical assay, 20 μl LDL (0.5 mg/ml) was added to 730 μl of borate buffer (200 mM, pH 8.5) and mixed during addition of 250 μl of fluorescamine (540 μM, in acetone). After incubation at room temperature for less than 120 min, fluorescence was measured (excitation: 390 nm, emission: 475 nm) using a Perkin-Elmer MPF-44A fluorimeter (Norwalk, CT). All samples were diluted appropriately in borate buffer to yield fluorescence below 600 arbitrary units, a range for which a linear relationship between fluorescence and amine concentration is observed (27). It was assumed that each LDL molecule contains 356 lysine residues (48).

Vitamin E (α- and γ-tocopherols) was measured by HPLC-electrochemical detection assay as previously reported (49). Typically, copper-mediated LDL oxidation was stopped by the addition of EDTA (100 μM, final concentration). Both copper- and HOCl-oxidized LDL were frozen at −80°C until extraction and vitamin E analysis.

Protein concentrations were determined using the Lowry assay (50) with bovine serum albumin (BSA) as the standard.

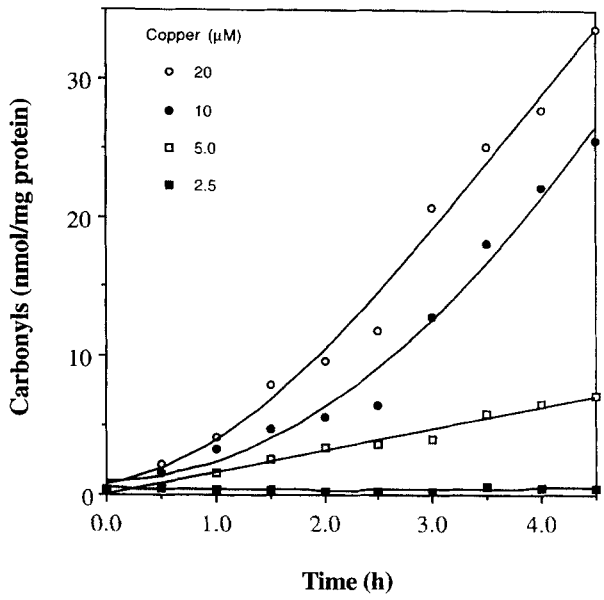

Fig. 1. Kinetics of apoB carbonyls formed during copper-mediated LDL oxidation. LDL (0.5 mg/ml) were incubated with varying $CuSO_4$ concentrations at 37°C in phosphate buffer (10 mM, pH 7.4) up to 4.5 h.

RESULTS

Kinetics of apoB carbonyl formation during Cu^{2+}- and HOCl-mediated LDL oxidation

ApoB carbonyls formed in a time-dependent manner during copper-mediated LDL oxidation (**Fig. 1**). All copper concentrations increased apoB carbonyls. During LDL oxidation, formation of apoB carbonyls was related to copper concentration in a hyperbolic manner; high copper concentrations (≥30 μM) resulted in saturation in apoB carbonyl content within 3 h (**Fig. 2**).

During HOCl-mediated LDL oxidation, apoB carbonyls plateaued in <2 h at most HOCl concentrations < 1000 μM (**Fig. 3**). During a 1 h incubation, HOCl-mediated apoB carbonyls increased linearly in an HOCl concentration-dependent manner below 600 μM (**Fig. 4**). Higher HOCl concentrations (up to 2 mM) resulted in a plateau in the formation of apoB carbonyls. At these HOCl concentrations apoB was highly fragmented, as assessed by SDS-PAGE (data not shown).

To estimate the maximum amount of apoB carbonyls that could be formed under the experimental conditions described for Figs. 2 and 3, we analyzed the data using double-reciprocal plots. For copper-dependent apoB carbonyl formation, when 1/carbonyl (nmol/mg) is plotted versus 1/copper (μM), the maximal theoretical carbonyl content, for a given LDL concentration

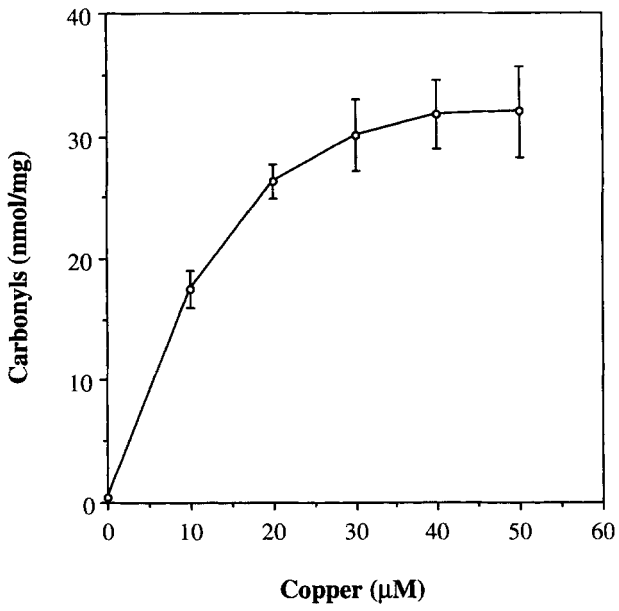

Fig. 2. ApoB carbonyl formation in response to increasing copper concentrations. Oxidation conditions were as in Fig. 1. The incubation time was 3 h. Shown are the mean ± SEM of triplicate assays.

and incubation time, is obtained. For example, at an LDL concentration of 0.5 mg/ml, at 3 h, the theoretical maximum apoB carbonyl content is 44 nmol/mg protein (1/0.0225 as shown in **Fig. 5A**). It should be pointed out that this maximum value is time-dependent. For instance, at 4 h, the maximum apoB carbonyl

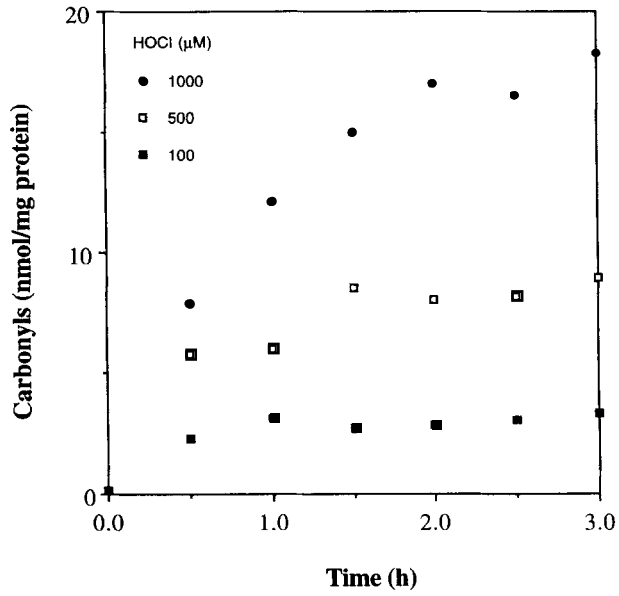

Fig. 3. Kinetics of apoB carbonyls formed during HOCl-mediated LDL oxidation. LDL (0.5 mg/ml) were incubated with varying HOCl concentrations at 37°C in phosphate buffer (10 mM, pH 7.4) up to 3 h.

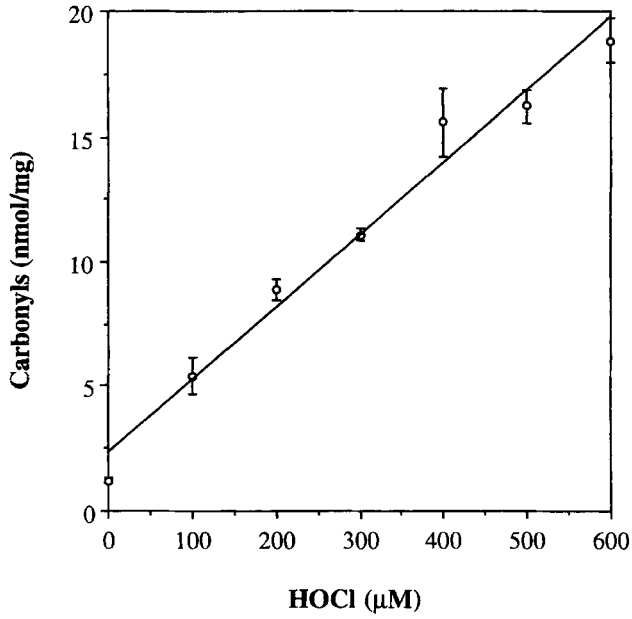

Fig. 4. ApoB carbonyl formation in response to increasing HOCl concentrations. Oxidation conditions were as in Fig. 3. The incubation time was 1 h. Shown are the mean ± SEM of triplicate assays; $r^2 = 0.976$.

content is 50 nmol/mg protein; while at 24 h, the maximum apoB carbonyl content is 70 nmol/mg protein (data not shown). Similarly, in the time course study of HOCl-induced LDL modification, when 1/carbonyl (nmol/mg) is plotted versus 1/time (h), at an LDL concentration of 0.5 nmol/mg and an HOCl concentration of 1 mM, the theoretical maximum apoB carbonyl content is 25 nmol/mg protein (1/0.04 as shown in Fig. 5B). This maximum carbonyl value is also HOCl concentration-dependent (Fig. 3). For instance, at HOCl concentration of 500 μM, the maximum apoB carbonyl content is 9.5 nmol/mg protein; while at HOCl concentration of 5 mM, the maximum apoB carbonyl content is 36 nmol/mg protein. These theoretical limits are also highly dependent on the LDL source and oxidation conditions.

Lipid peroxidation and apoB lysine loss during Cu^{2+}- and HOCl-mediated LDL oxidation

To investigate the relationship between apoB carbonyl formation and LDL lipid peroxidation, we measured TBARS. During copper-mediated LDL oxidation, apoB carbonyl formation correlated with LDL lipid peroxidation (**Fig. 6**). However, during HOCl-mediated LDL oxidation, TBARS did not increase above baseline (data not shown and (29)).

To further investigate whether apoB carbonyl formation parallels the loss of amino acids that are readily attacked by lipid peroxidation products (such as malon-

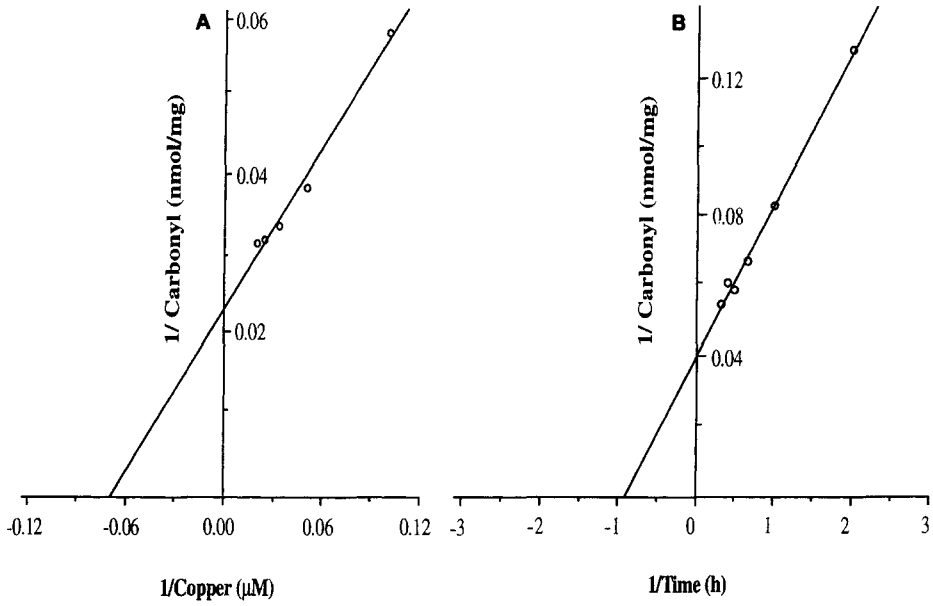

Fig. 5. Double reciprocal plots for (A) copper concentration-dependent apoB carbonyl formation. LDL (0.5 mg/ml) were incubated with varying concentrations of copper at 37°C in phosphate buffer (10 mM, pH 7.4) for 3 h; (B) time-dependent apoB carbonyl formation induced by HOCl. LDL (0.5 mg/ml) were incubated with 1 mM HOCl at 37°C in phosphate buffer (10 mM, pH 7.4) for varying lengths of time.

dialdehyde and 4-hydroxynonenal), we determined apoB lysine residues fluorometrically (27). During both copper- and HOCl-mediated LDL oxidation, the apoB lysine residues decreased with increasing oxidant concentrations (**Fig. 7**). The loss of apoB lysine residues were linearly related to apoB carbonyl formation (**Fig. 8**). Most striking were the differences between HOCl and copper. At low copper concentrations (<5 μM) there was nearly a 0.6:1 relationship between carbonyl formation and lysine amino groups lost. By contrast, during HOCl-mediated LDL oxidation, only 0.1 nmol of carbonyls was formed for each nmol of lysines lost. Thus, protein carbonyl formation is much more efficient at low copper concentrations. This further suggests that HOCl oxidation of proteins is so vigorous that it does not only attack lysine groups on apoB.

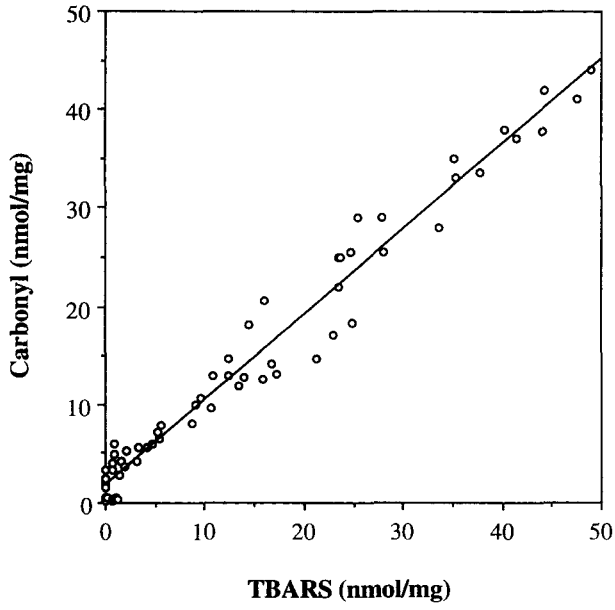

Fig. 6. Correlation of LDL lipid peroxidation versus apoB carbonyl formation in copper-mediated LDL oxidation. LDL aliquots were oxidized by varying copper concentrations up to 4.5 h as in Fig. 1; r^2 = 0.971.

Depletion of vitamin E during LDL oxidation

Vitamin E is a potent lipid-soluble antioxidant in LDL and is oxidized prior to polyunsaturated fatty acids (51, 52). Our experiments suggest that carbonyl formation is a result of lipid peroxidation during copper-mediated but not during HOCl-mediated LDL oxidation. Therefore, depletion of vitamin E (both α- and γ-tocopherols) during copper- and HOCl-mediated LDL oxidation was determined using conditions shown in Fig. 7, which caused major loss of amino groups. During copper-mediated oxidation, vitamin E was depleted almost completely by 10 μM copper during a 3-hour oxidation (**Fig. 9A**). By contrast, at HOCl concentrations (up to 300 μM) that caused significant apoB carbonyl formation and loss of amino groups, α-tocopherol concentrations were only slightly decreased and γ-tocopherol concentrations were virtually unchanged (Fig. 9B).

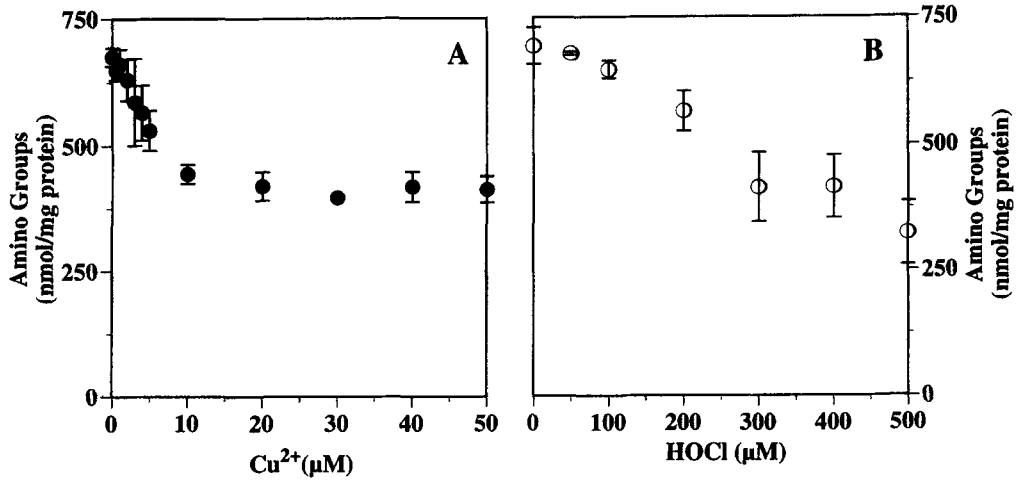

Fig. 7. Effects of Cu^{2+} and HOCl on the loss of apoB lysine residues. LDL (0.5 mg/ml) were incubated with copper for 3 h (A) or with HOCl for 3 h (B) at 37°C in phosphate buffer (10 mM, pH 7.4). ApoB amino groups were determined fluorometrically.

DISCUSSION

In this investigation the kinetics of apoB carbonyl formation induced by copper differed markedly from those induced by HOCl. ApoB carbonyl formation was saturated by μM copper concentrations but required mM HOCl concentrations. Lipid peroxidation correlated with apoB carbonyl formation during copper-mediated but not HOCl-mediated oxidation. For a given concentration of oxidant, apoB carbonyls reached a plateau with time during HOCl-mediated but not copper-mediated LDL oxidation. The number of apoB carbonyls was equivalent to the loss of apoB lysine residues during copper-mediated LDL oxidation at very low copper concentrations, but not during HOCl-mediated oxidation, suggesting that carbonyl formation by HOCl is inefficient. The differences in kinetics between copper- and HOCl-mediated LDL oxidation provide further support for the hypothesis that copper binds to specific sites on LDL where it causes oxidation (52, 53), and suggests that HOCl modifies apoB randomly.

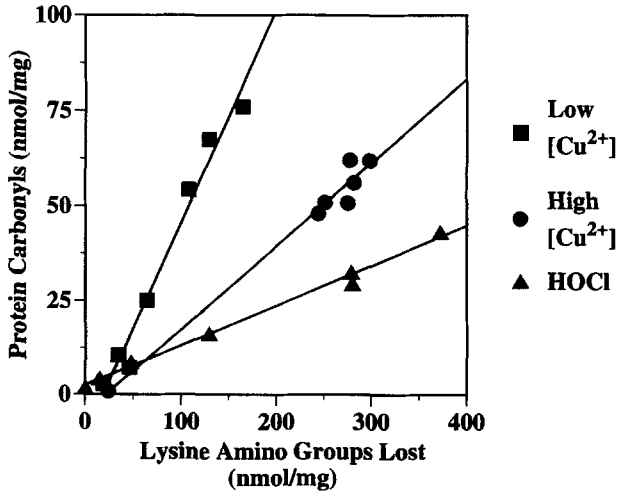

Fig. 8. Relationship between carbonyl formation and lysine amino groups lost. Carbonyl formation and amino group loss during LDL oxidation (as described in Fig. 7) mediated by HOCl (▲), high (>10 μM, ●) or low (<5 μM, ■) copper are shown. The correlation coefficients were for HOCl r^2 = 0.993, high copper r^2 = 0.977, and low copper r^2 = 0.973.

Copper-mediated LDL oxidation

The ratio of copper to LDL is one of the critical factors in determining the kinetics of copper-mediated LDL oxidation (16). A threshold copper concentration was required to initiate the oxidative modification of LDL protein; apoB carbonyl formation did not occur during the 4.5 h incubation at a copper concentration of 2.5 μM in freshly isolated LDL (Fig. 1). Storage of LDL did increase its oxidizability, supporting the role of LDL lipid hydroperoxides in its oxidation (29). In addition, for a given LDL concentration, apoB carbonyl formation reached saturation with increasing copper concentrations (Fig. 2), in agreement with Gieseg and Esterbauer (52). However, the parallel relationship between apoB carbonyls and TBARS formed during copper-mediated LDL oxidation up to 4.5 h (Fig. 6), was not observed at 24 h or 48 h because the TBARS decreased (data not shown). Therefore, measurements of protein carbonyls are a better index for evaluating the extent of LDL oxidation.

During copper-mediated LDL oxidation, exposed amino acid residues on apoB may react with oxidizing species to form carbonyl groups. This site-specific pro-

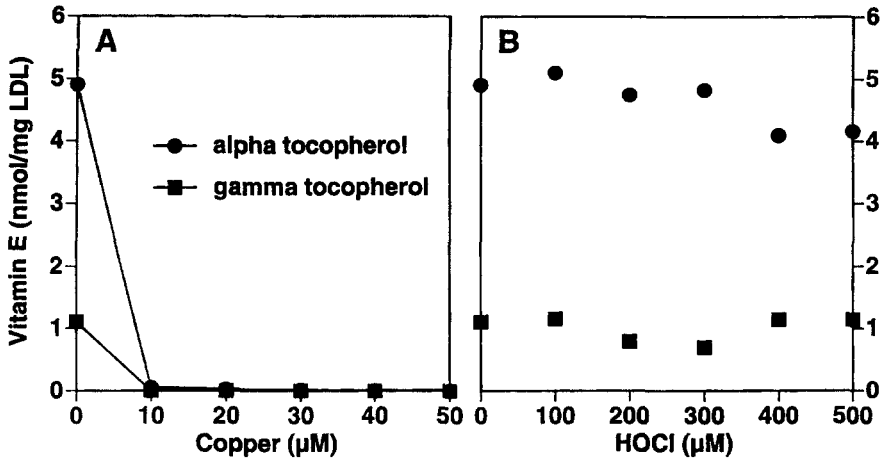

Fig. 9. Vitamin E concentrations in oxidized LDL. A) Copper-mediated oxidation: LDL (0.5 mg/ml) were incubated with increasing concentrations of copper at 37°C in phosphate buffer (10 mM, pH 7.4) for 3 h. B) HOCl-mediated oxidation: LDL (0.5 mg/ml) were incubated with increasing concentrations of HOCl at 37°C in phosphate buffer (10 mM, pH 7.4) for 1 h. Shown are the means of two determinations, representative of two experiments.

cess most likely involves hydroperoxides. These can further react with the copper–protein complex to yield an active oxygen species, such as the hydroxyl radical, at the copper binding site on the protein, with some amino acids being converted to carbonyl derivatives (54). The direct oxidation of amino acid residues, however, may not be the only mechanism for the generation of apoB carbonyl derivatives. Copper-mediated LDL oxidation begins first with a lag phase during which endogenous antioxidants are consumed (52). Then, the lipid radical-propagated chain reaction results in the formation of peroxyl radicals from the oxidation of polyunsaturated fatty acids (51). Carbonyl derivatives of apoB could arise through interaction of lysine, cysteine, or histidine residues with lipid peroxidation products such as malondialdehyde (38) and 4-hydroxynonenal (HNE) (39) via Michael additions and Schiff base adducts; the latter can also be measured by DNPH assay (55). In copper-mediated LDL oxidation, apoB carbonyl formation closely correlated with lipid peroxidation during short incubation periods (Fig. 6), and at low copper concentrations we found efficient conversion of lysines to carbonyls. However, with high copper concentrations or with HOCl, more lysines were destroyed than the number of protein carbonyls that were formed. It seems unlikely from the present study that apoB carbonyl formation will parallel the loss of other amino acid residues such as histidine and cysteine (total number in apoB: 115 and 25 respectively (48)) during LDL oxidative modification, although this aspect has not been explicitly investigated.

Why does the number of LDL lysines lost exceed the number of protein carbonyls formed? Hazell, van den Berg, and Stocker (28) showed that HOCl modification of LDL initially leads to formation of reversible lysine chlorohydrin, which decomposes to carbonyls and to other products. Loss of amino groups may result from reactions such as condensation and crosslinking, as well as direct attack by hydroperoxides or other oxidizing species. We also observed apoB aggregation and fragmentation by SDS-PAGE analysis (data not shown). Thus, some amino groups are converted to non-carbonyl species; hence the discrepancy between amino groups lost and protein carbonyls formed.

HOCl-mediated LDL oxidation

HOCl modifies apoB rapidly and randomly; apoB carbonyls plateaued in < 1 h at most HOCl concentrations < 1000 μM (Fig. 3). At concentrations up to 600 μM HOCl, apoB carbonyl formation was not saturated. These results are in sharp contrast to those of copper-mediated LDL oxidation in which apoB carbonyl formation saturated with < 50 μM copper (Fig. 2).

The differences in kinetics of protein carbonyl formation between the two oxidation systems can be attributed to differences in interactions of the oxidants with lipids or proteins. Protein carbonyls did not increase during a 3-h incubation of bovine serum albumin (fatty acid free) with copper (29), while incubation with HOCl resulted in an increase in protein carbonyls within minutes (56). Therefore, HOCl directly modifies proteins, while lipids, especially lipid hydroperoxides (even in trace amounts), are required to initiate copper-mediated protein oxidation (2, 29, 57). Despite the kinetic differences, the two oxidation systems do have similar biological consequences. These include an in-

crease in net negative surface charge and an accelerated uptake and apoB degradation by macrophages (27, 28). Also, antibodies raised against HOCl-modified LDL cross-react strongly with copper-modified LDL, and vice versa (58).

Why is lipid peroxidation virtually undetectable during HOCl-mediated LDL oxidation? HOCl is apparently more reactive with LDL proteins than with its antioxidants or lipids. Hazell et al. (28) blocked accessible lysine residues in apoB by methylating LDL. Subsequent HOCl treatment resulted in antioxidant depletion and cholesteryl ester hydroperoxide formation. LDL amino acid residues are directly modified by HOCl (59); concentrations >50 μM led to the oxidation of -SH, methionine, and tryptophan residues and formation of protein carbonyls (60). Lysine residues scavenge 68% of the added HOCl to form chloramines, while tryptophan and cysteine together account for reaction with a further 10% of the oxidant (41, 61). Chloramines of amino acids are unstable and spontaneously decompose to NH_3, Cl^-, and corresponding aldehydes (61). Hence the formation of aldehydes may lead to apoB carbonyls, not lipid peroxides.

HOCl modification may be an important mechanism for the in vivo oxidation of LDL. HOCl modifies apoB into a form that is readily taken up by macrophages despite the lack of lipid peroxidation (27, 28). Indeed, vitamin E that is depleted during lipid peroxidation was virtually unaffected during HOCl-mediated LDL oxidation (Fig. 8). This result is in accord with our previous findings that vitamin E does not prevent HOCl-induced protein carbonyl formation (56). Thus, the relatively large amounts of vitamin E and ascorbate that are present in atherosclerotic lesions (22) suggest that apoB oxidative modification alone, independent of lipid peroxidation, can be atherogenic.

In conclusion, the kinetics of apoB carbonyl formation mediated by copper differ markedly from that mediated by HOCl; apoB carbonyl formation correlates with lipid peroxidation during copper- but not HOCl-mediated oxidation. Furthermore, copper more efficiently converts lysines to carbonyls, but HOCl more rapidly causes random protein oxidation.

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