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Oxidative tyrosylation of high density lipoprotein by peroxidase enhances cholesterol removal from cultured fibroblasts and macrophage foam cells

GORDON A. FRANCIS*, ARMANDO J. MENDEZ*†, EDWIN L. BIERMAN*, AND JAY W. HEINECKE‡§

*Department of Medicine, University of Washington, Seattle, WA 98195; and ‡Department of Medicine, Washington University School of Medicine, St. Louis, MO 63110

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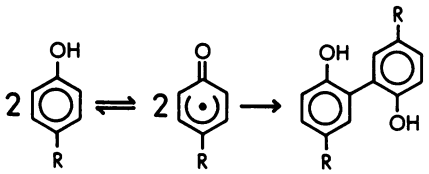
ABSTRACT **Lipoprotein oxidation is thought to play a pivotal role in atherogenesis, yet the underlying reaction mechanisms remain poorly understood. We have explored the possibility that high density lipoprotein (HDL) might be oxidized by peroxidase-generated tyrosyl radical. Exposure of HDL to L-tyrosine, H_2O_2, and horseradish peroxidase crosslinked its apolipoproteins and strikingly increased protein-associated fluorescence. The reaction required L-tyrosine but was independent of free metal ions; it was blocked by either catalase or the heme poison aminotriazole. Dityrosine and other tyrosine oxidation products were detected in the apolipoproteins of HDL modified by the peroxidase/L-tyrosine/H_2O_2 system, implicating tyrosyl radical in the reaction pathway. Further evidence suggests that tyrosylated HDL removes cholesterol from cultured cells more effectively than does HDL. Tyrosylated HDL was more potent than HDL at inhibiting cholesterol esterification by the acyl-CoA:cholesterol acyltransferase reaction, stimulating the incorporation of [^{14}C]acetate into [^{14}C]cholesterol, and depleting cholesteryl ester stores in human skin fibroblasts. Moreover, exposure of mouse macrophage foam cells to tyrosylated HDL markedly diminished cholesteryl ester and free cholesterol mass. We have recently found that myeloperoxidase, a heme protein secreted by activated phagocytes, can also convert L-tyrosine to *o,o'*-dityrosine. This raises the possibility that myeloperoxidase-generated tyrosyl radical may modify HDL, enabling the lipoprotein to protect the artery wall against pathological cholesterol accumulation.**

Reduction of low density lipoprotein (LDL) levels has been a major focus in the prevention of coronary artery disease. Evidence has accrued, however, that oxidized LDL rather than native LDL triggers the pathological events induced by hypercholesterolemia (for reviews, see refs. 1–3). In contrast, the consequences of high density lipoprotein (HDL) oxidation on atherogenesis have received scant attention, even though elevated levels of HDL appear to decrease the risk of vascular heart disease. HDL apparently inhibits LDL oxidation (4–7) but is itself susceptible to Cu^{2+}-stimulated lipid peroxidation (6, 8). Cu^{2+}-modified HDL loses its ability to promote cholesterol efflux from cultured macrophage foam cells, suggesting that oxidation might favor the formation of vascular lesions (8). The requisite free transition metal ions have not yet been detected in the arterial wall, however, and the structural attributes of oxidized lipoproteins that mediate their potent biologic effects are poorly understood.

A potential physiological catalyst of lipoprotein oxidation is myeloperoxidase, a heme protein abundant in phagocytes (9, 10). Myeloperoxidase uses H_2O_2 generated by activated monocytes, macrophages, and neutrophils as a substrate for oxidative reactions in the extracellular milieu. Binding of H_2O_2 to its heme group generates a ferryl π-cation radical that is reduced to the native state by oxidation of halides and other compounds (9, 10). A potential substrate for oxidation is L-tyrosine, a phenol present at 100–200 μM in human blood plasma. Chemical and photoexcitation studies have identified the initial product of L-tyrosine oxidation as tyrosyl radical (11–13). Phenolic coupling of tyrosyl radical yields *o,o'*-dityrosine (11, 12, 14, 15):



Dityrosine thus serves as a useful marker for tyrosine oxidation.

We have recently shown that myeloperoxidase converts L-tyrosine to dityrosine by a reaction that requires H_2O_2 but not halide (16). Dityrosine synthesis by myeloperoxidase may be physiologically relevant because it occurs at plasma concentrations of chloride and amino acids (16). In this study we use horseradish peroxidase, a well-characterized heme protein, as a model to explore the potential role of tyrosyl radical in the oxidative modification of HDL.

METHODS

Lipoproteins. HDL_3 (d = 1.125–1.210 g/ml, hereafter referred to as HDL) and LDL (d = 1.019–1.063 g/ml) were isolated from pooled plasma of healthy male volunteers by ultracentrifugation (17). HDL was subjected to heparin-Sepharose chromatography to remove particles containing apolipoproteins B and E (18). Acetyl-LDL was prepared by reaction of LDL with acetic anhydride (19).

Modification of HDL by Peroxidase. Reactions were carried out at 37°C for 24 h in buffer A [phosphate-buffered saline (PBS) supplemented with 100 μM diethylenetriamine pentaacetic acid, pH 8.0] under conditions where HDL oxidation was a linear function of enzyme concentration. Buffer A was passed over Chelex 100 resin (Bio-Rad) to remove transition metal ions potentially able to catalyze lipoprotein oxidation and diethylenetriamine pentaacetic acid was included to inhibit metal-ion-catalyzed oxidation reactions. The reaction

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Abbreviations: ACAT, acyl-CoA:cholesterol acyltransferase; BSA, fatty acid-free bovine serum albumin; HDL, high density lipoprotein; LDL, low density lipoprotein.

†Present address: Massachusetts General Hospital East, Cardiovascular Research Center, 149 13th Street, Charlestown, MA, 02129.
§To whom reprint requests should be addressed at: Division of Atherosclerosis, Nutrition and Lipid Research, Box 8046, 4566 Scott Avenue, St. Louis, MO 63110.

mixture contained HDL (1 mg/ml), 100 nM horseradish peroxidase (Boehringer Mannheim), 100 μM H_2O_2, and 100 μM L-tyrosine. Prior to analysis or cell studies, HDL was subjected to size-exclusion chromatography on a 10-DG column (Bio-Rad) equilibrated with buffer A or Dulbecco's modified Eagle's medium (DMEM; GIBCO/BRL) supplemented with 25 mM *N*-2-hydroxyethylpiperazine-*N'*-2'-ethanesulfonic acid (pH 7.5). Protein-associated dityrosine production was monitored by fluorescence using excitation and emission wavelengths of 328 nm and 410 nm, respectively (20, 21).

Analysis of Peroxidase-Modified HDL. SDS/PAGE was performed using 7–20% polyacrylamide gradient gels under nonreducing conditions as described (22). Immunoblot analysis was carried out with polyclonal antibodies monospecific for apolipoproteins A-I or A-II (23). Nondenaturing gradient gel electrophoresis was performed as described (24). Agarose gel electrophoresis was performed with Paragon Lipo gels (Beckman) by the manufacturer's instructions. Lipid composition of HDL was determined enzymatically (25).

HDL apolipoproteins were isolated after size-exclusion chromatography by precipitation with ice-cold 20% (wt/vol) trichloroacetic acid and delipidation with diethyl ether. Apolipoproteins (10 mg) were hydrolyzed by exposure to HCl vapor at 110°C for 24 h in argon-flushed reaction vials containing 6 M HCl with 1% phenol and then subjected to cellulose phosphate chromatography (20, 21). Amino acid analysis was performed on a Beckman model 7300 analyzer using an expanded sodium buffer system.

Cultured Cells. Human skin fibroblasts were cultured as described (24). Resident peritoneal macrophages were isolated from male Swiss–Webster mice (25–30 g; Tyler Laboratories, Bellevue, WA) by peritoneal lavage (26) and plated at 2×10^6 cells per 35-mm dish. Macrophages were cultured for 6–7 days in DMEM supplemented with 20% (vol/vol) fetal bovine serum, penicillin (100 units/ml), and streptomycin (100 μg/ml) and then used for experiments.

Cholesterol Efflux from Sterol-Loaded Fibroblasts. Confluent fibroblasts were cholesterol loaded by incubation with cholesterol (50 μg/ml) for 48 h in DMEM containing fatty acid-free bovine serum albumin (BSA; Sigma; 2 mg/ml) (27). After a 24-h equilibration in DMEM with BSA (1 mg/ml), the cells were incubated for 16 h in the same medium containing the indicated final concentration of HDL protein. Cells were then washed once with PBS, and acyl-CoA:cholesterol acyltransferase (ACAT) activity was estimated by incorporation of [^{14}C]oleate into ^{14}C-labeled cholesteryl ester (28). Radiolabel comigrating with cholesteryl oleate by TLC on silica gel was quantified by scintillation counting (28). Endogenous sterol synthesis was determined by [^{14}C]acetate incorporation into [^{14}C]sterol (29).

Coincubation of Fibroblasts with LDL and HDL. Fibroblasts (80% confluent) were incubated for 48 h with DMEM containing 10% lipoprotein-deficient serum to upregulate LDL receptor activity. Cells were then exposed for 24 h to DMEM containing BSA (1 mg/ml), LDL protein (50 μg/ml), and the indicated final concentration of HDL protein. After three washes with PBS, cellular lipids were extracted and separated by TLC (as above). Cholesteryl ester mass and free cholesterol mass were assayed as described (30).

Cholesterol Efflux from Macrophage Foam Cells. Mouse peritoneal macrophages were cholesterol-loaded using acetyl-LDL (50 μg/ml) in DMEM containing BSA (0.5 mg/ml) for 24 h. After a 24-h equilibration period in DMEM with BSA (1 mg/ml), macrophages were incubated in the same medium containing the indicated final concentration of HDL protein for 16 h. After three washes with PBS, cellular cholesteryl ester and free cholesterol mass were determined.

Other Assays. Dityrosine was prepared as described (21). Protein was measured by the Lowry method (31) using BSA as the standard.

RESULTS

Dityrosine is an intensely fluorescent amino acid that is stable to acid hydrolysis (20, 21). To determine whether HDL apolipoproteins exposed to tyrosyl radical undergo the phenolic coupling reaction, we exposed HDL to horseradish peroxidase, H_2O_2, and L-tyrosine in buffer A at 37°C in air. At the end of the incubation, we reisolated HDL by size-exclusion chromatography and monitored its fluorescence at the excitation and emission wavelengths characteristic of dityrosine. HDL modified by the complete system became markedly more fluorescent. The pH dependence of the fluorescence (16, 20) indicated that ≈90% of the fluorescence was likely to be due to dityrosine. We therefore used this property to characterize the reaction. HDL oxidation was linear for 4 h and continued for 24 h. Dityrosine synthesis was maximal at 100 μM H_2O_2. Higher peroxide concentrations inhibited the reaction, perhaps through enzyme inactivation or substrate inhibition. HDL fluorescence increased modestly in the absence of L-tyrosine, presumably due to direct oxidation of tyrosyl residues by horseradish peroxidase (32). Physiological concentrations of L-tyrosine (50–200 μM) in the reaction mixture markedly stimulated dityrosine formation.

HDL modified by the peroxidase/L-tyrosine/H_2O_2 (complete) system demonstrated crosslinked apolipoproteins on analytical SDS/PAGE under nonreducing conditions (Fig. 1*A*). There were prominent bands at apparent molecular masses of 47 kDa, 61 kDa, and 83 kDa, suggesting the formation of apolipoprotein A-I–$(A\text{-II})_2$ complexes, apolipoprotein A-I dimers, and apolipoprotein A-I trimers, respectively. Immunoblot analysis with anti-apolipoprotein A-I and anti-apolipoprotein A-II antibodies supported this analysis (data not shown). A modest increase in the apparent molecular masses of apolipoproteins A-I and A-II was also observed (Fig. 1*A*), perhaps due to covalent bonding between L-tyrosine and apolipoprotein. Catalase or heme protein inhibitors blocked dityrosine formation and apolipoprotein crosslinking. Peroxidase-modified HDL migrated with the same apparent molecular mass as control HDL on nondenaturing gradient gel electrophoresis (Fig. 1*B*); nonequilibrium gels showed no evidence of smaller pre-β-HDL particles or free apolipoproteins. On agarose gel electrophoresis, perox-

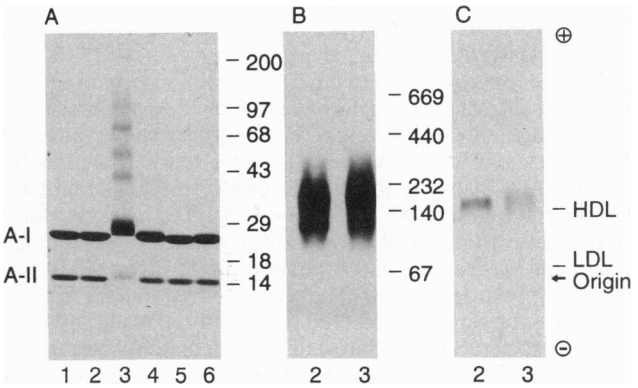

FIG. 1. SDS/PAGE (*A*), nondenaturing gradient gel electrophoresis (*B*), and agarose gel electrophoresis (*C*) of peroxidase-modified HDL. Lanes: 1, HDL incubated at 4°C for 24 h in buffer A; 2, HDL incubated at 37°C for 24 h in buffer A; 3, HDL modified by incubation at 37°C for 24 h with complete peroxidase/H_2O_2/L-tyrosine system; 4, complete system without L-tyrosine; 5, complete system without peroxidase; 6, complete system plus catalase (5 nM). Molecular mass and lipoprotein standards are indicated.

idase-modified HDL migrated slightly more rapidly than HDL (Fig. 1*C*). Low levels of conjugated dienes, a marker for early lipid peroxidation, were detected in crosslinked HDL. Similar levels were found in HDL modified by peroxidase in the absence of L-tyrosine, but there was little evidence of apolipoprotein crosslinking (Fig. 1*A*) or increased HDL-associated fluorescence. Chemical analysis of peroxidase-modified HDL showed little change in free cholesterol, cholesteryl ester, phospholipid, or triglyceride composition.

To test further the hypothesis that tyrosyl radicals modify HDL by inducing dityrosine crosslinking of its apolipoproteins, HDL was incubated with peroxidase, H_2O_2, and L-[^{14}C]tyrosine. After gel filtration, acid precipitation, and delipidation with diethyl ether, HDL apolipoproteins were acid-hydrolyzed. The resulting amino acids were analyzed by cellulose phosphate chromatography, which completely separates dityrosine from trityrosine and other fluorescent amino acids (20, 21). A major peak of radioactivity coeluted with a fluorescent reaction product (Fig. 2*A*). When authentic dityrosine was added to the hydrolysate, it also eluted with the radioactive peak. The excitation and emission spectra of the isolated reaction product were virtually identical with those of *o,o'*-dityrosine at acid, neutral, and alkaline pH values (Fig. 2 *B* and *C*). Amino acid analysis of the hydrolysate revealed a new peak that comigrated with dityrosine. These results indicate that HDL modified by the peroxidase/L-tyrosine/H_2O_2 system undergoes phenolic coupling of its apolipoprotein tyrosines.

Based on the increase in fluorescence, we estimate that ≈2.5% of the tyrosyl residues in peroxidase-modified HDL were converted to dityrosine. Since L-tyrosine represents 7 of 243 and 4 of 77 of the residues in apolipoproteins A-I and A-II, respectively, the changes in apparent molecular mass of the apolipoproteins seen on SDS/PAGE (Fig. 1*A*) may reflect other reactions. Indeed, crosslinking of the sea urchin fertilization envelope by peroxidase generates dityrosine, trityrosine, and pulcherosine (33, 34), and we have observed four products in L-tyrosine oxidized by myeloperoxidase (16). Multiple L-[^{14}C]tyrosine peaks were found in the acid hydolysate of HDL apolipoproteins (Fig. 2*A*). Moreover, dityrosine accounted for only 20% of the L-[^{14}C]tyrosine incorporated into HDL apolipoproteins. Thus, protein tyrosylation is the major reaction, and other tyrosine oxidation products are likely to be present in peroxidase-modified HDL.

To determine how peroxidase modification of HDL affects its ability to promote cholesterol efflux from cells, we exposed cholesterol-loaded human skin fibroblasts to tyrosylated HDL and then measured synthesis of cholesteryl ester. Depletion of an intracellular regulatory pool of free cholesterol would inhibit cholesterol esterification but stimulate cholesterol synthesis (35). Tyrosylated HDL was more potent than control HDL at blocking cholesterol esterification (Fig. 3*A*). The apparent K_m for inhibition of ACAT activity was 1.5 ± 0.4 μg/ml (mean ± SD, three experiments) for tyrosylated HDL and 24.0 ± 5.8 μg/ml for unmodified HDL. HDL incubated with heat-inactivated peroxidase was little affected, and HDL exposed to peroxidase and H_2O_2 in the absence of L-tyrosine was a less potent inhibitor of cholesterol esterification than control HDL, possibly due to nonspecific oxidative damage to the lipoprotein. HDL modified by the peroxidase system in the presence of aminotriazole or catalase failed to further inhibit cholesterol esterification compared with control HDL. Thus, inhibition of ACAT activity in sterol-loaded fibroblasts, like the crosslinking reaction, required HDL that had been modified by active peroxidase, L-tyrosine, and H_2O_2. We attributed decreased esterification to shrinkage of the free cholesterol substrate pool(s) for ACAT rather than to inhibition of ACAT by oxidation products because neither preexposure of cells to tyrosylated HDL nor inclusion of tyrosylated HDL in the reaction mixture affected ACAT activity in fibroblast detergent lysates supplied with exogenous cholesterol.

It is unlikely that tyrosylated HDL adversely affects cell viability. Fibroblasts incubated with serum-free medium, control HDL, or tyrosylated HDL showed identical trypan blue exclusion, incorporation of [^{14}C]oleate into [^{14}C]triacylglycerols, and protein content. Moreover, tyrosylated HDL was a more potent stimulator of [^{14}C]acetate incorporation into cellular [^{14}C]cholesterol than was HDL (Fig. 3*B*). This increased cholesterol synthesis and the decrease in cholesterol esterification strongly suggest that tyrosylated HDL depletes fibroblasts of excess cholesterol more effectively than HDL.

To investigate the ability of tyrosylated HDL to prevent the accumulation of lipoprotein-derived cholesterol, we ex-

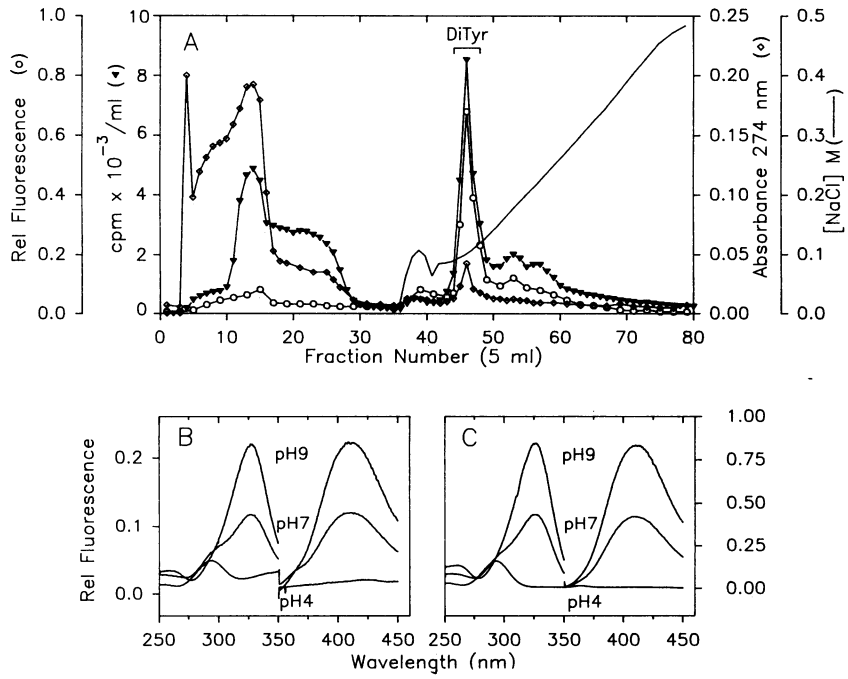

FIG. 2. Cellulose phosphate chromatography (*A*) and fluorescence excitation and emission spectra (*B*) of acid hydrolysate of peroxidase-modified HDL apolipoproteins. (*A*) HDL was incubated with peroxidase, H_2O_2, and 100 μM L-[^{14}C]tyrosine for 24 h. After isolation by size-exclusion chromatography, precipitation with trichloroacetic acid, and delipidation with diethyl ether, HDL apolipoproteins (10 mg) were hydrolyzed by HCl vapor at 110°C and then chromatographed on a 0.8 cm² × 12 cm cellulose phosphate column. The column was washed with 150 ml of 0.2 M acetic acid and material was eluted at 1 ml/min with a 250-ml linear NaCl gradient (0.0–0.5 M) in 0.2 M acetic acid. Fluorescence was measured after adjusting the pH of each fraction to 8.0 (○). Radioactivity (▼), A_{274} (◇), and salt concentration (solid line) were also monitored. L-Tyrosine and authentic dityrosine were eluted in the column flow-through and at the indicated ionic strength (DiTyr), respectively. (*B*) Amino acids eluting at the same ionic strength as dityrosine from a cellulose phosphate column were subjected to fluorescence spectroscopy at the indicated pH values with λ_{ex} at 328 nm and λ_{em} at 410 nm, respectively. (*C*) Fluorescence excitation and emission spectra of authentic dityrosine at the indicated pH values. Rel, relative.

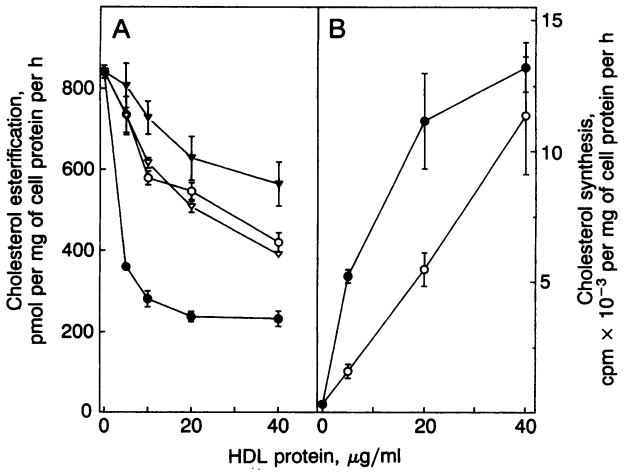

FIG. 3. Cholesterol esterification and cholesterol synthesis in human skin fibroblasts incubated with tyrosylated HDL. (*A*) Confluent cultures of fibroblasts loaded with nonlipoprotein cholesterol were incubated with the indicated final concentration of HDL. After a 16-h incubation at 37°C, cells were washed and incubated for 1 h with medium containing 9 μM [^{14}C]oleate bound to 0.2% BSA at 37°C. Cellular lipids were extracted, separated by TLC, and assayed for cholesteryl [^{14}C]oleate. (*B*) After a 24-h incubation with HDL, endogenous cholesterol synthesis was measured by exposing fibroblasts to 43 μM [^{14}C]acetate in DMEM containing BSA (1 mg/ml) for 2 h. Cellular lipids were extracted, separated by TLC, and assayed for [^{14}C]cholesterol. ○, Control HDL; ●, HDL modified with peroxidase, H_2O_2, and L-tyrosine; ▽, HDL incubated in the complete system with heat-inactivated peroxidase; ▼, HDL incubated in the complete system minus tyrosine. Each value is the mean ± SD of three determinations.

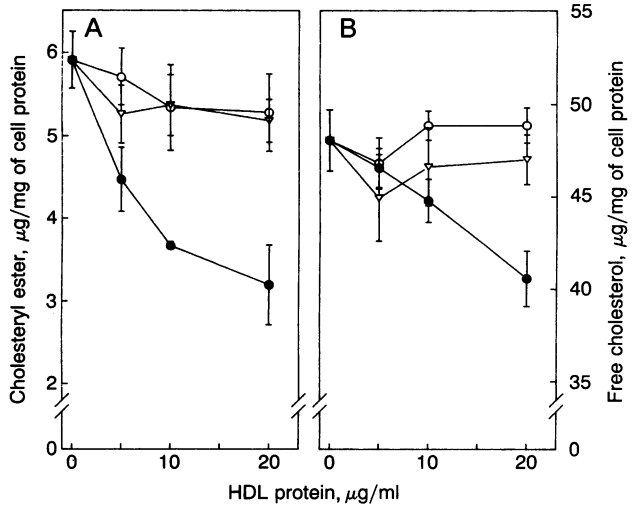

FIG. 4. Cholesterol mass in human skin fibroblasts coincubated with LDL and tyrosylated HDL. Fibroblasts (80% confluent) were incubated for 48 h with 10% lipoprotein-deficient serum to upregulate LDL receptor activity. Cells were then incubated for 24 h in DMEM containing BSA (1 mg/ml), LDL protein (50 μg/ml), and the indicated final concentration of lipoprotein. ○, Control HDL; ●, HDL modified by peroxidase, H_2O_2, and L-tyrosine; ▽, HDL modified by the complete system with heat-inactivated peroxidase. After the incubation, lipids were extracted from washed cells, separated by TLC, and then assayed for cholesteryl ester mass (*A*) and free cholesterol mass (*B*).

posed human fibroblasts with upregulated LDL receptor activity to both LDL and either tyrosylated HDL or control HDL and then measured free cholesterol and cholesteryl ester mass. Under these conditions, LDL delivers cholesterol to cells by receptor-mediated endocytosis (35), whereas HDL removes excess free cholesterol, probably from a pool(s) that regulates ACAT activity, thereby reducing cholesteryl ester formation (28, 35). After a 24-h incubation with LDL and increasing concentrations of tyrosylated HDL, fibroblast cholesteryl ester mass progressively declined (Fig. 4). HDL and HDL incubated with heat-inactivated peroxidase were much less effective at preventing cholesteryl ester accumulation. Free cholesterol mass in fibroblasts incubated with tyrosylated HDL at 20 μg/ml decreased by 5.0 ± 3.7 μg/mg (mean ± SD, three experiments). In contrast, control HDL produced a slight increase (0.6 ± 1.6 μg/mg) in free cholesterol mass.

To test the potential role of tyrosylated HDL in removing cholesterol from macrophage foam cells, we cholesterol-loaded mouse peritoneal macrophages by incubation with acetyl-LDL. A subsequent 16-h incubation with tyrosylated HDL decreased the macrophage cholesteryl ester mass (Fig. 5). Tyrosylated HDL was more potent than HDL at depleting the cells of esterified cholesterol, most notably at low lipoprotein concentrations where control HDL had no significant effect. In two experiments using lipoprotein at 10 μg/ml, tyrosylated HDL decreased macrophage cholesteryl ester levels by 46%, whereas control HDL induced a decrease of only 12%. With tyrosylated HDL at 40 μg/ml, free cholesterol also decreased significantly (5.49 ± 0.98 μg/mg, mean ± SD) compared with cells exposed to control HDL (1.36 ± 2.1 μg/mg).

DISCUSSION

Our results indicate that horseradish peroxidase can oxidize HDL apolipoprotein tyrosyl residues to dityrosine. The phenolic coupling reaction is independent of free metal ions but requires L-tyrosine, implying that tyrosyl radical is a diffusible catalyst that conveys oxidizing potential from the active site of the heme enzyme to protein tyrosyl residues. The mechanism of protein dityrosine formation is thus likely to be independent of the peroxidase used to oxidize L-tyrosine. The proposed intermediate in the crosslinking reaction—tyrosyl radical—might undergo several reactions. (*i*) The selectively reactive tyrosyl radical might crosslink with protein radicals generated from tyrosine or other amino acids, yielding tyrosylated protein. This may explain in part the modest increase in apparent molecular masses of apolipo-

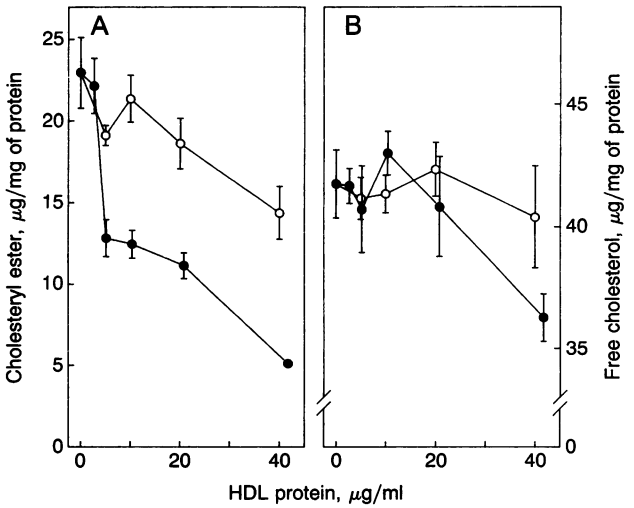

FIG. 5. Cholesterol mass in macrophage-derived foam cells exposed to tyrosylated HDL. Mouse peritoneal macrophages were cholesterol-loaded by incubation with acetyl-LDL (50 μg/ml). After a 24-h equilibration period, the cells were incubated for 16 h with the indicated final concentration of HDL (○) or HDL modified by peroxidase, L-tyrosine, and H_2O_2 (●). Cellular lipids were extracted and assayed for cholesteryl ester mass (*A*) and free cholesterol mass (*B*).

proteins A-I and A-II observed in tyrosylated HDL subjected to SDS/PAGE. (*ii*) Two protein tyrosyl radicals might productively interact to form intra- or intermolecular dityrosine crosslinks. The detection of both apolipoprotein A-I multimers and apolipoprotein A-I–(A-II) complexes in tyrosylated HDL is consistent with this suggestion. (*iii*) Tyrosyl radical might generate other protein and lipid oxidation products, including trityrosine and pulcherosine (20, 21, 34).

One hypothesis to explain the ameliorative effects of HDL on atherogenesis is that HDL promotes efflux of excess sterol from the artery wall into the blood, where cholesterol is esterified and transported to the liver for excretion (36). The observation that cholesterol-loaded cultured cells exposed to HDL exhibit a concentration- and time-dependent decrease in both cholesteryl ester mass and ACAT-catalyzed cholesterol esterification (29, 37) is consistent with this proposal. Reduced cholesteryl ester formation presumably reflects depletion of an intracellular regulatory pool of free cholesterol (35).

Several lines of evidence indicate that tyrosylated HDL enhances sterol removal from this intracellular regulatory pool(s). (*i*) Tyrosylated HDL was more effective than HDL at inhibiting cholesterol esterification in both human skin fibroblasts and mouse macrophage foam cells. (*ii*) Tyrosylated HDL was more potent than HDL at stimulating cholesterol synthesis, monitored as the incorporation of [^{14}C]acetate into [^{14}C]cholesterol. (*iii*) Cholesteryl ester mass decreased more in fibroblasts and macrophages exposed to low concentrations of tyrosylated HDL than in those exposed to HDL. At high tyrosylated HDL concentrations, intracellular free cholesterol also decreased, indicating that oxidized lipoprotein does not inhibit ACAT nonspecifically. These results suggest that tyrosylated HDL promotes cholesterol efflux from human skin fibroblasts and mouse macrophage foam cells more effectively than does HDL.

The mechanism underlying enhanced cholesterol efflux is unexplained, but it may involve changes in the conformation of HDL apolipoproteins or in the chemical potential for cholesterol diffusion (38). Phenolic coupling of apolipoproteins may facilitate interactions with regulatory domains of plasma membrane lipids (39) or putative HDL receptor proteins (28). We cannot exclude the possibility that oxidized lipids in tyrosylated HDL might regulate intracellular cholesterol trafficking (28). Tyrosylated HDL subjected to nondenaturing gradient gel electrophoresis exhibited no evidence of release of free apolipoproteins or significant alteration in particle size. Oxidative tyrosylation is thus clearly different both biochemically and biologically from the nitrosylation of tyrosyl residues produced by tetranitromethane, which induces lipoprotein aggregation and extensive crosslinking of HDL apolipoproteins and impairs HDL-mediated cholesterol efflux (24).

The physiological significance, if any, of the peroxidase-dependent oxidation of HDL tyrosines is unknown. We have recently found that myeloperoxidase can also catalyze the phenolic coupling of free L-tyrosine to dityrosine (16) and protein-dityrosine crosslinking (40). The reactions occur at plasma concentrations of chloride and L-tyrosine and can be executed by activated human neutrophils and macrophages (16, 40), suggesting that they might take place *in vivo*. This raises the possibility that the accumulation of sufficient numbers of myeloperoxidase-secreting phagocytes might help protect arterial wall cells against pathological cholesterol accumulation. Oxidized LDL, which induces monocyte chemoattractant protein(s) on endothelial cells (41) and converts cultured macrophages into lipid-laden foam cells (1–3), may play a pivotal role early in atherogenesis. We speculate that tyrosylation of HDL by phagocytes may counter the damaging effects of LDL oxidation, explaining in part HDL's ability to hinder the development of atherosclerotic lesions.

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