Malondialdehyde Modification of Lipoprotein(a) Produces Avid Uptake by Human Monocyte-Macrophages

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Increased plasma levels of the apoB-100-containing lipoprotein(a) (Lp(a)) are associated with an increased risk for atherosclerosis and myocardial infarction, but the mechanisms by which lipoprotein(a) may accelerate these processes remain obscure. In this study we have investigated the impact of the association of apoprotein(a) with the low density lipoprotein (LDL)-like Lp(a) particle upon specificity of receptor recognition after lipoprotein modification by malondialdehyde or transition metal-induced oxidation. We have determined that radioiodination labels both apoprotein components of Lp(a), that malondialdehyde modification produces an anionic lipoprotein comparable to native Lp(a) in Stokes' radius, and that N,N'-disubstituted 1-amino-3-iminopropene derivatives preferentially cross-link apoprotein(a) to apoB-100 protein. Like LDL, native Lp(a) is recognized in human monocyte-macrophages by the LDL receptor. Like LDL, progressive modification of Lp(a) by malondialdehyde abolishes lipoprotein recognition by the LDL receptor and produces uptake and hydrolysis by the scavenger receptor of human monocyte-macrophages. We propose that intimal retention of Lp(a) by extracellular components of the atherosclerotic reaction places the lipoprotein in a microenvironment favoring subsequent peroxidative modification. The chronic production of lipid peroxide-modified Lp(a) together with unmitigated cellular clearance by scavenger receptors may contribute to the accumulation of lipoprotein-derived lipid in macrophage-derived foam cells of the atherosclerotic reaction.

It is widely appreciated that increased plasma levels of lipoprotein(a) (Lp(a))† are associated with an increased risk for cardiovascular disease (1). Lp(a) resembles low density lipoprotein (LDL), the major carrier of plasma cholesterol, and contains 1 mol of apoB-100 protein per particle; it is distinguished from LDL by 1 or 2 mol of apoprotein(a) that are associated, presumably through disulfide linkage, with the apoB-100 protein (2-4). Apoprotein(a) demonstrates striking sequence homology to plasminogen (5, 6), and mediates interaction of Lp(a) with plasminogen cell surface sites on endothelial cells and monocytes (7-9). These and other studies have implicated Lp(a) as playing a prothrombotic role by interfering with the physiological functions of plasminogen.

The mechanism by which Lp(a) contributes to the acceleration of atherosclerosis, however, remain obscure. In part this can be attributed to controversy over the pathways that mediate cellular uptake of Lp(a) and might account for formation of macrophage-derived foam cells. Studies in human fibroblasts (10, 11) and human monocytes (12) in vitro, as well as transgenic mice expressing high levels of LDL receptors in vivo (13), have demonstrated uptake of Lp(a) by the LDL receptor. The plasma levels of Lp(a) in human subjects, however, remain inexplicably constant during drug therapies which effectively reduce plasma LDL through modulation of the LDL receptor (14). Adding further to the perplexity of cellular clearance of Lp(a), studies in the murine macrophage cell line P388D1 (15) have reported interaction of Lp(a) as well as recombinant apoprotein(a) with the scavenger receptor. The scavenger receptor, which is genetically and structurally distinct from the LDL receptor (16), has been implicated as a pathway for clearance of lipid peroxide-modified (oxidized) LDL in atheroma. The ability of scavenger receptors in the reticuloendothelial system to mediate rapid clearance (t1/2, 5 min) of ligands from the plasma (17), however, makes it difficult to reconcile persistence of elevated levels of Lp(a) with scavenger receptor-dependent removal as a physiologically relevant mechanism.

The role of Lp(a) modification in atherogenesis has yet to be examined. Accumulating evidence supports the proposal made by Fogelman et al. (18) in 1980 that modification of native LDL by malondialdehyde and other lipid peroxides released in situ may be a prerequisite to accumulation of lipoprotein-derived cholesterol by cells of the atherosclerotic reaction. We have previously demonstrated that malondialdehyde efficiently converts LDL to a form that produces intracellular cholesteryl ester accumulation in vitro (18) owing to recognition by the scavenger receptor of human monocyte-macrophages (19), and that malondialdehyde-modified LDL is selectively present in atheroma of WHHL rabbits in vivo (20). Malondialdehyde is a lipid peroxide product released during prostanoïd metabolism as well as chemical decomposition of polyunsaturated lipids (21, 22), and reacts with the positively charged ε-amino group of apoB-100 protein lysyl residues in a 1:2 molar ratio to form neutral intramolecular, N,N' -disubstituted 1-amino-3-iminopropene cross-links (19). In this study we have investigated the impact of the association of apoprotein(a) components with the LDL-like Lp(a)
particle upon specificity of receptor recognition after lipoprotein modification by malondialdehyde. We now demonstrate that malondialdehyde modification of Lp(a) induces avid uptake by the scavenger receptor of human monocyte-macrophages and that the parent native Lp(a) produces recognition by the LDL receptor.

**EXPERIMENTAL PROCEDURES**

**Materials.** Sodium $[^{251}]$iodide (15 mCi/µg) was purchased from Amersham Corp. All other supplies and reagents were obtained from sources previously reported (19, 23).

**Subjects.** Normal subjects at the University of California, Los Angeles, were recruited from the staff and student body as donors for blood donors, and Lp(a) was isolated as described (4). Previous studies at the University of Chicago served as donors for Lp(a). No one received drugs that might have affected serum cholesterol levels or lipid metabolism. All had normal hematocrits, leukocytes, and differential counts, serum cholesterol, and triglycerides. Informed consent was obtained in writing from each person at both institutions.

**Monocyte Isolation and Culture.** 500 ml of blood was taken after an overnight fast, and the monocytes were separated from 300 ml of venous blood by a modification of the Recalde method (24). Autologous serum for cell culture was prepared from the remaining 200 ml of blood as described (24). Human monocytes were suspended in 30% autologous serum in Iscove's modified Dulbecco's medium supplemented with 2 mM glutamine, 8 µg of insulin/ml, and 0.25 µg of fungizone/ml (designated medium B). Samples (0.5 ml) of the cell suspension containing 0.5 × 10⁶ cells were transferred to 2.0-cm² polystyrene wells and incubated at 37°C in a humidified, 5% CO₂ incubator. The medium was aspirated and replaced with fresh medium of the same composition twice weekly.

**Classification and Viability of Cells.** The cells were classified and their viability was determined as described (24). Because of the media changes and the washes prior to each experiment, the cells were 99% monocyte-macrophages before the radioactive lipoproteins were added. More than 95% of the cells were viable at the conclusion of experimentation.

**Lipoprotein Isolation.** Human LDL (d 1.019-1.063 g/ml) was isolated from the serum of individual fasted human subjects by ultracentrifugation (25), dialyzed against 0.1 M sodium phosphate, pH 7.4, containing 0.15 M sodium chloride and 0.01% EDTA (buffer A), and stored at 4°C.

Human Lp(a) was isolated at the University of Chicago from the plasma of individual fasted human subjects by sequential steps of ultracentrifugation, lysine-Sepharose chromatography, and density gradient ultracentrifugation in cesium chloride (12). Lp(a) preparations were dialyzed against 0.15 M sodium chloride, pH 7.4, containing 0.01% EDTA and 0.01% sodium azide, filter sterilized (0.45 µm), and stored at 5°C in vials filled to allow no airspace. Aliquots were removed with sterile tips, dialyzed against buffer A, and filtered (0.2 µm) (Acrodisc, Gelman) just before initiation of the cellular assay.

**Chemical Modification of Lipoproteins.** Labeling of LDL and Lp(a) with $[^{125}]$I was performed by the method of McFarlane (26) as modified by Bilheimer et al. (27). Modification of LDL (5 mg of protein/ml) by 100 mM malondialdehyde was conducted for 3 h at 37°C as previously described (19). Modification of Lp(a) (0.8 mg of protein/ml) by malondialdehyde was routinely conducted by the same protocol at a final concentration of 67 mM malondialdehyde. Acetylated LDL was produced by derivatization with acetic anhydride (28). Copper oxidation of LDL (22 h) and Lp(a) (17 h) was conducted essentially as described (29). Specifically, radioiodinated Lp(a) (0.1 mg of protein/ml) by malondialdehyde was dialyzed against EDTA-free phosphate-buffered saline, pH 7.2, and then was subjected to oxidation by 5 µM cupric sulfate in phosphate-buffered saline, pH 7.2, by incubation for 3.5, 17, and 22 h in plastic tissue culture dishes at 37°C in a humidified 5% CO₂ incubator. The reaction was quenched by addition of 0.02% EDTA and 0.22 M butylated hydroxytoluene followed by dialysis at 4°C against buffer A. Samples incubated for 3.5 and 17 h remained soluble as evidenced by full recovery of radioactivity, whereas the samples at 22 h demonstrated visible precipitation and loss of radioactivity in the supernatant. The conjugated diene levels were determined by absorbance at 233 nm and concentration calculated by a molar extinction coefficient of 27,500 (30).

All modified lipoproteins were immediately dialyzed at 5°C against buffer A. Lipoprotein solutions were sterile filtered (0.2 µm) upon completion of dialysis and stored at 4-5°C. Aliquots were removed for experimentation with sterile tips and filtered (0.2 µm) just before initiation of the cellular assays.

**Assay of Chemical Modification.** The malondialdehyde adducts of LDL and Lp(a) were quantitated by coelectrophoresis at 55% with thiobarbituric acid as described (19). Molar ratios were calculated assuming a total molecular weight of 51,500 for the apoB-100 protein of LDL (31). The total molecular weight of the protein components of Lp(a) was calculated as the sum of 1 mol of apoB-100 protein and 2 mol of apoprotein(a) isoform (assuming 28.1% carbohydrate, w/w) per mol of Lp(a) (3, 4). The molecular weights of the glycosylated apoprotein(a) isoforms, determined by sodium dodecyl sulfate-gel electrophoresis in 2-16% gels (Pharmacia LKB Biotechnology Inc.) with cross-linked phosphorlyase a as standard, have been previously described as follows: donor L. C., 280,000; donor L. T., 400,000 (4). Concentrations of protein samples used in cellular assay, given in molar terms, were calculated from the molecular weights given above and the protein concentrations determined by the method of Lowry et al. (32) with bovine plasma albumin as a standard.

**Cellular Assays.** Before initiating studies, cells in culture were washed three times with a 1-ml volume of Dulbecco's modified Eagle's medium containing 10 mM HEPES (medium C). For hydrolytic studies at 37°C, radioactive proteins were added to the cells together with a final concentration of 0.1% (w/w) bovine serum albumin (Pentax, Behring Diagnostics) in Iscove's modified Dulbecco's medium supplemented with 10% fetal calf serum. The cells were returned to the incubator for the indicated times. The proteolytic degradation of $[^{125}]$I-labeled protein was measured by assaying the amount of $[^{125}]$I-labeled trichloroacetic acid-soluble (noniodide) material formed by the cells and excreted in the culture medium as described for Goldstein and Brown (33). Concentrations of protein samples used in cellular assay, given in molar terms, were calculated from the small amounts (<0.01% of total radioactivity added) of $[^{125}]$I-labeled acid-soluble material that was formed in parallel incubations without cells. The protein content of cells solubilized by 0.1 M sodium hydroxide was measured by the method of Lowry et al. (32) with bovine plasma albumin as a standard. Statistical analyses were conducted by methods previously described (34).

**Heparin-Sepharose Chromatography.** Heparin-Sepharose was prepared from CBB-activated Sepharose 4B (Pharmacia) as previously described (19) and chromatography conducted as described by Armstrong et al. (11).

**Electrophoretic and Immunoblot Analyses.** Lipoproteins, containing 1 µg of protein, were electrophoresed on agarose gel (Corning Universal) as described by Noble (34) at 80 volts for 35 min at 10°C. The plastic backing was peeled off and electrophoretic transfer of lipoproteins from the agarose gel to Immobilon membrane (Millipore, Bedford, MA) was conducted as described by Towbin et al. (35) in a Bio-Rad Trans-Blot apparatus for 1 h at 230 volts and -15°C. Radioiodinated lipoproteins were visualized by exposure to XAR-5 x-ray film (Eastman Kodak, Rochester, NY) with an intensifying screen at -70°C. To visualize nonradioactive lipoprotein, a 0.22 µM tetrazolium chloride (Bethesda Research Laboratories) solution containing 1-2 µg of radioiodinated protein, were heated 5°C with rabbit anti-human Lp(a) antibody (1:100 dilution) (36). The blot was washed thoroughly and incubated with $[^{125}]$I-labeled donkey anti-rabbit Ig (0.6 mCi/ml) (Amersham) for 2 h at 22°C. Radiolabeled immune complexes were visualized as described above.

Aliquots of pooled fractions from heparin-Sepharose chromatography, containing 1-2 µg of radioiodinated protein, were heated 5 min at 90°C in a solution containing 0.1 M dithiothreitol, 1% sodium dodecyl sulfate, 0.09 M Tris-HCl, pH 6.8, and electrophoresed on 3-10% gradient polyacrylamide slab gels containing 0.1% sodium dodecyl sulfate as described by Laemmli (37). Electrophoretic transfer of lipoproteins to Immobilon membrane was conducted in a Bio-Rad Trans-Blot apparatus for 1 h at 60 volts and -15°C. Blots were incubated for 3 days at 5°C with 5% bovine serum albumin (BSA) in 0.1 M Tris-HCl, pH 8.0, 0.1 M NaCl, 0.05% bovine serum albumin, and 0.05% sodium azide. After incubation for 2 h at 22°C with rabbit anti-human Lp(a) antiserum (1:200 dilution) (38), the blot was washed thoroughly and incubated with $[^{125}]$I-labeled donkey anti-rabbit Ig (0.6 mCi/ml) (Amersham) for 2 h at 22°C. Radiolabeled immune complexes were visualized as described above.

**Virus Infection.** We now demonstrate that monocyte-macrophages before the radioactive lipoproteins were added. More than 95% of the cells were viable at the conclusion of experimentation.
RESULTS

The physicochemical and biochemical characterizations of Lp(a) preparations in this report have previously been described in detail (4). Several additional parameters relevant to our cell biology studies were examined. First, the distribution of radiolabeled mono-[125I]-iodotyrosine between apoB-100 protein and apoprotein(a) was determined. Dissociation of apoprotein(a) from the native Lp(a) particle to generate apoB-100 containing Lp(-a) (4) was achieved by reduction with the sulphydryl reagent dithiothreitol (11). The specific radioactivity of Lp(a-) isolated by heparin-Sepharose chromatography (11) was compared with that of native Lp(a). In the example shown in Fig. 1, the apoB-100 protein of Lp(a-) accounted for 62% of the radiolabel. This approach indicated that radioiodination of Lp(a) produced labeling of both the apoB-100 protein and apoprotein(a) components.

Next, several reports have described the liability of Lp(a) at pH values <6.2 (3) as well as propensity to precipitate upon modification by the lysine-specific reagents, acetic anhydride and malondialdehyde (38). We therefore tested the maintenance of soluble derivatized Lp(a) (280-kDa isoform) as a function of progressive modification by malondialdehyde (MDA) at pH 6.4. The conditions given in Table I produced progressively modified MDA-Lp(a) with no evidence of precipitation as assessed by absorbance at 600 nm and by recovery of >96% radioactivity after processing by an 0.2-μm filter.

Whether malondialdehyde neutralization of lysyl residues provided by both the apoB-100 and apoa proteins might lead to a change in physicochemical behavior of Lp(a) was examined by chromatography on Sepharose 4B-CL. The majority of [125I]-labeled Lp(a) (K. B.-1, molecular weight 4.2 × 10^6(3)) eluted prior to LDL (molecular weight 2.5 × 10^6 (25)) (Fig. 2). A small amount of radioactivity (4%) reflecting free [125I] eluted in the internal volume (V_i) determined by separate chromatographic analyses.

![Image](image_url)

**FIG. 1. Analysis of the distribution of radioactivity of thiol-reduced Lp(a) by heparin-Sepharose chromatography.** Radioiodinated Lp(a) (donor L. C., 280-kDa apoprotein(a) isoform) (1.24 mg, 743.8 cpm/ng) was reduced with 0.01 M dithiothreitol for 3 h at 37 °C in buffer containing 10 mM Tris-HCl at pH 7.6, 50 mM sodium chloride, 1 mM EDTA, and 0.2% sodium azide, and then applied to a column of heparin-Sepharose (0.9 × 5 cm). Elution was performed with the same buffer, and a step gradient of 0.5 M sodium chloride in 10 mM Tris-HCl at pH 7.6, 1 mM EDTA, and 0.2% sodium azide was applied at Fraction 75. Fractions (120 μl) were collected in siliconized tubes at a pumped flow rate of 7.2 ml/h, and aliquots (3 μl) taken for determination of radioactivity by γ-counting. Total recovery of the original radioactivity applied were Lp(a), 96.5%; MDA-Lp(a), 106.1%; and MDA-Lp(a) after treatment with 0.01 M dithiothreitol (●) to produce dissociation of apoprotein(a). Radioiodinated Lp(a) samples (20 μg, 420.1 cpm/ng) were subjected to gel chromatography on Sepharose 4B-CL (0.9 × 20 cm) in 0.01 M sodium phosphate, 0.15 M sodium chloride, 0.01% EDTA, pH 7.4. Fractions (560 μl) were collected in siliconized tubes at a pumped flow rate of 18 ml/h, and aliquots (150 μl) taken for determination of radioactivity by γ-counting. Total recoveries of the original radioactivity applied were Lp(a), 95.5%; MDA-Lp(a), 106.1%; and MDA-Lp(a) after treatment with dithiothreitol, 92.3%. Arrows indicate the peak fraction marking elution of dextran blue for the void volume (V_o), native LDL (5 mg), and lysozyme for the internal volume (V_i) determined by separate chromatographic analyses.

**FIG. 2. Effects of chemical modification of Lp(a) upon particle distribution in gel chromatography.** Samples for individual analyses include native Lp(a) (280-kDa apoprotein(a) isoform) (○), MDA-Lp(a) (●), and MDA-Lp(a) after treatment with 0.01 M dithiothreitol (●). Malondialdehyde was quantitated in samples (30 μg) by colorimetric reaction with thiobarbituric acid (18) and protein content was determined by Lowry analysis (32). The native parent Lp(a) (100 μg) demonstrated no endogenous malondialdehyde. The molar ratios were calculated on the basis of a molecular weight of 915,000 for the protein components of Lp(a).

| Table I | Progressive modification of Lp(a) by malondialdehyde as a function of concentration and time of incubation |
|---------|-------------------------------------------------------------------------------------------------|
| Lp(a) sample | Malondialdehyde concentration | Time at 37 °C | Malondialdehyde/ protein |
| mM | min | mol/mol* |
| 1 | 5 | 10 | 8 |
| 2 | 10 | 10 | 11 |
| 3 | 20 | 24 | 19 |
| 4 | 40 | 30 | 38 |
| 5 | 67 | 180 | 87 |

*Malondialdehyde was quantitated in samples (30 μg) by colorimetric reaction with thiobarbituric acid (18) and protein content was determined by Lowry analysis (32). The native parent Lp(a) (100 μg) demonstrated no endogenous malondialdehyde. The molar ratios were calculated on the basis of a molecular weight of 915,000 for the protein components of Lp(a).
dithiothreitol to produce dissociation of apoprotein(a) from the native lipoprotein particle (11) provided an additional opportunity to test whether thiol reduction could effect a change in elution behavior of MDA-Lp(a). As shown in Fig. 2, a trimodal pattern was generated, and approximately 11% of radiolabeled material was released to the internal volume. Immunoblot analyses revealed the presence of apoprotein(a) in the first and third peaks (data not shown). These findings indicated the sequential elution of the modified Lp(a), Lp(a-), and apoprotein(a) (280-kDa isoform). Since apoprotein(a) accounted for approximately 34% of the initial radioactivity, these results indicated that malondialdehyde derivatization produced cross-linking of the majority of apoprotein(a) to apoB-100 and that a portion (11% of the radioactivity) remained dissociable and eluted in the internal volume.

Agarose electrophoresis was also conducted and comparisons made between Lp(a) and LDL preparations (Fig. 3). As expected, both MDA-Lp(a) and MDA-LDL exhibited enhanced anodic mobility compared to the native lipoproteins, owing to neutralization of lysine residues by malondialdehyde derivatization. Thiol treatment of native Lp(a) produced dissociation of apoprotein(a) from the lipoprotein (Fig. 3, lane 6). Consistent with the results of gel filtration (Fig. 2), thiol reduction of MDA-Lp(a) generated three products; two of these were identified by immunoblot to contain apoprotein(a) (Fig. 3, lane 8).

These experiments together indicated that radiiodination labeled both apoprotein components of Lp(a). Malondialdehyde modification produced an anionic lipoprotein comparable to native Lp(a) in Stokes’ radius. Furthermore, since thiol reduction of MDA-Lp(a) released only one-third of the radiodinated apoprotein(a), these results indicated that malondialdehyde derivatization cross-linked the majority of apoprotein(a) to apoB-100 protein. Inspection of the amino acid sequence derived from cloned human apoprotein(a) cDNA (5) reveals the presence of 23 lysyl residues (translated molecular mass 500 kDa). Fortuitously for our studies, 20 lysyl residues reside in the invariant regions (plasminogen-like kringle V and serine protease domain) and thus remain constant throughout the spectrum of apoprotein(a) isoforms. By contrast, the primary sequence of apoB-100 protein (31) contains 358 lysine residues (translated molecular mass 515 kDa). Thus an Lp(a) particle would contain 381 or 404 total lysine residues, based upon 1 mol of apoB-100 protein and 1 or 2 mol of apoprotein(a), respectively.

The ability of human monocyte-macrophages to internalize and degrade 125I-Lp(a) was determined as a function of progressive lipoprotein modification by malondialdehyde (Fig. 4, A and B). The specificity of receptor recognition by the LDL receptor and scavenger receptor was determined concomitantly by addition of a 49-fold molar excess of native LDL or MDA-LDL, respectively. Experiments were also conducted with an equimolar concentration of 125I-LDL (39 pmol/ml, 20 μg of protein/ml) as a function of progressive modification to allow comparison between the two lipoproteins.

Human monocyte-macrophages demonstrated the ability to internalize and degrade native Lp(a) via the LDL receptor-dependent pathway. However, the rate of degradation was approximately 20% that of native LDL added at an equimolar concentration. Similar findings have been previously reported by Floren et al. (10) and Armstrong et al. (11) in human fibroblasts. Recent studies by Snyder et al. (12) have determined that the diminished cellular-dependent hydrolysis of Lp(a) in human monocyte-macrophages is due to impairment
in LDL receptor-mediated binding and lysosomal processing at 37 °C.

The total rate of hydrolysis remained low upon progressive addition of as much as 38 mol of malondialdehyde/mol of Lp(a) (Fig. 4A) and dramatically increased upon a higher incorporation ratio of 87 mol of malondialdehyde/mol of Lp(a) (Fig. 4B). The specificity of receptor recognition accounting for hydrolysis was determined by competition analyses. The consistent ability of nonradioactive native LDL to suppress hydrolysis of Lp(a) modified by as much as 19 mol of malondialdehyde/mol of lipoprotein indicated that the LDL receptor accounted for the majority of uptake of these particles. While native LDL proved ineffective at suppressing the degradation of more highly modified Lp(a), the ability of nonradioactive MDA-LDL to readily suppress hydrolysis indicated recognition of MDA35-Lp(a) and MDA47-Lp(a) by the scavenger receptor. We note here that, despite repeated efforts, lipoprotein containing molar ratios between 38 and 87 mol of malondialdehyde/mol of Lp(a) proved elusive, and propose that Lp(a), once converted by modification to a scavenger receptor-active form of modest uptake (MDA35-Lp(a)), undergoes a rapid rate of derivatization to generate a form producing rapid internalization and hydrolysis (MDA47-Lp(a)). Whatever the mechanism, it is clear that modification of Lp(a) by malondialdehyde produced avid uptake and hydrolysis by human monocyte-macrophages.

A direct comparison of the hydrolysis and specificity of receptor recognition of apoB-100-containing LDL as a function of progressive modification by malondialdehyde is shown in Fig. 4, C and D. As we have previously demonstrated (19), progressive modification of the LDL protein by malondialdehyde progressively abolishes LDL receptor-mediated uptake in human monocyte-macrophages (Fig. 4C). Modification of the apoB-100 protein lysines by 30 mol of malondialdehyde/mol of LDL or more (19, 39) abolishes recognition by the LDL receptor and triggers concomitant recognition by the scavenger receptor (Fig. 4D). Thus, modification of LDL by malondialdehyde produces threshold recognition upon derivatization of 16% of the apoB lysyl amino acids, whereas modification of Lp(a) appears to produce a two-stage response. Although the incomplete dissociation of apoprotein(a) from MDA-Lp(a) (Fig. 2) precludes an accurate assignment of derivatized lysine residues to the two different apoprotein components of Lp(a), the total percent of derivatized lysine residues is calculated to be 15–19% for MDA35-Lp(a) and 43–46% for MDA47-Lp(a).

Armstrong et al. (11) have demonstrated that Lp(a) is specifically bound, internalized, and degraded as efficiently as LDL by the LDL receptor pathway of human fibroblasts. Fig. 5A shows that 125I-Lp(a) (A), isolated by heparin-Sepharose chromatography after thiol reduction (Fig. 1), was hydrolyzed as efficiently as native 125I-LDL in human monocyte-macrophages. Moreover, the inefficient processing of 125I-Lp(a) noted in Fig. 4A was reproduced. The ability of native LDL, but not MDA-LDL, to suppress hydrolysis confirmed that the LDL receptor pathway mediated uptake of Lp(a) and Lp(a)-. Modification of LDL, Lp(a), and Lp(a)- by malondialdehyde produced avid uptake and hydrolysis by the scavenger receptor pathway in human monocyte-macrophages (Fig. 5B). Furthermore, 125I-MDA-Lp(a)- demonstrated an accelerated rate of hydrolysis as compared to 125I-MDA-Lp(a). These findings indicated that dissociation of the apoprotein(a) component(s) enhanced cellular processing of modified Lp(a)- by the scavenger receptor pathway.

The role of the scavenger receptor in the recognition of MDA-Lp(a) was examined by competition analyses with a panel of inhibitors of the scavenger receptor. Experiments were concomitantly conducted with 125I-MDA-LDL, a well-characterized ligand of the scavenger receptor (40). Addition of polyinosinic acid, MDA-LDL, and copper-oxidized LDL effectively inhibited 75%, and MDA-Lp(a) suppressed 65%, of the hydrolysis of 125I-MDA-LDL (Fig. 6A). Cross-competition analyses demonstrated that each inhibitor, including MDA-Lp(a), effectively suppressed 80–90% of the degradation of 125I-MDA-Lp(a) (Fig. 6B). A separate experiment was conducted with 125I-MDA-Lp(a) (7 pmol of protein/ml) in which competitors were added at a 50-fold molar excess and confirmed the ability of acetyl-LDL, copper oxidized LDL, and MDA-LDL to suppress 99, 96, and 94% of hydrolysis (data not shown). Native LDL, as previously demonstrated in Figs. 4B and 5B, and c-aminocaproic acid proved ineffective. These findings taken together indicate that recognition of MDA-Lp(a) in human monocyte-macrophages is mediated by the scavenger receptor. Furthermore, neither the LDL recep-
itor nor the plasminogen-binding site play a role in interaction of MDA-Lp(a) with human monocyte-macrophages.

The data presented thus far have described the cellular interactions of Lp(a) homozygous for the 280-kDa isoform of apoprotein(a). Studies were also conducted to determine the effect of larger isoforms upon hydrolysis by human monocyte-macrophages. As shown in Fig. 7A, Lp(a) homozygous for the 500-kDa isoform was compared to lipoprotein homozygous for the 280-kDa isoform. Native Lp(a) (500-kDa isoform) demonstrated both enhanced total hydrolysis and enhanced specific uptake by the LDL receptor as compared to lipoprotein containing the 280-kDa isoform. Similarly, modification by malondialdehyde revealed a rate of scavenger receptor-specific hydrolysis of modified Lp(a) (500-kDa isoform) nearly twice that of modified Lp(a) (280-kDa isoform) and was comparable to results obtained with MDA-LDL (Fig. 7B). Whether the size of the apo(a) isoform or cell donor variability accounted for these differences in hydrolysis was further examined.

Direct analyses were conducted to determine the total rate of degradation of lipoproteins as a function of protein concentration. The hydrolysis of Lp(a) heterozygous for the 330- and 370-kDa apoprotein(a) isoforms, after modification by malondialdehyde, demonstrated high affinity uptake and saturaibility and proved similar to the scavenger receptor-dependent processing of MDA-LDL (Fig. 8). The degradation of Lp(a) (280-kDa isoform), after modification by malondialdehyde, likewise demonstrated high affinity uptake (Fig. 9). However, the level of saturation for MDA-Lp(a) (280-kDa isoform) was approximately 80% that of MDA-LDL.

The ability of copper oxidation to convert LDL to a ligand recognized by the scavenger receptor, as well as an oxidized LDL receptor in murine peritoneal macrophages (41, 42), has implicated a role for transition metal-induced lipid peroxidation. We have asked whether, within the context of our studies of malondialdehyde derivatization of LDL and Lp(a), copper oxidation of Lp(a) produced a change in specificity of receptor recognition by human monocyte-macrophages. As described under "Experimental Procedures," each well received 0.5 ml of medium D containing the indicated concentration of radioiodinated human MDA-LDL (donor K. B., 280-kDa apoprotein(a) isoform; 441,437 cpm/pmol). After incubation for 4 h at 37 °C, the medium was removed and the content of 125I-labeled acid-soluble material was determined. The values shown are the mean ± 1 S.D. of triplicate determinations.
oxidized Lp(a) was consistent with that previously reported by Sattler et al. (43). As shown in Fig. 10, copper-oxidized Lp(a) displayed high affinity saturable uptakes. As previously shown for Lp(a) (280-kDa apoprotein(a) isofrom) derivatized by malondialdehyde (Fig. 9), the maximum rate of hydrolysis was lower than that of MDA-LDL. The specificity of receptor recognition of copper-oxidized Lp(a) was determined by competition analyses (Fig. 11). Inhibitors of the scavenger receptor suppressed >75% of the hydrolysis of 125I-labeled copper-oxidized Lp(a) while native LDL and ε-aminoacapric acid failed to compete. Cross-competition analyses conducted with 125I-MDA-LDL demonstrated similar results. These findings taken together indicate that copper-oxidized Lp(a), like MDA-Lp(a) and MDA-LDL, is recognized by the scavenger receptor of human monocyte-macrophages.

**DISCUSSION**

The association of an increased risk of cardiovascular heart disease in human subjects with increased levels of Lp(a) has prompted considerable interest in the role of this lipoprotein in both atherosclerosis and thrombosis (1, 14). The identification of apoB-100 protein as a constituent of Lp(a) has enabled comparison of the functional, biochemical, and physicochemical properties between LDL, the primary plasma carrier of cholesterol, and Lp(a). Lp(a) is chemically distinguished from LDL by the association of apo(a) glycoprotein with the apoB-100 protein. The presence of this additional plasminogen-like protein produces interaction of Lp(a) with the plasminogen receptor and likely interferes with thrombotic events (1, 14). In this report we have asked whether the association of apo(a) glycoprotein impacts upon lipoprotein modification, thought to be a key event in atherogenesis. The effect of apo(a) protein variants, recently shown to be encoded by at least 19 different alleles (45), upon lipoprotein conversion has also been examined. Our investigations demonstrate that the lipid peroxide product malondialdehyde readily modifies Lp(a) and produces avid uptake of the modified apoB-100-containing lipoprotein by the scavenger receptor of human monocyte-macrophages. Thus Lp(a) and LDL demonstrate functional similarity with respect to the recognition of modified lipoprotein by the scavenger receptor.

A number of challenges were apparent at the outset of these studies. Previous reports (38) had noted frank precipitation of >85-90% of Lp(a) upon derivatization of the lipoprotein by lysine-specific reagents such as acetic anhydride and malondialdehyde. Cryoprecipitation of Lp(a) preparations containing larger apoprotein(a) isoforms and lability of Lp(a) to pH and ionic strength (3, 4) further influenced experimental design. These challenges were met by modification of the protocols for lipoprotein derivatization as given in Table I and by conduct of cell biology analyses at 37°C. Experiments were also conducted to determine the distribution of radiolabel and lysyl derivatization between the two apoprotein components of Lp(a). Our data show that both apoB-100 and apoprotein(a) are radiolabeled, and they indicate that the cellular assays monitor the lysosomal degradation of both protein components. The demonstration that malondialdehyde derivatization produces anionic MDA-Lp(a) comparable to native Lp(a) in Stokes' radius indicates that the mechanism of cellular uptake involves scavenger receptor-mediated endocytosis of individual particles rather than aggregate-induced phagocytosis. These findings together allow direct comparison and analysis of the interactions of MDA-LDL and MDA-Lp(a) with the scavenger receptor of human monocyte-macrophages.

Since the apoB-100 protein contributes more than 89% of the total lysyl residues of the two apoprotein components of Lp(a), it likely provides the chief sites for modification of the lipoprotein by malondialdehyde. One might predict then that the ability of MDA-Lp(a) to interact with the scavenger receptor would depend upon both derivatization of essential lysines of apoB-100 protein to form the scavenger receptor-binding domains (19, 39) and the impact of apoprotein(a) upon steric accessibility of these domains to the scavenger receptor. Like LDL, native Lp(a) is recognized in human monocyte-macrophages by the LDL receptor. Like LDL, pro-
gressive modification of Lp(a) by malondialdehyde abolishes lipoprotein recognition by the LDL receptor and produces uptake and hydrolysis by the scavenger receptor of human monocyte-macrophages. While modified LDL demonstrates threshold recognition by the scavenger receptor upon addition of 30 mol of malondialdehyde/mol of LDL (19, 39), recognition of MDA-Lp(a) by the scavenger receptor exhibits a two-stage response. Our results suggest that progressive malondialdehyde derivatization first converts Lp(a) to a scavenger receptor-active form of modest uptake and then subsequently modifies this to a form producing avid uptake and hydrolysis.

The specificity of receptor recognition of native Lp(a) and the modified lipoprotein by human monocyte-macrophages has been determined by competitive inhibition analyses. Our data show that the native lipoprotein, like LDL, is recognized by the LDL receptor and that the cellular processing improves dramatically upon presentation of Lp(a)- which lacks apoprotein(a). Unlike studies in the murine P388D1 cells (15), we found no evidence to support interaction of native Lp(a) with the scavenger receptor. Instead, scavenger receptor recognition occurs only after modification of Lp(a) by malondialde-
hyde or by transition metal-induced oxidation. The cellular processing of modified Lp(a) is suppressed by inhibitors of the scavenger receptor, such as polyinosinic acid, MDA-LDL, copper-oxidized LDL, and acetyl-LDL, as well as copper-oxidized Lp(a) and MDA-Lp(a). Ligands of the LDL receptor, including native LDL and native Lp(a), as well as an inhibitor of the plasminogen-binding site fail to suppress the uptake of modified Lp(a). Since cellular processing of malondialdehyde-modified Lp(a)- by the scavenger receptor is enhanced upon removal of apoprotein(a), we suggest that, like modified LDL, the apoB-100 component is responsible for recognition of both modified Lp(a) and modified Lp(a)- by the scavenger receptor.

A series of studies (4, 46-49) has investigated the nature of the association between the apo(a) and apoB-100 proteins. The interaction has been characterized as involving a discrete apoprotein(a)-apoB-100 disulfide-linked complex (4, 46, 50, 51) as well as the hydrophobic interaction of the kringle IV domain, which demonstrates homology with the lysine-analog binding site of plasminogen (52, 53), with apoB-100-containing lipoprotein (49). We speculate that apoprotein(a) initially hinders interaction of the modified apoB-100-combining determinants of MDA-Lp(a) with the scavenger receptor, and that additional neutralization of the positively charged ε-amino group of apoB-100 lysine residues by malondialdehyde produces local dissociation of the apoproteins and allows access of the scavenger receptor to the binding determinants of MDA-Lp(a). The observed differences in maximal scavenger-receptor-dependent hydrolytic rate as a function of apoprotein(a) isoform size might be related to the efficiency of this proposed local dissociation or other interactions of apoB-100 yet to be described.

It has recently been demonstrated that Lp(a) accumulates in the subendothelial matrix of arterial lesions and saphenous vein grafts after coronary bypass surgery (54-56). The contents of plate apoprotein(a), much of it apparently in the form of intact Lp(a), exhibit a significant positive correlation with plasma apoprotein(a) levels (54, 55). The observed entrapment of lipoprotein likely reflects avid binding of Lp(a) to matrix components typically found in the atherosclerotic lesion such as fibrin, glycosaminoglycans, and fibronectin (57-61). Reports (54, 56) that the accumulation of apoprotein(a) exceeds that of apoprotein B in arterial plaques when the values were normalized for corresponding plasma concentrations further suggest that Lp(a) may contribute independently of LDL to atherosclerosis. We propose that the intimal retention of Lp(a) by extracellular components of the arterial intima plays a role in the lipoprotein in a microenvironment favoring interfacial chemical events of peroxidative modification. The chronic production of lipid peroxide-modified Lp(a) together with unmitigated cellular clearance by scavenger receptors may contribute to the accumulation of lipoprotein-derived lipid in macrophage-derived foam cells of the atherosclerotic lesion. It is further tempting to speculate that peptide scission accompanying peroxidative modification of lipoprotein (62) may act to release a portion of apoprotein(a) from the entrapped Lp(a). On the one hand, the resultant Lp(a)-, like Lp(a) and LDL, could participate in events of atherosclerosis while, on the other hand, the liberated apoprotein(a) could participate in modulation of thrombotic events.

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