Proc. Natl. Acad. Sci. USA
Vol. 79, pp. 1712–1716, March 1982
Biochemistry

Specificity of receptor-mediated recognition of malondialdehyde-modified low density lipoproteins

(scavenger receptor/low density lipoprotein receptor/human monocyte/atherosclerosis)

MARGARET E. HABERLAND*, ALAN M. FOGELMAN* AND PETER A. EDWARDS*†

*Division of Cardiology, Department of Medicine, and †Department of Biological Chemistry, School of Medicine, University of California at Los Angeles, Los Angeles, California 90024

Communicated by Robert L. Hill, November 9, 1981

ABSTRACT **Blood-borne human monocytes and macrophages derived from human monocytes *in vitro* express an active low density lipoprotein (LDL) receptor and an active receptor for negatively charged proteins, the scavenger receptor. When <15% of the lysine residues of human LDL were modified by malondialdehyde while the lipoprotein was in solution, recognition and uptake of the modified lipoprotein occurred via the LDL receptor. Further modification resulted in threshold recognition and uptake by the scavenger receptor with concomitant loss of recognition by the LDL receptor. The rate of degradation via the LDL receptor pathway was inversely related to the degree of modification whereas that mediated by the scavenger receptor was independent of the extent of incorporation of malondialdehyde once threshold recognition was achieved. In contrast to the interaction of LDL with malondialdehyde in solution, modification of <15% of the lysine residues of LDL adsorbed to heparin-Sepharose resulted in recognition and uptake by the scavenger receptor. The scavenger receptor-mediated uptake of malondialdehyde-modified LDL may be dependent on formation of recognition sites involving specific modified lysine residues or changes in the conformation of LDL induced by neutralization of specific lysine residues of the apoB polypeptides or both.**

It has been shown that interaction of low density lipoprotein (LDL) with malondialdehyde (MDA) abolishes the ability of the LDL receptor to recognize the modified lipoprotein (MDA-LDL) and concomitantly stimulates recognition of MDA-LDL by the receptor for negatively charged proteins, the scavenger receptor (1–4). The occurrence of these two distinct receptor-mediated pathways in human monocyte macrophages presents a unique opportunity to examine the effect of progressive modification of LDL by malondialdehyde on recognition by each receptor. Malondialdehyde is of particular interest because it is produced physiologically during catabolism of arachidonic acid by blood platelets at the site of arterial injury (5–7) and the peroxidative decomposition of unsaturated lipids (8, 9) by blood monocytes during phagocytosis. This paper presents data showing that malondialdehyde interacts with the lysine residues of the apoB polypeptides of LDL and that modification of a limited number of lysine residues results in threshold recognition of MDA-LDL by the scavenger receptor of human monocyte macrophages.

METHODS

Cells in Culture. Human monocytes were isolated from blood of normal individuals using methods B and 2B (2, 3, 10), classified and viability determined, and maintained as described (2, 10) in 30% autologous serum in Dulbecco's modified Eagle's medium (pH 7.4) supplemented with 24 mM $NaHCO_3$/10 mM Hepes, insulin at 8 μg/ml, glucose at 2 mg/ml, penicillin at 100 units/ml, streptomycin at 100 μg/ml, and fungizone at 0.25 μg/ml [designated medium B to be consistent with our previous publications (10)].

Isolation and Iodination of Lipoproteins. Human LDL (density, 1.019–1.063 g/cm^3) were prepared from sera of normal fasted subjects as described (11) and radioiodinated by the method of McFarlane (12) as modified by Bilheimer *et al.* (13).

Modification of Proteins by Malondialdehyde. Malondialdehyde was generated at room temperature by rapid acid hydrolysis (0.2 ml of 12 M HCl) of malonaldehyde bis(dimethyl acetal) (Aldrich; 0.165 ml); then, 4.8 ml of 0.1 M sodium phosphate buffer (pH 6.4) was added and the solution was adjusted to pH 6.4 with 10 M NaOH. LDL was modified by mixing equal volumes of protein (5–10 mg/ml) in 0.01 M sodium phosphate/0.15 M NaCl/0.01% EDTA, pH 7.4 (buffer A), with 0.2 M freshly prepared malondialdehyde in 0.1 M sodium phosphate (pH 6.4) and incubating the mixture at 37°C for 3 hr (1). Lower incorporation ratios were obtained by reducing both the initial concentration of malondialdehyde (0.02–0.1 M) and the time of incubation (10–60 min). The reaction was stopped by dialysis against buffer A for 16 hr at 4°C. The extent of incorporation was determined by the colorimetric thiobarbituric acid assay (1, 14). Molar ratios were calculated assuming a molecular weight for the particle protein content of LDL of 500,000 (15). All results are reported on the basis of protein determination by Lowry analysis (16). Control and modified proteins were characterized by electrophoresis in Tris/barbital/sodium barbital, pH 8.4, in agarose gel strips (Bio-Rad). Both untreated LDL and LDL incubated under the same conditions without malondialdehyde contained <0.2 mol of malondialdehyde per mol of LDL and behaved identically in chemical and cellular assays.

To determine the kinetics of incorporation of malondialdehyde into LDL, aliquots of the lipoprotein solution were removed at appropriate time intervals and heparin-manganese was added to precipitate the MDA-LDL adduct (1). The supernatant was discarded after centrifugation, and the precipitate was washed twice with heparin-manganese. Using radioiodinated lipoprotein, direct analysis showed that, regardless of the degree of incorporation of malondialdehyde into LDL, >94% of the lipoprotein remained in the precipitate. The lipoprotein was then analyzed for malondialdehyde by the colorimetric assay.

Heparin-Sepharose 4B was prepared by the method of Mitchell *et al.* (17) using Sepharose CNBr-4B (Pharmacia) and equilibrated in 10 mM sodium phosphate/0.01% EDTA, pH 6.4. A minicolumn (0.9 × 3 cm) was prepared and the packed

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U. S. C. §1734 solely to indicate this fact.

Abbreviations: LDL, low density lipoprotein; MDA, malondialdehyde; MDA_x-LDL, LDL modified by x mol of malondialdehyde per mol of LDL.

resin was gently suspended with ^{125}I-labeled native LDL (2.4 mg/ml of settled resin). The mixture was incubated for 60 min at 37°C, and unbound ^{125}I-labeled LDL ($\leq$10% of the total applied) was removed by repeated washing with 10 mM phosphate (pH 6.4). The buffer of choice was then added, and a buffer flow of 0.05 ml/min was initiated by a peristaltic pump (Gilson Minipuls). Fractions (12 min, 0.6 ml) were collected in ice, analyzed for radioactivity, and immediately pooled and dialyzed against buffer A at 4°C.

Cellular Assays. Cells were prepared for degradation assay by washing three times with 1.5 ml of Dulbecco's modified Eagles's medium/10 mM Hepes (medium C). All protein preparations were filtered through a 0.45-μm filter just prior to addition to cells in medium C/24 mM $NaHCO_3$ supplemented with glucose at 2 mg/ml (medium D). The proteolytic degradation of ^{125}I-labeled trichloroacetic acid-soluble (noniodide) material formed by the cells and excreted into the culture medium was determined as described (18). Corrections were made for the small amount (<0.01% of total radioactivity added) of ^{125}I-labeled acid-soluble material found in parallel incubations without cells.

Analysis of Lysine Content. Lipoproteins for amino acid analysis (100 μg) in 0.01% EDTA (pH 7) were lyophilized and delipidated by $CHCl_3$/MeOH, 2:1 (vol/vol) at 4°C, and the apoprotein residue was dried at reduced pressure. Hydrolyses were carried out at reduced pressure for 22 hr at 110°C in 6 M HCl. Analyses were kindly by Lynn Williams of the Department of Biological Chemistry on a Beckman model 120C amino acid analyzer (Beckman).

The extent of modification by malondialdehyde was determined by an indirect procedure, since acid hydrolysis regenerated free lysine (>98%) from the malonimyl derivative of lysine. The delipidated apoprotein, containing both unreacted and modified lysine residues, was treated with dinitrofluorobenzene (19, 20) to convert unreacted lysyl groups to the acid-stable dinitrophenyl derivative of lysine. Acid hydrolysis subsequently regenerated those lysyl groups modified by malondialdehyde, which were quantitated by amino acid analysis. More than 88% of the lysine residues of unmodified LDL from two different donors reacted with dinitrofluorobenzene, and this value was used to correct the results obtained with the modified lipoprotein to determine the extent of modification due to interaction with malondialdehyde.

Unreacted ε-amino groups were also determined by the colorimetric trinitrobenzenesulfonic acid assay (21). More than 90% of the lysine residues of unmodified LDL from four different donors reacted with the reagent. The number of lysine residues modified was determined as the difference in values obtained for modified and unmodified lipoproteins.

RESULTS

A detailed analysis of the kinetics of incorporation of malondialdehyde into LDL is shown in Fig. 1. The rate of formation of the MDA-LDL adduct reached an apparent maximum of 30–35 mol of malondialdehyde incorporated per mol of LDL at a time dependent on initial concentration of the dialdehyde. At least two different phases of incorporation were observed at each concentration in the plot of the inverse of the incorporation ratio versus time (data not shown). The reaction was linear for the first 10 min of incubation at 10 and 20 mM malondialdehyde and resulted in incorporation of 2 to 3 mol of malondialdehyde/mol of LDL (MDA_{2-3}-LDL). Based on these data, we propose that there are different sites in LDL that react with malondialdehyde at different rates. The more slowly reacting groups may represent sites of incorporation that become accessible to

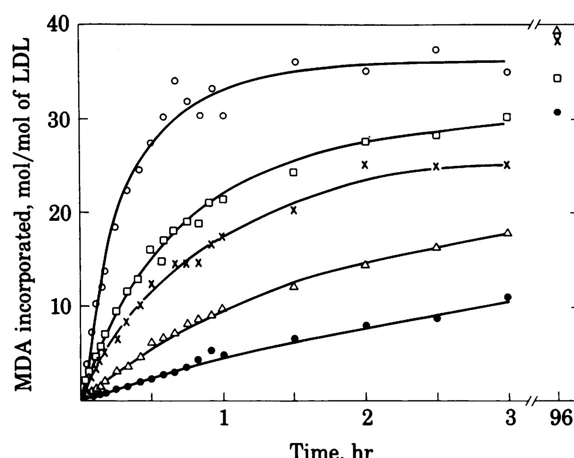

FIG. 1. Kinetics of incorporation of malondialdehyde into LDL. Samples of native LDL (5 mg/ml) were incubated with 10 mM (●), 20 mM (△), 38 mM (×), 50 mM (□), or 100 mM (○) malondialdehyde in 55 mM sodium phosphate/0.005% EDTA/75 mM NaCl, pH 6.5, at 37°C. Aliquots (10 μl, 50 μg) were removed as indicated, the MDA-LDL adduct was precipitated by heparin-manganese, and malondialdehyde was determined colorimetrically by the thiobarbituric acid assay.

the dialdehyde after the initial incorporation of 2 to 3 mol of malondialdehyde/mol of LDL.

The MDA-LDL adducts (3-hr reaction products of Fig. 1) were chilled in ice and dialyzed against buffer A at 4°C. Incorporation of malondialdehyde in each preparation, determined after precipitation of the MDA-LDL adduct with heparin-manganese (data not shown), was within 10% of that determined at 3 hr in the kinetic experiment (Fig. 1). These results showed that the linkages formed between malondialdehyde and LDL, regardless of the extent of incorporation, were relatively stable to dialysis at pH 7.4.

Omitting precipitation of the lipoprotein by heparin-manganese resulted in assayed values for bound malondialdehyde that were $\approx$20% higher. To avoid the effect of heparin-manganese on release of malondialdehyde, the molar ratios reported in this study were determined after dialysis of the MDA-LDL adducts to remove unreacted reagent and in the absence of precipitation by heparin-manganese.

The anodic mobility of each modified lipoprotein (3-hr reaction products of Fig. 1) in agarose electrophoresis relative to unmodified native LDL increased linearly as a function of extent of incorporation of malondialdehyde. In each case, a single band, detected by the lipid stain, fat red 7B, was observed. A similar study using radioiodinated lipoprotein preparations showed that the single band detected by the lipid stain strictly coincided with a single discrete band observed after autoradiography. We have assumed that the molar ratios for LDL preparations modified by malondialdehyde reported in this study reflect the extent of modification of the majority of the molecules.

Malondialdehyde has been shown to react primarily with the ε-amino groups of lysine residues of ribonuclease, resulting in formation of derivatives intra- and intermolecularly crosslinked by the conjugated imine, 1-amino-3-iminopropene (22). Since incorporation of malondialdehyde into LDL resulted in modified lipoprotein of the same molecular weight as native LDL, the interaction of malondialdehyde appeared to involve primarily intramolecular crosslinking of the lipoprotein (1). To determine whether lysine residues of the apoB polypeptides of LDL were the sites of modification by malondialdehyde, native LDL, MDA_{24}-LDL, and MDA_{64}-LDL were analyzed for amino acids. As given in Table 1, $\approx$2 mol of lysine residues was mod-

Table 1. Chemical analysis of effect of modification of human LDL by malondialdehyde

	Native LDL	MDA_{24}-LDL	MDA_{64}-LDL
Lysine residues modified,* no./mol of apoB protein			
Amino acid analysis†	0	2.8	9.0
Trinitrobenzenesulfonic acid‡	0	2.9	9.2
Lysine residues modified,§ mol/mol of LDL			
Amino acid analysis†	0	51	165
Trinitrobenzenesulfonic acid‡	0	52	163
MDA, mol/mol of LDL¶	0	24	64
Lysine residues modified, mol/mol of MDA			
Amino acid analysis†	0	2.1	2.6
Trinitrobenzenesulfonic acid‡	0	2.2	2.5
Anodic mobility‖	1.0	1.46	2.77

* Calculated by assuming 250 amino acid residues per mol of apoB protein to facilitate comparison with other studies (21); based on this assumption, there are 20.2 lysine residues per mol of protein (this study and ref. 21).

† Determined after acid hydrolysis of dinitrofluorobenzene-treated apoprotein.

‡ Difference in free amino groups between native and modified lipoprotein as determined by colorimetric assay.

§ Calculated by assuming a total protein component of 500,000 g/mol of LDL (15); based on this assumption, there are 367 lysine residues per mol of LDL.

¶ Colorimetric determination of MDA by thiobarbituric acid assay.

‖ Electrophoretic mobility relative to native LDL in agarose.

ified per mol of malondialdehyde incorporated. Direct determination of the reduction in number of free amino groups of each lipoprotein sample by colorimetric assay confirmed these results.

The amino acid composition of native LDL was in excellent agreement with that of Weisgraber *et al.* (20). Analyses conducted after acid hydrolysis of the malondialdehyde-modified apoproteins without treatment by dinitrofluorobenzene were identical to those of unmodified lipoprotein and showed no evidence of partial destruction of sensitive amino acids, such as methionine, histidine, or tyrosine residues, by malondialdehyde (22) or of a secondary effect of malondialdehyde on the determination of other amino acid residues, such as arginine (23).

A concentration previously shown to be within the high-affinity range of degradation for both the LDL receptor and the scavenger receptor, 10 μg/ml (1), was selected to examine the rate of degradation as a function of the extent of modification of LDL by malondialdehyde (Fig. 2). Incorporation of malondialdehyde into LDL resulted in a progressive and dramatic decrease in the rate of degradation until a malondialdehyde/LDL ratio of 28:1 was achieved. Additional modification of the lipoprotein resulted in a markedly accelerated rate of degradation that was independent of the degree of further modification. Addition of native LDL suppressed not only the lysosomal hydrolysis of ^{125}I-labeled native LDL but also the degradation of lipoproteins modified by as much as 28 mol of malondialdehyde/mol of LDL (Fig. 2). The inability of the added native LDL to suppress hydrolysis of modified lipoprotein containing >30 mol of malondialdehyde/mol of LDL suggested that these molecules were not processed by the LDL receptor pathway. Lysosomal hydrolysis of iodinated lipopro-

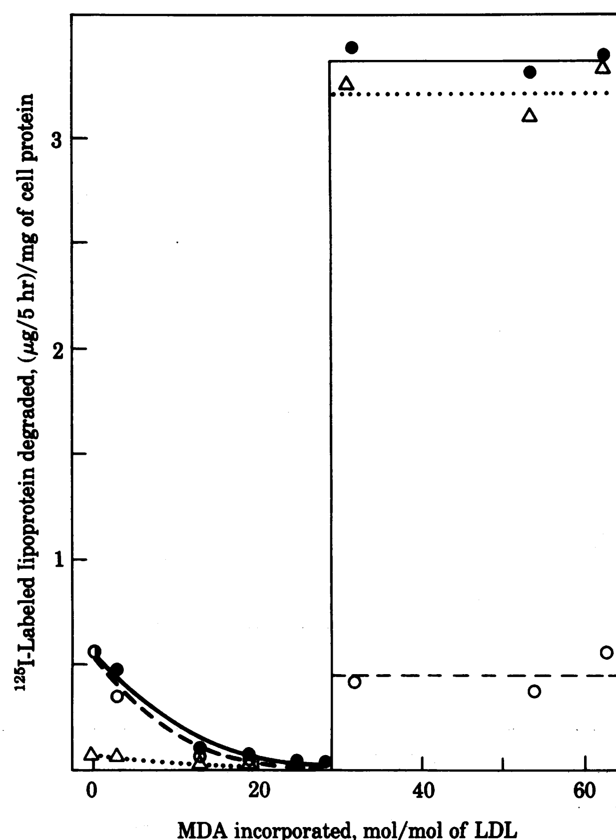

FIG. 2. Rate of degradation of ^{125}I-labeled lipoprotein as a function of incorporation of malondialdehyde into LDL. The degradation of radioiodinated native LDL and several MDA-LDL adducts (10 μg/ml, 90.3 cpm/ng) was determined in the absence (●) or presence of a 26-fold excess of nonradioactive native LDL (△) or of nonradioactive MDA_{66}-LDL (○). Monocyte macrophages maintained 15 days in culture in 30% autologous serum were washed and the indicated lipoproteins were added in a final volume of 1 ml of medium D. After 5 hr of incubation, the supernatant was assayed for noniodide radioactivity. The cellular protein content was 127.6 ± 11.8 μg per dish.

teins containing >30 mol of malondialdehyde/mol of LDL was effectively suppressed by MDA_{66}-LDL whereas that of ^{125}I-labeled native lipoprotein and lipoproteins modified by as much as 28 mol of malondialdehyde/mol of LDL was not (Fig. 2). Fucoidin (10 μg/ml), an inhibitor of the scavenger receptor (4), produced the same suppression as MDA_{66}-LDL (data not shown).

The differential competition by native LDL and MDA_{66}-LDL (Fig. 2) indicates that native LDL and lipoproteins in which <28 mol of malondialdehyde have been incorporated per mol of LDL are recognized, internalized, and degraded by the LDL-receptor pathway whereas lipoproteins modified by >30 mol of malondialdehyde/mol of LDL are processed by the scavenger-receptor pathway.

Degradation of ^{125}I-labeled native LDL, MDA_3-LDL, and MDA_{25}-LDL was also compared as a function of the concentration of radioiodinated lipoprotein (Fig. 3). Nonspecific degradation (hydrolysis observed in the presence of a 26-fold excess of nonradioactive native LDL) was the same for all three molecules. However, degradation in the absence of added nonradioactive LDL, as well as high-affinity degradation (the difference between hydrolysis in the absence or presence of a 26-fold excess of nonradioactive native LDL) of MDA_{25}-LDL, was markedly diminished in comparison with that of native LDL or MDA_3-LDL. Ligand binding by the LDL receptor is inhibited by addition of EDTA whereas that by the scavenger receptor

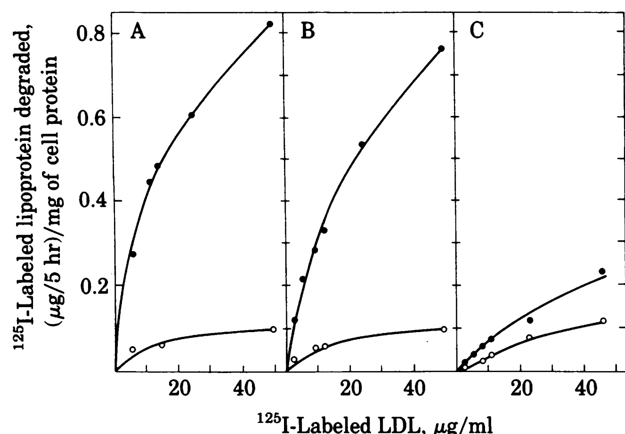

FIG. 3. Rate of degradation of native LDL (*A*), MDA_3-LDL (*B*), and MDA_{25}-LDL (*C*) as a function of concentration. The rate of hydrolysis of ^{125}I-labeled LDL (128.4 cpm/ng), MDA_3-LDL (116.4 cpm/ng), and MDA_{25}-LDL (116.4 cpm/ng) was determined in the absence (●) or presence (○) of a 26-fold excess of nonradioactive native LDL. Cultured monocyte macrophages maintained 11 days in 30% autologous serum were treated as in Fig. 2 and incubated for 5 hr. Cellular protein content was 262.2 ± 11.5 μg per dish.

is unaffected (2). In a separate experiment, degradation of native LDL, MDA_3-LDL, and MDA_{25}-LDL, but not of MDA_{69}-LDL, was abolished (>95%) by addition of EDTA (data not shown). These data, together with those of the competition by native LDL (Fig. 3), confirmed that the lysosomal hydrolysis of native LDL, MDA_3-LDL, and MDA_{25}-LDL occurred via the LDL-receptor pathway.

It has been reported that a portion of circulating LDL is entrapped in the arterial wall and, over a period of time, may be associated with a variety of components (24). We have selected heparin-Sepharose, which has been shown to bind LDL (17, 25), to study the incorporation of malondialdehyde into immobilized LDL. At a lipoprotein settled resin ratio of 20 μg/ml, > 91% of ^{125}I-labeled native LDL and <5% of ^{125}I-labeled MDA_{63}-LDL, was bound in 5 mM phosphate/0.01% EDTA, pH 6.4, within 30 min at 37°C. A minicolumn of heparin-Sepharose (0.9 × 3 cm) in 10 mM sodium phosphate/0.01% EDTA, pH 6.4, containing adsorbed ^{125}I-labeled native LDL was eluted with 50 mM malondialdehyde in 10 mM sodium phosphate (pH 6.4) at 37°C. Within 2 hr of treatment with the dialdehyde, 95% of the radioactive lipoprotein eluted from the column. The incorporation of dialdehyde into the eluted lipoprotein was 19 mol of malondialdehyde/mol of LDL. ^{125}I-labeled LDL maintained in solution and modified to the same degree was also prepared. As given in Table 2, LDL modified by malondialdehyde while adsorbed to heparin-Sepharose was taken up preferentially by the scavenger receptor whereas LDL modified while in solution was taken up preferentially by the LDL receptor. These results suggested that adsorption of LDL to heparin-Sepharose facilitated interaction of malondialdehyde with sites promoting recognition by the scavenger receptor, with a concomitant decrease in recognition by the LDL receptor.

In a series of related experiments, we have determined that ^{125}I-labeled native LDL adsorbed to heparin-Sepharose and subjected to the same protocol without malondialdehyde for as long as 19 hr did not elute from the column. Addition of 1 M NaCl to the buffer resulted in elution of 85% of the lipoprotein, which was recognized and degraded by the LDL-receptor pathway at the same rate as untreated native LDL (data not shown).

Highly negatively charged macromolecules, such as fucoidin and polyinosinic acid, have been reported to prevent binding of ^{125}I-labeled acetyl-LDL to the scavenger receptor of mouse peritoneal macrophages (26). We have confirmed that these molecules also inhibit degradation of ^{125}I-labeled MDA_{38}-LDL in human monocyte macrophages. The presence of carbohydrate in several of these inhibitors prompted us to test directly the ability of neoglycoproteins to displace modified LDL from the scavenger receptor of monocyte macrophages. None of the bovine serum albumin conjugates tested, β-L-fucose$_{44}$-albumin, β-D-galactose$_{42}$-albumin, α-D-mannose$_{40}$-albumin, or β-D-*N*-acetylglucosamine$_{35}$-albumin competed with the degradation of ^{125}I-labeled MDA_{52}-LDL (4 μg/ml) at molar ratios >400:1. Similarly, no effect in the rate of degradation of ^{125}I-labeled native LDL (8 μg/ml) was observed, confirming previous reports that carbohydrate was not a requirement for recognition by the LDL receptor (27). These results also suggest that the scavenger-receptor-mediated recognition of MDA-LDL differs from the recognition of mannose/glucose/*N*-acetylglucosamine-terminal glycoproteins by alveolar macrophages (28).

DISCUSSION

Uptake of malondialdehyde-modified LDL by the scavenger receptor of human monocyte macrophages occurs at a threshold level of ≈30 mol of malondialdehyde/mol of LDL. LDL that has been modified to a lesser degree, on the other hand, is processed by the LDL-receptor pathway.

The concept of clearance as a threshold event was used by Ashwell and Morell (29) to describe the uptake of asialoglycoproteins by the hepatic galactosyl receptor. It was determined that desialyation of any 2 of the 10 penultimate galactosyl groups

Table 2. Specificity of receptor-mediated uptake of MDA-LDL as a function of preparation

^{125}I-Labeled lipoprotein	Specific radio-activity, cpm/ng	Preparation	MDA,* mol/mol of LDL	Lysine modified,† mol/mol of LDL	^{125}I-Labeled lipoprotein degraded,‡ (μg/4 hr)/mg of cell protein: No added nonradioactive lipoprotein	Nonradioactive native LDL added	Nonradioactive MDA_{69}-LDL added
Native LDL	228.4	None	0	0	0.569 ± 0.025	0.063 ± 0.009	0.476 ± 0.062
MDA_{17}-LDL	228.4	Solution	17	36	0.140 ± 0.014	0.027 ± 0.004	0.097 ± 0.008
MDA_{19}-LDL	117.5	Resin adsorbed	19	42	0.133 ± 0.024	0.082 ± 0.007	0.018 ± 0.003
MDA_{66}-LDL	228.4	Solution	66	165	1.93 ± 0.10	1.77 ± 0.17	0.19 ± 0.01

* Determined by colorimetric thiobarbituric acid assay.

† Determined by colorimetric trinitrobenzenesulfonic acid assay.

‡ Normal monocyte macrophages were maintained in culture for 8 days in 30% autologous serum. Incubation was in 1 ml of medium D containing ^{125}I-labeled lipoprotein at 15 μg protein/ml in the absence (no added nonradioactive lipoprotein) or presence of added nonradioactive native LDL (360 μg) or MDA_{66}-LDL (360 μg). After 4 hr, ^{125}I-labeled acid-soluble material was assayed. Results are mean ± SD for quadruplicate samples. Protein content of the cells was 221.4 ± 12.8 μg per dish.

of ceruloplasmin sufficed to stimulate recognition by the galactosyl receptor (30). The interaction of a minimum of 30 mol of malondialdehyde/mol of LDL (60 lysine residues) is required to stimulate recognition by the scavenger receptor of human monocyte macrophages, if the modification is conducted with LDL maintained in solution. However, LDL adsorbed to heparin-Sepharose and modified *in situ* by malondialdehyde to a level of 19 mol of malondialdehyde/mol of LDL is preferentially degraded by the scavenger-receptor pathway. Unlike uptake of asialoceruloplasmin by the galactosyl receptor, scavenger-receptor-mediated recognition of altered LDL may be dependent on modification of specific residues; interaction of malondialdehyde with these lysyl groups may be facilitated by adsorption of LDL to heparin-Sepharose. It also appears that uptake by the scavenger receptor may be a direct response to the altered charge of the lipoprotein. Neutralization of the positive charge on the lysine residues by malondialdehyde, acetic anhydride, or glutaraldehyde (1, 2, 4, 31) results in uptake by the scavenger receptor whereas preservation of the positive charge of the lysyl groups by reductive methylation does not (2). Thus, recognition of malondialdehyde-modified LDL by the scavenger receptor may be dependent on formation of recognition site(s) involving specific modified lysine residues or changes in the conformation of the apoB polypeptides of LDL induced by neutralization of certain lysyl groups.

It is clear that the interaction of native LDL with the LDL receptor of human monocyte macrophages is dependent on the presence of a limited number of lysine residues. As few as 2 to 3 mol of malondialdehyde/mol of LDL reproducibly results in a decreased rate of degradation of the lipoprotein; further modification, up to 15% of the lysyl groups, produces a progressive decrease in the rate of degradation via the LDL receptor. These data support the conclusions of Weisgraber *et al.* (20), who determined that chemical modification of 15% of the lysine residues of LDL by carbamylation or of 20% by acetoacetylation prevented the modified LDL from binding to the LDL receptor of cultured human fibroblasts.

We have shown that only modified lipoproteins internalized by the scavenger receptor promote cholesteryl esterification in human monocyte macrophages and have proposed that modification by malondialdehyde or other physiological process is a prerequisite to the formation of the foam cells (1, 2). These and other data (31–33) suggest that the scavenger receptor of macrophages and related scavenger cells is responsible for *in vivo* clearance of modified LDL. The observation that the basal level (35 μM) of plasma malondialdehydelike material increases in patients suffering from intravascular thrombosis (subarachnoid hemorrhage, cerebral thrombosis, transient ischemic attacks) to >50 μM (34) suggests that malondialdehyde may be generated locally in sufficiently high concentration to effect such modification. LDL in which <15% of the lysine residues have been modified by malondialdehyde is recognized, but degraded at considerably slower rates than native LDL, by the LDL receptor of human monocyte macrophages and presumably by the LDL receptor of other cell types as well. Thus, it is anticipated that, in disease states in which modification of LDL may occur, such as atherosclerosis or diabetes, a small but significant proportion of circulating LDL may contain partially modified lipoprotein.

We thank Dr. Robert J. Delange for use of his protein chemistry laboratory facilities; Mr. Mark Lehrman for providing the neoglycoproteins; and Ms. Jan Seager, Mrs. Martha Hokom, Mr. Daniel Flores, and Mrs. Mercedes Limon for expert technical assistance. This work was supported in part by U.S. Public Health Service Grants HL 25590, HL 19063, 1T32 H1 07412, and RR 865; a grant from the American Heart Association, Greater Los Angeles Affiliate (649-P5); and the Laubisch Funds. A.M.F. is the recipient of a U.S. Public Health Service Research Career Development Award (HL-00426). P.A.E. is an Established Investigator of the American Heart Association.

1. Fogelman, A. M., Shechter, I., Seager, J., Hokom, M., Child, J. S. & Edwards, P. A. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 2214–2218.
2. Shechter, I., Fogelman, A. M., Haberland, M. E., Seager, J., Hokom, M. & Edwards, P. A. (1981) *J. Lipid Res.* **22**, 63–71.
3. Fogelman, A. M., Haberland, M. E., Seager, J., Hokom, M. & Edwards, P. A. (1981) *J. Lipid Res.* **22**, 1131–1141.
4. Brown, M. S., Basu, S. K., Falck, J. R., Ho, Y. K. & Goldstein, J. L. (1980) *J. Supramol. Struct.* **13**, 67–81.
5. Hamberg, M., Svensson, J., Wakabayashi, T. & Samuelson, B. (1974) *Proc. Natl. Acad. Sci. USA* **71**, 345–349.
6. Hammerstrom, S. & Falardeau, P. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 3691–3695.
7. Smith, J. B., Interman, C. M. & Silver, M. J. (1976) *J. Lab. Clin. Med.* **88**, 167–172.
8. Barber, A. A. & Bernheim, F. (1967) *Advances in Gerontological Research*, ed. Strehler, B. L. (Academic, New York), Vol. 2, pp. 355–403.
9. Stossel, T. P., Mason, R. J. & Smith, A. L. (1974) *J. Clin. Invest.* **54**, 638–645.
10. Fogelman, A. M., Seager, J., Hokom, M. & Edwards, P. A. (1979) *J. Lipid Res.* **20**, 379–388.
11. Fogelman, A. M., Seager, J., Edwards, P. A. & Popjak, G. (1977) *J. Biol. Chem.* **252**, 644–651.
12. McFarlane, A. S. (1958) *Nature (London)* **182**, 53.
13. Bilheimer, D. W., Eisenberg, S. & Levy, R. I. (1972) *Biochim. Biophys. Acta* **260**, 212–221.
14. Patton, S. & Kurtz, G. W. (1951) *J. Dairy Sci.* **34**, 669–674.
15. Steele, Jr., J. H. & Reynolds, J. A. (1979) *J. Biol. Chem.* **254**, 1639–1643.
16. Lowry, O. H., Rosebrough, M. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275.
17. Mitchell, C. D., King, W. C., Applegate, K. R., Forte, T., Glomset, J. A., Norum, K. R. & Gjone, E. (1980) *J. Lipid Res.* **21**, 625–634.
18. Goldstein, J. L. & Brown, M. S. (1974) *J. Biol. Chem.* **193**, 265–275.
19. Wofsy, L. & Singer, S. J. (1963) *Biochemistry* **2**, 104–116.
20. Weisgraber, K. H., Innerarity, T. L. & Mahley, R. W. (1978) *J. Biol. Chem.* **253**, 9053–9062.
21. Habeeb, A. F. S. A. (1966) *Anal. Biochem.* **14**, 328–336.
22. Chio, K. S. & Tappel, A. L. (1969) *Biochemistry* **8**, 2827–2832.
23. King, T. P., (1966) *Biochemistry* **11**, 3454–3459.
24. Hoff, H. F. (1979) *Artery* **6**, 178–187.
25. Mahley, R. W., Weisgraber, K. H., & Innerarity, T. L. (1979) *Biochim. Biophys. Acta* **575**, 81–91.
26. Brown, M. S., Ho, Y. K., & Goldstein, J. L. (1980) *J. Biol. Chem.* **255**, 9344–9352.
27. Shireman, R. B. & Fisher, W. R. (1979) *Biochim. Biophys. Acta* **572**, 537–540.
28. Stahl, P. D., Rodman, J. S., Miller, M. J. & Schlesinger, P. H. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 1399–1403.
29. Ashwell, G. & Morell, A. G. (1974) *Adv. Enzymol. Relat. Areas Mol. Biol.* **41**, 99–128.
30. Van Den Hamer, C. J. A., Morell, A. G., Scheinberg, I. H., Hickman, J. & Ashwell, G. (1970) *J. Biol. Chem.* **245**, 4397–4402.
31. Goldstein, J. L., Ho, Y. K., Basu, S. K. & Brown, M. S. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 333–337.
32. Mahley, R. W., Innerarity, T. L., Weisgraber, K. H. & Oh, S. Y. (1979) *J. Clin. Invest.* **64**, 743–750.
33. Mahley, R. W., Weisgraber, K. H., Innerarity, T. L. & Windmueller, H. G. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 1746–1750.
34. Santos, M. T., Valles, J., Aznar, J. & Vilches, J. (1980) *J. Clin. Pathol.* **33**, 973–976.