Role of Lysines in Mediating Interaction of Modified Low Density Lipoproteins with the Scavenger Receptor of Human Monocyte Macrophages*

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The ability of the scavenger receptor of human monocyte macrophages to recognize human low density lipoproteins (LDL) progressively modified by three lysine-specific reagents, malondialdehyde, acetic anhydride, or succinic anhydride, has been investigated. Regardless of the reagent utilized, receptor-mediated uptake was dependent upon modification of >16% of the peptidyl lysines rather than upon the net negative charge of derivatized LDL. Rates of lysosomal hydrolysis of acetyl-LDL and succinyl-LDL increased as a function of progressive modification and reflected the amount of derivatized LDL binding to the receptor. Succinylation or acetylation of >60% of the lysines was necessary to attain maximal ligand binding, internalization, and degradation. In contrast, modification of only 16% of the peptidyl lysines by malondialdehyde resulted in maximal levels of binding, uptake, and hydrolysis. The expression of receptor recognition site(s) appears to depend upon the charge modification of critical lysine residues of the LDL protein rather than the net negative charge of the lipoprotein complex. Malondialdehyde, a bifunctional reagent, may modify surface and sequestered lysines concomitantly and thus promote efficient formation of the recognition site(s).

There is now substantial evidence that blood monocytes are precursors of certain foam cells in the early stages of atherogenesis (1-7). The development of foam cells characteristically includes the massive deposition of cholesteryl esters in lipid droplets within the cytoplasm (8, 9). Although the molecular mechanisms involved in this cellular transition have yet to be defined, human blood monocytes and macrophages derived from monocytes in culture express several membrane receptors which mediate the internalization of certain cholesteryl ester-rich lipoproteins. These include the LDL receptor, the scavenger receptor, and the β-VLDL receptor (10-15).

The scavenger receptor mediates the endocytosis of modified LDL which are anionic in character (16, 17). This receptor is present on human monocytes and macrophages (11, 18), macrophages of lower animals (16), endothelial cells of bovine origin (19, 20), as well as foam cells obtained from explants of rabbit atherosclerotic aorta (21). The activity of the scavenger receptor, in contrast to the activities of the LDL and β-VLDL receptors (22, 23), is not subject to down regulation by the intracellular content of cholesterol (16, 18). Thus, continued endocytosis of cholesterol-rich lipoproteins via the scavenger receptor of macrophages and subsequent lysosomal hydrolysis generate large quantities of intracellular cholesterol, of which equal amounts are either re-esterified in the cytoplasm or secreted into the medium (24). As a consequence, massive accumulation of esterified cholesterol occurs in macrophages incubated with certain anionic lipoproteins in vitro (25). These cells have some of the characteristics of foam cells of atherosclerotic lesions (25).

Anionic LDL which are internalized by the scavenger receptor have been produced by a variety of methods. Chemical modification of LDL by acetic anhydride (16), diketene (26), or malondialdehyde (10, 27), incubation of LDL with endothelial cells (28, 29), and extraction of cholesteryl ester-rich particles from atherosclerotic aorta (30) each results in lipoproteins which are recognized by the scavenger receptor. Competitive displacement experiments have demonstrated that acetyl-LDL and malondialdehyde-LDL compete on an equimolar basis for binding and uptake by the scavenger receptor of human monocyte macrophages and murine peripheral macrophages (11, 17), whereas endothelial cell-modified LDL and the arterial extracted particles, although preferentially recognized by the scavenger receptor, only partially compete with the chemically modified lipoproteins (28, 30). Since the scavenger receptor recognizes not only these several modified lipoproteins but also a diverse group of polyanionic macromolecules, including maleyl-human albumin, polyinosinic acid, and fucoidin, it has been suggested that a specific configuration of multiple negative charges is necessary to induce receptor binding of these different ligands (17, 31).

We have previously demonstrated that neutralization of ≤16% of the lysine residues of the apo-B polypeptides of LDL by malondialdehyde, or ≤60 mol lysine/mol of LDL, results in recognition and uptake of the modified lipoprotein by the LDL receptor in human monocyte macrophages (27). Further modification of the LDL results in threshold recognition and endocytosis by the scavenger receptor with concomitant loss of recognition by the LDL receptor. The rate of degradation of the lipoprotein via the LDL receptor pathway is inversely related to the degree of modification. In contrast, the uptake of the modified lipoprotein mediated by the scavenger receptor...
and subsequent rate of hydrolysis are independent of the extent of incorporation of malondialdehyde into LDL once receptor recognition has been achieved (27).

On the basis of these results we suggested that scavenger receptor-mediated uptake of malondialdehyde-LDL was dependent upon the formation of recognition site(s) involving specific modified lysine residues and/or as a consequence of changes in the conformation of the apo-B protein in the lipoprotein complex (27). The possibility also existed that a general electrostatic mechanism requiring only modified LDL of a certain net negative charge accounted for the threshold recognition of malondialdehyde-LDL. In this report we have determined the effects of chemical modification of LDL by several lysine-specific reagents upon recognition of the altered lipoproteins by the scavenger receptor of human monocyte-macrophages.

**EXPERIMENTAL PROCEDURES**

**Materials**—Sodium I[125]Iodide (15 mCi/μg) and [1,4-14C]succinic anhydride (115 mCi/mg) were purchased from Amersham Corp. Perkin-Elmer I[125]Iodine was purchased from Sigma. All other supplies and reagents were obtained from sources previously reported (32).

**Subjects**—Normal subjects were recruited from the staff and student body at the University of California. No one received drugs that might alter the cholesterol or triglyceride content of their plasma or lipid metabolism. The subjects had normal hematocrits, white blood cell and differential counts, serum cholesterol, and triglycerides. Informed consent was obtained in writing from each person.

**Separation of Cells**—Five hundred ml of blood was taken after an overnight fast and the monocytes were separated from 300 ml of blood using counterflow centrifugation (method BB in Ref. 18).

**Cell Culture**—Cells were suspended in 30% autologous serum in Dulbecco's modified Eagle's medium supplemented with NaHCO3 (24 mM), Hapes (10 mM), insulin (8 μg/ml), and fungizone (0.025 μg/ml), hereinafter referred to as Medium B in order to be consistent with our previous publications (18, 32). One-ml samples of the cell suspension containing 1.2 × 10⁶ cells were transferred to 9.6-cm² polystyrene wells (Falcon 3046) for studies dealing with cellular binding or cellular association of lipoproteins, or 0.5-ml samples of the cell suspension containing 0.5 × 10⁶ cells were transferred to 2.0-cm² polystyrene wells (Falcon 3047) for studies dealing with lysosomal hydrolysis of lipoproteins, and incubated at 37 °C in an atmosphere of 5% CO₂, 95% incubator. The medium was aspirated and replaced with fresh medium of the same composition twice weekly.

**Classification and Viability of the Cells**—The cells were classified and their viability determined as previously described (33). Because of the nature and the washing procedures being performed prior to the determination, the cells were ≥90% monocyte macrophages before the radioactive lipoproteins were added. More than 95% of the cells were viable at the end of the incubation.

**Preparation of Sera**—Autologous serum was prepared from 200 ml of blood as previously described (18).

**Lipoproteins**—Human LDL (d 1.019–1.063 g/ml) was isolated from the serum of individual healthy human subjects by ultracentrifugation (method BB in Ref. 18). LDL was modified by malondialdehyde and the adduct was quantitated by colorimetric assay with thiobarbituric acid as previously reported (10, 27).

**RESULTS**

We have previously demonstrated that modification of 60 mol of lysine of the LDL protein by malondialdehyde is sufficient to produce threshold recognition of the lipoprotein by the scavenger receptor (27). To discriminate between net charge change and the number of modified lysyl residues of the lipoprotein required for recognition of chemically derivatized LDL by the scavenger receptor, succinic anhydride was selected as a second chemical reagent for the modification. Whereas interaction of malondialdehyde with peptidyl lysine modified by malondialdehyde, and a charge change of ≥2 occurs upon interaction of succinic anhydride with lysine. The number of modified lysines were critical for inducing recognition of the chemically derivatized LDL by the scavenger receptor, we would predict that succinylation of >60 mol of lysine/mol of LDL would be necessary to promote ligand-receptor interaction. Alternatively, if the charge change reflected by the electrophoretic mobility of the chemically derivatized LDL protein were the major requisite for receptor recognition of malondialdehyde-LDL, the same charge change could be achieved by succinylation of only 30 mol of lysine/mol of LDL.
complex measured by colorimetric assay corresponded with the number of modified lysyl residues of the apo-B polypeptides determined by amino acid analysis (27). The progressive incorporation of [1,4-14C]succinic anhydride into LDL directly related to an equimolar loss of free amino groups as determined by colorimetric assay.

Scaven et al. (38) had previously characterized LDL in which >90% of the lysine groups had been succinylated. Even at this high degree of modification, the lipid composition, ultraviolet and far-ultraviolet circular dichroic spectra, and molecular weight of the succinyl-LDL were identical to those determined for the unmodified lipoprotein. In view of these earlier detailed studies, we consider it likely that succinylation of LDL described in this report resulted primarily in the formation of succinyllysine derivatives of the apo-B polypeptides without disruption of the lipoprotein complex.

Comparison of the anodic mobilities of succinyl-LDL and malondialdehyde-LDL in agarose electrophoresis is shown in Fig. 1 as a function of progressive modification of lysines of the LDL protein. As would be predicted by the chemical reactions of these lysine-specific reagents (Table I), it was necessary to modify twice as many lysyl residues by malondialdehyde as by succinic anhydride in order to achieve the same electrophoretic mobility of the lipoprotein complex. The linear relationships shown in Fig. 1 have been confirmed over a range of 10-200 mol of lysine modified per mol of LDL for lipoprotein isolated from four separate subjects (data not shown). In each preparation, a single discrete band was observed, whether detected by the lipid stain, fat red 7B, or by autoradiography (data not shown). We have assumed then that the molar ratios reported in this study reflected the extent of modification of the majority of the molecules within each given preparation.

The rate of degradation of 125I-lipoprotein as a function of progressive modification by succinic anhydride is shown in A of Fig. 2. Since the human monocyte macrophages express both the LDL and scavenger receptors (11), it is also necessary to determine the specificity and degree of recognition by each receptor by competition analyses. The data obtained for 125I-native LDL demonstrated the typical pattern for ligands selectively recognized and internalized by the LDL receptor (Fig. 2A). The rate of hydrolysis of 125I-labeled LDL was effectively suppressed by addition of a 26-fold molar excess of nonradioactive native LDL, and was not affected by the addition of competitors of the scavenger receptor, i.e. malondialdehyde-LDL or polyniosinic acid. Likewise, the typical competitive analysis obtained for ligands bound and internalized by the scavenger receptor is shown for 125I-labeled LDL in which 180 mol of lysine/mol of lipoprotein have been modified by malondialdehyde (Fig. 2B). The rate of hydrolysis of 125I-malondialdehyde-LDL was selectively suppressed by the addition of either a 26-fold molar excess of nonradioactive malondialdehyde-LDL or polyniosinic acid, and was not affected by the addition of an excess of nonradioactive native LDL.

The designation ▲ denotes the onset of threshold recognition by the scavenger receptor which occurs upon neutralization of 60 mol of lysine/mol of LDL by malondialdehyde (27). The designation ◆ denotes the degree of succinylation of LDL necessary to achieve an electrophoretic mobility comparable to the electrophoretic mobility of malondialdehyde-LDL which results in the onset of threshold recognition. It is clear that net negative charge of the succinylated lipoprotein is insufficient to promote recognition by the scavenger receptor. LDL in which 37 or 56 mol of lysine had been succinylated per mol of LDL were preferentially recognized by the LDL.

### Table I

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Modification</th>
<th>Charge Change/Lysine</th>
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<td>Malondialdehyde</td>
<td>R-N=CH_2CH_2CH_2-CH=O</td>
<td>▲ neutral; △ change = -1</td>
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<tr>
<td>Succinic anhydride</td>
<td>R-NH-C_2CH_2CH_2-CH=O</td>
<td>▲ ▲ human; △ change = -2</td>
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<tr>
<td>Acetic anhydride</td>
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### Table II

<table>
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<tr>
<th>Initial molar ratio</th>
<th>Final molar ratio</th>
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<tr>
<td>3.1</td>
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<td>146.6</td>
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<tr>
<td>12.2</td>
<td>212.6</td>
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</tbody>
</table>

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*The [1,4-14C]succinic anhydride (118 or 59 mCi/mmol) was made 0.02 M in chloroform prior to aliquoting as described under "Experimental Procedures." LDL (1.5 mg, 3 nmol) was calculated to contain a total of 1104 nmol of lysine for determination of the initial molar ratio.

*Calculated assuming $M_r = 500,000$ for the total protein component of LDL (27, 35).

* Determined as the difference in lysyl content of the original unmodified LDL and the [1,4-14C]succinyl-LDL by colorimetric assay (34).
receptor. The lower rates of hydrolysis of these two succinyl-LDL preparations compared to 125I-native LDL were consistent with the effect of modification of lysine residues upon binding by the LDL receptor (27, 39).

Recognition of succinyl-LDL by the scavenger receptor occurred upon modification of >106 mol of lysine/mol of LDL, based upon the finding that both nonradioactive malondialdehyde-LDL and polyinosinic acid suppressed the hydrolysis of these preparations of 125I-succinyl-LDL to the same degree. These and other data from similar experiments demonstrated that charge modification of a minimal number of lysyl residues of the apo-B polypeptides of LDL was necessary to induce recognition by the receptor. Although an increased anionic electrophoretic mobility of the lipoprotein necessarily occurred with charge modification, the net addition of negative charge provided by succinylation was insufficient to account for receptor recognition.

In contrast to the threshold recognition observed for the hydrolysis of malondialdehyde-LDL (27), the rates of degradation of succinyl-LDL attributed to high affinity uptake by the scavenger receptor increased as a function of progressive succinylation. It was necessary to modify >67% of the lysines of the LDL protein by succinylation (S248 in A, Fig. 2) in order to produce an altered lipoprotein which was hydrolyzed at the same rate as malondialdehyde-LDL (Fig. 2B). To elucidate if factors such as steric hindrance or electrostatic repulsion of the succinyllysine derivatives contributed to the reduced rates of degradation and/or prevented interaction of the ligand binding site(s) with the scavenger receptor, the effect of progressive neutralization of lysines of the LDL protein by reaction with acetic anhydride (Table I) upon receptor recognition and rates of hydrolysis was examined. Acetic anhydride has previously been shown by Goldstein et al. (16) to be an effective reagent for the modification of LDL. The reaction typically has been conducted at a ratio of 40 mol of acetic anhydride/mol of LDL (16). Acetyl-LDL prepared in this manner competed on an equimolar basis with malondialdehyde-LDL for scavenger receptor-mediated uptake and degradation in both human monocyte macrophages (11) and murine peritoneal macrophages (17), and thus appeared particularly suitable for the purposes of this study.

Progressive modification of 125I-labeled LDL was conducted at ratios of 1.4, 2.9, 9, and 20 mol of acetic anhydride/mol of LDL lysine. It was determined by colorimetric analysis that modification of 167, 147, 188, and 225 mol of lysine/mol of LDL protein had occurred under these conditions. As shown by the competition data in Fig. 3, each of the 125I-acetyl-LDL preparations was specifically recognized by the scavenger receptor of human monocyte macrophages. Moreover, the results were similar to those previously obtained by succinylation of LDL (Fig. 2). The rates of degradation of acetyl-LDL due to specific uptake were lower than the hydrolytic rate of malondialdehyde-LDL, and increased as a function of progressive acetylation. A rate of hydrolysis comparable to that obtained with malondialdehyde-LDL was obtained after acetylation of 61% of the lysyl residues (Ac225 in Fig. 3).

Thus both acetylation and succinylation resulted in formation of the receptor recognition site(s) on LDL, but neither method of chemical derivatization promoted threshold recognition of the modified LDL. The rates of hydrolysis of the acetyl- and succinyl-LDL preparations mediated by the scavenger receptor were directly related to the number of modified lysines, whether the peptidyl lysines had been neutralized by acetylation or succinylation.
acetylation or charge substituted by succinylation. The direct relationship of the degree of succinylation of LDL to the subsequent rate of hydrolysis dependent upon the scavenger receptor persisted over a concentration range of 5-100 μg of lipoprotein/ml (Fig. 4). The kinetics of specific hydrolysis of the several preparations of succinyl-LDL were biphasic and characteristic of cellular degradation dependent upon high-affinity uptake. In contrast, the saturation kinetics and rate of hydrolysis of malondialdehyde-LDL mediated by the scavenger receptor were independent of the degree of chemical modification (Fig. 5), as would be expected to occur for threshold recognition of this ligand (27).

The data of Fig. 4 suggested that receptor recognition of succinyl-LDL increased as the degree of modification increased, and that this should be reflected in the cross-competition analyses. The ability of preparations of succinyl-LDL to inhibit the degradation of 125I-malondialdehyde-LDL is shown in Fig. 6. Progressive succinylation of LDL produced modified lipoproteins which became increasingly more effective in preventing the hydrolysis of 125I-malondialdehyde-LDL.

Rates of hydrolysis of 125I-modified lipoprotein measure the full spectrum of cellular events initiated by receptor-mediated uptake, and include binding of the ligand at the cell surface, internalization of the ligand-receptor complex, and lysosomal hydrolysis of the ligand (16). As shown in Fig. 7, progressive succinylation of 125I-LDL resulted in an increase in the amount of lipoprotein specifically bound to the cell at 4 °C. A maximal level of binding equivalent to that of malondialdehyde-LDL was achieved with succinylation of >69% of the lysines of the LDL protein (S253 and S262 in Fig. 7). The increase in binding at 4 °C as a function of progressive succinylation was paralleled by a similar increase in cellular-associated radioactivity at 37 °C (Fig. 8). Thus the rates of lysosomal hydrolysis for LDL modified by succinic anhydride or by malondialdehyde (Figs. 2, 4, and 5) were proportional

**Fig. 4.** Specific degradation of 125I-modified LDL as a function of the extent of modification by succinic anhydride or malondialdehyde. Normal human monocytes were cultured in 30% autologous serum in 0.5 ml of Medium B for 8 days and were prepared for studies at 37 °C as described under “Experimental Procedures.” Each well received 0.5 ml of Medium D containing the indicated concentrations of 125I-lipoprotein (104 cpm/ng) in which 70, 177, or 220 μg of lysine had been succinylated per mol of LDL, designated S70 (x), S177 (X), or S220 (C), respectively, or in which 238 μg of lysine had been modified by malondialdehyde/mol of LDL, designated M238 (0). After incubation for 4 h at 37 °C, the medium was removed and the content of 125I-labeled acid-soluble material was determined. Specific degradation was determined by the difference in rates of degradation obtained in the absence or presence of polyinosinic acid at 30 μg/ml. Each point represents the mean of three determinations.

**Fig. 5.** Specific degradation of LDL as a function of progressive modification by malondialdehyde. Normal human monocytes were cultured in 30% autologous serum in 0.5 ml of Medium B for 7 days and were prepared for studies at 37 °C as described under “Experimental Procedures.” Each well received 0.5 ml of Medium D containing the indicated concentrations of 125I-lipoprotein (72 cpm/ng) in which 89 (0), 171 (C), 182 (A), or 213 (x) mol of lysine/mol of LDL had been modified by malondialdehyde. After incubation for 4 h at 37 °C, the medium was removed and the content of 125I-labeled acid-soluble material was determined. Specific degradation was determined by the difference in rates of degradation obtained in the absence or presence of polyinosinic acid at 100 μg/ml. The values shown are the mean ± 1 S.D. of triplicate wells.

**Fig. 6.** Competitive inhibition of the degradation of 125I-malondialdehyde LDL by LDL modified by succinic anhydride by malondialdehyde. Normal human monocytes were cultured in 30% autologous serum in 0.5 ml of Medium B for 7 days and were prepared for studies at 37 °C as described under “Experimental Procedures.” Each well received 0.5 ml of Medium D containing 10 μg/ml of 125I-malondialdehyde LDL (180 μg of lysine modified by malondialdehyde/mol of LDL, 128 cpm/ng) together with the concentration of nonradioactive lipoprotein indicated on the abscissa. Nonradioactive lipoprotein preparations included LDL in which 136, 156, 218, or 238 mol of lysine had been succinylated per mol of LDL, designated S136 (0), S156 (C), S218 (A), or S238 (C), respectively, and malondialdehyde LDL in which 171 mol of lysine had been modified per mol of LDL, designated M171 (x). After incubation for 4 h at 37 °C, the medium was removed and the content of 125I-labeled acid-soluble material was determined. The 100% value for the degradation of 125I-malondialdehyde LDL in the absence of added proteins was 1.4 μg degraded 4 h−1 mg of cell protein−1.
Scavenger Receptor Uptake of Chemically Modified LDL

FIG. 7. Specific binding at 4 °C of 125I-modified LDL as a function of the extent of modification by succinic anhydride or malondialdehyde. Normal human monocytes were cultured in 50% autologous serum in 1 ml of Medium B for 10 days and were prepared for studies at 4 °C as described under "Experimental Procedures." Each well received 1.0 ml of Medium C containing 10 μg/ml of 125I-lipoprotein (157 cpm/pg) in which 126, 196, 253, or 262 mol of lysine had been succinylated per mol of LDL, designated S126, S196, S253, or S262, respectively, or in which 218 mol of lysine had been modified by malondialdehyde per mol of LDL, designated M218. After gentle rotation for 90 min at 4 °C, the medium was removed and the 125I-lipoprotein bound to the cells was determined as described under "Experimental Procedures." Specific degradation was determined by the difference in binding obtained in the absence or presence of polyinosinic acid at 100 μg/ml. The values shown are the mean ± 1 S.D. of quadruplicate wells.

FIG. 8. Specific association of 125I-modified LDL with cells at 37 °C as a function of the extent of modification by succinic anhydride or malondialdehyde. Normal human monocytes were cultured in 50% autologous serum in 1 ml of Medium B for 10 days and were prepared for studies at 37 °C as described under "Experimental Procedures." Each well received 1 ml of Medium D containing 50 μg/ml of 125I-lipoprotein (200 cpm/pg) in which 92, 190, or 209 mol of lysine had been succinylated per mol of LDL, designated S92, S190, or S209, respectively, or in which 175 mol of lysine had been modified by malondialdehyde/mol of LDL, designated M175. After incubation for 2 h at 37 °C, the medium was removed and the 125I-lipoprotein associated with the cells was determined as described under "Experimental Procedures." Radioactivity specifically associated with the cells was determined as the difference obtained in the absence or presence of polyinosinic acid at 100 μg/ml. The values shown are the mean ± 1 S.D. for quadruplicate wells.

to the amount of modified lipoprotein initially bound by the scavenger receptor.

Experiments to determine the specific binding of ligand at 4 °C as a function of protein concentration were conducted to learn if changes in the equilibrium dissociation constant or maximal binding capacity accounted for the differences in cellular processing of LDL as a function of progressive succinylation. It became apparent that the low levels of specific binding for those lipoprotein preparations containing from 125 to 200 mol of succinyllysine/mol of LDL precluded accurate determination of the dissociation constant and maximal binding capacity. Whether or not these parameters can be determined by modification of this assay for such studies in macrophages derived from human monocytes has yet to be resolved.

DISCUSSION

The relationship of anionic character to ligand recognition by the scavenger receptor of human monocyte macrophages has been determined for several negatively charged LDL produced by chemical derivatization. It is clear from these studies that the net negative charge of the altered lipoprotein complex alone is insufficient to induce ligand-receptor interaction. Receptor-mediated uptake is more specifically dependent upon alteration of the charge of a limited number of the ε-amino groups of lysines of the apo-B polypeptides. Modification of >16% of the peptidyl lysines by malondialdehyde, or >29% by succinic anhydride or by acetic anhydride promotes recognition of the modified lipoprotein by the scavenger receptor.

Of the three lysine-specific reagents, malondialdehyde is particularly efficient in generating altered LDL which is recognized by the receptor. Charge neutralization of 60 mol, or 16%, of the lysyl residues of the LDL protein by malondialdehyde is sufficient to result in maximal levels of receptor binding and, consequently, of lysosomal hydrolysis. What might account for the efficient modification of LDL by malondialdehyde? We propose that several classes of lysyl residues exist which differ in reactivity toward modification by a given reagent. As presented in 1970 by Meighen and Schachman (40), a relatively electrophoretically homogeneous species of derivatized protein would be obtained if this were the case. We have consistently observed a homogeneous, discrete band in agarose electrophoresis for LDL modified by malondialdehyde, succinic anhydride, or acetic anhydride regardless of the degree of modification. The interaction of malondialdehyde with the lipoprotein involves modification of 2 mol of lysine/mol of malondialdehyde (27). If one considers that the reaction of the first aldehydic function occurs with a readily accessible and reactive lysine, then reaction of the second aldehydic function is necessarily confined to lysyl residues within the radius of the first lysyl adduct. The opportunity thus exists for modification of lysyl residues of lower reactivity which may be sequestered within the tertiary structure of the apo-B polypeptides. Both the physical constraint imposed by the three-carbon unit of malondialdehyde and its bifunctional character may result in concomitant modification of lysyl residues from two classes of differing reactivity. Efficient formation of the site(s) resulting in threshold recognition and binding by the receptor may be due to the ability of malondialdehyde to interact with certain less reactive lysyl residues.

In contrast, both acetic anhydride and succinic anhydride interact with the ε-amino group of lysine on an equimolar basis. Acetylation or succinylation of LDL could proceed by sequential modification of that class of more reactive lysines, presumably those at or near the surface of the lipoprotein complex, before proceeding to the next class of lower reactivity. A similar mode of reaction with LDL may also exist for other monofunctional, lysine-specific reagents, including di-
ketene, cyanate, and maleic anhydride. Although acetylation or succinylation of >29% of the lysyl residues of the LDL protein promotes ligand-receptor interaction, modification of >60% of the ε-amino groups is necessary to achieve maximal rates of receptor-mediated hydrolysis (Figs. 2 and 3).

Both malondialdehyde-LDL and polyinosinic acid effectively suppress the receptor-mediated binding and lysosomal hydrolysis of the anhydride-modified lipoproteins to the same degree. The converse, however, does not occur: the ability of succinyl-LDL to compete with malondialdehyde-LDL is dependent upon the degree of succinylation. Progressive succinylation results in an increasingly more effective competitor of the degradation of malondialdehyde-LDL (Fig. 6). Similar results have been obtained for the scavenger receptor-mediated uptake of other negatively charged lipoprotein complexes, i.e. cholesteryl ester-rich particles extracted from human atherosclerotic aorta (30) and endothelial cell-modified LDL (28, 29).

The molecular features of the recognition determinant(s) on modified low density lipoprotein have yet to be defined. We propose that a conformational change in the tertiary structure of the apo-B polypeptides of the lipoprotein complex occurs in response to charge modification of specific, critical lysyl residues and results in the spatial clustering of amino acyl residues comprising the recognition determinant(s). The residues forming the recognition domain(s) may be present in a single, continuous portion of the amino acid sequence (sequence-dependent domain) or may be comprised of several distant regions of the primary sequence spatially located in close proximity (conformation-dependent domain). Brown and Goldstein and their colleagues (17, 31) have proposed that a specific cluster of negatively charged groups provided by the ligand is essential for interaction with the scavenger receptor. Such a cluster of negative charge could either serve as a critical structural element of the recognition determinant(s) or play a role in the orientation of the ligand with respect to binding to the receptor.

It is important to consider that charge modification of the peptidyl lysines may be but one mechanism for promoting expression of the recognition determinant(s). It is possible, for example, that a change in conformation of the apo-B polypeptides may also occur in response to changes in the lipid composition of the lipoprotein complex, e.g. as occurs with endothelial cell-modified LDL (28, 29), or in response to interaction of other macromolecules with low density lipoprotein.

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REFERENCES