Vol. 137, No. 1, 1986 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
May 29, 1986 Pages 29-35

SCAVENGER RECEPTOR FOR MALONDIALDEHYDE-MODIFIED HIGH DENSITY LIPOPROTEIN ON RAT SINUSOIDAL LIVER CELLS

Masaji Murakami, Seikoh Horiuchi*, Kyoko Takata and Yoshimasa Morino

Department of Biochemistry, Kumamoto University Medical School, Honjo, 2-2-1, Kumamoto 860, Japan

Received March 11, 1986

SUMMARY: We report here the presence of a membrane-associated receptor which mediates endocytic uptake of malondialdehyde-modified high density lipoprotein (MDA-HDL) on sinusoidal liver cells. Binding of [^{125}I]MDA-HDL to the cells was followed by internalization and degradation in lysosomes. The binding and lysosomal degradation of [^{125}I]MDA-HDL were effectively inhibited by unlabeled MDA-HDL and acetyl-HDL. However, formaldehyde-treated serum albumin or low density lipoprotein modified either by acetylation or malondialdehyde, ligands known to undergo receptor-mediated endocytosis by sinusoidal liver cells, did not affect the binding of [^{125}I]MDA-HDL to the cells. These results indicate that a receptor for MDA-HDL is described as a distinct member among the scavenger receptors for chemically modified proteins.

Receptor-mediated endocytosis of chemically modified proteins generally called "Scavenger function", is one of the prominent features of macrophages or macrophage-derived cells, and has recently been paid special attention from its potential link to disease processes such as atherosclerosis (1) and diabetes mellitus (2). Ligands known to belong to this category so far include maleylated albumin (3-5), acetyl-LDL (3, 6-8), MDA-LDL (5, 9-12), and aldehyde-modified proteins such as f-Alb and glycolaldehyde-modified albumin (13-16). From the established fact that sinusoidal liver cells, major scavenger cells in vivo, express on their surface membranes the receptors which mediate the endocytic uptake of these chemically modified proteins (14, 17, 18), it is relevant to use these cells to elucidate molecular mechanism for the scavenger function of macrophages or macrophage-derived cells. Our previous studies using these cells revealed that endocytic uptake of these ligands was mediated by at least two

*To whom correspondence should be addressed.

Abbreviations used are: HDL, high density lipoprotein; LDL, low density lipoprotein; MDA-HDL, malondialdehyde-modified HDL; MDA-LDL, malondialdehyde-modified LDL; acetyl-HDL, acetylated HDL; acetyl-LDL, acetylated LDL; f-Alb, formaldehyde-modified albumin; TCA, trichloroacetic acid.

0006-291X/86 $1.50

different receptors, one for acetyl-LDL or MDA-LDL and another for aldehyde-modified proteins such as f-Alb (19).

During our studies on the scavenger function for chemically modified proteins, we noted that MDA-HDL was also endocytosed by sinusoidal liver cells. In this communication, we provide evidence for the presence on sinusoidal liver cells of a new receptor-mediated pathway, in which MDA-HDL is bound to a cell surface receptor, internalized, transported to lysosomes, and degraded.

MATERIALS AND METHODS

Lipoproteins. HDL (d = 1.063 - 1.21) and LDL (d = 1.019 - 1.063) were isolated by ultracentrifugation from fresh human plasma of normolipidemic subjects after overnight fasting as described previously (19), and these lipoproteins were dialyzed against 0.15 M NaCl and 1 mM EDTA, pH 7.4. The purity of the LDL preparation was the same as that reported previously (19). The HDL preparation was further treated by heparin-Sepharose affinity chromatography (20) to remove traces of apolipoprotein E and B. The HDL preparation thus obtained was found to contain no apolipoprotein E or B upon sodium dodecyl sulfate polyacrylamide gel electrophoresis. Protein was measured as described previously (14, 21).

Chemical Modification of Lipoproteins and Iodination. MDA-HDL and MDA-LDL were prepared by incubation with malondialdehyde generated from malonaldehyde bis(dimethyl acetal) (Aldrich) according to the method of Fogelman et al. (9). Acetyl-LDL and acetyl-HDL were prepared by acetylation with acetic anhydride according to the method of Basu et al.(22). MDA-HDL and acetyl-HDL were iodinated with [^{125}I] as described previously (14). The extent of lysine modification by acetylation or malondialdehyde treatment was determined with trinitrobenzenesulfonic acid as the difference in lysyl residues of modified and unmodified HDL according to the method of Habeeb (23) as described previously (16). The extents of lysine modification of these ligands were as follows: MDA-HDL, 92.0 %; MDA-LDL, 86.2%; acetyl-HDL, 85.2%; acetyl-LDL, 91.5%. f-Alb was prepared as described previously (14).

Binding Assay. Sinusoidal liver cells were prepared from male Wistar rats as described previously (19, 24). The isolated cells were suspended in Eagle's essential medium containing 3% bovine serum albumin buffered with 20 mM 2-(4-hydroxyethyl)-1-piperazineethanesulfonic acid to pH 7.4 (buffer A). Binding assay was performed in 1.6-ml of polyethylene centrifuge tube (Eppendorf) as described previously (19). Briefly, a reaction mixture contained, in a total volume of 0.1 ml of buffer A, 3 x 10^6 cells and a fixed amount of [^{125}I] MDA-HDL in the absence or presence of an unlabeled ligand to be tested. The reaction was initiated by the addition of [^{125}I] MDA-HDL. Incubation was performed for 90 min at 0°C with several intervals on a Vortex mixer. Upon termination of the reaction, 1.0 ml of ice-cold buffer A was added to each tube, followed by centrifugation at 12800 x g for 25 sec at 4°C. The supernatant was discarded and the pelleted cells were resuspended in 1.0 ml of ice-cold buffer A. After the cells were washed twice more, the cell-associated radioactivity was measured as described previously (19).

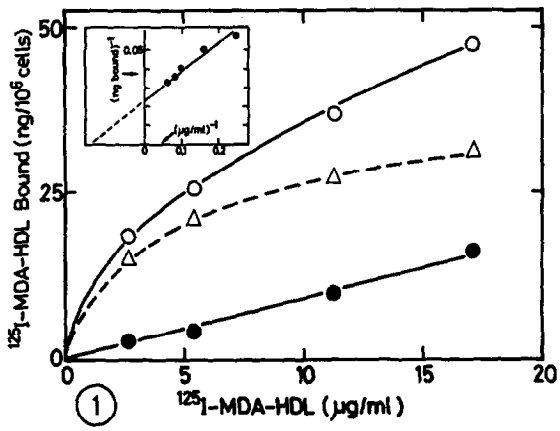
Endocytosis and Subsequent Intracellular Degradation. After preincubation for 10 min at 37°C, sinusoidal liver cells (1.2 x 10^7) were incubated, in a total volume of 2.0 ml of buffer A, at 37°C with 4.7 µg of [^{125}I]MDA-HDL or [^{125}I]-acetyl-HDL in 2.0 ml of buffer A. Time aliquots (0.2 ml) were withdrawn at

indicated times and placed on the top of mixture of dinonylphthalate and dibutylphthalate (1:3) in a polyethylene tube, followed by centrifugation at 12800 x g for 1 min. A portion (0.1 ml) of the resulting supernatant was determined for TCA-soluble radioactivity as described previously (25). The plot of an increase in TCA-soluble radioactivity in the medium against incubation time gave a straight line from which the amounts of [^{125}I] MDA-HDL degraded by the cells were determined and expressed as ng of [^{125}I] MDA-HDL/h/10^6 cells. Parallel incubation with [^{125}I]HDL did not produce any appreciable increase in TCA-soluble radioactivity. To examine the effect of chloroquine, the cells were preincubated at 37°C for 20 min with 300 uM chloroquine and incubated with a radiolabeled ligand in the same way as described above.

RESULTS AND DISCUSSION

Binding of [^{125}I]MDA-HDL to Sinusoidal Liver Cells

The binding of [^{125}I]MDA-HDL to the isolated sinusoidal liver cells reached an equilibrium within 90 min at 0°C. In the presence of 100-fold excess of unlabeled MDA-HDL, the binding of [^{125}I]MDA-HDL was reduced by more than 70% (data not shown), indicating that unlabeled MDA-HDL and radiolabeled MDA-HDL were competing for a limited number of common binding sites. The binding of [^{125}I]MDA-HDL to the cells at 0°C as a function of its concentration exhibited a typical saturation curve (Fig. 1). The double reciprocal plot of its specific binding gave a straight line indicating the involvement of a single binding mode with an apparent $K_d = 2.5 \times 10^{-8}$ M and $B_{max} = 43.5$ ng/10^6 cells (Fig. 1, inset). These kinetic data strongly suggest that a membrane-associated receptor is involved in the interaction of MDA-HDL with the cells. Since it is established that sinusoidal liver cells contain at least two scavenger receptors for chemically modified proteins; one for aldehyde-modified proteins such as f-Alb and another for acetyl-LDL or MDA-LDL (19), we have compared the MDA-HDL receptor with those receptors under the same conditions. Fig. 2 shows effects of several ligands on the binding of [^{125}I]MDA-HDL to the cells. The binding of [^{125}I]MDA-HDL to the cells was inhibited in a dose-related manner by unlabeled MDA-HDL. Although less effective, unlabeled acetyl-HDL inhibited significantly the binding of [^{125}I]MDA-HDL. However, neither of f-Alb, acetyl-LDL nor MDA-LDL did affect this binding process. Thus, it is evident that the MDA-HDL receptor is distinct either from the f-Alb receptor or the acetyl-LDL receptor.



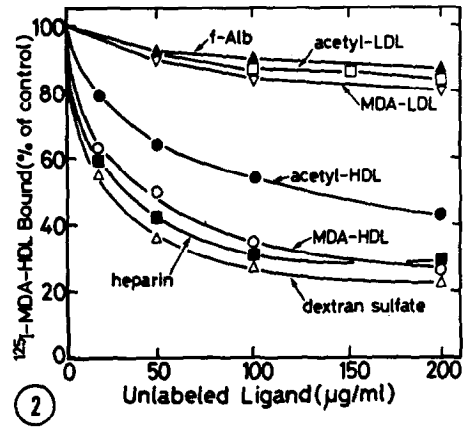


Fig. 1. Binding of [^{125}I]MDA-HDL to sinusoidal liver cells as a function of its concentration. Each tube contained, in a final volume of 0.1 ml of buffer A, 3 x 10^6 cells and increasing amounts of [^{125}I]MDA-HDL (320 cpm/ng). After incubation for 90 min at 0°C, the total binding (○) was determined as described under "Materials and Methods." Nonspecific binding (●) was determined by parallel incubation in the presence of 100 μg of unlabeled MDA-HDL. Specific binding (△) was obtained by subtracting nonspecific binding from the total binding. Assays were run in duplicate. The inset shows the double reciprocal plot of the specific binding versus ligand concentrations.

Fig. 2. Effect of several unlabeled ligands on binding of [^{125}I]MDA-HDL to sinusoidal liver cells. Each tube contained, in a total volume of 0.1 ml of buffer A, 3 x 10^6 cells, 6.0 μg/ml of [^{125}I]MDA-HDL (320 cpm/ng), and indicated concentrations of either of the following unlabeled ligands; MDA-HDL (○), acetyl-HDL (●), MDA-LDL (▽), acetyl-LDL (□), f-Alb (▲), heparin (■), and dextran sulfate (△). After incubation for 90 min at 0°C, the amount of the cell-associated radioactivity was determined as described under "Materials and Methods." The 100% value for [^{125}I]MDA-HDL binding was determined in the absence of any competing ligands. Assays were run in duplicate.

Previous studies using mouse peritoneal macrophages (1, 6), human monocyte macrophages (10, 11) and sinusoidal liver cells (19) have revealed that the scavenger receptors for chemically modified proteins share a common property of being sensitive to polyanions such as dextran sulfate and fucoidin. As Fig. 2 shows, [^{125}I]MDA-HDL binding to the cells was effectively inhibited by dextran sulfate and heparin. From the ligand specificity revealed by these binding studies, it seems likely that a receptor for MDA-HDL might represent a new member among the scavenger receptors expressed by sinusoidal liver cells.

Endocytic Uptake of [^{125}I]MDA-HDL by Sinusoidal Liver Cells

To examine the endocytic uptake of MDA-HDL by sinusoidal liver cells as well as to test further whether MDA-HDL was endocytosed via a route identical with acetyl-HDL, the cells were incubated at 37°C with either [^{125}I]MDA-HDL or [^{125}I]acetyl-HDL and TCA-soluble radioactivity released into the incubation

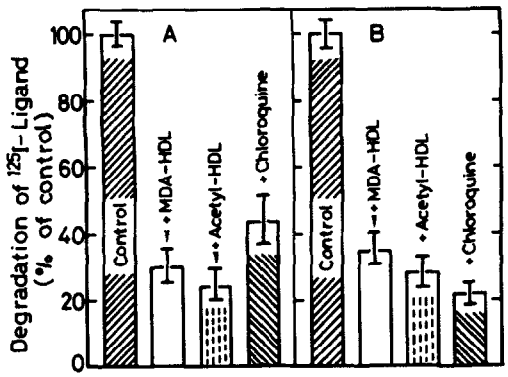


Fig. 3. Endocytic uptake of [^{125}I]MDA-HDL by sinusoidal liver cells. Each tube contained, in a total volume of 2.0 ml of buffer A, 1.2 x 10^7 cells, and 4.7 μg of [^{125}I]MDA-HDL (320 cpm/ng) or [^{125}I]acetyl-HDL (1600 cpm/ng). After incubation for 1 h at 37°C in the absence (control) or presence of a 50-fold excess of unlabeled MDA-HDL or acetyl-HDL, the rate of the hydrolysis of [^{125}I] MDA-HDL (A, left panel) or [^{125}I] acetyl-HDL (B, right panel) was determined. Effect of chloroquine was determined as described under "Materials and Methods." Assays were run in triplicate. Each bar represents the range. Similar experiments at 4°C did not result in appreciable increase in TCA-soluble radioactivity in the medium.

medium was determined at timed intervals. TCA-soluble radioactivity derived from each radiolabeled ligand was detected 10 - 15 min postincubation and continued to increase proportionally with time thereafter (data not shown). As Fig. 3 shows, degradation of either [^{125}I]MDA-HDL (Fig. 3A) or [^{125}I]acetyl-HDL (Fig. 3B) during 1 h incubation was effectively inhibited by the presence of chloroquine, indicating that those ligands were endocytosed and transported to lysosomes for proteolytic digestion. This lysosomal degradation of these radiolabeled ligands was inhibited by excess unlabeled MDA-HDL as well as acetyl-HDL to a similar degree. Thus, it appears that scavenger function of sinusoidal liver cells for MDA-HDL and acetyl-HDL is mediated by the same receptor.

Recognition by MDA-HDL Receptor versus Extents of the Chemical Modification

We next examined correlation between the extent of malondialdehyde modification of HDL and the subsequent generation of the ligand activity for the MDA-HDL receptor. Six HDL preparations modified by malondialdehyde to different extents were labeled with [^{125}I] and their endocytic degradation was compared at the same protein concentration (2.5 μg/ml). As shown in Fig. 4, the modification of HDL up to 42% of its total lysines did not produce any ligand activity. Further modification of HDL resulted in a sharp increase in the

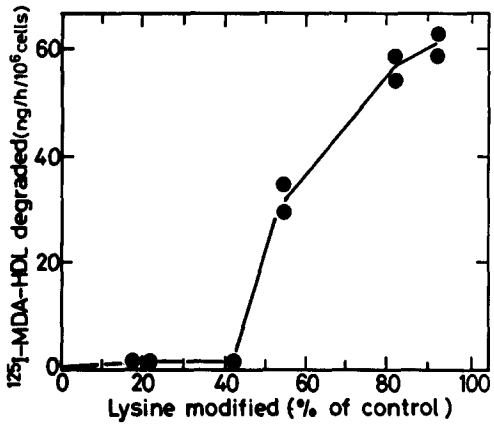


Fig. 4. Rate of degradation of [^{125}I]MDA-HDL as a function of extents of malondialdehyde modification. Six preparations of MDA-HDL with different degrees of modification were prepared as described under "Materials and Methods." Each preparation was iodinated with [^{125}I] and tested for its intracellular degradation. Each tube contained, in a final volume of 2.0 ml of buffer A, 1.2 x 10^7 cells, and 2.5 μg/ml of each [^{125}I]MDA-HDL preparation (300-900 cpm/ng). The reaction mixture was incubated for 1 h at 37°C and the rate of degradation was determined as described under "Materials and Methods." The extent of lysine modification of each MDA-HDL preparation was as follows: 18.6, 22.8, 41.8, 55.6, 81.8 and 92.0%. Assays were run in duplicate.

lysosomal hydrolysis of the endocytosed ligand and the modification by more than 60% led to the generation of an almost maximal ligand activity. Haberland et al. (10, 11) observed a similar threshold recognition of MDA-LDL by the acetyl-LDL receptor of human monocyte macrophages; incorporation of >30 mol malondialdehyde/mol of LDL produced a maximal ligand activity whereas the modification less than 28 mol/mol of LDL did not generate an active ligand for the receptor. Likewise, also in the case of the MDA-HDL receptor, the receptor recognition appears to require the modification of a specific lysine residue(s) of HDL, rather than a simple increase in net negative charge upon malondialdehyde modification. This notion was also supported by the ligand specificity of the MDA-HDL receptor that the binding and degradation of MDA-HDL were unaffected by MDA-LDL or acetyl-LDL, but significantly influenced by acetyl-HDL (Figs. 2 & 3).

Our preliminary experiment indicated that MDA-HDL and acetyl-HDL were endocytosed by a receptor-mediated mechanism by rat peritoneal macrophages via a route distinct from acetyl-LDL or MDA-LDL. Thus, the MDA-HDL receptor revealed from the present study using sinusoidal liver cells could cooperate with the acetyl-LDL receptor in scavenging lipoproteins chemically modified in situ.

ACKNOWLEDGMENTS

We are grateful to Dr. Seiji Fukuda for valuable discussion and to Prof. Yasuhiko Matsukado for pertinent criticism of the manuscript. This work was supported in part by a grant-in-aid for scientific research from the Ministry of Education, Science and Culture of Japan, and by a grant from Japan Heart Foundation.

REFERENCES

1. Brown, M. S., and Goldstein, J. L. (1983) Annu. Rev. Biochem. 52, 223-261.
2. Vlassara, H., Brownlee, M., and Cerami, A. (1985) Proc. Natl. Acad. Sci. (USA) 82, 5588-5592.
3. Goldstein, J. L., Ho, Y. K., Basu, S. K., and Brown, M. S. (1979) Proc. Natl. Acad. Sci. (USA) 76, 333-337.
4. Johnson, W., Pizzo, S. V., Imber, M. J., and Adams, D. O. (1982) Science 218, 574-576.
5. Haberland, M. E., and Fogelman, A. M. (1985) Proc. Natl. Acad. Sci. (USA) 82, 2963-2967.
6. Brown, M. S., Basu, S. K., Falck, J. R., Ho, Y. K., and Goldstein, J. L. (1980) J. Supramol. Struct. 13, 67-81.
7. Mahley, R. W., Weisgraber, K. H., Innerarity, T. L., and Windmueller, H. G. (1979) Proc. Natl. Acad. Sci. (USA) 76, 1746-1750.
8. Henriksen, T., Mahoney, E. M., and Steinberg, D. (1981) Proc. Natl. Acad. Sci. (USA) 78, 6499-6503.
9. Fogelman, A. M., Shechter, I., Seager, J., Hokom, M., Child, J. S., and Edwards, P. A. (1980) Proc. Natl. Acad. Sci. (USA) 77, 2214-2218.
10. Haberland, M. E., Fogelman, A. M., and Edwards, P. A. (1982) Proc. Natl. Acad. Sci. (USA) 79, 1712-1716.
11. Haberland, M. E., Olch, C. L., and Fogelman, A. M. (1984) J. Biol. Chem. 259, 11305-11311.
12. Fogelman, A. M., Haberland, M. E., Seager, J., Hokom, M., and Edwards, P. A. (1981) J. Lipid Res. 22, 1131-1141.
13. Nilsson, M., and Berg, T. (1977) Biochim. Biophys. Acta 100, 136-143.
14. Horiuchi, S., Takata, K., and Morino, Y. (1985) J. Biol. Chem. 260, 475-481.
15. Horiuchi, S., Takata, K., and Morino, Y. (1985) J. Biol. Chem. 260, 482-488.
16. Horiuchi, S., Murakami, M., Takata, K., and Morino, Y. (1986) J. Biol. Chem. in press.
17. Nagelkerke, J. F., Barto, K. P., and van Berkel, T. J. C. (1983) J. Biol. Chem. 258, 12221-12227.
18. Blomhoff, R., Drevon, C. A., Eskild, W., Helgerud, P., Norum, K. R., and Berg, T. (1984) J. Biol. Chem. 259, 8898-8903.
19. Horiuchi, S., Takata, K., Maeda, H., and Morino, Y. (1985) J. Biol. Chem. 260, 53-56.
20. Weisgraber, K. H., and Mahley, R. W. (1980) J. Lipid Res. 21, 316-325.
21. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951) J. Biol. Chem. 193, 265-275.
22. Basu, S. K., Goldstein, J. L., Anderson, R. G. W., and Brown, M. S. (1976) Proc. Natl. Acad. Sci. (USA) 73, 3178-3182.
23. Habeeb, A. F. S. A. (1966) Anal. Biochem. 14, 328-336.
24. Berry, M. N., and Friend, D. S. (1969) J. Cell Biol. 43, 506-520.
25. Horiuchi, S., Kamimoto, Y., and Morino, Y. (1985) Hepatology 5, 376-382.