Vol. 88, No. 2, 1979 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
May 28, 1979 Pages 485-490

ROLE OF NET CHARGE OF LOW DENSITY LIPOPROTEINS IN HIGH AFFINITY BINDING AND UPTAKE BY CULTURED CELLS

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Received March 29, 1979

Summary: Selective modification of arginine residues of LDL by cyclohexanedione or acetylation of lysine residues of LDL deminishes their high affinity binding and internalisation by human skin fibroblast up to 50% as compared with native LDL. The enhanced negative charge of the modified LDL particles results in an accelerated electrophoretic mobility towards the anode. Neuraminidase treatment of cyclohexanedione-modified LDL and acetyllysine-LDL normalizes not only their electrophoretic mobility, but also restores more than 80% of the original binding and uptake capacity, the specificity of this effect being indicated by using fibroblasts deficient in LDL receptor and by competitive binding and internalization experiments.

Introduction: The high affinity receptor mediated uptake of homologous LDL by cultured human arterial smooth muscle cells or human skin fibroblasts requires the interaction between specific receptor sites located in coated pits on the cell surface and a recognition site of the apoprotein of the LDL particles (1,2).

Arginine and lysine residues of the apolipoprotein B and E have been proposed as the recognition marker for binding of LDL to the cell surface receptor (3,4). This conclusion was derived from the observation that selective chemical modification of arginine or lysine residues almost totally abolishes binding of LDL to cell surface receptors. On the other hand, it has been shown (5) that the sialic acid content of LDL particles also controls receptor mediated binding and uptake of LDL by cultured cells.

Abbreviations used: LDL, low density lipoprotein; CHD, cyclohexanedione

0006-291X/79/100485-06$01.00/0

The quoted results suggest that the surface net charge contributes to the regulation of binding and internalization of LDL. This assumption is confirmed by the present report, which will demonstrate that modification of either arginine residues or lysine residues of LDL apoprotein depresses their high affinity binding and uptake by the cells, but that this effect can be abolished by sialic acid removal from the modified LDL.

Material and Methods: Source of chemicals: Cow's milk lactoperoxidase (160 U/mg) was purchased from Boehringer (Mannheim), neuraminidase from Vibrio comma cholerae (500 U/ml) from Behringwerke (Marburg), 1,2-cyclohexanedione from Serva (Heidelberg) and ^{125}I, carrier-free, from Radiochemical Centre (Amersham) U.K. Lipoproteins: Human LDL (d 1.02-1.055) were isolated and labelled with ^{125}I as described previously (6), yielding specific activities of 8-12 x 10^4 cpm/µg LDL protein. Modification of the arginyl residues of LDL apoproteins with 1,2-cyclohexanedione (CHD-LDL) and regeneration by hydroxylamine were performed according to (7). Acetylation of ε-amino groups of lysine was done as described (8), this procedure resulting in a specific acetylation of about 1/2 of the lysine residues of LDL (acetyl-LDL). Protein-bound arginine and lysine residues were determined according to (9) and (10). Native LDL were desialized as described previously (5). Free sialic acid and LDL-bound sialic acid were determined according to (11), the latter after acid hydrolysis. LDL protein was determined by the method of Lowry et al. (12).

Cells: Human fibroblasts were grown from skin explant from a normal infant as described previously (5) and were used for incubation experiments between 3 and 8 passages. Human skin fibroblasts deficient in LDL receptor (GM 1915) were obtained from the Institute for Medical Research (Camden, N.J., U.S.A.).

Incubation experiments: Cell monolayers were washed twice with Hank's solution, after which 3 ml of fresh medium containing 10% lipoprotein-free human serum and 10 µg LDL protein/ml, either native LDL, CHD-LDL, acetyl-LDL or the desialized form of one of them, was added. After a 6 h incubation period at 37°C the medium was removed, and cell monolayers were washed 7 times with 3 ml of Hank's solution containing 0.1% bovine serum albumin.

Assays for binding, internalisation and degradation: The binding (surface bound plus internalized) and degradation assays were performed at 37°C according to (13). Binding studies were performed at 4°C as described by (14).

Results: Chemical modification of either arginine or lysine residues of LDL alters their net charge and enhances their electrophoretic mobility. 1,2-cyclohexanedione treatment diminishes the number of free guanidino groups of arginine (CHD-LDL), while a subsequent 1,2-cyclohexanedione and acetic anhydride treatment followed by a

Table 1

	A		B			
			ng LDL protein/mg cell protein/6h			
	Mol/ mol LDL		untreated		Neuraminidase treated	
LDL-modification	Arg	Lys	binding + uptake	degra- dation	binding + uptake	degra- dation
none (native LDL)	8.2	28.1	a 686	1440	1143	2357
			b 226	474	302	729
CHD - LDL	4.3	27.8	a 350	706	524	1194
			b 202	450	281	691
LDL regenerated from CHD - LDL	7.4	28.0	a 581	1336	975	2186
			b 229	486	297	748
Acetyl - LDL	7.3	16.4	a 406	852	688	1434
			b 214	458	278	683

Free arginine and lysine residues (A) and high affinity binding and uptake and degradation of native, modified and/or desialized [^{125}I]LDL by cultured normal (a) and LDL receptor deficient (b) human skin fibroblasts (B). Amino acid residues were calculated as mol per mol LDL, assuming 250 residues per mol (3). Cells were incubated with a medium containing 10 µg lipoprotein protein/ml for 6 h at 37°C. Afterwards the radioactivity associated with the cells and degraded was determined as described under Methods. Data are mean values of 4 experiments.

removal of the cyclohexanedione groups produces LDL particles with free arginine residues but acetylated ε-amino groups of lysine (acetyl-LDL). Either modification results in an accelaration of electrophoretic mobility toward the anode and a corresponding loss of free guanidino and ε-amino groups (Table 1). Neuraminidase treatment of the modified LDL, however, removes > 85% of negatively charged sialic acid residues (data not shown) and hence normalizes their electrophoretic mobility.

At low LDL concentration, high affinity binding to specific receptor sites is rate limiting in the process by which cultured fibroblasts internalize LDL. Therefore all LDL binding and uptake

experiments were performed at a concentration of 10 µg LDL protein/ml medium. These experiments revealed a clear correlation between the net charge of native or modified LDL and the rate of their high affinity binding and uptake by fibroblasts (Table 1). CHD-LDL and acetyl-LDL were bound and taken up at a rate 45 and 40% slower than native LDL. After partial desialisation by neuraminidase, however, binding and uptake of both CHD-LDL and acetyl-LDL proceeded at almost normal rates. As described by Mahley et al. (3), regeneration of CHD-LDL by incubation with hydroxylamine restores more than 80% of the original binding and uptake, which may be increased even more by subsequent neuraminidase treatment (Table 1). Desialized native LDL are internalized up to 100% faster than untreated LDL (4).

The specificity of the observed effects is evident from binding and internalisation experiments with human skin fibroblasts deficient in LDL receptor. It appears from the figures of Table 1 (lines b) that the mutant fibroblasts internalize LDL at a reduced rate but that chemical modification of LDL does not influence this process.

Competetive binding and internalisation studies (Figure 1) revealed that non-labelled CHD-LDL does not compete with native [^{125}I]LDL for binding and internalisation by the cells. Partially desialized CHD-LDL, however, exhibits a competition for binding and uptake in the order of native LDL. The most effective competitor is desialized native LDL.

Discussion: The observation (3) that modification of half of the arginine residues of LDL apolipoprotein almost totally abolished the high affinity binding of LDL to the cell surface receptor sites of cultured human skin fibroblasts suggested that arginine is involved in the interaction of LDL with specific cell surface.

However, the concept that arginine residues function as recognition markers of LDL is questioned by the following results:

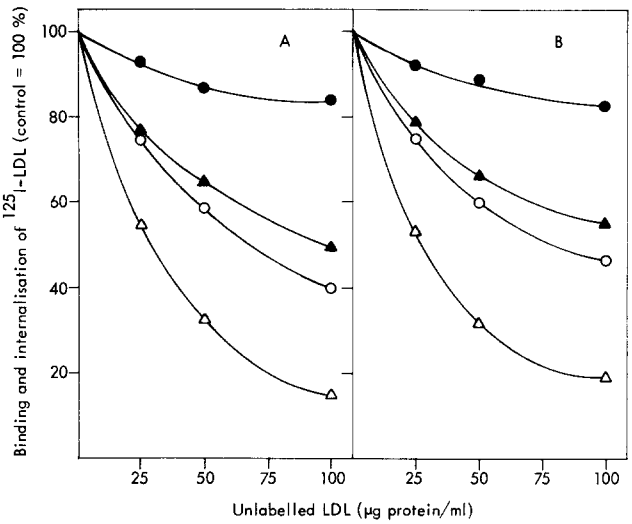

Figure 1. Competition of native LDL (o), CHD-LDL (●), partially desialized LDL (Δ) and partially desialized CHD-LDL (▲) for binding (A) and uptake (B) by cultured human skin fibroblasts. Binding was determined at 4°C, uptake at 37°C after trypsinisation of the cells. Cells were incubated for 6 h at 37°C in a medium containing 10 μg [^{125}]LDL protein/ml and the specified concentrations of nonlabelled native, modified and/or desialized LDL.

(a) CHD-LDL recovers its ability to be bound and internalized by fibroblasts almost completely after reducing the excess of its negative charges by partial desialisation. This desialized CHD-LDL competes successfully with native LDL for binding and internalisation by fibroblasts.

(b) LDL with acetylated ε-amino groups of lysine residues but free (regenerated) arginine residues is bound and internalized at a rate reduced by 40% as compared with native LDL, although its arginine groups are available as recognition markers. Under these conditions, again neuraminidase treatment restores original binding and uptake rates.

These results point out the importance of the net charge of the LDL and its possible role in maintaining the conformation of the recognition site. Removing or adding of surface-associated positively or negatively charged residues are suggested to alter the spatial

arrangement of the recognition site on the lipoprotein which is represented by as yet unidentified residues. This assumption is suggested by our previous finding (4) that (a) desialized LDL are internalized by smooth muscle cells up to 100 % faster than native LDL and that (b) LDL modified by entrapped gangliosides are internalized by arterial smooth muscle cell at a rate up to 80% lower than native LDL.

The physiological significance of our results lies in the fact, that LDL from hyperlipaemic subjects show a charge heterogeneity (15) that may influence their biological half life and hence their "atherogenicity".

ACKNOWLEDGEMENTS: This work was supported by the Deutsche Forschungsgemeinschaft (SFB 104).

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