CHANGES IN SIZE AND PROPERTIES OF LIPOPROTEIN PARTICLES IN THE EXTRACELLULAR MEDIUM ON INCUBATION OF LOW-DENSITY LIPOPROTEINS WITH HUMAN FIBROBLASTS

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Thanks to the investigations of Goldstein et al. and Brown et al. [5, 10] the mechanisms of receptor-mediated endocytosis of low-density lipoproteins (LDL) have now been studied in adequate depth. Meanwhile the principles governing nonspecific interaction between LDL and cells under conditions of hypercholesteremia still remain largely unexplained. The fact that during long-term incubation of LDL with fibroblasts the quantity of cholesterol accumulating in the cells exceeds the quantity of LDL protein degraded and assimilated by the cell, as shown by their ratio in LDL [8, 9], demonstrates the necessity for a study of the processes of extracellular conversion of LDL. In the investigation described below the properties of LDL in the extracellular medium were studied during incubation with fibroblasts.

EXPERIMENTAL METHOD

Experiments were carried out on human embryonic lung tissue fibroblasts at the 10th subculture, grown in parallel cultures in six Pavitskii flasks. The cells were used in the experiments after they had formed a monolayer. Light-microscopic examination of the cell culture in the course of the experiment showed that it remained viable. The incubation medium consisted of Eagle's medium containing human serum proteins with a density of over 1.250 g/ml (5 mg/ml) and human LDL in a concentration of 50 μg/ml as protein. The solutions were sterilized by passage through a millipore filter with a pore diameter of 0.25 μ. Penicillin (100 units/ml), streptomycin (100 μg/ml), and kanamycin (50 units/ml) were added to the incubation medium. Fibroblasts were incubated at 37°C in medium containing LDL for 48 h. After the end of the experiments changes taking place in LDL in the incubation medium were analyzed by disc electrophoresis in polyacrylamide gel (PAG) [3], by microzonal electrophoresis on cellulose acetate [7], and by electron microscopy. Lipoproteins (LP) for electron-microscopic study were applied to a carbon supporting film and stained with 1% phosphotungstic acid solution. The material was examined in the IEM-7A electron microscope. LDL (1.019-1.055 g/ml) were isolated by ultracentrifugation [11], and centrifuged twice to remove other proteins present as impurities.

EXPERIMENTAL RESULTS

On investigation in the electron microscope the native LDL had the appearance of round or irregularly shaped particles (Fig. 1a). The diameter of most particles was between 19 and 27 nm (Fig. 1b), in agreement with values obtained by other workers [1, 13]. During incubation of the LDL in culture medium without the addition of fibroblasts for 48 h at 37°C, besides particles of the usual size, larger formations, which varied considerably in size, were found. LDL incubated under the same conditions but in the presence of fibroblasts were distributed mainly on the supporting film as clusters or chains consisting of individual particles (Fig. 1a). Many of these particles were smaller in size than native LDL (Figs. 1a

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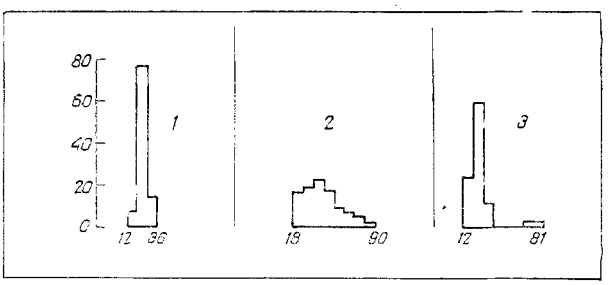

Fig. 1. Electron microscopy of human LDL. a) Electron micrograph of LDL stained with phosphotungstic acid, 100,000 μ; b) distribution of LDL by size on the basis of electron-microscopic data. 1) Original LDL; 2) LDL incubated for 48 h at 37°C in culture medium without fibroblasts; 3) LDL incubated with fibroblasts for 48 h at 37°C. Abscissa, diameter of particles (in nm); ordinate, number of particles (in %).

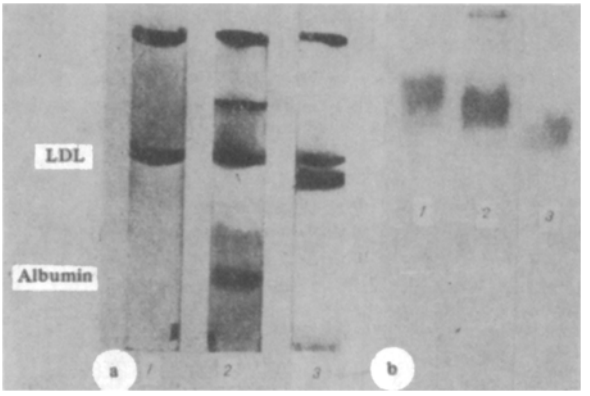

Fig. 2. Electrophoresis of human LDL: a) disc electrophoresis of LP in PAG (stained with Sudan IV); b) microzonal electrophoresis of LP on cellulose acetate (stained with Oil red O). 1) Original LDL; 2) LDL incubated for 48 h in culture medium at 37°C; 3) LDL incubated with fibroblasts for 48 h at 37°C.

and b). Besides clusters of particles, round formations with rather indistinct outlines also were found in the culture fluid.

The results of disc electrophoresis of LP in PAG agreed with those of electron microscopy. On incubation of LDL without cells at 37°C for 48 h, LP accumulated in the region corresponding to the position of pre-β-LP and in the zone between pre-β-LP and β-LP, whereas in the presence of cells this phenomenon was not observed; moreover, a subfraction of β-LP with a smaller size appeared (Fig. 2a).

Microzonal electrophoresis of LDL in the incubation medium showed that the subfraction of LP particles formed during incubation at 37°C without cells possesses lower electrophoretic mobility. In the presence of cells this subfraction did not appear in the incubation medium, but a subfraction of LP with higher electrophoretic mobility, i.e., with a larger negative charge appeared (Fig. 2b).

On incubation of LDL with fibroblasts in the extracellular medium, LP particles of smaller size, with increased ability to form aggregates, and with higher electrophoretic mobility thus appeared. The increase in size of the LDL particles in the control experiments reflects their ability to aggregate. A similar tendency was observed with respect to high-density LP during incubation of human blood plasma [14].

The following suggestions can be made regarding possible mechanisms for the change in properties of LDL in the extracellular medium on incubation with fibroblasts: 1) partial proteolytic and lipolytic degradation of LDL under the influence of cell enzymes either in the incubation medium or on the cell surface; 2) selective entry of LDL lipids into the cells either during reversible endocytosis or as a result of metabolism of LDL particles on the surface of fibroblasts; 3) the high rate of uptake of larger LDL particles with a positive charge by the cells.

The disparity observed between the quantity of lipids entering the cell and the quantity of protein supplied and degraded, on the basis of their ratio in LDL [4, 8, 9], and also data showing an increase in the hydrated density of LDL during their circulation in the blood plasma [2] and under the influence of endothelial cells [12], are evidence in support of the view that besides the uptake of intact LDL particles by receptor-mediated endocytosis, selective entry of the lipid moiety of LDL into the cell also takes place.

Data on the ability of macrophages to take part in uncontrolled uptake of modified LDL with an increased negative charge [6], and also the increase in the negative charge of LDL under the influence of fibroblasts, discovered in the present investigation, suggest that the macrophages in the body as a whole (and in a focus of atherosclerosis, in particular), may play the role of a system for the elimination of partially degraded LDL. This conclusion is supported by data showing increased uptake by macrophages by LDL which have been partly metabolized under the influence of endothelial cells [12].

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