INTERACTION OF LIPID MICELLES WITH BLOOD SERUM LIPOPROTEINS

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Recently protein-free models of low-density lipoproteins (LDL) have been described in the literature [8, 10, 11] in the form of complex lipid micelles. It is suggested that lipid micelles can also be used as carriers of drugs for oriented targeting of tissues [5]. Lipid micelles have been shovn to interact with blood plasma lipoproteins, including LDL [5, 12]. It is not yet clear whether the content of different components of the micelles affects their interaction with LDL. The aim of this study was to investigate interaction of lipid micelles with LDL, depending on the lipid composition of the micelles.

EXPERIMENTAL METHOD

Total cholesterol, phospholipids, triglycerides, and total protein were analyzed on a "CentrifiChem 400" automatic analyzer ("Baker," USA), Isolation of high-density lipoprotein (HDL), very low-density lipoprotein (VLDL), and LDL fractions from blood serum was carried out by the methods described in [3, 4, 9]. Human blood serum lipoproteins were analyzed by polyacrylamide gel electrophoresis by the method [6] in the modification [1]. Concentrations of 7-hydroxycholesterol in fractions obtained by gel-chromatography were determined by quantitative thin-layer chromatography as described in [13] on "Silufol" plates (Kavalier, Czechoslovakia), in a solvent system of ethyl acetate—heptane (1:1 by volume). Gel chromatography of the serum, dispersion of the lipid micelles and mixture of the serum with the micelles were carried out on a 50 × 1 cm column with sepharose CL-4B. The dispersion of lipid micelles was incubated witt human blood serum (0.2 ml of micellar dispersion + 0.2 ml serum) for 1 h at 37°C. A mixture of 0.2 ml of the micellar dispersion or 0.2 ml of serum with 0.2 ml of buffer was used as the control, and incubated under the same conditions. The lipid micelles were obtained from cholesterol oleates, cholesterol ("Sigma," USA), and phosphatidylcholine ("Nattermann," West Germany) in different molar proportions. The mixture of lipids was suspended in medium containing 150 mM NaCl, 10 mM Tris-HCl, pH 7.4. The suspension of lipids was dispersed on a "Sonic-300" ultrasonic disintegrator (Fisher, USA). The resulting suspension was centrifuged at 20,000*g* for 1 h.

EXPERIMENTAL RESULTS

Dependence of interaction of lipid micelles with human blood serum lipoproteins on the content of free cholesterol with molar fractions of 0.14, 3, 20.3, and 33.3% was investigated by polyacrylamide gel electrophoresis. Compared with the control there was a decrease in the intensity of staining of the LDL band, depending on the content of free cholesterol in the micelles (Fig. 1). The least intensity of staining of the LDL band was observed in a mixture of serum with micelles containing the lergest quantity of cholesterol. Meanwhile the intensity of staining of the LDL bands was virtually unchanged. Weakening of staining thus observed can be explained on the grounds that as a result of interaction of the micelles with LDL, micelle-LDL complexes were formed, and remained at the starting point during electrophnresis. Thus interaction of LDL with micelles was potentiated

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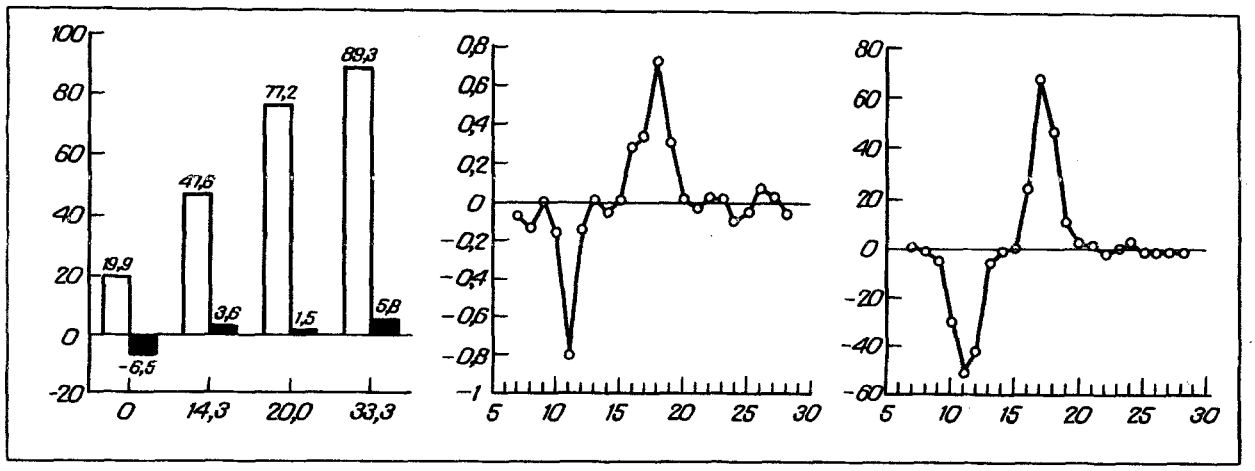

Fig. 1 Fig. 2 Fig. 3

Fig. 1. Dependence of interaction of micelles with lipoproteins on cholesterol content in micelles. abscissa, concentraticn (in M, %) of cholesterol in micelles; ordinate, interaction (E) of LDL and HDL with micellar preparation (decrease in intensity of staining of LDL and HDL bands in polyacrylamide gel columns compared with control, as a result of incubation of serum with micelles). Unshaded columns — LDL, black columns — HDL.

Fig. 2. Redistribution of cholesterol on interaction of lipid micelles with serum. Abscissa, No. of fraction during sel chromatography; ordinate, change in concentration (in mg %) of cholesterol in fraction (ΔC):

where C_m is the cholesterol concentration in the fractions in the experiment, C_p the cholesterol concentration in fractions in the preparation, and C_s the cholesterol concentration in serum fractions.

Fig. 3. Transfer of 7-hydroxycholesterol from micelles to LDL. Abscissa, No. of fraction during gel chromatography; ordinate, change in concentration of 7-hydroxycholesterol in the given fraction (in relative units).

with an increase in their molar fraction of cholesterol. This result is evidence of the high selectivity of interaction of cholesterol-saturated micelles with LDL.

Similar interaction of lipoproteins also was observed with micelles containing triolein in the core instead of cholesterol oleate. The selectivity of interaction of the micelles with LDL evidently depends only on the content of free cholesterol in the micelles and is virtually independent of the core composition of the micelles.

Interaction of lipid micelles with blood plasma lipoproteins also was studied by gel chromatography. As a result of incubation with serum the cholesterol content in the micelles (fraction 11) was reduced, whereas in HDL (fractions 17 and 18) it was increased, and in HDL (fraction 21) virtually unchanged. The results are given in Fig. 2 in differential form, enabling the redistribution of cholesterol between the different fractions to be judged. Clearly an increase of cholesterol in LDL and a decrease in cholesterol in the micelles were observed. It can be concluded thet on incubation of the preparation with blood serum cholesterol is transferred from micelles to LDL.

Analysis of the redistribution of phospholipids showed that the phospholipid is transferred from LDL to micelles probably as a result of their fusion, with the formation of complexes. Simultaneously phospholipid is transferred from micelles or from micelle—LDL complexes to HDL and to protein. The increase in the micellar phospholipid is evidently the difference between its gain as a result of fusion with LDL and its loss as a result of transfer to HDL and protein.

Transfer of triglycerides can take place only on contact between particles and not through the aqueous phase. Loss of triglyerides into LDL and their increased content in micelles are therefore unequivocal evidence of interaction between micelles and LDL by fusion. The triglyceride level in HDL remains unchanged in this case.

An experiment was carried out to study oriented transfer of oxidized cholesterol from the preparation to LDL. For this purpose, micelles were prepared in which half of the cholesterol (expressed in moles) was replaced by 7-hydroxycholesterol. The results in Fig. 3 confirm the appearance of a 7-hydroxycholesterol peak in LDL, which was not present in the original serum

(fraction 17), and a decrease in its content in the preparation. Transfer of 7-hydroxycholesterol from micelles to LDL thus demonstrated is evidence that the micellar preparation can be used to supply oxidized cholesterol to target cells, through the intervention of LPL, including, evidently, by their receptor uptake by peripheral organs and tissues.

Analysis of the results obtained by electrophoresis and gel-chromatography, reveals that on incubetion of the micellar preparation with blood serum two processes take place simultaneously: 1) fusion of a large part of the LDL with micelles, with the formation of complexes; 2) transfer of part of the cholesterol and of oxidized cholesterol from micelles to LDL, amd of phospholipid to HDL. Transfer of cholesterol and hydroxycholesterol from micelles to LDL is quantitatively on a muck larger scale (by about 4.6 times) than hansfer of phospholipid to HDL.

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