The interaction of very-low-density lipoproteins from pig and rat plasma with immobilized heparin

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Human VLD lipoprotein*, the major vehicle for the transport of endogenous triacylglycerol in the plasma (Robinson, 1970), shows a characteristic interaction with immobilized heparin (Iverius, 1972). Thus chromatography of whole ultracentrifugally prepared VLD lipoprotein on columns of heparin covalently linked to agarose gel (Iverius, 1971) yields two fractions, one of which binds to the gel in the presence of 0.12 m NaCl. This bound fraction can be eluted from the gel by using a gradient of ascending ionic strength. These subfractions of human VLD lipoprotein have been characterized by their specific apoprotein compositions. In particular, the apoprotein E (nomenclature of Alaupovic et al., 1972) present has been suggested to be either a major (fraction of higher affinity) or a relatively minor component (fraction of lower affinity) (Shelburne & Quarfordt, 1977) of the VLD-apoprotein present. Isolated apoprotein E also shows a high affinity for immobilized heparin, and this affinity can be prevented by specific modification of the protein’s arginine residues (Shelburne & Root, 1975). This apparent apoprotein-E-mediated binding of VLD lipoprotein to the sulphated glycosaminoglycan is also abolished when whole VLD-lipoprotein preparations are acetylated (Iverius, 1972). The possibility that apoprotein E may be functionally important in the interactions between VLD lipoprotein and the endothelial cell surface, a major site of VLD-lipoprotein catabolism, is lent credence by these and further observations on the molecular nature of the endothelial cell’s surface. This cell type has a high concentration of sulphated glycosaminoglycans associated with it, particularly a heparan sulphate moiety, closely related structurally to heparin (Buonassissi & Root, 1975). The existence in vitro of a VLD-lipoprotein species that interacts strongly with heparin may signify the presence of a lipoprotein species that behaves in a similar fashion with endothelial-cell-surface glycosaminoglycans in vivo (Zilversmit, 1973).

The present study extends these observations on human material with particular reference to another atherosclerosis-susceptible species, the pig (Thomas et al., 1970), and an atherosclerosis-resistant species, the rat (Kottke & Subbiah, 1978).

VLD lipoproteins were isolated from pig and rat plasma by the ultracentrifugal flotation method of Havel et al. (1955). Each fraction was washed twice by re-centrifugation at 40 000 rev./min (113 000 g, for 16 h at 12°C in a solution of density 1.006 g/ml. Heparin–Sepharose affinity chromatography of the washed VLD lipoproteins was carried out in the manner of Shelburne & Quarfordt (1977). This used a column (9.0 cm x 2.5 cm) containing heparin covalently linked to Sepharose 6B-CL agarose gel (400 μg of heparin/ml of gel) by the method of Iverius (1971). Both rat and pig VLD lipoproteins were applied to the column in 0.05 m-NaCl/2 mm-sodium phosphate adjusted to pH 7.4 with 0.1 m-NaOH, and eluted by using a linear gradient of NaCl from 0.05 to 0.5 m (total volume 120 ml). The eluting gradient contained the 2 mm-sodium phosphate buffer, pH 7.4, and was applied to the column at a rate of 24 ml/h. Fractions (4 ml) were collected and their absorbance was recorded. Protein content of the fractions was assessed by the method of Lowry et al. (1951), with crystalline bovine serum albumin as a standard and with the addition of sodium deoxycholate to a final concentration of 0.1% (w/v) to clear any lipid present (Petersburg et al., 1975). Triacylglycerol concentrations were determined by the method of Martin (1975).

Under the conditions described, 80–95% of VLD-lipoprotein protein of both species became bound to the column. Subsequent elution of the bound pig VLD lipoprotein yielded two fractions, one eluted in the 0.12–0.18 m NaCl region of the NaCl gradient, the other in the 0.25–0.3 m region. Rat VLD lipoprotein, however, was eluted from the column in only one fraction, corresponding to the 0.18 m region of the ionic gradient. Quantitative recovery of the applied lipoprotein was achieved for both types of preparation. Pig VLD lipoprotein was also applied to the affinity column in 0.12 m-NaCl buffered with phosphate at pH 7.4 as described above and eluted with the gradient as before. Under these conditions 55% of the applied VLD-lipoprotein protein was bound by the column, and this was subsequently eluted in the 0.25–0.3 m NaCl region of the gradient. The higher-affinity fraction from pig VLD lipoprotein showed a somewhat lower triacylglycerol/protein ratio than the fraction of lower affinity. When rat VLD lipoprotein was applied to the column in 0.2 m-NaCl, 95% of the applied lipoprotein protein was eluted directly from the column before gradient initiation. From the results it would appear that, as with human ultracentrifugally prepared VLD lipoprotein, pig VLD lipoprotein prepared in the same manner interacted with heparin-Sepharose in a characteristic manner to yield high- and low-affinity fractions. By contrast, rat ultracentrifugally prepared VLD lipoprotein showed only a comparatively weak interaction with heparin–Sepharose. Qualitative analysis of the delipidated VLD-lipoprotein apoproteins from the pig and rat sources, by sodium dodecyl sulphate/polyacrylamide-gel electrophoresis, revealed further evidence compatible with the possible role of apoprotein E in the higher-affinity binding of the particles to immobilized heparin.

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* Abbreviation: VLD lipoprotein, very-low-density lipoprotein.