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CHARACTERISATION AND CELLULAR UPTAKE OF AN APO(a)-FREE LIPOPROTEIN OBTAINED ON REDUCTION OF HUMAN LIPOPROTEIN(a)

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Lipoprotein(a) which was first detected in 1963 by Berg (1) was initially thought to be a genetic variant of low-density lipoproteins (LDL). It was subsequently shown, however, that Lp(a) is different to LDL in several chemical, physicochemical and immunochemical properties. Thus Lp(a) is isolated in the ultracentrifuge at a higher density gradient range than is LDL and on agarose gel electrophoresis it has pre-beta-mobility as opposed to the beta mobility of LDL. In addition to apoprotein-B the major apoprotein of LDL, Lp(a) contains an additional high molecular weight glycoprotein (a) which is attached to apo-B through disulfide bonds (2, 3, 4). Three different molecular weight species of apo-(a) have been detected having molecular weights greater than, equal to and smaller than apo-B. This may in part be responsible for the reported (4) heterogeneity of Lp(a). Although the lipid composition of Lp(a) is very similar to that of LDL the former has a higher protein content due to the extra protein moiety. Lp(a) also has a higher polysaccharide content than LDL (5).

Previous studies have mainly investigated the disulfide linkage between apo-B and apo-(a) in the presence of detergents such as sodium dodecyl sulfate (SDS) which disrupt the native lipoprotein structure. We therefore decided to investigate the possible cleavage of this disulfide under non-denaturing conditions (6). Reduction of Lp(a) with dithiothreitol in a Tris buffer (pH 7.4) at 37°C for 3 hr yielded both a lipoprotein and a lipid-free protein component. These two components could be separated by either ultracentrifugation or heparin-sepharose chromatography (6).

Immunochemical characterisation of the lipid-free protein component, whether purified by ultracentrifugation or heparin-sepharose chromatography, showed only an antigenic reaction against anti-Lp(a) and not against anti-B. Two protein bands were observed on SDS-polyacrylamide gel electrophoresis having molecular weights similar to and slightly less than the molecular weight of apo-B. This lipid-free protein component would therefore appear to be (a).

The lipoprotein component from reduced Lp(a) was similar to LDL in many of

its properties and was quite distinct from normal unreduced Lp(a) (Table I). Thus it had beta-mobility on agarose gel electrophoresis, it contained only apo-B as its major apoprotein and it had a molecular weight similar to that of LDL as evidenced by gel-exclusion chromatography on sephacryl S-400. The protein-lipid composition was also comparable to that of LDL rather than normal unreduced Lp(a).

TABLE I

Chemical and physicochemical properties of normal unreduced Lp(a), LDL and the lipoprotein component of reduced Lp(a).

Property	Lp(a)	LDL	reduced Lp(a)*
electrophoretic mobility	pre-beta	beta	beta
density g/ml	1.05-1.12	1.02-1.063	<1.063
apoprotein content	B-100, (a)	B-100	B-100
gel-exclusion volume	256 ml	290 ml	286 ml
% lipid-protein composition by weight			
free cholesterol	7.9	8.5	9.5
cholesteryl ester	37.1	40.7	42.8
phospholipids	19.0	21.3	21.2
triglycerides	5.0	7.1	4.6
protein	30.9	22.4	21.8

* purified by heparin-sepharose chromatography

Further evidence for the similarity of the lipoprotein component from reduced Lp(a) to LDL was obtained from studies on its uptake through the apo-B-mediated receptor pathway in cultured human fibroblasts (7). Both LDL and reduced Lp(a) showed virtually identical concentration-dependent saturation curves for the specific binding, internalisation and degradation steps by the LDL-receptor. Normal unreduced Lp(a) on the other hand, was a much poorer ligand for the receptor. At a protein concentration of 100µg, binding of Lp(a) was only 15% of LDL, internalisation was 20% of LDL and degradation was 18% of LDL (6). Analysis of the data from the degradation curves revealed that normal unreduced Lp(a) had a four-fold higher Km than the other two lipoproteins while its maximum degradation capacity was only 25% that of LDL and unreduced Lp(a).

Our present findings show that Lp(a) is in essence an LDL particle to which the protein (a) is attached by disulfide bonds to apo-B. After removal of apo-(a) by reductive cleavage, the resultant lipoprotein is comparable to LDL in its chemical and physicochemical properties and in its uptake by the LDL-receptor pathway in cultured human fibroblasts. Apo-(a) probably does not interact to any great extent with lipids since it is readily released from the lipoprotein by reductive cleavage. This would support the postulate of Fless et al. (4) that only apo-B is necessary to stabilise the lipids while the more amphiphilic apo-(a) interacts with the aqueous environment. Finally, although normal unreduced Lp(a) can be cleared by the LDL-receptor pathway in cultured human fibroblasts, it is a much poorer ligand than either LDL or the lipoprotein component from reduced Lp(a).

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