APOLIPOPROTEIN A-II CAN INCORPORATE INTO PLASMA VERY LOW DENSITY LIPOPROTEINS

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1. Introduction

Apolipoproteins A-I and A-II represent >90% of the protein mass of human plasma high density lipoproteins (HDL) [1,2]. By contrast, apoA-I and apoA-II constitute <1% of the protein in very low density lipoproteins (VLDL) [1]. ApoA-I and apoA-II in intestinal chylomicrons could serve as precursors for apoA proteins in HDL [3,4]. The exact processes by which apoA-I or apoA-II are assembled into HDL in vivo have not yet been completely elucidated. Although the interaction of apoA-I and apoA-II with phospholipid and their lipid binding sites have been studied [5,6], very little information regarding the associations of apoA-I and apoA-II with VLDL is available. Using VLDL derived from type III hyperlipoproteinemic patients, apoA-II were shown to react with apoE, a major apolipoprotein of VLDL to form a stable apoE-ApoA-II complex [7]. Here, we report that an in vitro incubation (16 h at 37°C) of apoA-I and apoA-II with VLDL results in significant incorporation of only apoA-II into VLDL. Incorporation of apoA-II was accompanied by marked displacement of apoE (>90%) from VLDL. Since apoC proteins were not as readily displaced by apoA-II, results suggest that apoE may be more loosely bound to VLDL than the apoC proteins.

2. Materials and methods

2.1. *Lipoprotein preparation*

VLDL were isolated from plasma pooled from 5 normal men or from 2 male patients with type V hyperlipoproteinemia by the method in [7]. The VLDL were washed by recentrifugation at $d = 1.006$ and then dialyzed against a buffer containing 0.01 M Tris, 0.1 M NaCl, 0.01 M NaN_3, and 0.01% EDTA (pH 7.4). HDL obtained by ultracentrifugation [8] were delipidated with diethyl ether–ethanol (3:1, v/v) and the resultant apolipoproteins were fractionated on Sephadex G-150 (2.6 × 200 cm) in the presence of 4 M guanidine–HCl [2]. The purified apoA-I and apoA-II appeared as single bands following application of 100 μg samples to sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS–PAGE) [9].

2.2. *Incorporation experiment*

The initial experiment was carried out using VLDL isolated from type V subjects. ApoA-I (1 mg) and apoA-II (0.1 mg) were incubated (37°C, 16 h) with 1 ml VLDL containing 0.6 mg VLDL apolipoproteins, 5.7 mg triacylglycerol, and 0.79 mg cholesterol. The mixture was then applied, in a final volume of 25 ml, to a KBr density gradient: 1 ml of the mixture was adjusted to $d = 1.3$ at the bottom and a step-density gradient was constructed from the bottom of the tube to the top with an additional 3.5 ml of $d = 1.25$, 8.5 ml of $d = 1.063$, and 8 ml of $d = 1.006$. Following ultracentrifugation (55 000 rev./min for 3 h at 4°C), the sample was fractionated from top to bottom into 29 tubes and the density was determined by a refractometer.

2.3. *Radioimmunoassay*

Double radioimmunoassays (RIAs) were performed as in [10]. To detect the maximal immunoreactivity of apoA-I, Tween-20 was added to a final concentration of 0.38% [10]. As shown in [10], the RIAs are specific for apoA-I or apoA-II since they do not cross-react with apoC-I, apoC-II, apoC-III, apoB, or apoE present at 1000-fold excess.

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3. Results and discussion

While apoA-II is only a minor protein constituent of human plasma VLDL [1], the presence of an apoA-II–apoE complex in VLDL has been reported [7]. The complex ($M_r > 65\,000$) appears to be stable in 6 M urea and 7.5% SDS as shown in PAGE.

This study was designed to explore in vitro interaction of apoA-I and apoA-II with VLDL during incubation of VLDL with an excess of apoA-I and apoA-II. In preliminary experiments we have incubated VLDL with apoA-I and apoA-II, respectively, at 37°C for 16 h and found either apoA-I or apoA-II could be incorporated into VLDL (not shown). As demonstrated by radioimmunoassay, apoA-II was incorporated into VLDL 5-times more efficiently than apoA-I at equivalent concentrations. In addition, when apoA-I and apoA-II were 'co-incubated' with VLDL at equivalent concentrations, only apoA-II associated with VLDL. In subsequent experiments VLDL obtained from patients with type V hyperlipoproteinemia were incubated with apoA-I and apoA-II at a ratio of 10:1 (w/w) at 37°C for 16 h followed by density gradient ultracentrifugation. As shown in fig.1, the apoA-I and apoA-II treated VLDL migrated to the top of the gradient. Only apoA-II was found to be associated with VLDL to a significant degree as judged by specific radioimmunoassays. Virtually no apoA-I was incorporated when the incubation media contained apoA-I and apoA-II at a ratio of 10:1 (w/w).

The possibility that apoA-I incorporated into VLDL might dissociate during ultracentrifugation or following the exposure to high concentrations of KBr was examined by utilizing an alternate VLDL isolation technique. VLDL pre-treated with apoA-I and apoA-II were isolated by column chromatography using Bio-Gel A-15m [11]. The results were similar to the density-gradient ultracentrifugation. No apoA-I was found to be associated with VLDL in the presence of apoA-II.

Moreover, SDS–polyacrylamide gel electrophoretic patterns of apoA-I/apoA-II-treated and untreated VLDL (fig.2) clearly demonstrate that untreated VLDL contain the major apolipoproteins B, E and C, whereas the reisolated apoA-I/apoA-II-treated VLDL contain only apolipoproteins B, A-II and C (pooled fraction no. 1 and 2, from fig.1). The bottom of the gradient contains primarily apoA-I and a little apoE (pooled fraction no. 28 and 29, from fig.1).

ApoA-II incorporation into VLDL obtained from

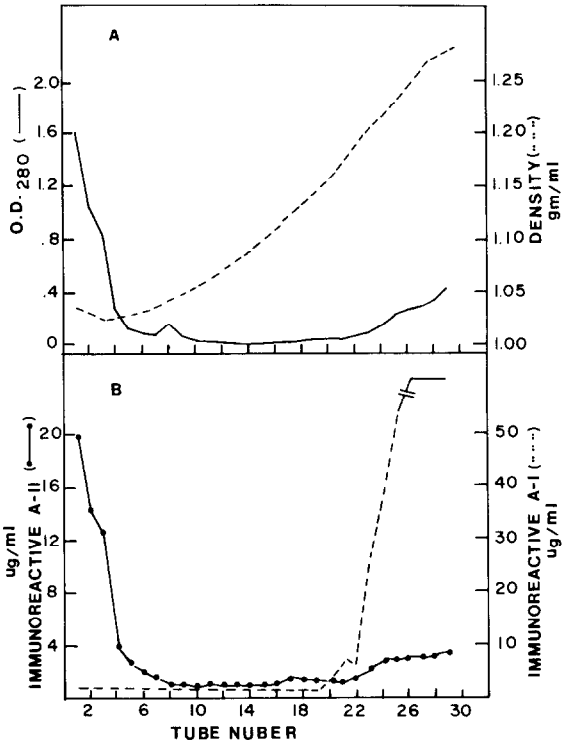

Fig.1. Ultracentrifugational profile of apoA-I- and apoA-II-treated VLDL in a KBr density gradient. (A) VLDL (0.6 mg protein) obtained from type V subjects were incubated with apoA-I (1 mg) and apoA-II (0.1 mg) at 37°C for 16 h. The density of each fraction was determined by a refractometer. Each fraction contained a 0.8 ml aliquot. (B) Radioimmunoassay of apoA-I and apoA-II on each fraction.

normal subjects was also examined. After incubating apoA-II (1 mg) with VLDL (0.5 mg protein) for 16 h at 37°C, the VLDL were reisolated by a standard ultracentrifugation technique [8] at $d = 1.006$. Fractions from $d < 1.006$ and $d > 1.006$ were electrophoresed on SDS–polyacrylamide gel. As shown in fig.2 (gels 4–6), apoB and apoC remained associated with apoA-II in the VLDL fraction, whereas no apoE was detected in this fraction. The apoE released from VLDL was recovered together with the excess of apoA-II in the fraction with $d > 1.006$ (fig.2, gel 6). These results suggest that the orientation of apoE within VLDL may be quite different from that of apoC.

From the present study VLDL appears to be a better 'acceptor' for apoA-II than for apoA-I. The effect of apoA-II incorporation on the lipid–protein stoichiometry of VLDL is shown in table 1. Follow-

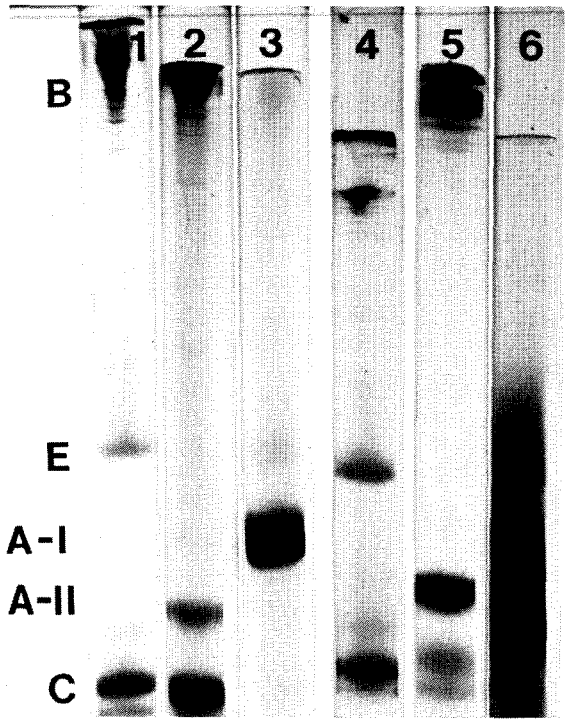

Fig.2. SDS–PAGE of apoA-I and apoA-II or apoA-II-treated VLDL: (1) VLDL (20 μg) obtained from type V subjects prior to incubation with apoA-I and apoA-II; (2) pooled sample from fractions 1 and 2 from fig.1; (3) pooled sample from fractions 28 and 29 from fig.1; (4) VLDL (20 μg) from normals; (5) $d < 1.006$ fraction of apoA-II-treated VLDL; (6) $d > 1.006$ fraction of apoA-II-treated VLDL.

ing incubation of normal VLDL (500 μg) with a limited amount of apoA-II (0–400 μg) for 2 h there was no significant change observed in total lipids (triglycerides) or total protein of reisolated VLDL. The ratio of triglycerides to protein remained constant following apoA-II incorporation. On the other hand, significant loss of apoE occurred during incorporation of apoA-II with little or no decrease in the content of apoC-II and apoC-III. The finding suggests that the surface area of VLDL surface area available for apoprotein deposition is limited and that apoE associates loosely with the lipids of the VLDL particles. At present, we do not know if the displacement of apoE by apoA-II is due to an apolipoprotein exchange process or due to the lipid–protein interaction.

It was shown in [12] that isolated exogenous apoA-I can incorporate into HDL and displace apoA-I but not apoA-II. Therefore, these results seem to suggest that the binding affinity between apoA-II and HDL is higher than that of apoA-I. Kinetically, the apolipoprotein–lipid association is quicker for apoA-II than for apoA-I [13]. These data suggest that the apoA-I molecule binds to HDL more loosely than apoA-II, and are consistent with the observations in [14,15]. Incorporation of apoA-II into canine HDL has been studied [16] and it was observed that 1 mol apoA-I can be displaced by 2 mol apoA-II. A similar result is reported using human HDL showing that apoA-II progressively displaces apoA-I from HDL, whereas the lipid composition and some physical properties of lipids are not affected [17].

Table 1
Loss of apoE, apoC-II and apoC-III following the incorporation of apoA-II into VLDL in 2 h

No.	ApoA-II added (μg)	Recovery of triglycerides in 1 ml of $d = 1.006$ top (μg)	Recovery of total protein in 1 ml of $d = 1.006$ top (μg)	Triglyceride/protein ratio	μg ApoA-II incorp.[a]	% Loss of		
						ApoE[b]	ApoC-II[a]	ApoC-III[a]
1	0	1290	159	8.1	0.90	0	0	0
2	25	1340	151	8.9	10.42	<10	<10	0
3	50	1490	172	8.7	21.35	65	0	0
4	100	1600	195	8.2	28.05	80	0	0
5	200	1340	164	8.2	30.22	85	<10	0
6	400	1650	218	7.6	41.5	95	0	0

[a] Apoprotein levels were determined by specific radioimmunoassays. Immunoreactivity of each apoprotein was in close agreement with the scanning profile obtained from SDS-gel electrophoresis

[b] ApoE level (relative) was determined by scanning SDS–polyacrylamide gels

Normal VLDL (500 μg) was incubated with different amounts of apoA-II at 37°C for 2 h in a total volume of 1.0 ml (pH 7.4). The sample volume was then adjusted to 10 ml with $d = 1.006$ and centrifuged for 16 h

The interaction of apoA-II with VLDL has not yet been reported. The exact mechanism by which apoA-II displaces apoE is not clear from this study, although it might be explained by its loose association with VLDL. For example, rat apolipoprotein E (30–40%) is displaced upon a single ultracentrifugation and is found affiliated with water-soluble proteins of $d > 1.21$ [18]. On the other hand, in humans with alcoholic hepatitis or lecithin–cholesterol acyltransferase (L-CAT) deficiency, HDL is elevated and apoE constitutes 40% of the HDL protein mass [19,20]. In these patients no apoE is found incorporated with VLDL. However, when L-CAT-deficient plasma is incubated with L-CAT, apoE is transferred from HDL to VLDL [19,20]. These studies suggest that once apoE enters the circulation, it associates with nascent HDL and is then transferred to VLDL during the cholesterol esterification process. Nevertheless, it is not clear why cholesterol esterification would result in a drastically dynamic change of apoprotein composition in lipoproteins. It appears that the molecular interaction of apolipoproteins with lipoproteins and lipids with lipoproteins may establish the lipid–apoprotein composition of plasma lipoproteins. Whether or not the net transfer of apolipoproteins to VLDL observed in this study has some physiological significance remains unclear. It would be of interest to carry out experiments using nascent HDL particles (apoE-rich HDL) in the presence of isolated apoA-II to follow the fate of apoE in the circulation.

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