

Studies on the apoproteins of rat lymph chylomicrons: characterization and metabolism of a new chylomicron-associated apoprotein

Noel H. Fidge¹ and Peter J. McCullagh

Baker Medical Research Institute, Commercial Road, Prahran, Victoria, Australia 3181 and
Department of Immunology, John Curtin School of Medical Research,
Australian National University, Canberra, ACT, Australia 2650

Abstract The apoprotein composition of rat lymph chylomicrons and very low density lipoprotein (VLDL) was investigated following isolation and purification by preparative gel electrophoresis. The medium molecular weight apoproteins in the 27,000–60,000 range were characterized by electrophoretic mobility, immunochemical identification, amino acid composition, and molecular weight determination. In addition to three previously identified apoproteins, A-I, E, and A-IV, present in both rat serum high density lipoproteins (HDL) and lymph chylomicrons, a fourth peptide of molecular weight 59,000 was always a consistent major component of lymph VLDL washed free of serum protein contaminants by repeated ultracentrifugation. The protein formed complexes with phosphatidylcholine vesicles, was amphipathic in nature, and, when injected into rats, became associated with serum HDL and lymph chylomicrons. It was removed from chylomicrons after gel filtration through agarose columns but not after repeated ultracentrifugation; it differed slightly in amino acid composition and did not show immunochemical identity against antisera to any other apoprotein. The results of *in vivo* studies suggest that the protein preferentially associates with lymph chylomicrons and thus may play some important role in the metabolism of exogenous triglyceride transport. Since A-I, A-II, and A-IV apoproteins are also integral components of lymph chylomicrons, we suggest that the protein be designated A-V apoprotein. The distribution of A-I, E, A-IV, and A-V in serum HDL and lymph VLDL was approximately 54, 10, 12, and 0%, and 22, 3, 19, and 22%, respectively. — **Fidge, N. H., and P. J. McCullagh.** Studies on the apoproteins of rat lymph chylomicrons: characterization and metabolism of a new chylomicron-associated apoprotein. *J. Lipid Res.* 1981. **22**: 138–146.

Supplementary key words VLDL · HDL · apo E · apo A-I · apo A-IV · apo A-V

The composition and metabolism of lymph apoproteins is less clearly understood than that of the corresponding plasma apoproteins. Although the composition of lymph lipoproteins may be partly influenced by filtration of apoproteins from plasma, and therefore of non-intestinal origin, there are still

quite notable differences between the lymph and plasma apoprotein profile. For instance, apo A-I, the principal protein of triglyceride-deficient plasma HDL, is a major component of triglyceride-rich lymph chylomicrons of both rat (1, 2) and man (3, 4), and recent studies have demonstrated the existence of significant proportions of apo A-IV in chylomicrons of both human (5) and rat lymph (6). We have consistently found apoproteins of higher molecular weight in lymph chylomicrons than those previously characterized, and we noted that the apoprotein composition varied with different isolation methods. The present study was carried out to investigate further the nature of lymph apoproteins, particularly those of medium molecular weight, ranging between $30\text{--}70 \times 10^3$ daltons that are associated with rat lymph chylomicrons and very low density lipoprotein.

EXPERIMENTAL PROCEDURE

Lymph collection

Lymph was collected from 250–300 g male rats (John Curtin School, Canberra strain) previously fed a commercial chow diet. Either the thoracic duct or the main mesenteric lymph duct was cannulated under ether anaesthesia by the methods of Bollman, Cain, and Grindlay (7) or Warshaw (8). The rats were placed in restraining cages and allowed free access to food and water. They were also periodically infused with 0.5 ml of 0.5% saline through an indwelling femoral vein cannula. Lymph was collected during the day and separately overnight into 100-ml sterile flasks

Abbreviations: VLDL, very low density lipoprotein; LDL, low density lipoprotein; HDL, high density lipoprotein; SDS, sodium dodecyl sulfate; PC, phosphatidylcholine.

¹ Reprint requests to: Dr. N. Fidge, Baker Medical Research Institute, Commercial Road, Prahran, Victoria, Australia 3181.

containing sodium azide (to provide a final concentration of 0.01%) to minimize bacterial contamination.

Lipoprotein separation

Ultracentrifugal isolation. For the initial isolation procedure, 200–300 ml lymph was layered beneath 0.15 M NaCl, pH 7.4, containing 1 mM EDTA (hereafter called 0.15 M NaCl) in polycarbonate bottles and centrifuged at 16,000 rpm for 30 min in the 35 rotor of the Beckman L5-60 ultracentrifuge. The $S_f > 400$ lipoproteins (hereafter referred to as chylomicrons) formed a packed layer near the top of the tube and the infranate was collected by vacuum aspiration into 500-ml Erlenmeyer flasks and recentrifuged at the same conditions to yield any remaining chylomicrons which were pooled with the original top fraction. The infranate was then centrifuged at 35,000 rpm (35 rotor, d 1.006 g/ml) for 12–16 hr in order to isolate the S_f 20–400 fraction, referred to hereafter as VLDL. In later preparations, since most of the remaining lymph triglyceride could be isolated by shorter centrifugation periods, with the conditions suggesting that the bulk of the VLDL was in the S_f 100–400 range, the infranate was centrifuged for 6 hr to provide lymph VLDL. Both chylomicrons and VLDL were resuspended in 0.15 M NaCl and washed as described below until all immunodetectable traces of albumin, which by electroimmunoassay could not have exceeded 0.1% of the total protein, were removed. Chylomicrons were washed twice by resuspension in five volumes of 0.15 M NaCl and centrifuged in the 40.3 rotor at 17,000 rpm for 30 min, followed by one further wash for 6 hr at 50,000 rpm in the Beckman 50 rotor. VLDL was washed by three to four centrifugations at 50,000 rpm for 6 hr. The necessity for repeated ultracentrifugation or gel filtration to remove contaminating serum proteins from chylomicrons has also been reported by other workers (3, 4).

Isolation by gel filtration. Chylomicrons were also freed of plasma protein contaminants by gel filtration through Biogel A5m columns (Biorad Labs) as described previously (9). Briefly, 1–2 ml of chylomicrons containing 2 mg protein per ml was loaded onto 2.5 × 90 cm columns equilibrated with 0.15 M NaCl, pH 7.4, containing 1 mM EDTA and 0.01% sodium azide. Fractions were monitored by absorbance at 280 nm.

Rat serum lipoproteins were separated from serum of rats fasted overnight prior to blood collection. They were purified by ultracentrifugation as described previously (10).

Delipidation of lipoproteins

Purified chylomicrons and VLDL and serum lipoproteins were dialyzed against 5 mM NH_4HCO_3 , pH 8.2, and lyophilized. Delipidation was performed

with chloroform methanol and ether as described previously (10). After the final ether wash, the protein was dried under N_2 and dissolved in 0.05 M NH_4HCO_3 , pH 8.2, containing 0.1 M sodium dodecyl sulfate (SDS). Protein concentration was determined by the method of Lowry et al. (11).

Analytical procedures

Electrophoresis was performed on polyacrylamide gels containing 0.1% sodium dodecyl sulfate and on slab gels containing 0.1% SDS. Disc gel electrophoresis in 15% polyacrylamide gels containing SDS was performed according to the method described by Weber and Osborn (12) which contains Tris-HCl rather than phosphate buffers. The same system was used for the 0.1% SDS, 15% acrylamide slab gel which was set up in the Biorad model 1100 electrophoresis cell (Biorad Labs, Richmond, CA). During all electrophoresis procedures, gels were cooled by circulating cold tap water through a glass coil immersed in the tank buffers. SDS gels were first fixed in 7% acetic acid in 10% methanol before staining for 1 hr with Coomassie blue (12). Gels were then destained for 2 days in 7% acetic acid, 5% methanol.

Apoprotein separation

Separation of the medium molecular weight apoproteins of rat HDL or lymph chylomicrons was achieved by preparative gel electrophoresis on 15% acrylamide gels containing 0.1% SDS. In earlier separations, 12 disc gels (0.6 × 8 cm) were loaded with 200–300 μg of protein and the bands located by staining one-half of one notched gel. Later separations were carried out on slab gels. The areas of gel corresponding to stained bands were sliced out, pooled and macerated with a glass rod, and eluted with ten volumes of 0.1% SDS in 0.05 M NH_4HCO_3 , pH 8.0, containing 0.1 mM sodium azide. After a 12-hr incubation at 37°C in a shaking water bath, the gels' fragments were centrifuged and eluted again with five volumes of eluting buffer which was then pooled with the first supernatant. The combined eluates were dialyzed for 3 days against 5 mM NH_4HCO_3 with changes twice daily and then lyophilized. The dried apoproteins were redissolved in 0.05 M NH_4HCO_3 , pH 8.0, and aliquots were taken for protein assay, analytical gel electrophoresis, amino acid analysis, electroimmunoassay, and molecular weight determination. Corresponding slices from gels not loaded with protein were used as blanks for amino acid analysis.

Immunochemical analysis

Antisera to rat albumin (Calbiochem, La Jolla, CA) and apoproteins B (the tetramethylurea-insoluble

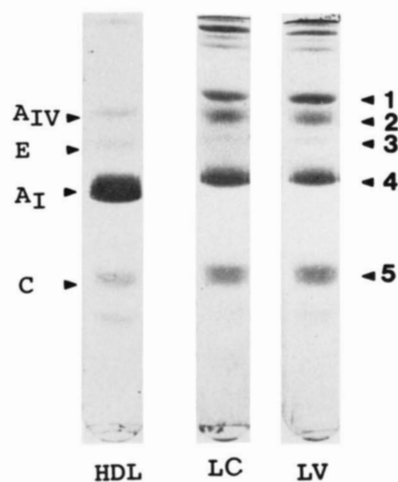


Fig. 1. Electrophoresis of lymph apo VLDL and rat apo HDL on 15% acrylamide gels containing 0.1% SDS. From left, rat apo HDL, lymph chylomicron apoprotein, and lymph apo VLDL.

fraction of rat LDL), A-I, E, and A-IV (from rat HDL, isolated according to Swaney, Reese, and Eder (13)), were prepared by conventional immunological techniques. Rabbits, weighing 2–3 kg were injected with 50–100 μ g protein per kg body weight and booster injections given every 14 days until the antisera produced clear “rocket” immunoprecipitin lines against the respective antigen in the electroimmunoassay system. Each antisera was monospecific in that no “rockets” were produced when other antigens were tested at high concentrations. Electroimmunoassay was performed according to Laurell (14) on 1% agarose impregnated with varying concentrations of antisera, ranging from 0.2% for rat albumin to 3.0% for rat apo A-I. Electrophoresis was performed in a Biorad Model 1400 immunoelectrophoretic apparatus using the barbital-acetate buffer systems described in the Biorad operations manual. After electrophoresis, the agarose gels were washed in saline and distilled H_2O , dried, and stained with Coomassie blue, and the protein concentration calculated by comparison with known standards included on each plate.

Immunodiffusion studies were performed by the Ouchterlony technique (15) on 1% agarose in 0.05 M barbital buffer, pH 8.2.

Amino acid analysis

Samples were dialyzed against distilled H_2O , lyophilized, and hydrolyzed in 4 N methanesulfonic acid in evacuated sealed tubes. Two hundred μ g of protein were hydrolyzed for analysis by the two-column procedure in the Beckman 120 B analyzer or 50 μ g were hydrolyzed for analysis on the Beckman 121 M analyzer.

Lipid-protein interactions

The interaction of the unidentified protein with lipid was compared with rat albumin and A-I apoprotein. Dispersions of egg yolk L- α -phosphatidylcholine, 99% pure by thin-layer chromatography (Sigma Chemical Co., St. Louis, MO) were prepared by prolonged sonication (16) and the bilamellar vesicles (second peak, ca. 70%) were separated from multilamellar vesicles (first peak) by gel filtration through a Biogel A-5m column (16). The vesicles were identified by electron microscopy. Radiolabeled proteins were incubated with phospholipid vesicles (ratio 1:5 w/w) in a final volume of 1 ml at 40°C for 10 hr and the phospholipid-protein complexes were separated from unassociated protein by chromatography over Biogel A5-m columns (1.5 \times 90 cm) equilibrated with 0.15 M NaCl, pH 7.4.

In vivo studies

^{125}I -Labeled proteins were prepared as described previously (10). Less than 1 g-atom of iodine was bound per mole of protein. Approximately 5 μ Ci of each protein was injected into the tail vein of fat-fed lymph (thoracic) duct-cannulated rats and blood or lymph samples taken at various times after injection. Lipoproteins were then isolated as described above.

Charge-shift electrophoresis

Immuno-electrophoresis of A-V apoprotein was performed in the presence of Triton X-100, Triton X-100 plus sodium deoxycholate, or Triton X-100 plus cethyltrimethylammonium bromide, as described by Beisiegel and Utermann (17).

RESULTS

Heterogeneity of the medium molecular weight group of apoproteins of lymph VLDL was confirmed by SDS disc gel electrophoresis in 15% acrylamide gels as shown in **Fig. 1**. This method also clearly shows that three of the lymph VLDL apoproteins (bands 2, 3, and 4) have similar migration to three HDL apoproteins A-IV, E, and A-I while another major apoprotein present in lymph VLDL (band 1) is absent from rat serum HDL.

Further studies were required to characterize and identify these apoproteins. To obtain purified peptides, serum HDL and lymph VLDL apoproteins were separated by preparative SDS gel electrophoresis as described in the Methods section. Using this technique, serum HDL apoproteins A-I, A-IV, and E were isolated and their amino acid compositions determined. From both amino acid composition data and molecular weight determinations, it was found that

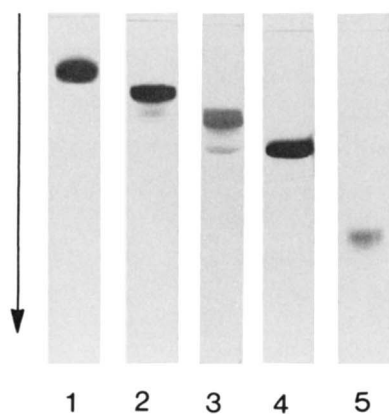


Fig. 2. Isolation of medium molecular weight apoproteins following preparative SDS gel electrophoresis of lymph VLDL apoprotein. Gels 1, 2, 3, 4, and 5 correspond to proteins eluted from gels and which correspond to bands 1–5 of Fig. 1.

the purified rat apoproteins were identical to the A-I, A-IV, and E apoproteins described by Swaney et al. (13). Antisera were then prepared to each apoprotein as described above.

Lymph VLDL and chylomicron apoproteins, separated by preparative SDS gel electrophoresis are shown in **Fig. 2**. All of the proteins in the medium molecular weight range were separated by this method and total recovery of protein varied between 68–81% of that loaded onto the gels. In some cases it was necessary to repeat the preparative electrophoresis procedure to obtain electrophoretically homogeneous bands. Each of the purified lymph apoproteins was then tested, by electroimmunoassay, against antisera to rat A-IV, A-I, and E apoproteins and albumin and the results are shown in **Fig. 3**. This study demonstrated that fraction 4 was immunologically identical to apo A-I, fraction 3 to apo E, and fraction 2 to apo A-IV. There was no reactivity of any fraction (or of whole lymph VLDL) to anti-rat albumin. Since fraction 1 (which was the other major lymph VLDL apoprotein not present in serum HDL) did not react against A-I, E, A-IV, B (not shown), or albumin, a separate antisera against this unidentified protein was produced in rabbits. As seen in **Fig. 3**, this antisera only reacted against fraction 1 and showed no immunological reactivity with apoproteins A-I, E, A-IV, or serum albumin.

The amino acid compositions and molecular weights of the lymph apoproteins are compared with apo A-I, A-IV, and E compositions in **Table 1**, and it describes the similarities between fraction 4 and apo A-I, 3 and apo E, 2 and apo A-IV. The amino acid composition of fraction 1 was not similar to any of the other apoproteins and its molecular weight of approximately 59,000 was considerably higher than the others. It

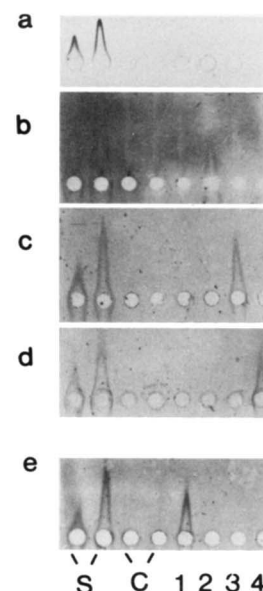


Fig. 3. Electroimmunoassay of lymph VLDL apoproteins. S, standards, ca. 100 and 200 ng, respectively. C, control wells, containing 0.15 M NaCl. a, b, c, d, and e contain antisera to rat albumin, apo A-IV, apo E, apo A-I, and the high molecular weight protein shown in gel 1 of **Fig. 2**. Wells 1–4 contain purified proteins eluted from SDS gels and corresponding to bands 1–4 of **Fig. 2**.

was further studied to investigate its possible apoprotein properties as described below.

Characterization of new chylomicron associated protein

After incubating apo A-I with phosphatidylcholine (PC) vesicles a lipid protein complex was formed (**Fig.**

TABLE 1. Amino acid analysis of rat apoproteins

MW	45000	45000	35000	35000	27500	27500	59000
	2 ^a	A-IV	3	E	4	A-I	1
Lys	6.7	7.3	5.9	4.7	10.8	9.5	4.0
His	1.3	2.2	1.5	1.1	3.2	2.4	2.4
Arg	3.3	3.8	9.3	9.6	6.3	5.2	2.4
Trp	0.5	1.0	1.2	1.3	2.1	2.0	0.7
Asp	12.7	12.0	10.4	9.8	12.1	13.6	7.8
Thr	5.1	5.4	4.7	5.2	4.3	4.9	5.6
Ser	6.0	6.1	6.9	4.6	6.0	4.3	14.7
Glu	20.8	18.9	20.9	19.5	17.4	18.4	15.7
Pro	4.2	3.6	2.2	3.4	2.2	2.3	6.1
Gly	5.8	5.7	7.4	6.0	5.5	5.1	14.6
Ala	8.0	7.1	7.5	8.4	6.6	7.6	8.3
Half Cys	Tr	Tr		0.8			
Val	5.8	5.4	4.5	5.4	4.1	4.3	5.2
Met	2.3	2.4	1.6	3.0	1.8	0.9	0.5
Ile	2.0	2.6	2.1	2.5	1.9	1.7	2.7
Leu	10.3	11.0	8.6	10.4	11.2	12.6	4.3
Tyr	1.5	2.4	2.4	2.1	1.9	2.1	1.9
Phe	2.9	2.9	2.9	2.2	2.6	3.1	3.0

^a Mol/100 mol protein. Mean of two analyses of each apoprotein. A-I, E, and A-IV apoproteins were isolated from rat HDL and the numbered column refers to chylomicron apoprotein corresponding to the gel band shown in **Fig. 1**.

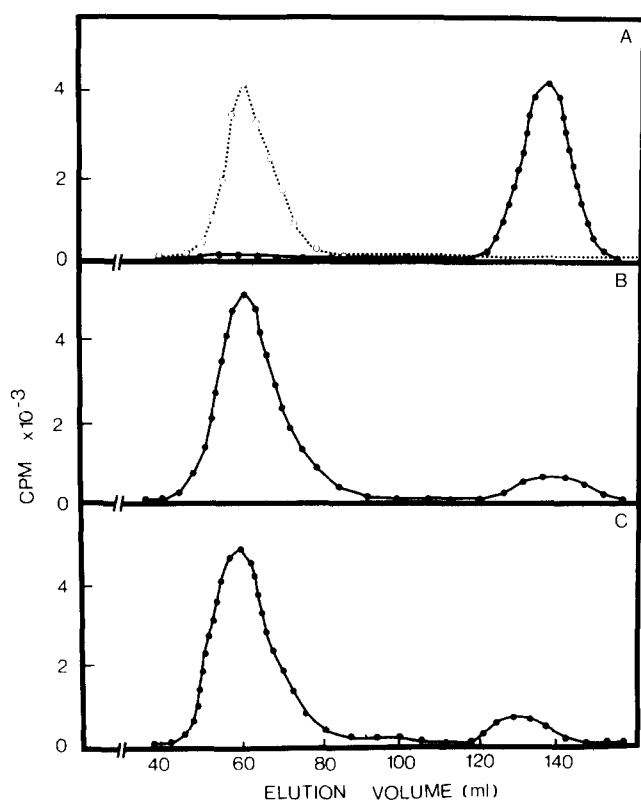


Fig. 4. Separation of complexes formed between protein and phosphatidylcholine (PC) bilamellar vesicles. A, Elution characteristics of PC vesicles (broken line) loaded separately and of ^{125}I -labeled rat albumin after 10-hr incubation at 40°C with PC vesicles on a Biogel A5 column, 1.5×90 cm eluted with 0.15 M NaCl , pH 7.4. B, First peak shows complex formed after incubating ^{125}I -labeled rat A-I apoprotein with PC vesicles (1:5 w/w ratio) for 10 hr at 40°C . Second peak is unbound apo A-I. C, Complex formed between new apoprotein and PC after incubation as for B.

4B) which eluted near the same volume as PC vesicles alone (Fig. 4A). As shown in Fig. 4C, the new apoprotein also almost completely associated with PC to form a lipid-protein complex which eluted in a similar position as the A-I-PC complex. However, albumin remained unassociated after incubation with PC (Fig. 4A). During the course of this study, it was reported that a serum protein, β_2 -glycoprotein-1, exhibited binding properties to human serum lipoproteins (18), was amphipathic in nature, and thus resembled the apoprotein-like properties of the new protein described in this report. In the absence of a rat β_2 -glycoprotein-1 antisera, we are unable to determine whether the new apoprotein described above is identical to this serum protein. However the distinctive lipid binding properties of the chylomicron-associated protein together with the unusual metabolic behavior (described below) suggests a specific role for this peptide in lipid transport and we have tentatively designated the protein A-V apoprotein to distinguish it from other nonspecific serum proteins. Evidence of a

distinct physiological role in triglyceride transport for this apoprotein is suggested by the results described below.

Metabolic studies with A-V-apoprotein

When injected into rats, ^{125}I -labeled A-V apoprotein became associated with serum and lymph lipoproteins, in contrast to ^{125}I -labeled albumin which remained unassociated. **Fig. 5** compares the partitioning of radioactivity between lymph and plasma at various times after the simultaneous intravenous administration of approximately $10\text{ }\mu\text{g}$ each of ^{125}I -labeled apo A-V and ^{131}I -labeled albumin into lymph duct-cannulated rats. Labeled A-V apoprotein rapidly transferred from the blood to lymph, so that 10 min after injection, the lymph label (in cpm/ml) had reached 60% of the blood radioactivity, whereas blood albumin equilibrated more slowly with lymph and reached similar levels as lymph approximately 2 hr after injection. **Table 2** shows that A-V apoprotein had become associated with several lipoprotein fractions in serum whereas albumin (not shown) remained in the $d > 1.21$ g/ml fraction. At 5 min after injection, 33% of the total serum radioactivity was associated with HDL, while approximately 4% was recovered in the $d < 1.006$ g/ml fraction. This proportion increased up to 14% 19 hr after injection. Of the total radioactivity transferred into lymph, only a small proportion of albumin (0.3–4.3%) was recovered in lymph chylomicrons compared to 19.4% (after 2–3 hr) of the ^{125}I -labeled A-V (**Table 3**). SDS-polyacrylamide gels confirmed that the label was present in the A-V protein band.

Labeled A-V apoprotein was injected into two lymph duct-cannulated rats and lymph was collected for 6 hr

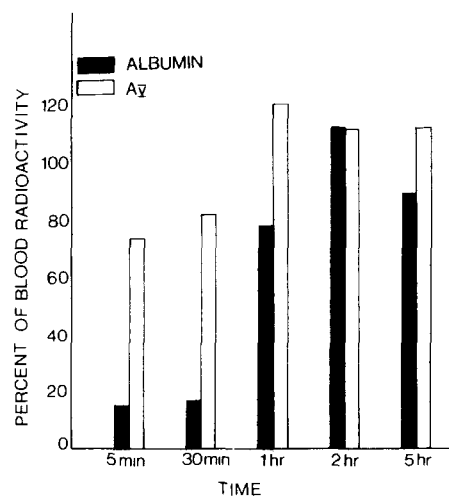


Fig. 5. Relative proportion of ^{125}I -labeled albumin and apo A-V in lymph (expressed as a percent of blood radioactivity) after injecting radiolabeled proteins into separate rats (two at each time point).

TABLE 2. Distribution of radioactivity in serum after injecting ^{125}I -labeled apo A-V into rats

Time after Injection	Lipoprotein Fraction			
	d < 1.006	d 1.006–1.063	d 1.063–1.21	d > 1.21
	% distribution ^a			
5 min	3.9	7.1	33.2	55.7
1 hr	4.5	11.9	17.4	66.0
5 hr	8.2	13.4	17.5	60.9
19 hr	14.0	12.1	14.7	59.1

^a Mean data from two rats at each time point.

after injection. Chylomicrons were harvested and washed once by ultracentrifugation (see above) to obtain in vivo ^{125}I -labeled A-V chylomicrons. Ninety percent of labeled apoproteins remained with chylomicrons after ultracentrifugation (Table 4). However, after in vitro incubation with serum or injection into rats, considerable label was found in both the HDL and infranate fractions, suggesting that clearance of chylomicron-A-V apoprotein proceeds via transfer into the HDL and lipoprotein-free fractions of plasma. It was interesting to note that only a small proportion of ^{125}I -labeled A-V associated with the LDL fraction, whereas when injected as the unbound apoprotein (Table 2) considerable label associated with this fraction. This discrepancy may be due to different mechanisms involved in catabolism of associated and unassociated apoprotein.

Effect of isolation procedure on chylomicron apoprotein composition

Repeated ultracentrifugation of lymph chylomicrons and VLDL did remove albumin and some higher molecular weight components (not identified) but not A-V apoprotein as seen in Fig. 6. There was no apparent change in the proportion of apoproteins A-V, A-IV, E, and A-I from the first wash (Fig. 6B) up to the fourth wash (Fig. 6C) by ultracentrifugation as described in the Methods section. Even prolonged ultracentrifugation for 16 hr at 50,000 rpm did not alter the proportion of these medium molecular weight

TABLE 3. Association of radioactivity with lymph chylomicrons after injecting simultaneously either ^{131}I -labeled rat albumin or ^{125}I -labeled apo A-V into lymph duct-cannulated rats

Period of Lymph Collection Time	Percent of Radioactivity in Lymph Associated with d < 1.006 g/ml fraction	
	Apo A-V	Albumin
0–1 hr	12.0 ^a	0.3 ^a
1–2 hr	15.6	1.8
2–3 hr	19.4	1.4
3–18 hr	29.6	4.3

^a Mean data from two rats at each time point.TABLE 4. Distribution of radioactivity in serum after in vitro incubation or in vivo injection of lymph chylomicrons labeled with ^{125}I

	Time	Lipoprotein Fraction			
		d < 1.006	d 1.006–1.063	d 1.063–1.21	d > 1.21
		% distribution ^b			
In vitro					
Control ^a	5 min	89.0	2.1	1.8	7.1
Serum ^a	5 min	51.3	2.0	14.9	31.8
Serum	40 min	54.7	0.2	12.2	32.9
In vivo	5 min	45.1	1.0	16.7	37.2
	10 min	37.4	2.2	23.4	37.0
	40 min	17.2	1.8	37.6	43.4

^a ^{125}I -labeled A-V chylomicrons (labeled by in vivo exchange described in text) incubated with 4 ml 0.15 M NaCl, pH 7.4, or 4 ml serum as shown.^b Mean data from two rats at each time point.

proteins, although approximately equal amounts dissociated and were recovered from the d 1.006 g/ml infranate as found by electroimmunoassay (not shown). However, one passage through a Biogel A5m column removed albumin and apo A-V as observed in Fig. 6D.

Charge-shift electrophoresis

Binding of deoxycholate and cetyltrimethylammonium bromide detergents by A-V was clearly demonstrated by the marked change in electrophoretic mobility found in the immunoelectrophoresis experiments (data not shown).

Quantitative distribution of apoproteins

The percentage proportion of each lymph VLDL and chylomicron apoprotein was estimated by scan-

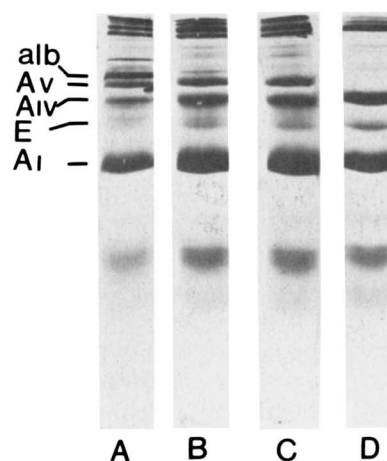


Fig. 6. SDS gel patterns of chylomicron apoprotein. A, First ultracentrifugal isolation; B, second wash (third centrifugation) showing decrease in albumin and other bands above it; C, fourth wash (fifth centrifugation) showing complete removal of albumin but not A-V; D, one passage of chylomicrons, showing apoprotein composition similar to A, through Biogel A5-m.

ning SDS gels (15) after staining with Coomassie blue and was compared with the proportion of apoproteins of serum HDL in **Table 5**.

DISCUSSION

Three of the four major chylomicron apoproteins characterized in this report, namely A-I, A-IV, and E apoproteins are also found in serum HDL. The fourth protein of higher molecular weight, (around 59,000) has not been found associated with any other rat serum lipoprotein but in the present study was shown to possess the biochemical features of an apoprotein. Using SDS gel electrophoresis, Glickman and Kirsch (2) separated chylomicron apoprotein and reported a gel pattern almost identical to the patterns shown in this paper. Four proteins of medium molecular weight were also observed (2) but the protein with highest molecular weight (R_f 0.41, mol wt ~60,000) corresponding to the new apoprotein reported here was described as a possible plasma protein contaminant. After elution from SDS gels, this peptide (R_f 0.41) was shown to aggregate and remained on top of gels containing 6M urea. Holt, Wu, and Clark (19) observed a chylomicron protein on SDS gels of mol wt ~60,000 which they found to be removed or greatly reduced after gel filtration through agarose and recently, Polz and Kostner (18) reported that a serum protein, β_2 -glycoprotein-1, present in VLDL, was capable of binding to human lipoproteins and also possessed amphiphatic properties. Without an antiserum to rat β_2 -glycoprotein-1, we were unable to confirm the identity of a similar plasma protein in the rat, but it appears that both proteins have similar properties. Additionally, we have studied the *in vivo* metabolism of this protein and found that it preferentially associates with triglyceride-rich lipoproteins and particularly binds to those present in the lymph. Antisera to the new apoprotein did not react with apoproteins A-I, E, A-IV, or B and presumably, therefore, is not representative of an A-I, E, or A-IV polymer or association complex of any combination of these apoproteins. We suggest that since this new chylomicron-associated protein exhibited the properties of an apolipoprotein and may possibly be specifically involved in chylomicron transport, the peptide be designated A-V apoprotein, to distinguish it from other nonspecific plasma proteins.

In the presence of thiol reducing agents, A-V remained as one single band on SDS gels indicating that it existed as a monomeric polypeptide unit. The triglyceride-rich lipoproteins of lymph were separated into two subclasses, $S_r > 400$ (termed chylo-

TABLE 5. Distribution of rat serum HDL, lymph chylomicron, and lymph VLDL apoproteins, determined by densitometry of three stained 15% acrylamide gels

Gel Fraction (Fig. 5)	Identity	Lipoprotein		
		Serum HDL	Lymph Chylomicrons	VLDL
		% of total absorbance		
	B + high mol wt proteins	0	17	16
1	A-V	trace ^a	22	22
2	A-IV	12	17	19
3	E	10	4	3
4	A-I	54	25	22
5	C	24	15	18

^a When gels are loaded with higher amounts of apo HDL, a band in the A-V position becomes visible. Also, due to slight differences in chromogenicity, quantitation by this gel technique should be considered semiquantitative.

microns) and S_r 20–400 (called lymph VLDL). The apoproteins of both groups were qualitatively and quantitatively similar, a finding which has also been described in detail for the apoproteins of human (4) and rat chylomicrons (2). The composition was identical in chylomicrons and VLDL obtained from lymph of both thoracic and mesenteric lymph duct.

Estimation of the distribution of chylomicron, VLDL, and HDL apoproteins showed considerable differences between lymph VLDL and serum HDL. Whereas A-I was the major serum HDL protein, apoproteins A-IV, A-V, and A-I were more equally distributed in the triglyceride-rich lymph lipoproteins. The amino acid pattern of A-V showed some differences when compared with the other peptides but, like other rat apoproteins (13), was not characterized by the absence of any residue. Apo A-V was higher in glycine and serine and lower in leucine and lysine content than A-I, A-IV, or E apoproteins. The possible contamination of serine and glycine from SDS gels has been discounted since analyses were corrected by subtracting blank gel values.

The heterogeneity of lymph chylomicrons and VLDL apoproteins demonstrated in this study and by others (2–6) raises some interesting questions regarding the origin, role, and fate of each apoprotein. This is a difficult question, due mainly to the complicated organization of the lymphatic and capillary supply at the intestinal absorptive cell location, so that apoproteins appearing in lymph lipoproteins may have been derived by filtration from the blood. Other laboratories have reported labeling of A-I and B apoproteins of lymph chylomicrons following the feeding of radioactively labeled amino acids to rats (1, 2), although Glickman and Kirsch (2) failed to find labeling of the high molecular weight protein (R_f 0.41) cor-

responding to A-V in this present study. In addition, proteins of similar molecular weights have been noted in gel patterns of apoproteins isolated from the serum of subjects suffering from several forms of liver disease, particularly in alcoholic hepatitis (20), and we have noted proteins of similar electrophoretic mobility in the VLDL of rats in which the clearance of triglyceride has been blocked by Triton treatment (21).

Several experiments demonstrated the apolipoprotein nature of A-V. It had an affinity for phospholipid vesicles similar to other A apoproteins, it showed amphipathic properties as demonstrated by charge-shift electrophoresis and in contrast to albumin, when administered in vivo to rats, rapidly transferred into lymph and associated with lymph chylomicrons. This suggests a specific role for A-V in chylomicron transport or metabolism. Whether the A-V apoprotein is synthesized, assembled, and secreted with chylomicrons from the intestinal mucosal cell, or added to chylomicrons in the lymph after secretion is yet to be determined. However, other important apoproteins are probably transferred to lipoproteins after their secretion, and available evidence suggests that E and some C apoproteins, which are present in chylomicrons, are not synthesized in the gut (6) and so are apparently transferred to these triglyceride-rich lipoproteins after their secretion from the mucosal cells.

Of further interest is the recent finding that some apoproteins may occur in plasma not associated with lipoproteins. Wong and Rubinstein (22) found 40–50% of apo E in the $d > 1.21$ g/ml fraction of control rat serum but virtually none in cholesterol-fed rats, suggesting that in this situation, apo E was more firmly bound to lipoproteins. High concentrations of E apoprotein in the lipoprotein-free fraction of plasma have also been reported elsewhere (21, 23). A large proportion of total plasma A-IV apoprotein is also found unassociated with lipoproteins in both plasma (17, 24) and lymph (6). Thus evidence is accumulating to suggest that a pool of unassociated apolipoproteins exists in plasma that may have an important physiological role in the lipoprotein transport system. Perhaps some apoproteins are secreted independently of nascent lipoproteins and transferred to lipoproteins during a certain phase of their biochemical formation or degradation. In fact, the transfer of A-V from chylomicrons into the HDL and lipoprotein-free fractions of plasma closely resembled the in vivo behavior of A-IV apoprotein recently reported from this laboratory (24).

Apoprotein A-V remained firmly bound to lymph chylomicrons throughout extensive ultracentrifugation, but was removed by one passage through an agarose column. The fact that agarose gel chroma-

tography is commonly used to purify chylomicrons may explain why this higher molecular weight protein has not been studied in more detail in previous studies. Perhaps A-V associated differently with phospholipid vesicles than chylomicrons, and remained bound during passage through the Biogel A5 column. Other apoproteins, for example A-IV, E, A-I, and B apoproteins are dissociated from lipoproteins during repeated centrifugation. As noted in the present study, the relative proportion of the medium molecular weight apoproteins did not alter during repeated ultracentrifugation, which suggests that A-V did not become relatively concentrated during repeated washing. Further studies are in progress to investigate the origin, role, and properties of this interesting apoprotein. ■

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