The N-terminal 17% of apoB binds tightly and irreversibly to emulsions modeling nascent very low density lipoproteins

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Abstract **The N-terminal 17% of apolipoprotein B (apoB-17) readily associates with dimyristoylphosphatidylcholine (DMPC) multilamellar vesicles (MLV) to form large (240-Å diameter) discoidal particles. Because apoB is normally secreted with triacylglycerol (TAG)-rich lipoproteins, we studied the binding of apoB-17 to triolein-rich emulsions modeling nascent TAG-rich very low density-like lipoproteins. Emulsions with the following composition (by weight) were prepared: 85–89% triolein, 1.1–1.4% cholesterol, and 10–14% phosphatidylcholines (PC) including either egg yolk (EY)-, dimyristoyl (DM)-, or dipalmitoyl (DP)-PC representing (at 25°C), respectively, a fluid surface, a surface at transition, and a mainly solid surface. The respective sizes were 1,260 ± 500, 1,070 ± 450, and 830 ± 300 Å mean diameter. The emulsions were incubated with conditioned medium containing apoB-17, and then reisolated by ultracentrifugation. Analysis of the emulsion-bound proteins by gel electrophoresis showed that all three emulsions bound primarily apoB-17. The DPPC emulsions bound more apoB-17 than EYPC or DMPC emulsions. Immunoaffinity-purified apoB-17 exhibited saturable, high affinity binding to EYPC and DPPC emulsions. The respective K_d values were 32 ± 23 and 85 ± 27 nM and capacities (N) were 10 and 58 molecules of apoB-17 per particle. When apoB-17 bound to emulsions was incubated with DMPC MLV at 26°C for 18 h, it remained bound to the emulsions, indicating that once bound to these emulsions it is unable to exchange off and solubilize DMPC into discs. In contrast, apoE-3 bound to emulsions dissociated from the emulsions when incubated with DMPC MLV and formed discs. Thus, apoB-17 binds strongly and irreversibly to emulsions modeling nascent lipoproteins. It therefore may play an important role in the stabilization of nascent VLDL and chylomicrons.**—Herscovitz, H., A. Derksen, M. T. Walsh, C. J. McKnight, D. L. Gantz, M. Hadzopoulou-Cladaras, V. Zannis, C. Curry, and D. M. Small. **The N-terminal 17% of apoB binds tightly and irreversibly to emulsions modeling nascent very low density lipoproteins.** ***J. Lipid Res.*** **2001.** 42: **51–59.**

Supplementary key words truncated forms of apoB • multilamellar vesicles • EYPC • DPPC

Human apolipoprotein B-100 (apoB-100), a major risk factor for the premature development of coronary heart disease (1, 2), is a large (4,536 amino acids, molecular mass 550 kDa) (3–6) secretory glycoprotein with unique structural properties. It directs the formation and secretion of very low density lipoproteins (VLDL) in the liver (7) and mediates the clearance of plasma low density lipoproteins (LDL) (8). A unique mRNA editing in the small intestine generates a truncated form of apoB corresponding to its N-terminal 48% (apoB-48) (9), which directs the formation of chylomicrons (10). A number of other truncated forms of apoB corresponding to the N-terminal 28% to 90%, are found in plasma of human subjects with hypobetalipoproteinemia, a condition that is characterized by low levels of plasma apoB-containing lipoproteins (11, 12). These truncations result from mutations in the apoB gene. Analysis of truncated apoB-containing lipoproteins isolated from patients' plasma showed that forms of apoB containing as little as 37% of the N-terminal region (apoB-37) appear to have the necessary structural information to direct the formation of triacylglycerol (TAG)-rich lipoproteins such as VLDL (11). However, forms corresponding to 31% of the N-terminal region of apoB (apoB-31), or shorter, lose their ability to recruit enough TAG to form VLDL, and unlike the longer forms, are secreted in both lipid-associated (on high density lipoprotein-like particles) and lipid-poor states (11). These findings therefore suggest that amino acid sequences beyond apoB-31 may be more lipophilic, and thereby capable of recruiting TAG. Notably, several lines of evidence are consistent with the notion that the structure of the N-terminal region of apoB is fundamentally different from the rest of the molecule.

Abbreviations: DM, dimyristoyl; DP, dipalmitoyl; EY, egg yolk; HDL, high density lipoproteins; LDL, low density lipoproteins; MLV, multilamellar vesicles; PC, phosphatidylcholine; TAG, triacylglycerol; VLDL, very low density lipoproteins.

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First, the N-terminal 17% is thought to be globular (13, 14), and it contains a cluster of six disulfides (of eight identified in apoB-100) (15). Second, this region is composed of similar amounts of amphipathic α-helices and β-strands (13, 14, 16, 17). On the basis of the crystal structure of lipovitellin (18) Mann et al. (14) suggested that 13 strands in amino acids 21–263 form a β-barrel surrounding a central helix, and that amino acids 440–592 form 17 α-helices. Segrest, Jones, and Dashti (19) suggested that β-strands in amino acids 615–676 are highly homologous to the first of the three domains enriched in amphipathic β-clusters lining the "lipid pocket" of lipovitellin (14). In contrast, the region between apoB-21 and apoB-41 appears to be highly enriched in amphipathic β-strands (13, 16), which, if arranged as 11-mers, could form antiparallel amphipathic β-sheets in which the hydrophobic face could bind TAG (16). Third, the N-terminal 22% of apoB bound to LDL is largely trypsin releasable, while regions beyond apoB-22 are composed of both trypsin-releasable and nonreleasable domains (20), indicating that the N-terminal region of apoB is only weakly associated with the LDL particle surface and/or core (20). Fourth, analysis of intact LDL by cryoelectron microscopy (21) suggests that the N-terminal segment of apoB-100 protrudes out of the particle (22), supporting the idea that this region is globular and perhaps not in intimate contact with the surface of LDL particles.

Nevertheless, the N-terminal region of apoB plays a key role in the intracellular folding of apoB into its secretion-competent form. First, proper pairing of cysteines to form S-S bonds within the N-terminal 11% is critical for the folding and secretion of apoB (23–27). Second, internal domains enriched in β-sheets, which are more lipophilic and therefore thought to be essential for recruiting the bulk of TAG, are unable to be secreted when expressed without the N-terminal region of apoB (28). Third, microsomal triglyceride transfer protein (MTP), an essential player in the process of lipoprotein assembly (29, 30) is dependent on the N-terminal 17% of apoB for its activity (28). This is attributed to the fact that the binding site for MTP has been localized to the N-terminal region of apoB. In fact, two binding sites have been identified. One was localized by Hussain et al. (31) to the region encompassing residues 430–570, and by Bradbury et al. (32) to the region located between residues 512 and 721. The second binding site was located by Mann et al. (14) to amino acid residues 2–154. The binding of MTP to apoB (33–35) presumably allows MTP to mediate the recruitment of TAG and phospholipids to initiate the formation of TAG-rich lipoproteins. Thus, given the critical role of the N-terminal domain of apoB in the initiation of lipoprotein assembly, it is conceivable that this region is in direct contact with the surface (and perhaps even the core) of nascent VLDL and chylomicrons, thereby stabilizing their structure. As VLDL is catabolized to LDL, the N terminus presumably rearranges itself so that it is no longer in intimate contact with the lipoprotein surface (22). Several studies are consistent with this idea, as they suggest that the organization of apoB on VLDL is different from that on LDL (36–38). To test the hypothesis that apoB-17 binds to nascent VLDL, we determined whether it can bind with high affinity to TAG-rich emulsions modeling the lipid composition of nascent VLDL, and if so, whether it behaves like full-length apoB and therefore cannot be exchanged off of the emulsion surface. To that end, we utilized the well-characterized emulsion system with defined composition (39–41) developed by Miller and Small (39) to serve as a model for nascent TAG-rich lipoproteins. The emulsions are composed of triolein (TO), phosphatidylcholines (PC), and low levels of free cholesterol simulating nascent lipoproteins (42). These emulsions have been used to study the binding of the exchangeable apolipoproteins apoA-I and apoE-3 (43–45), thereby allowing for a direct comparison with apoB-17. The findings presented in this article show that apoB-17 exhibits high affinity and irreversible binding to these emulsions, consistent with a possible role for apoB-17 in the stabilization of nascent TAG-rich lipoproteins such as VLDL and chylomicrons.

EXPERIMENTAL PROCEDURES

Materials

Triolein, cholesterol, egg yolk phosphatidylcholine (EYPC), dimyristoylphosphatidylcholine (DMPC), and dipalmitoylphosphatidylcholine (DPPC) were purchased from Nu Check Prep (Elysian, MN). Nitrocellulose membranes were from Schleicher & Schuell (Keene, NH). Goat polyclonal antibodies to apoB were purchased from Biodesign International (Kennebunk, ME). ApoE-3 was kindly provided by Dr. K. Weisgraber (Gladstone Foundation, University of California, San Francisco). [^{35}S]methionine (specific activity > 1,000 Ci/mmol) and phosphatidylcholine (L-α-dipalmitoyl, [2-palmitoyl-9,10-^{3}H(N)]) were purchased from DuPont-NEN (Boston, MA); [9,10-^{3}H]trioleoylglycerol, [4-^{14}C]-cholesterol, and phosphatidylcholine (L-α-1-palmitoyl-2-oleoyl-[oleoyl-1-^{14}C]) were obtained from Amersham (Arlington Heights, IL). All other reagents were of the highest purity available.

Methods

Preparation of TAG-rich emulsions. Emulsions composed of TO cholesterol and EYPC, DMPC, or DPPC were prepared as described (43). Briefly, mixtures containing lipids dried from chloroform were suspended in phosphate-buffered saline (PBS), pH 7.4, and then sonicated at temperatures above the transition of the PC. Sonicated mixtures were then overlayered with water and centrifuged in an SW41 rotor (Beckman, Fullerton, CA) at room temperature for 11 min at 24,000 rpm. Isolated emulsions floating to the top of the tubes were recovered by tube slicing.

The composition of the isolated emulsions was determined by two alternative methods: *a*) liquid scintillation spectrometry of the lipids obtained after their separation by thin-layer chromatography (43), and *b*) colorimetric assays of TAG (46), cholesterol (47), and PC, determined as inorganic phosphate (48). The size of the isolated emulsions was estimated both according to the method described by Miller and Small (42) and by negative-stain electron microscopy after osmium fixation (41, 43).

Preparation of protein. Near-confluent mammary-derived C127 cells expressing apoB-17 were incubated in serum-free medium for 40 h (17). Twenty-five to thirty 100-mm-diameter dishes were pooled, and concentrated 20- to 40-fold, using a stirred cell (Amicon, Danvers, MA) equipped with a membrane of 30-kDa molecular weight cutoff. The unlabeled concentrated media were

mixed with conditioned media containing ^{35}S-labeled proteins obtained after an 18-h labeling period of a single 60-mm-diameter dish with [^{35}S]methionine. The specific radioactivity of apoB-17 bound to emulsions was determined as described below.

Alternatively, C127 cells expressing apoB-17 were grown in a continuous culture, in a bioreactor (from Kinetek System). In this system, cells are attached onto hollow fibers that provide a large surface area. Conditioned media collected from these cells were concentrated 50- to 100-fold as described above. Concentrated media were then mixed with media harvested from cells grown in a 60-mm-diameter dish and labeled for 18 h with [^{35}S]methionine.

The combined media were then purified by immunoaffinity chromatography as described (49), except that apoB-17 bound to the immunoadsorbent was washed in 1% Triton X-100 to remove all the lipids associated with apoB-17 prior to its elution with 0.1 M glycine, pH 2.5. Purified apoB-17 was analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (50) and Western blotting (17).

Characterization of purified apoB-17 by circular dichroism. Purified apoB-17 was dialyzed into 5 mM phosphate buffer, pH 7.4. Far-UV circular dichroic (CD) spectra were recorded on an Aviv 62DS CD spectropolarimeter (Aviv Associates, Lakewood, NJ) at 25°C from 250 to 200 nm as described (17).

Binding protocols. For apoB-17 in conditioned media, fixed amounts of emulsion-PC were mixed with increasing amounts of conditioned medium in a 1-ml final volume. Incubation was at 25°C for 30 min. The density of the mixtures was adjusted to 1.01 g/ml by the addition of sucrose and samples were then spun in an SW55 rotor for 1 h at 40,000 rpm at 25°C. The emulsions floating to the top of the tubes were recovered by tube slicing. The reisolated emulsions were then solubilized in sample buffer (50) and resolved by 8% SDS-PAGE followed by Coomassie blue staining, and autoradiography. Proteins from a duplicate gel were electrotransferred onto a nitrocellulose membrane, and then probed with polyclonal antibodies to apoB (17). The mass of apoB-17 bound to emulsions was determined as follows: The Coomassie blue dye associated with the apoB-17 band was extracted with 25% pyridine, and the absorbance was measured at 605 nm (51). The mass of apoB-17 was calculated from a reference curve constructed by plotting the absorbance of the dye associated with increasing amounts of albumin separated on the same gel. The radioactivity associated with apoB-17 band was determined by liquid scintillation spectrometry as described (52).

For immunoaffinity-purified apoB-17, binding was carried out essentially as described (43). Briefly, fixed amount of emulsion-PC were added into tubes containing increasing amounts of ^{35}S-labeled apoB-17 and the volume was adjusted to 175 μl. Incubation was carried out at 25°C for 30 min. Separation of emulsion-bound proteins was accomplished by centrifugation in a Beckman Airfuge for 10 min at 20 psi. Aliquots of 100 μl were removed from the bottom of the tube, using an airtight Hamilton syringe, and transferred into a counting vial. The remaining contents were transferred into a separate vial and the radioactivity was determined by liquid scintillation spectrometry.

Calculation of binding parameters. Calculations were carried out essentially as described (43). The data were plotted according to equation 1 (53):

$$K_d = (N[PC] - P_b)P_f / P_b \qquad Eq.\ 1)$$

K_d is the dissociation constant, and N represents the number of binding sites in relation to phospholipids. PC, P_b, and P_f represent the respective concentration of PC and of bound and free protein.

Incubation of emulsion-bound apoB-17 or emulsion-bound apoE-3 with DMPC multilamellar vesicles (MLV) to estimate exchange between emulsions and DMPC MLV. First, ^{35}S-labeled apoB-17 or purified unlabeled apoE-3 was incubated with emulsions for 30 min. The proteins bound to the emulsions were then isolated by tube slicing after centrifugation in an SW55 rotor as described before. The isolated emulsions were then incubated for 18 h at 26°C with buffer (PBS) alone or with buffer containing DMPC MLV prepared as described (17). The ratio of DMPC to emulsion-PC did not exceed 1:4, respectively. After incubation, aliquots were spun in an Airfuge for 10 min at 20 psi. Aliquots of 100 μl, recovered from the bottom, were analyzed for their protein content either by colorimetry (for apoE-3) (54) or by liquid scintillation spectrometry (for apoB-17).

Aliquots recovered from the bottom were also analyzed by negative-stain electron microscopy (EM) to determine whether small discoidal or round particles were formed after the incubation with DMPC MLV.

RESULTS

Characterization of triolein-rich emulsions

To study the binding of apoB-17 to TAG-rich nascent lipoproteins, we prepared emulsions composed of TO, free cholesterol, and different PC (EYPC, DMPC, or DPPC). The composition and size of the isolated emulsions are shown in **Table 1**. These emulsions contain TO as their major component (accounting for more than 80% of the total mass). The size of the isolated emulsions calculated from their composition was in good agreement with the size measured by negative-stain EM, and ranged between 830 and 1,260 Å (mean diameter) (Table 1). The size distribution was broad, as reflected by the large standard deviations, but similar in size distribution to large nascent VLDL. The binding of apoB-17 to the emulsions did not change their morphology.

ApoB-17 in conditioned media binds to model TAG-rich lipoproteins

To determine whether apoB-17 secreted into conditioned media binds to TAG-rich emulsions, fixed amounts of emulsion-PC were incubated with increasing amounts of concentrated media for 30 min at 25°C. The mixtures were then centrifuged to separate the emulsion-bound proteins

TABLE 1. Average lipid composition and size of isolated emulsions

	Lipid Composition			Particle Diameter	
				Method	
Emulsion	TO	PC	Cholesterol	Lipid Composition[a]	EM[b]
	Weight %			*Å*	
EYPC	89.1	9.6	1.3	1,200	1,260 ± 500
DMPC	88.2	10.7	1.1	1,100	1,070 ± 450
DPPC	84.8	13.8	1.4	840	830 ± 300

[a] Particle diameters were calculated from the composition data, using the program developed by Miller and Small (42) and assuming spherical emulsion structure. The appropriate partition of TO and cholesterol into the core and surface was taken into account.

[b] Means ± SD of randomly chosen negatively stained particles.

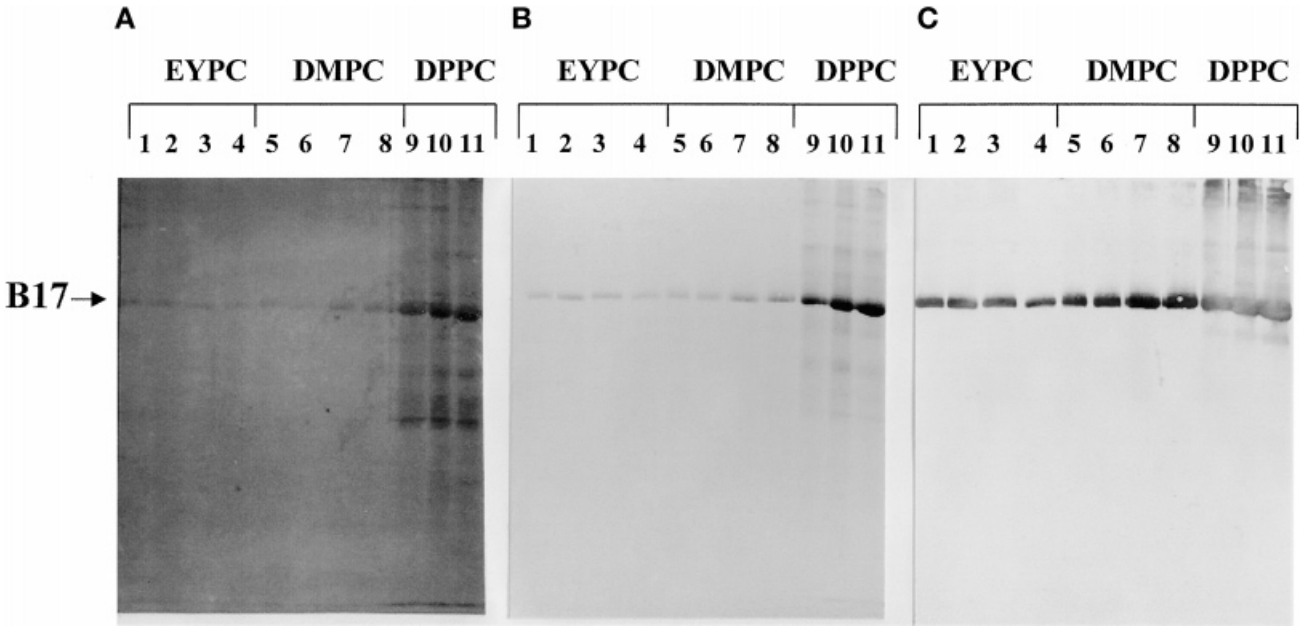

Fig. 1. ApoB-17 in conditioned medium binds to EYPC-, DMPC-, and DPPC-containing emulsions. Cells were incubated in serum-free medium, and conditioned medium was collected and concentrated as described in Methods. Increasing amounts of concentrated medium were incubated for 30 min at 25°C with a fixed amount of emulsion-PC. Emulsion-bound proteins were isolated by ultracentrifugation and then analyzed by SDS-PAGE followed by Coomassie blue staining (A), autoradiography (B), and Western blotting (C) using polyclonal antibodies to human apoB. The lanes indicate micrograms of added apoB-17 per 500 μg of emulsion-PC: lanes 1, 5, and 9, 6 μg; lanes 2, 6, and 10, 12 μg; lanes 3 and 7, 24 μg; and lanes 4, 8, and 11, 34 μg of apoB-17.

from the unbound proteins. The proteins bound to the emulsions were analyzed by SDS-PAGE followed either by staining, autoradiography, or Western blotting. **Figure 1** shows that apoB-17 was bound to all three emulsions, as determined by Coomassie blue staining (Fig. 1A), autoradiography (Fig. 1B), and immunoblotting (Fig. 1C). The amount of apoB-17 that bound to the emulsions depended on the surface PC. Thus, the emulsions with the mostly solid surface (DPPC) bound the highest amount of apoB-17, while that with the fluid surface (EYPC) bound the least. Furthermore, of all the proteins present in the media, apoB-17 was the only protein that bound to EYPC- and DMPC-containing emulsions (Fig. 1A and B, lanes 1–4 and 5–8, respectively). Although the DPPC-containing emulsion bound minor amounts of other proteins in addition to apoB-17, apoB-17 was by far the most abundant protein (Fig. 1A and B, lanes 9–11). As shown on the blot, a small fraction of apoB-17 on the surface of the DPPC-containing emulsions seemed to be aggregated (Fig. 1C, lanes 9–11). The aggregation is presumed to be due to the relatively high concentration of apoB-17 on the emulsion surface.

Characterization of immunoaffinity-purified apoB-17

Immunoaffinity chromatography of conditioned media yielded a single protein band detected by Coomassie blue staining of SDS-polyacrylamide gels (**Fig. 2**, inset). The protein was identified to be apoB-17 by Western blotting (data not shown). Purified apoB-17 was analyzed by far-UV CD to determine its secondary structure. Analysis of the spectra depicted in Fig. 2 showed that purified lipid-free apoB-17 contained 30% α-helix, 27% β-sheet, 18% β-turn, and 25% random coil.

Purified apoB-17 binds with high affinity to EYPC- and DPPC-containing emulsions

The parameters of binding of apoB-17 to EYPC- and DPPC-containing emulsions were derived from experiments in which increasing amounts of immunoaffinity-purified apoB-17 were incubated with fixed amounts of emulsion-PC.

Figure 3 shows the profile of binding of apoB-17 to EYPC- and DPPC-containing emulsions. The binding of apoB-17 to both emulsions was saturable. However, the binding to EYPC-containing emulsions reaches saturation at concentrations that are about 10 times lower than those required to saturate the DPPC-containing emulsions. This is consistent with the ability of DPPC-containing emulsions to accommodate much greater amounts of apoB-17 when incubated with apoB-17 in conditioned media (Fig. 1). The binding parameters were derived from the regression analysis (see Fig. 3, inset) of the data depicted in Fig. 3 (43). As shown in **Table 2**, the binding capacity, N, is 1.85 ± 0.74 g/100 g EYPC and 23.47 ± 4.50 g/100 g DPPC. The number of protein molecules per average emulsion particle was 10 and 58 for EYPC- and DPPC-containing emulsions, respectively. These differences are significant at $P = 0.0002$. The respective affinities of apoB-17 for both emulsions, defined by the K_d values, was derived from the intercept of the linear regression. The K_d derived for binding of apoB-17 to EYPC-containing emulsions was 32 ± 23 nM, while that for DPPC-containing

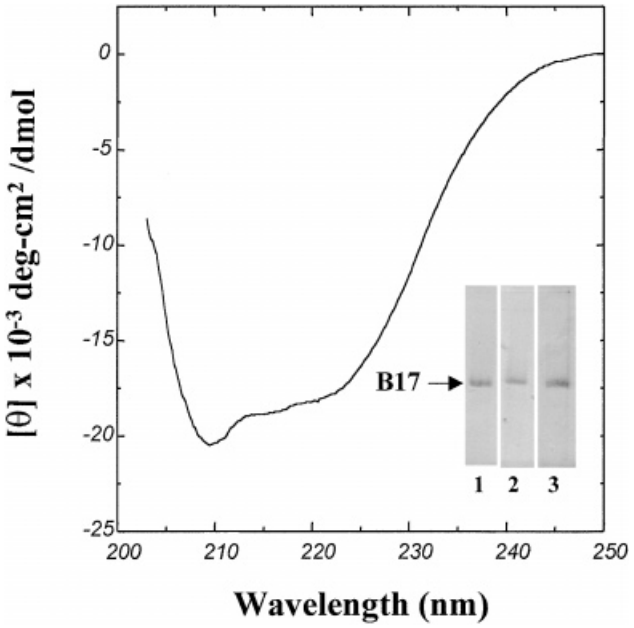

Fig. 2. Far-UV CD spectrum of purified lipid-free apoB-17. ApoB-17 in conditioned media was immunoaffinity purified and then dialyzed into 5 mM phosphate buffer prior to recording the spectrum. Purified apoB-17 was also analyzed by SDS-PAGE followed by Coomassie blue staining. Three different preparations are depicted (inset).

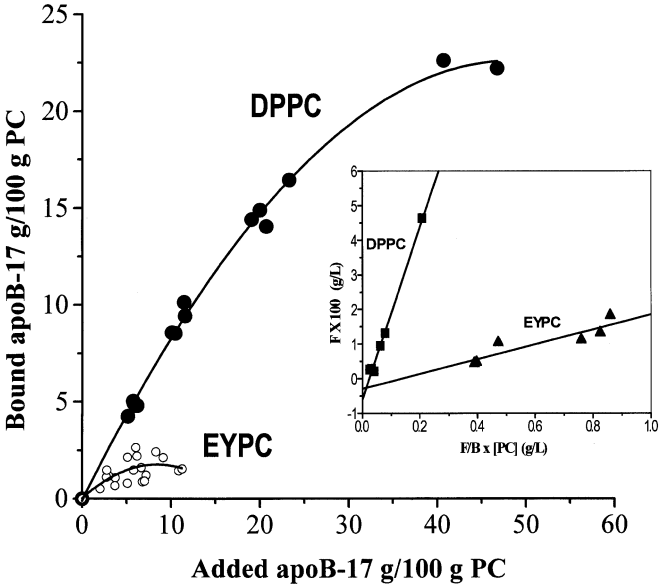

Fig. 3. ApoB-17 binds to EYPC- and DPPC-containing emulsions in a saturable manner. Increasing amounts of immunoaffinity-purified apoB-17 were incubated with a fixed amount of emulsion-PC for 30 min at 25°C. The amount of apoB-17 bound to the emulsions was determined as described in Methods and plotted against the added amount of apoB-17 relative to the amount of PC. The closed symbols represent DPPC emulsions; the open symbols represent EYPC emulsions. The inset is a linearized plot of the binding data. Filled squares represent DPPC, and filled triangles represent EYPC emulsions. The curves shown in inset depict one representative experiment for each emulsion.

emulsions was 85 ± 27 nM. These differences are significant at $P = 0.03$. On the basis of these parameters it is clear that apoB-17 exhibited high affinity binding for both emulsions regardless of the physical state of the PC acyl chains. ApoB-17 binds much more strongly compared with apoA-I and apoE-3, whose K_d values are in the micromolar range (43).

Can apoB-17 bound to model emulsions be exchanged off of the surface?

The binding experiments shown above clearly demonstrated high affinity binding of apoB-17 to TO-rich emulsions containing different surface PCs. It was therefore important to determine whether apoB-17 that bound to these emulsions can be exchanged off of the surface. To address this question we took advantage of the property of apoB-17 to bind to DMPC MLV to form discoidal particles (17). Thus, it was expected that apoB-17 that dissociated from the emulsion's surface would bind to DMPC vesicles, and form discoidal particles. These studies also included apoE-3 for comparison because like apoB-17, it binds to triolein-rich emulsions containing EYPC (43) and it readily associates with DMPC MLV to form discoidal particles (55). In this series of experiments EYPC- or DPPC-containing emulsions were first incubated with purified apoB-17 or apoE-3 for 30 min, and then reisolated by ultracentrifugation. The isolated emulsions were recovered by tube slicing and then incubated for 18 h at 26°C in buffer, or in buffer containing DMPC MLV. **Figure 4** shows that after incubation in buffer for a period of 18 h at 26°C, a small fraction, about 25% and 9% of the emulsion-bound apoB-17 dissociated from

TABLE 2. Parameters for the binding of purified apoB-17 to EYPC- and DPPC-containing emulsions

		N		
Emulsion	K_d	Protein/PC	Protein/PC	Protein Molecules/Particle[a]
	nM	*mol/mol* ($\times 10^{-3}$)	*g/g* ($\times 10^{-2}$)	
EYPC (n = 4)	32 ± 23	0.16 ± 0.06	1.85 ± 0.74	10
DPPC (n = 3)	85 ± 27	1.93 ± 0.37	23.47 ± 4.50	58

Binding parameters were derived from the linearized plots of the data presented in Fig. 3 according to Equation 1 in text. The values represent means ± SD. K_d, the dissociation constant, was derived from the intercept; and N, the upper limit of binding, was derived from the slope of the linear regression as described (43).

[a] An average particle size as described in Table 1.

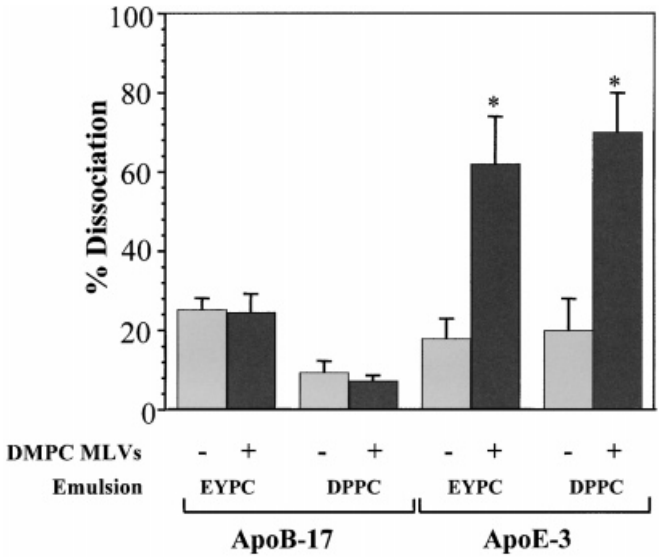

Fig. 4. ApoB-17 bound to emulsions, unlike apoE-3, cannot be exchanged off of the emulsions by DMPC MLV. ApoB-17 and apoE-3 were incubated with EYPC- and DPPC-containing emulsions for 30 min. The emulsion-bound apoB-17 and apoE-3 were isolated by centrifugation as described in Methods, and then incubated for 18 h in PBS (gray columns), or PBS containing DMPC MLV (black columns). The emulsions were separated by centrifugation and the amount of protein in the infranatants was quantified and expressed as a percentage of total emulsion-bound protein. The means of three experiments ±SD are depicted. * $P < 0.04$.

EYPC- and DPPC-containing emulsions, respectively. Similarly, about 18% and 20% of emulsion-bound apoE-3 dissociated from EYPC- and DPPC-containing emulsions, respectively. However, there was no increase in the fraction of apoB-17 that dissociated from either emulsion in the presence of DMPC MLV (Fig. 4). In contrast, there was a significant increase in the fraction of apoE-3 that dissociated from either EYPC- or DPPC-containing emulsions ($P < 0.04$), when DMPC MLV were present. Furthermore, the fraction of apoE-3 that dissociated from the emulsions bound to DMPC to form lipid-rich complexes of round and discoidal morphology as determined by negative-stain EM (data not shown). These findings demonstrate a clear difference between apoB-17 and apoE-3 and are consistent with the higher affinity of apoB-17 for EYPC emulsions (K_d = 32 nM) compared with that of apoE-3 (K_d = 1,170 nM) (43).

DISCUSSION

The present study demonstrates that apoB-17 readily binds to emulsions modeling nascent TAG-rich lipoproteins. Both nonpurified and immunoaffinity-purified apoB-17 bound to emulsions containing either a fluid or a solid surface (e.g., EYPC- or DPPC-containing emulsions, respectively). The binding of apoB-17 to these emulsions occurs rapidly, almost instantly, as determined in an experiment in which purified apoB-17 was added to the emulsions just before centrifuging the mixtures to separate the emulsion-bound from the unbound apoB-17. The amount of apoB-17 that bound to the emulsions was similar to that obtained after a 30-min incubation with the emulsions (data not shown). The affinity of apoB-17 for both EYPC- and DPPC-containing emulsions is high, as the K_d is in the nanomolar range (Table 2). However, the affinity of apoB-17 for EYPC-containing emulsions is significantly higher than that for DPPC-containing emulsions (K_d = 32 and 85 nM, respectively) (P = 0.03). Furthermore, the affinity of apoB-17 for EYPC emulsions is 25- and 35-fold higher, respectively, than that determined for either apoE-3 (K_d = 1,170 nM) or apoA-I (K_d = 740 nM) (43). The high affinity of apoB-17 for both EYPC- and DPPC-containing emulsions presumably renders it nonexchangeable, as demonstrated in Fig. 4. Thus, incubation of apoB-17 bound to either EYPC- or DPPC-containing emulsions overnight in buffer resulted in the dissociation of a small fraction of apoB-17. However, the presence of DMPC MLV did not promote increased dissociation of apoB-17 over control (buffer alone). The fraction that dissociated could represent a subpopulation of molecules that are only loosely bound to the emulsions. Nonetheless, the major fraction of apoB (e.g., >75%) remained bound to both emulsions. In contrast, the dissociation of emulsion-bound apoE-3 was greatly enhanced on incubation with DMPC MLV (Fig. 4). The fraction that dissociated consequently solubilized the large MLV into small round and discoidal particles (not shown). Similarly, Tall and Small (56) reported that on incubation of HDL with DMPC MLV, the majority of apoA-I molecules dissociated from HDL and solubilized the large MLV into discs. These findings are consistent with the exchangeable nature of apoA-I and apoE-3 and with other in vitro studies demonstrating that apoE-3 was displaced from VLDL by synthetic amphipathic helical peptides (57), while apoA-I was displaced from monolayers by apoA-II (58). Thus, the loosely folded structure of apoA-I and apoE-3 (59) is consistent with lower affinity for lipoprotein surfaces, which in turn allows them to transfer between lipoproteins.

In contrast to the high affinity of apoB-17 for EYPC emulsions, the maximum capacity (N) of apoB-17 bound to EYPC emulsions is much lower than that of apoA-I and apoE-3, as only 1.9% of apoB-17 relative to phospholipids bound to EYPC emulsions compared with 15% and 17% of apoA-I and apoE-3, respectively (43). Thus, only 10 molecules of apoB-17 were accommodated on the surface of EYPC emulsions compared with more than 200 molecules of either apoA-I or apoE-3 (43). These differences cannot be accounted for by the difference in the size of apoB-17 compared with these apolipoproteins. Instead, they reflect substantial differences in the three-dimensional structure and their mode of interaction with the emulsions. For example, on the basis of its thermodynamic properties, lipid-free apoA-I was proposed to be in a molten globular-like state under near-physiological conditions (59). This model was proposed for the other exchangeable apolipoproteins as well (59–61). Such a structure presumably confers the exchangeable apolipoproteins, flexibility to accommodate different conformations on binding to the surface of different lipoprotein particles.

Their interaction with lipoprotein surfaces was proposed to occur by insertion of amphipathic α-helices between the acyl chains of the PC (62, 63). If, however, only a few helices are engaged in binding to the emulsion surface, then some regions of the molecules may protrude out, consequently allowing more molecules to be accommodated on the emulsion surface. ApoB-17, on the other hand, has similar amounts of amphipathic α-helices and β-sheets (14, 17), which increased from 30% to 39%, and from 27% to 37%, respectively, on its binding to DMPC MLV to form lipid-rich discoidal particles [Fig. 2 and ref. (17)]. It is conceivable that both α- and β-structures are involved in binding to the emulsion surface (13, 16, 17). A possible model of the structure of apoB-17 bound to the emulsion surface is shown in **Fig. 5**. It is based on the crystal structure of lipovitellin (18) and its homology to the N-terminal 20% of apoB (14). This model shows that the β-barrel region encompassing amino acids 21–263 (14) protrudes out of the surface, and therefore is minimally engaged in binding. However, part of the α-helical domain (residues 294–592), and the β-sheet domain (residues 615–676) which is homologous to the A sheet of lipovitellin, are intimately associated with the emulsion core. The helical domain could also interact with the acyl chains of the PC, while the β-sheets have been proposed to interact directly with TAG (16). Because apoB-17 has only one of the three sheets proposed to line the lipid-binding pocket in lipovitellin (18), it could be more flexible to accommodate itself onto the TAG surface of the emulsions. The possibility of direct interaction between regions in apoB-17 and the core is consistent with its high affinity binding and with its nonexchangeability (Fig. 4). Finally, it is consistent with the study reported by Weinberg et al. (64), in which the interfacial properties of apoB-17 and apoA-I were measured by pulsating air bubble and oil drop tensiometry. They found that although both bound to the oil, apoB-17 increased elasticity to 19 mN/m while apoA-I increased it to less than half, 7.6 mN/m (64). Thus, apoB-17 has a higher capacity to stabilize the structure of the oily core of lipoproteins.

Although only 1.9% of apoB-17 relative to phospholipids bound to EYPC emulsions, about 10 times more protein, 23.5%, bound to DPPC emulsions (Table 2). This striking increase can be explained by the potential difference in available surface area for protein binding on the two different emulsions. The DPPC emulsions were sonicated slightly above 41°C, the transition temperature of DPPC. Thus, when the emulsions were formed, the chains were in a liquid-like expanded state and DPPC covered the surface of the TO. The area of DPPC at the surface has been calculated to be about 54 Å^2 per molecule (45). On cooling from 41°C to 25°C there is a slight reduction in the volume of the triglyceride (45) but not enough to make a significant difference in the surface area covered by the DPPC. Thus at 25°C the DPPC still covers about 54 Å^2 per molecule (45). We have shown that the DPPC is partly crystalline at 25°C and most probably lays at an angle (estimated to be about 44°) normal to the tangent of the surface, which allows it to cover 54 Å^2 (45). If DPPC stood straight up, that is, was normal to the tangent of the surface, it would cover only an area of 40–42 Å^2 per molecule. Thus, by standing up the DPPC could uncover 12–14 Å^2 per DPPC molecule of potential hydrophobic core surface or about 22% to 25% of the total core surface. We suggest that the large amount of apoB-17, which occupies approximately 23% of the surface, probably changes the angle of tilt so that the DPPC molecules are packed nearly normal to the tangent of the sphere, thus creating a large amount of TAG surface to which the B-17 amphipathic helices and β-sheets can bind. On the other hand, the EYPC surface is a highly compressed two-dimensional liquid. Because it does not crystallize and contract on cooling, little space is available for apoB-17 to insert and, therefore, only a few molecules bind to these emulsions.

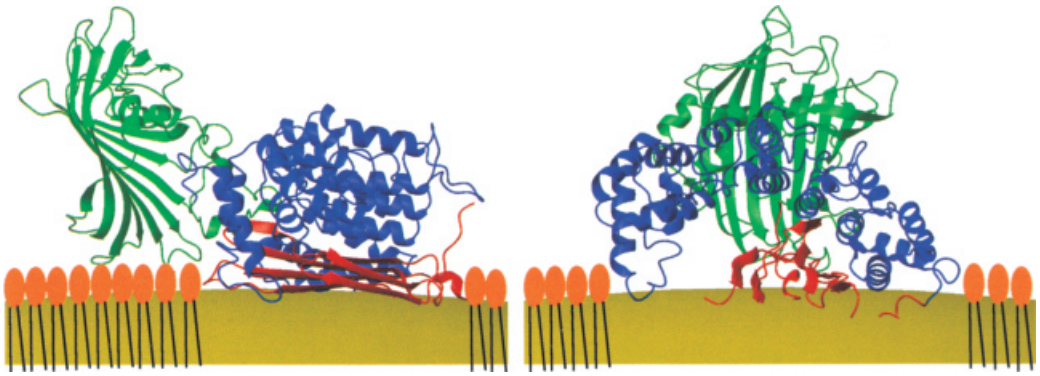

Fig. 5. A model of the three-dimensional structure of apoB-17 bound to emulsions. The three-dimensional structure of apoB-17 bound to emulsions was created on the basis of the crystal structure of lipovitellin (18) and its homology to apoB (14). To construct the apoB-17 model, the sequences of apoB-17 and lipovitellin were first aligned with BLAST (67). Then, the alignment and the crystal structure of lipovitellin were used as input for MODELLER (68) to calculate the apoB-17 model. This figure was created using MOLMOL (69). The emulsion surface is shown as a yellow sphere with a 1,000-Å diameter. Phospholipids are shown in orange. The β-barrel region of apoB-17 (shown in green) appears to protrude out of the surface. Thus, the regions beyond the β-barrel are involved in the interaction of apoB-17 with the emulsion surface. These include part of the α-helical domain (shown in blue) and β-sheet domain (shown in red), which is homologous to the A sheet of lipovitellin proposed to line the lipid-binding pocket (14). The image on the right is rotated 90 degrees from the left.

In summary, the findings presented in this article provide evidence that in addition to its essential role in the initiation of lipoprotein assembly, apoB-17 could play an important role in the stabilization of nascent TAG-rich lipoprotein such as VLDL and chylomicrons. This should be viewed in light of the events leading to the formation of TAG-rich lipoproteins. During translation, as the N terminus of nascent apoB emerges through the translocon into the lumenal face of the endoplasmic reticulum membrane it appears to bind phospholipids (65) and some TAG, which are shuttled from the β-barrel region of MTP to the homologous β-barrel of apoB to form a "proteolipid" intermediate (19). This step may be essential for subsequent MTP-mediated recruitment of primarily phospholipids, diacylglycerol (66), and some TAG by α-helices and β-sheets clustered within the N-terminal 29%, which is necessary for recruitment of the bulk TAG by β-sheets between apoB-29 and apoB-41 (66) to form the "primordial" particles. Subsequent acquisition of TAG and phospholipids by these precursor particles leads to the formation of nascent VLDL. Such a scenario supports the possibility that the N terminus of apoB is tightly associated with the surface of nascent VLDL and contributes to its stabilization.

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