Self-association and Lipid Binding Properties of the Lipoprotein Initiating Domain of Apolipoprotein B*

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The amino-terminal 20.1% of apolipoprotein B (apoB20.1; residues 1–912) is sufficient to initiate and direct the formation of nascent apoB-containing lipoprotein particles. To investigate the mechanism of initial lipid acquisition by apoB, we examined the lipid binding and interfacial properties of a carboxyl-terminal His6-tagged form of apoB20.1 (apoB20.1H). ApoB20.1H was expressed in Sf9 cells and purified by nickel affinity chromatography. ApoB20.1H was produced in a folded state as characterized by formation of intramolecular disulfide bonds and resistance to chemical reduction. Dynamic light scattering in physiological buffer indicated that purified apoB20.1H formed multimers, which were readily dissociable upon the addition of nonionic detergent (0.1% Triton X-100). ApoB20.1H was incapable of binding dimyristoylphosphatidylcholine multilamellar vesicles, unless its multimeric structure was first disrupted by guanidine hydrochloride. However, apoB20.1H multimers spontaneously dissociated and bound to the interface of naked and phospholipid-coated triolein droplets. These data reveal that the initiating domain of apoB contains solvent-accessible hydrophobic sequences, which, in the absence of a hydrophobic lipid interface or detergent, engage in self-association. The high affinity of apoB20.1H for neutral lipid is consistent with the membrane binding and desorption model of apoB-containing lipoprotein assembly.

apoB is the major structural protein of hepatic very low density lipoproteins and intestinal chylomicrons (1, 2). The assembly of apoB-containing lipoproteins proceeds in two steps: cotranslational incorporation of the nascent apoB polypeptide into a precursor lipoprotein particle in the rough ER followed by the additional posttranslational lipidation via fusion with lipid droplets in the ER and/or the Golgi (3–6). Both steps of apoB-containing lipoprotein assembly require MTP, a dedicated cofactor composed of a unique 97-kDa subunit complexed with the ubiquitous ER-localized folding enzyme, protein-disulfide isomerase (7, 8). During the first step of lipoprotein assembly, MTP may transfer lipid molecules from the ER membrane or other donor sites directly to apoB and/or may bind to apoB and promote its proper folding and autonomous lipid acquisition (7, 9). MTP also participates in bulk transmembrane lipid transport from the cytosol into the ER-Golgi, a process critical for second step apoB particle expansion (10–12).

Several models of the first initiating step of lipoprotein assembly have been proposed. The earliest and most commonly accepted theory posits that the apoB polypeptide interacts with the inner leaflet of the ER membrane during translation, causing the nucleation and desorption of an ~20-nm diameter emulsion particle upon the completion of translation (13–17). A variation of this model proposes that the interfacial binding and lipoprotein desorption process is completed upon translocation of only the first ~1000 amino-terminal residues of apoB, giving rise to a small, high density lipoprotein-like emulsion particle precursor (1, 18). An alternative hypothesis of lipoprotein initiation is based on the modeling of the amino-terminal ~1,000 amino acids of apoB as a closed three-sided, funnel-shaped lipid-binding cavity, similar to that in the apoB paralog, lipovitellin (LV), the processed form of vitellogenin (19–22). Transfer of predominantly phospholipid molecules into this cavity is predicted to form a discoidal proteolipid intermediate, which is subsequently converted to a TG-rich lipoprotein upon further translation and MTP-mediated lipid transfer (21, 23).

New insights into the mechanism of initial lipid acquisition by apoB come from the observation that the amino-terminal 20.1% of apoB (apoB20.1; residues 1–912) is the minimum sequence necessary and sufficient to initiate the first step of TG-rich lipoprotein particle assembly (18). The ability to express and purify this functional domain has allowed us to examine its biochemical and interfacial properties as a means to understand how it functions in vivo. Our data suggest that apoB20.1H contains solvent accessible hydrophobic surfaces that have a particularly high affinity for neutral lipid interfaces. These properties argue that the initiating domain of apoB may catalyze a lipid desorption reaction during the initial formation of nascent apoB-containing lipoproteins in the ER.

MATERIALS AND METHODS

Expression of Recombinant ApoB20.1H—ApoB20.1H (18) was cloned into the pFastBac1 vector, and the resulting plasmid was transposed into DH10Bac competent cells (Invitrogen). The production of apoB20.1H in Sf9 cells was achieved as described previously (24). Viral supernatants containing recombinant apoB20.1H were cleared by centrifugation at ~1,000 × g, filter-sterilized, and stored at 4 °C in the presence of 0.05% sodium azide, 0.1 mM phenylmethylsulfonyl fluoride, and 1 μg/ml pepstatin and leupeptin.

Purification of ApoB20.1H—All steps were performed at 4 °C. One liter of apoB20.1H viral supernatant was placed in a 2-liter Erlenmeyer flask and adjusted to 325 mM NaCl and 0.5% Triton X-100. Twenty ml of nickel-nitritoltriacetic acid-agarose (Qiagen), pre-equilibrated with 10 mM Tris-HCl, 300 mM NaCl, 1 mM MgCl2, 5% glycerol, pH 8.0, was added and incubated for 1.5 h with orbital shaking at 130 rpm. The slurry was poured into a glass column, the resin was washed with five bed volumes of wash buffer 1 (50 mM NaH2PO4, 300 mM NaCl, 15 mM

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§The abbreviations used are: apoB, apolipoprotein B; DMPC, 1,2-dimyristoylphosphatidylcholine; DTT, dithiothreitol; ER, endoplasmic reticulum; GdnHCl, guanidine hydrochloride; LV, lipovitellin; MTP, microsomal triglyceride transfer protein; TBS, Tris-buffered saline; TG, triglyceride; mN, millinewton.
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imidazole, pH 8.0), followed by 15 bed volumes of wash buffer II (50 mM NaH₂PO₄, 300 mM NaCl, pH 8.0). Protein was eluted by running 1 bed volume of elution buffer (50 mM NaH₂PO₄, 300 mM NaCl, 250 mM imidazole, pH 8.0) into the column. The flow was stopped, and the column was incubated for 5 min before restoring the flow. Two successive elutions were performed. Purified protein was concentrated and exchanged into 10 mM Tris, pH 7.4, 140 mM NaCl, 3 mM EDTA, 1 mM benzamidine, 1 mM phenylmethylsulfonyl fluoride, and 1.5 mM sodium azide (TBS) with a Centricon-30 centrifugal concentrator (Millipore) or with a stirred ultrafiltration cell utilizing nitrogen pressure and a YM30 regenerated cellulose membrane (Millipore). Final protein concentration was determined using the bicinchoninic acid method (Pierce).

Molecular Weight Determination by Mass Spectrometry—The mass of purified apoB20.1H was determined using a Bruker AutoFlex MALDI-TOF instrument. Purified protein was diluted to a final concentration of 1 μg/ml in a saturated solution of sinapinic acid (3,5-dimethoxy-4-hydroxycinnamic acid), 50% acetonitrile, and 0.1% trifluoroacetic acid. One-μl samples were spotted on a stainless steel target plate, and spectra were obtained in the linear mode using the high molecular weight program. For the analysis shown, >600 laser shots were summed, and peaks were found using the centroid method. Calibration was performed using a 5-point protein calibration mixture composed of protein A [m + 2H]²⁺, 22,306 Da; trypsinogen, 23,982 Da; bovine serum albumin [m + 2H]²⁺, 33,216 Da; protein A, 44,613 Da; and bovine serum albumin, 66,431 Da.

Assessment of Disulfide Bond Formation—Three μg of purified protein was incubated at various temperatures in the absence or presence of 50 mM DTT in a final volume of 20 μl. The samples were incubated for 10 min and then adjusted to 200 mM iodoacetamide, to alkylate free sulfhydryls and to inactivate the DTT. After further incubation at 37 °C for 20 min, concentrated SDS-PAGE sample buffer (without the reducing agent) was added, and samples were boiled for 5 min and analyzed by 8% SDS-PAGE. To assay for the possible presence of free sulfhydryls, 2 μg of purified protein in 10 μl of SDS (0.5%), with or without 5 mM DTT, was heated at 65 °C for 10 min. Samples then received 8 μl of TBS and either 2 μl of dimethylformamide or 2 μl of 100 mM fluorescein-5-maleimide (Pierce) dissolved in dimethylformamide. After incubation at room temperature for 30 min, samples were precipitated by adjusting to 10% trichloroacetic acid. After washing the pellets with acetone, samples were dissolved in SDS-PAGE sample buffer containing 0.1 M DTT, boiled for 5 min, and fractionated by 8% SDS-PAGE. The gel was viewed and photographed under UV illumination.

Binding of ApoB20.1H to Phospholipid Vesicles—DMPC multilamellar vesicles were prepared as described previously (25). For DMPC clearing assays, purified apoB20.1H was mixed with DMPC vesicles in phosphate-buffered saline at final concentrations of 175 and 500 μg/ml, respectively. Samples were incubated at 24.5 °C with inversion, and the turbidity was monitored at 325 nm at the indicated times. ApoA-I, which was added at the same protein:lipid mass ratio, served as a positive control. In some experiments, the mixtures were incubated for 20 h, adjusted to a density of 1.25 g/ml with KBr, and centrifuged at 100,000 rpm in a Beckman TL.100 Topletop Ultracentrifuge (Beckman Coulter) for 18 h at 15 °C, as described previously (18). The top 1 ml (d < 1.25 g/ml) and bottom 2 ml (d > 1.25 g/ml) fractions were recovered by tube slicing. One-fifth of the top and bottom fractions was precipitated with 10% trichloroacetic acid and analyzed by 8% SDS-PAGE. In other DMPC binding studies, 100 μg of purified apoB20.1H was mixed with 2 mg DMPC in a final concentration of 0, 0.7, or 2.0 mM GdnHCl (final volume of 600 μl). The mixtures were incubated for 20 h at 24 °C with constant dialysis against phosphate buffered saline to remove the

GdnHCl. The samples were adjusted to a density of 1.25 g/ml with KBr and analyzed by density gradient centrifugation as described above.

Light Scattering Analysis of ApoB20.1H—The hydrodynamic radius of apoB20.1H was measured using a Malvern Instrument ZEN 1600 at a wavelength of 633 nm. One hundred μl of purified protein at a concentration of 0.25 mg/ml was centrifuged at 14,000 × g for 10 min to pellet any particulate matter. The supernatant was transferred to a quartz cuvette, and 10–15 readings at 20 °C were averaged per sample. The hydrodynamic radius of the protein was also measured after adjusting the sample to 0.1% Triton X-100.

Interfacial Activity of ApoB20.1H at the Triolein/Water Interface—The interfacial behavior of apoB20.1H at a triolein/water interface was examined using a Tracker® oil-drop tensiometer (IT Concept, Parc de Chancolan, Longessaigne, France) (26, 27). Ten-μl drops of pure triolein were rapidly formed into a cuvette containing apoB20.1H at a concentration of 25 μg/ml in 41.3 mM Tris, pH 7.4, and the binding of the protein to the triolein/water interface was measured as the decrease in interfacial tension with time. In some experiments, before apoB20.1H was injected into the cuvette, a triolein droplet was formed and then coated with a monolayer of phosphatidylyceroline, obtained by centrifugation of Intralipid® (Fresenius Kabi Clayton) in an SW27 rotor (Beckman Coulter) at 27,000 rpm for 35 min at 4 °C followed by collection of the infranatant fractions. Binding rate constants were derived by log transformation of the tension versus time data.

RESULTS

Expression and Purification of ApoB20.1H—A recombinant apoB20.1H-containing bacmid genome was transfected into S9 insect cells to generate infectious viral particles. A series of subsequent infections, using successively harvested viral supernatants, were performed to achieve a maximal level of expression. apoB20.1H secreted from infected cells displayed SDS-PAGE mobility consistent with its predicted molecular mass of 102,475 Da (Fig. 1A, lane 2). To further characterize the status of apoB20.1H expressed in this system, viral supernatants were subjected to density gradient ultracentrifugation. As

![Figure 1](image-url)
baculovirus replicates via a lytic life cycle, considerable cellular lipid is released during the course of viral infection. As observed in Fig. 1B, apoB20.1H secreted into this milieu binds sufficient lipid to float in the $d < 1.25$ g/ml buoyant lipoprotein fraction (Fig. 1B, lane 1). This behavior is consistent with previous analyses of apoB17, which revealed an avid affinity for exogenous lipids (28, 29). These data also revealed that anti-human apoB antibodies specifically recognized the $\sim 100$-kDa band during immunoblot analysis, further confirming its identity. Denaturing analysis of the Coomassie-stained gels, using bovine serum albumin standards, indicated an $\sim 4.0$ mg/liter concentration of apoB20.1H in the crude culture supernatant. A single round of nickel affinity chromatography yielded $\sim 3$ mg of purified protein (Fig. 1C). Upon concentration and buffer exchange into TBS, the purified protein remained soluble and stable for at least 28 days when stored at $4 \, ^\circ \text{C}$ (data not shown).

Initial Characterization of Purified ApoB20.1H—MALDI-TOF mass spectrometry of purified apoB20.1H revealed a molecular mass of 103,361 Da (Fig. 2A), a value in close agreement with the calculated molecular mass of 102,475 Da. Although apoB20.1H contains one N-linked glycosylation site that is utilized in human plasma apoB100 (Asn-158) (30, 31), the absence of an electrophoretic mobility shift upon $N$-glycanase treatment and lack of binding to concanavalin A (data not shown) suggest that apoB20.1H may not contain $N$-linked carbohydrate; however, $O$-linked glycosylation cannot be ruled out. Six intramolecular disulfide bonds form cotranslationally within apoB20.1H, independently of its lipidation state (32–34). To assess the state of the disulfide bonds in apoB20.1H, purified protein was incubated without or with 50 mM DTT for 10 min at varying temperatures followed by alkylation of the free sulfhydryls and analysis by nonreducing SDS-PAGE. At both 22 and $50 \, ^\circ \text{C}$ in the absence of DTT, all apoB20.1H was in a disulfide-bonded form as evidenced by the faster gel mobility relative to the fully reduced control (Fig. 2B, compare lanes 1 and 5 with lane 7). At $22 \, ^\circ \text{C}$, addition of DTT did not alter the mobility of apoB20.1H (Fig. 2B, compare lanes 1 and 2), consistent with the existence of a native folded state in which intramolecular disulfide bonds are solvent inaccessible (35, 36). However, as the temperature was raised from 22–$50 \, ^\circ \text{C}$, increased access to disulfide bonds caused a corresponding increase in the ratio of reduced:folded protein (Fig. 2B, lanes 2, 3, 4, and 6).

To examine the possibility that apoB20.1H binds DMPC vesicles, apoA-I or apoB20.1H were incubated with DMPC vesicles for 20 h followed by equilibrium density gradient centrifugation at $d = 1.25$ g/ml. The lipid-containing top (T) and lipid-poor bottom (B) fractions were analyzed by SDS-PAGE and staining with Coomassie Blue.

Purified ApoB20.1H Fails to Bind DMPC Vesicles—Previous studies showed that apoB17 rapidly binds to DMPC vesicles forming small micellar particles (24, 25, 29). In addition, we showed that apoB20.1H recovered directly from baculovirus supernatants was associated with sufficient cellular lipid to float at $d < 1.25$ g/ml (Fig. 1B). However, upon purification away from lipid and detergent, apoB20.1H failed to decrease the turbidity of a solution of DMPC vesicles (Fig. 3A, closed circles); under the same conditions apoA-I mediated rapid vesicle clearing (Fig. 3A, open circles). To examine the possibility that apoB20.1H can bind to DMPC vesicles but cannot convert them into smaller recombinant lipoproteins, incubations were performed for 20 h followed by the analysis of free and DMPC-bound protein by density gradient centrifugation and SDS-PAGE. As expected, all of the apoA-I bound to the DMPC vesicles and was recovered in the $d < 1.25$ g/ml top density gradient fraction (Fig. 3B, lane 1). In contrast, all of the apoB20.1H was found in the lipid poor bottom fraction (Fig. 3B, lane 4), confirming that in purified form, apoB20.1H has little affinity for DMPC vesicles.

Purified ApoB20.1H Exists in Multimeric Form—To better understand the solution properties of apoB20.1H and why it failed to bind to
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DMPC, purified protein was subjected to dynamic laser light scattering analysis (38–40). Transformation of the raw scattering intensity spectrum (Fig. 4A) to volume percent (38) (Fig. 4B) revealed that apoB20.1H existed in TBS in a broad size distribution with a mean diameter of 12.8 nm (Fig. 4B, closed circles). Assuming that the protein exists in a roughly spherical conformation, these data indicate a molecular mass of ~640-kDa (39) suggesting that on an average, apoB20.1H may exist in solution predominantly as hexamers, with a smaller percentage of both higher and lower orders of multimers. Addition of 0.1% Triton X-100 shifted the entire population to a smaller, more mono-disperse, distribution with a mean diameter of 7.2 nm, corresponding to a molecular mass of 110-kDa, close to the monomeric molecular mass of apoB20.1H (Fig. 4B, open circles). The fact that this relatively hydrophobic surfactant dissociates apoB20.1H multimers suggests that the monomers associate via hydrophobic interactions. In agreement with this, exposure to high ionic strength buffers failed to dissociate apoB20.1H into monomers (data not shown).

Activation of ApoB20.1H DMPC Binding by Multimer Dissociation—Considering the multimeric state of apoB20.1H in solution, we examined the possibility that self-association masked its lipid-binding domains and therefore prevented its interaction with DMPC vesicles. To examine this possibility, purified apoB20.1H was incubated with 0, 0.7, or 2 M GdnHCl followed by the addition of DMPC vesicles. After dialysis, the vesicles were subjected to density gradient centrifugation to separate bound and free protein. Incubation with 0.7 M GdnHCl resulted in ~90% conversion of apoB20.1H into a buoyant, lipid-associated form (Fig. 5, lanes 5 and 6), and 2 M GdnHCl resulted in almost quantitative DMPC binding (lanes 7 and 8). These data suggest that dissociation of apoB20.1H multimers by GdnHCl exposed binding sites that enabled it to interact with DMPC vesicles.

ApoB20.1H Multimers Spontaneously Dissociate and Bind to a Triolein Interface—Using an oil drop tensiometer, we analyzed the behavior of apoB20.1H at a triolein/water interface (26, 27). Upon injection of a 10-μl triolein droplet into apoB20.1H solution, in the absence of detergent or denaturants, the interfacial tension at the drop surface rapidly fell from its base-line value of 32 to ~15 mN/m (Fig. 6). This indicates that apoB20.1H has a high affinity for the triolein/water interface, a property shared by apoA-I, apoA-IV, and apoA-V (26). Analysis of the time-tension curve yielded an exponential binding rate of 2.4 × 10⁻³ s⁻¹, a value similar to that noted for apoA-IV, which has a binding rate constant of 3 × 10⁻³ s⁻¹ (26). The fact that the apoB20.1H multimers were able to bind to the triolein/water interface in the absence of either detergents or denaturants suggests that the interaction between the lipid-binding sequences in apoB20.1H and the hydrophobic aqueous/neutral lipid interface, unlike their interaction with the relatively polar surface of DMPC vesicles, is sufficiently strong so as to spontaneously disrupt its multimeric state.

To more closely model the binding of apoB to a nascent emulsion particle, a triolein droplet was first coated with egg phosphatidylcholine before exposure to apoB20.1H. As shown in Fig. 7, binding of phospholipid to the triolein droplet reduced its interfacial tension from ~32 to ~20 mN/m (arrow a). With the removal of excess phospholipid from the cuvette by continuous buffer exchange, the surface tension increased slightly to ~23 mN/m (Fig. 7, arrow b). Subsequent addition of apoB20.1H to the cuvette caused a prompt decrease in surface tension to ~12 mN/m (Fig. 7, arrow c) reflecting its capacity to effectively bind to a phospholipid monolayer on a triglyceride substrate.

DISCUSSION

We have established, by carboxyl-terminal truncation mutagenesis, that the amino-terminal 912 amino acids of apoB constitutes a domain capable of initiating the assembly of TG-rich lipoproteins (18). Here we report the expression and purification of a His₆-tagged form of this functional domain and describe its structure and interaction with lipid. As discussed in detail below, our data indicate that apoB20.1H contains solvent-accessible sequences that preferentially bind to hydrophobic lipid interfaces, particularly those that contain TG. This behavior suggests that apoB may initiate lipoprotein particle formation via an ER membrane nucleation and desorption mechanism to form nascent emulsion particle precursors.
FIGURE 7. Spontaneous binding of apoB20.1H to a phospholipid/triolein/water interface. Using the oil drop tensiometer, a 10-μl triolein droplet was injected into a cuvette containing Tris buffer, and the tension at the oil-water interface was continuously monitored with constant stirring. At time a, 2 ml of a micellar solution of phosphatidylcholine (750 μg/ml) was added to the cuvette. At time b, excess phosphatidylcholine was removed from the cuvette by initiating continuous buffer exchange at 4 ml/min. At time c, ApoB20.1H was injected into the aqueous phase to achieve a final concentration of 25 μg/ml. Vertical displacements in the trace beginning at ~3050, 6800, and 7900 s reflect microdroplet volume oscillations used for surface elasticity measurements (not shown).

The conceptual framework for TG-rich lipoprotein assembly via a membrane desorption mechanism derives in part from biophysical studies demonstrating that triolein dissolved in a phospholipid bilayer can “oil-out” to form a separate phase when its solubility limit is exceeded (41, 42). The formation of such lens-like blebs within a membrane bilayer in vivo may constitute an intermediate in the process of lipoprotein assembly and be facilitated by specialized lipoprotein-forming proteins such as apoB and MTP. The earliest conceptualization of this model has invoked the cotranslational association of the entire apoB polypeptide with the inner leaflet of the ER membrane, a process that induces site-specific TG accumulation within the membrane bilayer and the blebbing off of a relatively large (∼10–20 nm) lipoprotein precursor (13–17). However, this model has been challenged on a number of grounds, including the high thermodynamic cost associated with extensive membrane dismantling and remodeling, and the fact that the process has never been observed morphologically (21, 43).

An alternative model, which circumvents some of these theoretical shortcomings, posits that only the amino-terminal domain of apoB is required to form nascent lipoprotein particles, which thereafter undergo additional maturation and lipidation by both co- and post-translational mechanisms. Two different variants of this concept have been proposed. In one paradigm, the amino-terminal ∼1,000 amino acids of apoB can fold into a lipid-binding cavity similar to that of its evolutionary progenitor, LV (21). The cavity is then filled, by an unspecified process, with phospholipid and a small amount of TG, thereby forming a phospholipid-rich, proteolipid intermediate (23). A second alternative model, which we have advanced, is that an even shorter amino-terminal sequence of apoB initiates and completes a membrane desorption reaction, resulting in the direct formation of a small, dense, emulsion-like precursor (1, 18).

The ability to express and purify the lipoprotein initiating domain of apoB has allowed us to assess its properties with regard to these alternative models of assembly. The desorption model of assembly requires a surface active protein capable of interacting directly with lipid interfaces, whereas the LV homology model requires that apoB adopt a folded conformation in which its lipid-associating sequences are buried in the interior of a lipid-binding cavity (19, 44). Our tensiometer data reveal that apoB20.1H, which is fully capable of initiating lipoprotein formation in vivo (18), is indeed highly surface active and can rapidly bind to and lower the interfacial tension of hydrophobic lipid interfaces.

Interestingly, the surface activity of apoB20.1H is masked in the classical DMPC binding assay by its ability to form multimers in aqueous solution. This behavior, which is also a characteristic of the exchangeable apolipoproteins (45), is evidence for the presence of secondary structural elements of sufficient hydrophobicity to require shielding from the aqueous milieu. The fact that apoB20.1H achieves this in vitro by the formation of oligomers, which cannot interact with polar lipids (i.e. DMPC vesicles) without prior dissociation by detergents or denaturants, further argues that it is a surfactant apoprotein rather than a monomeric, globular, lipid transport protein.

In further support of this conclusion was the finding that although apoB20.1H multimers could not spontaneously bind to the surface of DMPC vesicles, when exposed to either pure TG or a phospholipid monolayer on a TG substrate (which models the likely circumstance, in vivo) they spontaneously dissociated and rapidly bound to the lipid interface (Figs. 6 and 7). Hence, the lipid-binding elements in the lipoprotein initiating domain of apoB may exhibit selective preference for more hydrophobic interfaces and in vivo may seek the hydrophobic environment of the ER membrane interface. This behavior, in conjunction with the action of MTP and possibly other accessory proteins, may induce the membrane TG phase transition that is the initiating event in the lipoprotein desorption process.

If the initiating domain of apoB does function by such a desorption reaction, it is unclear how its structural homology to LV relates to the lipoprotein assembly process (19, 44). Modeling of apoB based on the crystal structure of LV, which contains a closed, three-sided, lipid-binding cavity, reveals that the β-sheet structure in the amino terminus of apoB can form only two sides of an LV-like cavity (21). To model apoB with a three-sided cavity, it is necessary to postulate that MTP binds to apoB, thus providing a third β-sheet needed to reconstitute a complete LV-like cavity (46). A more recent iteration of this model proposes that a helix-turn-helix motif within apoB could close a lipid-binding cavity (23, 47). However, an alternative hypothesis, which is supported by our present data, is that the amino-terminal β-sheets in the LV homology domain of apoB form an interface with the ER membrane during translation, which in cooperation with MTP leads to the desorption of a nascent emulsion particle. Indeed, the lipid composition of apoB20.1H secreted from MTP-expressing cells is consistent with that of a lipoprotein containing a hydrophobic lipid core, although conflicting results have been obtained using different expression and purification procedures (1, 18, 23). The specific role of MTP in facilitating the early stages of apoB-containing lipoprotein assembly are not known in detail. In one model, MTP performs a chaperone-like function to facilitate the conformational transitions within apoB that drive initial lipoprotein formation (9, 48). Alternatively, it has been proposed that MTP may transfer so-called catalytic lipids to apoB, thereby facilitating the capacity of apoB to engage in autonomous lipid recruitment (7). In either case, using the technologies described, it will be interesting to study how the presence of MTP may alter the interfacial behavior of apoB, thereby providing additional clues as to how these two proteins cooperate to form nascent lipoproteins.

In conclusion, the large size and hydrophobicity of apoB have impeded the application of biochemical and biophysical techniques commonly employed to explore apolipoprotein structure and function. The appreciation that the amino-terminal domain of apoB is an autonomously folded functional unit (18, 33, 49) has justified its analysis independently of the full-length protein. The studies of apoB20.1 described here provide the first functional characterization of the lipoprotein initiating domain of apoB and reveal its potent lipid-binding properties, especially its preference for neutral versus polar lipids. These properties...
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are fully consistent with the membrane desorption model of lipoprotein assembly. Additional structural, biochemical, and biophysical studies, built on approaches and insights presented here, promise a clearer picture of how apoB and perhaps other members of the large lipid transfer gene family (1, 50), function to acquire, sequester, and reorganize hydrophobic lipids within the secretory pathway of lipoprotein-producing cells.

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