Apolipoprotein A-V (apoA-V), the newest member of the plasma apolipoprotein family, was recently discovered by comparison of the mouse and human genomes. Studies in rodents and population surveys of human apoA-V polymorphisms have noted a strong effect of apoA-V on plasma triglyceride levels. Toward the elucidation of the biologic function of apoA-V, we used spectroscopic and surface chemistry techniques to probe its structure and interfacial activity. Computer-assisted sequence analysis of apoA-V predicts that it is very hydrophobic, contains a significant amount of α-helical secondary structure, and probably is composed of discrete structural regions with varying degrees of lipid affinity. Fluorescence spectroscopy of recombinant human apoA-V provided evidence of tertiary folding, and light scattering studies indicated that apoA-V transforms dimyristoylphosphatidylcholine vesicles into discoidal complexes with an efficiency similar to that of apoA-I. Surface chemistry techniques revealed that apoA-V displays high affinity, low elasticity, and slow binding kinetics at hydrophobic interfaces, properties we propose may retard triglyceride-rich particle assembly. Metabolic labeling and immunofluorescence studies of COS-1 cells transfected with human apoA-V demonstrated that apoA-V is poorly secreted, remains associated with the endoplasmic reticulum, and does not traffic to the Golgi. Given that overexpression of the apoA-V gene lowers plasma triglycerides in mice, these data together suggest that apoA-V may function intracellularly to modulate hepatic VLDL synthesis and/or secretion.

Lipoprotein metabolism is regulated by the plasma apolipoproteins, a family of surface-active lipid binding proteins. The smaller, exchangeable members of this family evolved from a single primordial gene to control various processes in intravascular lipid transport (1). The largest member of the family, apoB,1 evolved from ancient lipid transport proteins involved in oogenesis (2) to play a central role in the intracellular assembly of triglyceride-rich lipoproteins in the intestine and liver. ApoA-V is the most recently described member of the plasma apolipoprotein family. Unlike all other apolipoproteins, which were identified in human plasma, apoA-V was discovered by comparative sequence analysis of the human and murine genomes as a gene on chromosome 11, downstream of the A-I/A-IIIA-AIV gene cluster, displaying homology to apoA-IV (3). Concomitantly and independently, apoA-V was identified as a gene up-regulated in the early phase of hepatic regeneration in the rat (4). Mature human apoA-V is a 39-kDa protein with 343 residues and 27% sequence identity with human A-IV.

apoA-V was found to have a powerful effect on plasma triglycerides. Overexpression of human apoA-V in transgenic mice (3) or by use of adenoviral vectors (5) lowers plasma triglyceride levels, whereas inactivation of the apoA-V gene by homologous recombination causes a 4-fold increase in plasma VLDL triglycerides (3). Several single nucleotide polymorphisms in the apoA-V gene have been identified and shown to constitute three distinct haplotypes, two of which exhibit a strong association with elevated fasting VLDL triglycerides in several populations (6–9). Interestingly, the apoA-V gene is expressed only the liver, and its plasma concentration is very low, less than 0.1% of that of apoA-I (4). Thus, it is possible that apoA-V affects plasma triglyceride levels by modulating hepatic VLDL assembly and secretion, rather than the intravascular metabolism of triglyceride-rich lipoproteins.

Over the past 3 decades, structure-function studies have been instrumental in elucidating the biologic function of the plasma apolipoproteins and the mechanisms by which they control lipid metabolism (10). Now, in the genomics era, a new apolipoprotein, apoA-V, has been discovered by computational methods, and its physiological effects have been explored in transgenic animals and with population genetic approaches. However, the apoA-V protein itself has not been isolated, and little is known about its biochemical and biophysical properties. Given the strong association of plasma triglycerides and the risk for atherosclerotic cardiovascular disease (11), we used computational, spectroscopic, and surface chemistry techniques to investigate the structure, lipid binding, and interfacial behavior of recombinant human apoA-V.

EXPERIMENTAL PROCEDURES

Lipids—EPC, DMPC, and triolein were purchased from Sigma. EPC was diluted to 0.1 mg/ml in high performance liquid chromatography grade CHCl3 (Aldrich) and stored under nitrogen at –20 °C. Phospholipid concentration was determined by phosphorous analysis (12).

Preparation of Apolipoproteins—Recombinant human apoA-V was expressed in E. coli and purified as described.2 A 17-amino acid MHHHHHLGLVPRGSIDAGIDDDN was appended to the N terminus to facilitate
purification by nickel affinity chromatography. Human apoA-IV was isolated from donors with the A-V/1-1 genotype (14). Human apoA-I was isolated from high density lipoproteins (15) or produced in E. coli (16). Protein concentration was determined by bicinchoninic acid (17).

All apolipoproteins were homogeneous by SDS-PAGE.

**Computational Analysis of Apolipoprotein A-V Structure**—The amino acid sequence of mature human apoA-V (NCBI entry AAF25662) was subjected to computational analysis. Mean residue hydrophobicity, (H), and mean residue helical hydrophobic moment, \( \mu \), were calculated using a consensus hydrophobicity scale (18). Residue hydrophobicity plots were smoothed using a median filter (19). Secondary structure was computed using the Chou-Fasman, Garnier, NNSSP, Preditor, and PHD programs available on the World Wide Web from www.expasy.org. Analysis of the sequence was analyzed by Fourier transformation of mean residue helical hydrophobic moment versus residue rotation angle along the protein sequence (20). Amino acid variability among the human, rat, and mouse apoA-V sequences was analyzed using a substitution matrix-median filter algorithm (19).

**Fluorescence Spectroscopy**—Fluorescence studies were performed on an SLM 8000C spectrofluorometer (21). Spectra of apoA-V in 10 mM sodium citrate buffer, pH 3.0, were recorded at 25 °C using a 1-cm cell with excitation at 280 nm, 1-s integration, and 4-nm slits for both monochromators. Quenching studies were performed by the addition of citrate-buffered 6 M KI. Stern-Volmer quenching constants, \( K_q \), were obtained from plots of \( I/I_0 \) versus \( [Q] \), where \( I \) and \( I_0 \) are the emission intensities in the absence or presence of 200 \( \mu \)M KI. The slope was calculated as \( K_q = [Q]/I(I_0 - I) \).

**Results**

**Computational Analysis of Apolipoprotein A-V Structure**—The calculated molecular mass of human apoA-V was 38,905 Da. The calculated pI values of native and His-tagged recombinant apoA-V are 5.99 and 6.20, respectively. Human apoA-V has four tryptophan residues (at positions 5, 97, 147, and 325), five tyrosine residues (at positions 7, 87, 109, 171, and 270), and a single cysteine at position 204. There are no predicted N-linked glycosylation sites in human apoA-V.

All structural analysis programs utilized predicted that apoA-V is a highly \( \alpha \)-helical protein, with the following percentages of \( \alpha \)-helical structure: Chou-Fasman, 73%; Garnier, 47%; NNSSP, 76%; Preditor, 61%; PHD, 83% (Fig. 1A).

Calculation of mean residue hydrophobicity indicates apoA-V is more hydrophobic than either apoA-I or apoA-IV, although it contains an equivalent amount of total amphipathic structure, as measured by mean residue helical hydrophobic moment (Table I). However, a hydrophobicity plot revealed an asymmetric distribution of hydrophobic residues along the apoA-V sequence; the amino terminus residues 85 and the carboxyl terminal third of the molecule were relatively hydrophilic, whereas the middle region, from residue 85 to 220 was much more hydrophobic (Fig. 1B). Calculation of the product of predicted \( \alpha \)-helicity and mean molecular helical hydrophobic moment, \( F(\alpha)(\mu) \), which correlates with interfacial exclusion pressure (32), suggests that the lipid affinity of human apoA-V is similar to that of apoA-I (33).

Fourier transform of mean residue helical hydrophobic moment as a function of residue rotation angle along a protein sequence identifies regions of amphipathic \( \alpha\)-helical structure as maxima between 80 and 120°, \( \beta \)-sheet structure as maxima between 140 and 180°, and random coil structure as maxima between 0 and 40°. Analysis of apoA-V revealed that, within the region of a hydrophobic, dominantly amphipathic region of the molecule, from residues 169 and 246 (Fig. 1C), the amphipathic \( \alpha \)-helical structure in apoA-V is less well defined than that of other human apolipoproteins.

**Intracellular Secretory Trafficking of Apo-A-V**—Human apoA-V and two control secretory proteins, HSA and apoB6.6F (amino acids 1–300) of human apoA-I, c-terminal FLAG epitope tagged 130–300 residues were transfected into COS-1 cells using FuGene 6 (30). Twenty-four hours later, cells were trypsinized and replated into two sets of dishes. Cells in the first set of dishes were metabolically radiolabeled with \( ^{35} \text{S} \)-labeled Met and Cys for 3 h, followed by immunoprecipitation of media and lysates with rabbit anti-HSA (Roche Applied Science), goat anti-human apoA-I (Academy Biosciences), or goat anti-human apoA-V. Immune complexes were recovered with protein G-Sepharose, fractionated by 12.5% SDS-PAGE, and visualized by fluorography (31). Cells in the second set of dishes were fixed with formaldehyde, permeabilized with 0.1% saponin in phosphate-buffered saline, blocked with 1% bovine serum albumin, and incubated with primary antibodies at the following dilutions: goat anti-apoA-V (1:100); goat anti-human apoB-100; rabbit anti-HSA, 1:400. Cells were then incubated with rhodamine-conjugated rabbit anti-goat IgG or goat anti-rabbit IgG secondary antibodies (Jackson ImmunoResearch) at a concentration of 25 \( \mu \)g/ml. Cells were post-fixed, mounted in 90% glycerol, and viewed using a Zeiss Axioplan 2 microscope with a \( \times 63 \) oil objective. Images were captured with a Zeiss AxioCam with a gain setting of 3.

**Results**

**Computational Analysis of Apolipoprotein A-V Structure**—The calculated molecular mass of human apoA-V was 38,905 Da. The calculated pI values of native and His-tagged recombinant apoA-V are 5.99 and 6.20, respectively. Human apoA-V has four tryptophan residues (at positions 5, 97, 147, and 325), five tyrosine residues (at positions 7, 87, 109, 171, and 270), and a single cysteine at position 204. There are no predicted N-linked glycosylation sites in human apoA-V.

All structural analysis programs utilized predicted that apoA-V is a highly \( \alpha \)-helical protein, with the following percentages of \( \alpha \)-helical structure: Chou-Fasman, 73%; Garnier, 47%; NNSSP, 76%; Preditor, 61%; PHD, 83% (Fig. 1A). All programs also predicted a break in the \( \alpha \)-helical structure between residues 10 and 13, 191 and 194, 240 and 249, and 293 and 299, and all but PHD predicted breaks between residues 54 and 62 and 158 and 162. A prominent feature of apoA-V is that most of these intervals are punctuated by multiple proline residues, including a unique tetraproline sequence at residues 293–296.

Calculation of mean residue hydrophobicity indicates apoA-V is more hydrophobic than either apoA-I or apoA-IV, although it contains an equivalent amount of total amphipathic structure, as measured by mean residue helical hydrophobic moment (Table I). However, a hydrophobicity plot revealed an asymmetric distribution of hydrophobic residues along the apoA-V sequence; the amino terminus residues 85 and the carboxyl terminal third of the molecule were relatively hydrophilic, whereas the middle region, from residue 85 to 220 was much more hydrophobic (Fig. 1B). Calculation of the product of predicted \( \alpha \)-helicity and mean molecular helical hydrophobic moment, \( F(\alpha)(\mu) \), which correlates with interfacial exclusion pressure (32), suggests that the lipid affinity of human apoA-V is similar to that of apoA-I (33).

Fourier transform of mean residue helical hydrophobic moment as a function of residue rotation angle along a protein sequence identifies regions of amphipathic \( \alpha \)-helical structure as maxima between 80 and 120°, \( \beta \)-sheet structure as maxima between 140 and 180°, and random coil structure as maxima between 0 and 40°. Analysis of apoA-V revealed that, within the region of a hydrophobic, dominantly amphipathic region of the molecule, from residues 169 and 246 (Fig. 1C), the amphipathic \( \alpha \)-helical structure in apoA-V is less well defined than that of other human apolipoproteins.

**Considered together,** these analyses suggest that, unlike other members of the exchangeable apolipoprotein family, apoA-V has a multidomain structure. Residues 1–60 constitute a hydrophilic, moderately amphipathic region typical of the N-terminal globular domains of apoA-I, apoA-IV, and apoE. Residues 61–170 constitute a region that is very hydrophobic but weakly amphipathic. The interval between residues 171 and 245 contains three very hydrophobic, strongly amphipathic \( \alpha \)-helices, which are thus predicted to have high surface activity. Finally, the intervals between residues 246 and 295 and residues 296 and 343, which are delineated by multiple pro-
lines, constitute relatively hydrophilic regions that have no predominant secondary structural motif. Analysis of amino acid variability among the human, rat, and mouse apoA-V sequences revealed that the intervals between residues 155 and 222 and residues 254 and 290 are highly conserved (Fig. 1D).

Fluorescence Spectroscopy—ApoA-V displayed a maximum tryptophan fluorescence emission at 339 nm, which is less blue-shifted than apoA-I and apoA-IV (Table II). Tryptophan fluorescence was resistant to iodide quenching, as reflected by a fractional exposure of 0.26. The tryptophan emission anisotropy ratio with excitation at 305 and 270 nm, a qualitative measure of tyrosine→tryptophan energy transfer efficiency that reflects the compactness of folding (22), was 2.01. As with apoA-IV, these values suggest that apoA-V adopts a tertiary conformation in which its tryptophan residues reside, on average, in a hydrophobic environment within energy transfer range of nonvicinal tyrosines (22).

Binding of ApoA-V to Phospholipid Multilamellar Vesicles—The addition of apoA-V to DMPC vesicles induced a time-dependent decrease in light scattering intensity (Fig. 2); the time required for a 50% decrease (T) was 209 s. Human apoA-I induced a similar decrease in light scattering, with a slightly longer T value of 380 s. These data indicate that the kinetics of apoA-V-dependent transformation of DMPC vesicles into discoidal complexes are roughly equivalent to that of apoA-I. When apoA-V-DMPC discoidal complexes generated at pH 3.0 were brought to pH 7.4, apoA-V remained tightly bound on their surface. Moreover, in contrast to the behavior of lipid-free apoA-V, apoA-V complexed with DMPC remained soluble across a broad pH range, suggesting that under physiological conditions in vivo, apoA-V exists in a lipid-associated state, both in the intracellular and intravascular compartments.

Activity of ApoA-V at the Phospholipid/Water Interface—Adsorption of apoA-V to EPC monolayers, measured as the change in surface pressure, increased as a function of subphase concentration and reached a maximum at concentrations of 5.0×10⁻⁵ g/dl and above. Analysis of the binding curve yielded a Kᵦ₀ of 1.9×10⁻⁵ g/dl, a value similar to Kᵦ₀ values observed for apoA-I and apoA-IV (23). Binding of apoA-V to the EPC/water interface decreased linearly with increasing initial pressure (Fig. 3); extrapolation of the Π₋ΔΠ curve yielded an exclusion pressure of 32.3 mN/meter. Exclusion pressures of other apolipoproteins are as follows: apoA-IV, 29.1 mN/meter (23);
solubilization kinetics. A solution of 500 μg of apoA-V was injected beneath EPC monolayers spread at increasing initial surface pressures, and the change in surface pressure, ΔP, due to its adsorption to the interface was determined. The solid line is a least squares regression of the data; extrapolation to zero yields an exclusion pressure of 32.3 mN/meter. The dashed lines are data, similarly determined, for human apoA-I and apoA-IV (25).

**TABLE II**

| Fluorescence spectroscopy parameters | λmax | Amax/λem | Kq/K\text{NATA} |
|-------------------------------------|------|----------|-----------------|
| ApoA-V                              | 339  | 2.01     | 0.26            |
| ApoA-I                              | 333  | ND       | 0.29            |
| ApoA-IV                             | 332  | 2.00     | 0.24            |

λmax: maximum fluorescence emission, excitation at 280 nm.
Amax/λem: ratio of emission anisotropy with excitation at 305 and 270 nm.
Kq/K\text{NATA}: Stern-Volmer iodide tryptophan quenching constant, normalized to the quenching of N-acetyltryptophanamide.

**Fig. 2.** Effect of apolipoproteins A-V and A-I on DMPC vesicle solubilization kinetics. A solution of 500 μg of DMPC bilayer vesicles in 50 mM sodium citrate, 150 mM NaCl, pH 3.0, was incubated at 24 °C in the absence or presence of 200 μg of apolipoprotein A-V or apoA-I, and right angle light scattering at 580 nm was continuously monitored. Data are the average of two experiments. Drop lines indicate the time for 50% clearance (T).

apoA-I, 33.2 mN/meter (33); apoA-II, 34 mN/meter (33); and apoC-II, 33.6 mN/meter (34).

**Dynamic Behavior of ApoA-V at the Air/Water Interface**—Surface area-tension loops of human apoA-V at the air/water interface displayed an increase in surface tension with an expansion of bubble surface area (Fig. 4), indicating that apoA-V undergoes changes in surface conformation in response to changes in interfacial geometry, albeit with a much lower elastic modulus than human apoA-IV (Table III). The surface viscosity of apoA-V, however, was similar to apoA-IV. Area-tension loops recorded at 5–80 cycles/min (Fig. 5) revealed that, unlike apoA-I or apoA-IV, which display rate-dependent increases in elasticity, the elasticity of apoA-V was rate-insensitive, which implies a more rigid and less adaptable interfacial conformation.

**Dynamic Behavior of ApoA-V at the Oil/Water Interface**—ApoA-V adsorbed avidly to the oil/water interface, as evidenced by the decrease in surface tension from a base-line value of 32 mN/meter to a nadir of 16.3 mN/meter (Fig. 6). These data are consonant with the prediction that apoA-V possesses high affinity for hydrophobic interfaces. Interestingly, in contrast to its interaction with phospholipid vesicles, apoA-V bound to the oil/water interface at a rate more than 10-fold slower than apoA-I. Analysis of the apoA-V time-tension curve yielded a binding rate constant of 3.5 × 10^{-3} s^{-1}, whereas the corresponding rate constants for apoA-I and apoA-IV were 50.4 × 10^{-3} and 45.9 × 10^{-3} s^{-1}, respectively (Table III).

**Fig. 3.** Determination of the interfacial exclusion pressure of apolipoprotein A-V. ApoA-V was injected beneath EPC monolayers spread at increasing initial surface pressures, and the change in surface pressure, ΔP, due to its adsorption to the interface was determined. The solid line is a least squares regression of the data; extrapolation to zero yields an exclusion pressure of 32.3 mN/meter. The dashed lines are data, similarly determined, for human apoA-I and apoA-IV (25).

**Fig. 4.** Dynamic behavior of human apolipoprotein A-V at the air/water interface. Surface tension of a solution of apoA-V at the air/water interface was measured with a pulsating bubble surfacitometer as bubble surface area was sinusoidally oscillated at 20 cycles/min. The dashed lines are data similarly determined for human apoA-I and apoA-IV (27).
liter drops of triolein were rapidly injected into a cuvette containing 25 mM Tris, pH 7.5, and surface tension was continuously monitored at 1-s intervals.

The decrease in surface tension consequent to apolipoprotein binding at the triolein/buffer interface was measured with a Tracker automatic oil drop tensiometer. Ten-micro-liter drops of triolein were rapidly injected into a cuvette containing 25 \text{mM} \text{Tris, pH 7.5}, and surface tension was continuously monitored at 1-s intervals.

The Golgi and are consistent with low secretion efficiency.

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ent with localization primarily to the ER (35). These data suggest that apoA-V may not traffic efficiently from the ER to the Golgi and are consistent with low secretion efficiency.

**DISCUSSION**

It is thought that exchangeable apolipoproteins bind to lipid interfaces by a cooperative interaction of multiple repeated amphipathic \(\alpha\)-helical domains (36). The largest of these, apoA-I, apoA-IV, and apoE, display a repeating pattern of homologous 11- and 22-mer amphipathic \(\alpha\)-helical segments, which are demarcated by proline residue “hinges” that increase the cooperativity of binding (36). Our computational analysis suggests that, like other apolipoproteins, human apoA-V contains a significant amount of \(\alpha\)-helical structure. Far UV circular dichroism analysis of apoA-V\(^{-}\) supports this prediction but also indicates the presence of a significant component of \(\beta\)-sheet and \(\beta\)-turn structure. Our analysis further predicts that, unlike apoA-IV or apoA-I, the \(\alpha\)-helical regions in apoA-V are not composed of homologous repeating 11- or 22-mer units but rather display considerable heterogeneity in both length and hydrophobicity. These regional differences in hydrophobic properties, together with the unusual clustering of proline residues in apoA-V, raises the possibility that discrete independently folded domains exist within apoA-V that have distinct functional roles.

Comparison of the kinetic data from the light scattering and oil drop experiments supports this hypothesis. As apolipoprotein-induced solubilization of DMPC vesicles requires both binding and penetration of amphipathic helices into the vesicle surface (26), the present data suggest that, like human apoA-I, some amphipathic helices in apoA-V are able to rapidly bind and penetrate the phospholipid interface. However, at the more hydrophobic triolein/water interface, apoA-V displayed much slower binding kinetics, implying a different mode of binding. As interfacial unfolding, reorientation, and integration of hydrophobic domains is necessary to generate full surface activity, and as there is an inverse relationship between conformational stability and adsorption rate (37), the slow binding of apoA-V to the oil/water interface implies a molecular structure that is either very stable, and/or lacks cooperativity among distinct domains.

As predicted, the monolayer data establish that apoA-V has an interfacial exclusion pressure similar to apoA-I, which suggests that in plasma, apoA-V should be tightly bound to lipoproteins. However, the slope of the \(IL - \Lambda II\) curve was much shallower than that observed for other apolipoproteins. Considering the predicted structure of apoA-V, and given our observation that interfacial activity may be sensitive to the hydrophobic properties of only one or two amphipathic repeats (24), this finding suggests that the binding of apoA-V to lipid interfaces may be determined by only a few discrete domains. A candidate for such a role is residues 171–188, a predicted \(\alpha\)-helical segment that is both very hydrophobic and highly amphipathic. It is noteworthy that this region is highly conserved among mammalian apoA-V sequences.

**TABLE III**

|                  | Air/water interface | Lipid/water interface |
|------------------|---------------------|-----------------------|
|                  | \(e^a\)  | \(\phi^b\) | \(e^c\) | \(K_A^d\) | \(f K_{EX}^e\) | \(K_A^f\) |
| Apo A-V          | 12.9          | 24.4          | 9.7  | 1.31   | 32.3          | 3.5  |
| Apo A-IV         | 7.6           | 12.4          | 1.6  | ND^g   | 33.2          | 50.4 |
| Apo A-I          | 21.7          | 28.0          | 10.2 | 1.10   | 29.0          | 45.9 |

\(^a\) \(e\), interfacial elasticity.

\(^b\) \(\phi\), \(\gamma\)-area loop phase angle.

\(^c\) \(e^c\), viscoelastic component.

\(^d\) \(K_A\), equilibrium dissociation constant for interaction with phospholipid monolayers.

\(^e\) \(f K_{EX}\), exclusion pressure for phospholipid monolayers.

\(^f\) \(K_A\), rate constant for binding to a triolein/buffer interface.

\(^g\) \(n\), not determined.

**FIG. 5.** Calculated total elasticity of human apolipoproteins A-V, A-IV, and A-I at the air/water interface as a function of oscillation cycle rate.

**FIG. 6.** Dynamic interfacial behavior of human apolipoprotein A-V at the oil/water interface. The decrease in surface tension consequent to apolipoprotein binding at the triolein/buffer interface was measured with a Tracker automatic oil drop tensiometer. Ten-micro-liter drops of triolein were rapidly injected into a cuvette containing 25 \(\mu \text{g/ml} \text{apoA-V (10 mM citrate, pH 3.0)}\) or 25 \(\mu \text{g/ml} \text{apoA-IV or apoA-I (43 mM Tris, pH 7.5,)}\) and surface tension was continuously monitored at 1-s intervals.
Dynamic tensiometry provides insight into the interfacial behavior of proteins that is not revealed by static techniques (27). The elastic response to changing interfacial geometry is particularly informative, as interfacial elasticity is a critical factor in determining the abilities of proteins to stabilize oil/water emulsions (38). At the air/water interface, apoA-V displayed a lower elastic modulus than apoA-IV. Moreover, whereas apoA-IV and apoA-I displayed increased elasticity in response to a more rapid change in surface area, the elastic behavior of apoA-V was insensitive to rate. Our studies with apoA-IV suggest that elasticity is a function of multiple amphipathic α-helices that adopt an expanded interfacial conformation such that the more hydrophilic helices undergo reversible squeeze-out in response to surface pressure (23, 27). Thus, in apoA-V, the absence of the tandem, proline-punctuated helices characteristic of apoA-IV and apoA-I, or the lack of cooperativity between domains with very different amphipathic-hydrophobicity profiles, may result in a molecule that is relatively stiff and inelastic.

An interesting and unique feature of lipid-free apoA-V is its poor solubility between pH 3.5 and 9.5, a property that led us to conduct the present studies at pH 3.0. Removal of the N-terminal His-tag used to facilitate purification had no detectable effect on the solubility properties of lipid-free apoA-V. Considering that 3.5–9.5 is the pH range for optimal interresidue hydrogen bonding, this behavior suggests that, at physiological pH, apoA-V may require association with lipid to fully stabilize its tertiary conformation. Indeed, lipid-associated apoA-V is soluble over a broad pH range. Nevertheless, the fluorescence data presented here and the circular dichroism data of Beckstead et al. indicate that, even at pH 3.0, lipid-free apoA-V adopts an ordered tertiary structure and displays interfacial behavior consistent with a lipid binding protein. By extension, these data imply that, intracellularly, apoA-V is likely to be tightly membrane bound.

Given the apparent insolubility of lipid-free apoA-V at physiological pH, and the fact that its plasma concentration is in the μg/ml range (4) (i.e., <1% of that of apoA-IV and <0.1% of that of apoA-I) it is possible that the negative impact of apoA-V gene expression on plasma triglyceride levels is mediated intracellularly rather than intravascularly. In this regard, we have proposed that apolipoproteins with moderate lipid affinity and high interfacial elasticity, such as apoB and apoA-IV, stabilize and facilitate nascent particle expansion during the second stage of assembly, whereas surfactants or apolipoproteins with high lipid affinity and low elasticity impede this process (27). Thus, the finding that apoA-V exhibits high affinity, low elasticity, and slow binding kinetics at hydrophobic interfaces suggests that it may retard the second step of VLDL assembly, possibly by constraining particle expansion, or by binding to and retarding the movement of nascent VLDL through the secretory pathway.

The findings that very little apoA-V is secreted from the transfected COS-1 cells (under conditions that promote efficient secretion of HSA and apoB6.6), and that little apoA-V appears to traffic to the Golgi, support this hypothesis, and are consistent with an intracellular role in hepatic lipid metabolism. Moreover, these findings provide an explanation for low apoA-V levels in plasma (4), and agree with the prediction that apoA-V is membrane bound in vivo. This atypical behavior raises the question as to whether apoA-V functions as a classical apolipoprotein. That apoA-V escapes transport to the cell surface can be explained by several mechanisms. It is accepted that movement of proteins from ER to Golgi occurs by a combination of bulk flow and active sorting in the ER and the ER-Golgi intermediate compartment (39, 40). Proteins that lack anterograde sorting signals may be excluded from ER export sites and thus secreted slowly an/or inefficiently. Furthermore, proteins that lack their own retention or recycling signals can interact with proteins that do. For example, the 97 kDa subunit of microsomal triglyceride transfer protein interacts with the KDEL-containing protein, PDI, a process that in part underlies its retention within the ER (41). Interestingly, a BLAST search revealed 55% identity between the C-terminal domain of apoA-V and residues 239–260 of mouse microsomal triglyceride transfer protein.

A biological role for such behavior is suggested by the observation that apoA-V gene expression is activated in the early phase of hepatic regeneration that precedes the onset of DNA synthesis, and is injury specific (i.e., not an acute phase response) (4). The liver is the only organ capable of regeneration after a toxic or infectious insult, and one of its first metabolic responses to injury is a rapid decline in apoB and triglyceride secretion (42). Thus, if indeed apoA-V can modulate VLDL assembly, in the setting of acute hepatic injury, apoA-V might serve as an “intracellular brake” on VLDL assembly and secretion that would conserve lipid for required membrane biogenesis and as an energy source during regeneration. This function might also operate in the normal state to modulate hepatic lipid export in response to diet and metabolic demands.

In summary, our data demonstrate that apoA-V displays distinctive properties that are different from other members of the exchangeable apolipoprotein family. Whereas apoA-V displays phospholipid vesicle transformation kinetics and monolayer binding avidity similar to apoA-I, it displays low interfacial elasticity and slow binding kinetics at the more hydrophobic oil/water interface. These dynamic interfacial properties are consistent with the hypothesis that apoA-V impedes triglyceride-rich particle assembly. Further studies on the intracellular distribution of apoA-V, the impact of apoA-V gene expression on intracellular VLDL assembly, and the effect of human apoA-V genetic variations on plasma VLDL metabolism will be needed to reveal whether, and in what circumstances, apoA-V modulates hepatic VLDL synthesis and secretion.
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