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Apolipoprotein C-I Binds More Strongly to Phospholipid/Triolein/Water than Triolein/Water Interfaces: A Possible Model for Inhibiting Cholesterol Ester Transfer Protein Activity and Triacylglycerol-Rich Lipoprotein Uptake

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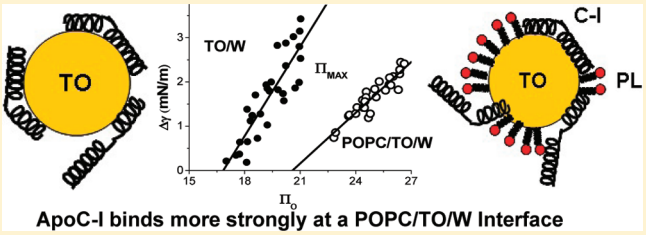
ABSTRACT: Apolipoprotein C-I (apoC-I) is an important constituent of high-density lipoprotein (HDL) and is involved in the accumulation of cholesterol ester in nascent HDL via inhibition of cholesterol ester transfer protein and potential activation of lecithin:cholesterol acyltransferase (LCAT). As the smallest exchangeable apolipoprotein (57 residues), apoC-I transfers between lipoproteins via a lipid-binding motif of two amphipathic α-helices (AαHs), spanning residues 7−29 and 38−52. To understand apoC-I's behavior at hydrophobic lipoprotein surfaces, oil drop tensiometry was used to compare the binding to triolein/water (TO/W) and palmitoyloleoylphosphatidylcholine/triolein/water (POPC/TO/W) interfaces. When apoC-I binds to either interface, the surface tension (γ) decreases by ∼16−18 mN/m. ApoC-I can be exchanged at both interfaces, desorbing upon compression and readsorbing on expansion. The maximal surface pressures at which apoC-I begins to desorb (Π_{max}) were 16.8 and 20.7 mN/m at TO/W and POPC/TO/W interfaces, respectively. This suggests that apoC-I interacts with POPC to increase its affinity for the interface. ApoC-I is more elastic on POPC/TO/W than TO/W interfaces, marked by higher values of the elasticity modulus (ε) on oscillations. At POPC/TO/W interfaces containing an increasing POPC:TO ratio, the pressure at which apoC-I begins to be ejected increases as the phospholipid surface concentration increases. The observed increase in apoC-I interface affinity due to higher degrees of apoC-I−POPC interactions may explain how apoC-I can displace larger apolipoproteins, such as apoE, from lipoproteins. These interactions allow apoC-I to remain bound to the interface at higher Π values, offering insight into apoC-I's rearrangement on triacylglycerol-rich lipoproteins as they undergo Π changes during lipoprotein maturation by plasma factors such as lipoprotein lipase.



Apolipoprotein C-I (apoC-I) is synthesized in the liver and circulates in the plasma bound to the surface of high-density lipoproteins (HDLs), very low-density lipoproteins (VLDLs), intermediate-density lipoproteins (IDLs), and chylomicrons.[1] As the smallest exchangeable apolipoprotein (57 amino acids), apoC-I transfers between lipoproteins and chylomicrons, altering their metabolic properties.[2,3] On nascent HDL, apoC-I is a potent activator of lecithin:cholesterol acyltransferase (LCAT), which allows for the accumulation of cholesterol ester (CE) and HDL particle maturation. ApoC-I serves as a secondary activator of LCAT, activating the enzyme with 10−45% efficacy relative to its primary activator, apolipoprotein A-I.[4−6] ApoC-I is a potent inhibitor of cholesterol ester transfer protein (CETP), which promotes exchange of CE and triacylglycerols (TAGs) between HDL and VLDL or LDL.[7−9] On the surface of HDL, apoC-I displaces hepatic lipase (HL), which hydrolyzes HDL phospholipids and mono- and diglycerides.[10−12] In activating LCAT and inhibiting CETP and HL, apoC-I aids in the synthesis and stabilization of mature HDL particles.

Comparatively, apoC-I is known to inhibit activity of lipoprotein lipase (LPL), an enzyme critical for hydrolysis of VLDL triacylglycerols and conversion of VLDL to LDL.[13] ApoC-I further inhibits clearance of TAG-rich lipoproteins and their remnants by impairing interactions with the cellular receptors mediating their uptake.[14,15] Impaired uptake of β-VLDL has been linked to apoC-I-induced displacement of apoE, the ligand for the low-density lipoprotein receptor-related protein (LRP).[16−18] Together, these findings suggest that apoC-I lipoprotein surface modifications could displace apoE or HL, while the structure of apoC-I on the lipoprotein surface may allow for a portion of the peptide to interact with and regulate the function of LCAT or CETP. Understanding of the structure of apoC-I is therefore central to understanding its functional effects on the surface of lipoproteins.

ApoC-I, as a lipid-free monomer in solution, is largely unfolded exhibiting an average helical content of 31%.[19,20] The helical content increases to 65−75% in lipid-bound or self-associated states.[20−22] Nuclear magnetic resonance (NMR) and circular dichroism (CD) studies show that apoC-I contains four 11mer repeats, predicted to produce two lysine-rich class A

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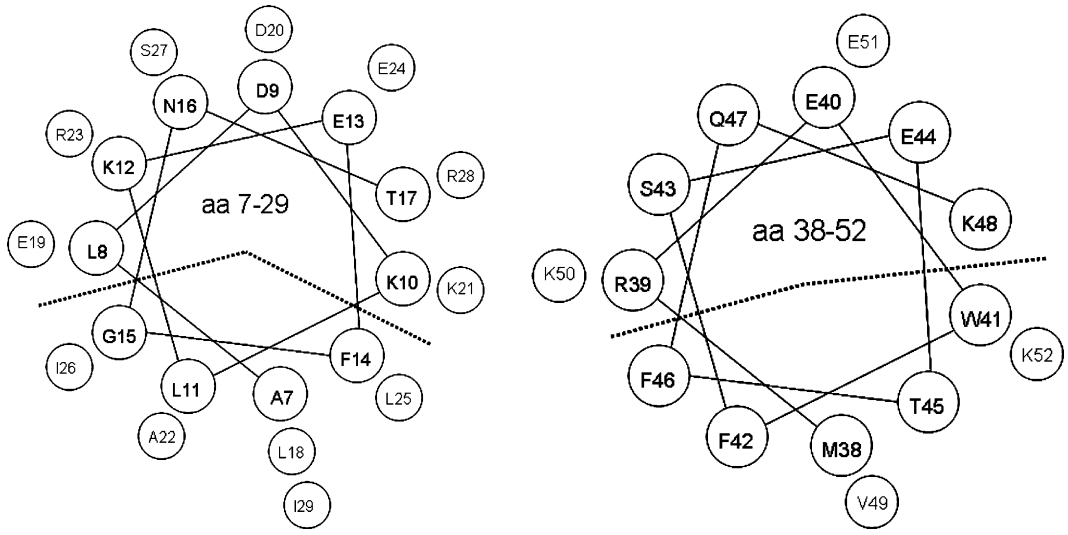

Figure 1. Helical wheel representation of the apoC-I α-helices as α11/3 helices. Hydrophobic helical faces are represented by dotted lines. The N-terminal AαH hydrophobic face (left) is comprised of nine residues (GLAFIALLI), and the C-terminal AαH hydrophobic face (right) is comprised of six residues (FFMTWV).

amphipathic α-helices (AαH), spanning residues 7−29 and 38−52.[23−28] A common lipid surface-binding motif among apolipoproteins and synucleins, the class A AαH is characterized by a large (30−50%) apolar face essential for interactions with apolar lipid moieties and positive residues distributed in the polar−nonpolar interface, which interact with phospholipid headgroups and solvent molecules.[29−33]

Figure 1 depicts the N-terminal (left) and C-terminal (right) AαH helix wheel diagrams, with a dashed line demarcating their respective hydrophobic faces. The lysine residue (K52) at the end of the C-terminal AαH likely snorkels up out of the hydrophobic face.[30,31] Because of its structural similarity to apoA-I and α-synuclein, apoC-I's AαHs are modeled as α11/3 helices.[32,33] Such helices are slightly unwound compared to the standard α-helix, subsuming a rotation of 98.18° as opposed to 100° per residue.

To elucidate the interaction of apoC-I AαHs with phospholipid on the lipoprotein surface, several studies monitored the effect of point mutations on binding of apoC-I to and stabilization of phospholipid vesicles.[20,28,34−36] Thermal denaturation and renaturation of apoC-I−DMPC complexes, monitored via CD, revealed the greatest reduction in apoC-I α-helical content and the ability to reconstitute apoC-I−DMPC complexes for mutations interrupting the apolar face of either AαH.[20,28] These studies indicate an essential cooperativity in binding of the α-helices to the phospholipid surface.[20] Additional studies, utilizing DMPC clearance assays and electron microscopy, showed that the C-terminal AαH contains aromatic residues essential for stabilizing the structure of the entire peptide and mediating phospholipid interactions.[36]

Apolipoprotein monolayers spread at an air/water (A/W) interface compared with apolipoprotein deposition at phospholipid/air/water (PL/A/W) interfaces provide additional insight into the interaction between apolipoproteins and phospholipid.[37−43] The surface pressure−area (Π−A) isotherms generated for compression of apoC-I monolayers at an A/W interface reveal a phase transition at a Π of ∼37 mN/m and a collapse at a Π of ∼47 mN/m.[44,45] Likewise, compression of apoC-I deposited on DPPC monolayers spread at an A/W interface revealed a phase transition beginning at a Π of ∼24−27 mN/m and a collapse at a Π of ∼49 mN/m.[46−48] Brewster angle microscopy revealed that each phase transition could be attributed to different phases of apoC-I.[44−48] These results suggest a two-step desorption of apoC-I from A/W and DPPC/A/W interfaces, wherein one AαH desorbs at lower pressures (Π = 37 and 24−27 mN/m, respectively) before complete peptide desorption from the interface at higher pressures (Π = 47 and 49 mN/m, respectively).

Together, previous work with apoC-I and DMPC vesicles and apoC-I at a DPPC/A/W interface highlights the structural changes in apoC-I upon lipid binding and the conformational changes of the peptide as Π changes, which may happen in the transition of nascent HDL to mature, spherical HDL or of VLDL to LDL. Such studies are limited, however, by the lack of a truly physiologic interface, the ability to monitor quantitative, real-time surface modifications induced by apolipoprotein binding, and a full analysis of the effects of phospholipids on apolipoprotein binding.

To better ascertain how apolipoproteins change conformations or modify lipoprotein surfaces upon binding, the oil/water surface provides a model for peptide interactions with a TAG core, while a phospholipid/oil/water interface more closely resembles the lipoprotein surface. Both interfaces can be further refined by adding other molecules known to be in lipoprotein surfaces (e.g., cholesterol, fatty acids, etc.). To date, only a few studies[37,49−55] have examined the surface behavior of apolipoproteins or their consensus peptides at oil/water interfaces. Even fewer focused on the surface behavior of apolipoproteins at phospholipid/oil/water interfaces.[56,57]

In this study, we examined the interfacial properties of native apoC-I at TO/W and the more physiological palmitoyloleoylphosphatidylcholine/triolein/water (POPC/TO/W) interfaces. POPC better represents the phospholipid content at a lipoprotein surface than DMPC or DPPC, as POPC is found in abundance in TAG-rich lipoproteins, while DMPC and DPPC do not appear significantly on the surface of lipoproteins.[57] Our goal was to understand how apoC-I, via its amphipathic α-helices, binds triacylglycerols (represented here by triolein) and/or phospholipids (POPC) and how

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surface properties vary based on constituents on the modeled TO/PC surface.

To monitor the modifications induced by apoC-I at the lipoprotein surface, we used an oil-drop tensiometer[53,58,59] to measure and compare the adsorption isotherms, desorption behaviors, and elasticity properties of apoC-I at both interfaces. From this, we were able to see how interactions of the apoC-I AαH with POPC alter the binding of apoC-I to and its affinity for the interface. On the basis of the protocol of Mitsche et al.,[57] we varied the POPC surface concentration and quantitatively determined the effects on apoC-I surface retention as Π changes. From these results and comparison with other peptides we have studied, notably the AαH-rich apoA-I,[52,55,57] we propose a model in which apoC-I binds and increases Π to such a degree that molecules, such as apoE, HL, or CETP apolipoprotein activators, would be displaced from the surface.

MATERIALS AND METHODS

Materials. Triolein (>99% pure) was purchased from NU-CHEK PREP, Inc. (Elysian, MN), and its interfacial tension (γ) was 32 mN/m.

POPC (1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine) was purchased from Avanti Polar Lipids (Alabaster, AL) dissolved in chloroform to a concentration of 25.0 mg/mL and stored at −20 °C. The POPC was dried under nitrogen and resuspended in 2 mM phosphate-buffered saline (PBS, pH 7.4) at a concentration of 2.5 or 5.0 mg/mL. Using a probe sonicator, the POPC in PBS was constituted into SUVs. POPC SUVs, using the method of Mitsche and Small,[57] were injected at varied concentrations into the aqueous phase and given sufficient time to adsorb to the preformed TO drop, such that the interfacial tension fell to 23−25 mN/m. At these γ values, roughly 42−37% of the TO drop was covered with POPC, based on calculations by Mitsche and Small.[57]

Full-size human apoC-I (57 amino acids) was obtained via solid-state synthesis and purified by high-performance liquid chromatography to 95−98% purity as described previously.[20,28] The N- and C-termini of the peptide were not blocked. SDS gels, CD, and electron microscopy revealed the structure and stability of wild-type (WT) apoC-I prepared by this method closely resemble those of the plasma protein (data not shown). A stock solution of peptide was made by adding 2 mM phosphate buffer (PB, pH 7.4) to a final peptide concentration of 1.0 mg/mL. Varied amounts of apoC-I stocks could be added to the aqueous phase to obtain different protein concentrations (from 7.5×10^{-8} to 1×10^{-6} M at a TO/W interface and from 1×10^{-7} to 5×10^{-7} M at a POPC/TO/W interface).

Interfacial Tension (γ) Measurement. The interfacial tension (γ) of TO/W and POPC/TO/W interfaces in the presence of varied concentrations of apoC-I in the aqueous phase was measured with an I. T. Concept (Longessaigne, France) Tracker oil-drop tensiometer,[53,58,59] with the goal of observing and quantifying the surface activity of apoC-I at either interface. A 16 μL triolein drop was formed in 6.0 mL of gently stirred 2 mM PB (pH 7.4) containing no or varied concentrations of POPC. Varied amounts of apoC-I were added to the aqueous phase when TO/W interfaces had reached an equilibrium γ (γ_e) of 32 mN/m or when POPC/TO/W interfaces had reached a γ between 23 and 25 mN/m. After POPC had adsorbed to the surface, the bulk buffer with POPC SUVs was exchanged with 250 mL of 2 mM POPC-free PB (pH 7.4) prior to addition of apoC-I to the aqueous phase. This buffer exchange was done using the protocol of Mitsche and Small[57] and resulted in the removal of >99.9% of the original buffer. The γ of apoC-I at either interface was recorded continuously until it approached a γ_e. The surface pressure (Π) was calculated from the surface tension of the TO/W interface (γ_{TO} = 32 mN/m) minus the surface tension of the interface with POPC and apoC-I (γ), such that Π = 32 mN/m − γ. The temperature was 25.0 ± 0.1 °C for all experiments.

Desorption and Readsorption Processes. Following adsorption of apoC-I to either a TO/W or a POPC/TO/W interface, the drop underwent a series of compressions and re-expansions with the goal of determining if apoC-I completely or only partially desorbed from the respective interface. Once γ approached a γ_e, the TO drop (16 μL) was compressed by rapidly decreasing the volume by different ratios: 6.25% (1 μL), 12.5% (2 μL), 25% (4 μL), 37.5% (6 μL), 50% (8 μL), or, when possible, 62.5% (10 μL). This sudden decrease in volume induced a decrease in drop surface area, resulting in a sudden compression and abrupt decrease in γ. The oil drop was held at this reduced volume for 5−10 min, with γ recorded continuously. If apoC-I readily desorbed, γ increased to a γ_e, observed as a desorption curve. After 5−10 min, the interface was expanded by increasing the volume of the drop by equivalent ratios back to its initial volume (16 μL). As the surface area increased upon expansion, γ abruptly increased. If apoC-I adsorbed from the bulk phase and adhered to the newly formed extra surface, γ decreased to the initial γ_e, observed as a readsorption curve. This process of stress compression and re-expansion was repeated after the bulk buffer was exchanged with 150 mL of 2 mM PB (pH 7.4) devoid of apoC-I.

Values of Π_{max}. The desorption and readsorption protocol provided information about not only the nature of ejection of the peptide from either interface but also the Π at which such ejection occurs. Π_{max} is the maximal pressure (Π) that a peptide can withstand before all or part of the molecule is ejected from the surface. Once a γ_e had been reached following adsorption of apoC-I to TO/W or POPC/TO/W interfaces, we conducted the series of experiments highlighted above in which the drop volume was decreased abruptly, thereby decreasing γ and increasing Π to a given value, Π_o. The change in tension ($\Delta\gamma$) over the following 5−10 min as peptide desorbed from the surface was plotted against Π_o. Regression of a linear fit to the plot reveals Π_{max} as the point at which $\Delta\gamma$ = 0, such that not even part of the peptide desorbs from the surface upon compression.[51,52]

Equilibrium Oscillations of the Interface and the Elasticity Analysis. Oscillating TO/W and POPC/TO/W interfaces with apoC-I bound allowed for comparison of viscoelasticity. After γ approached γ_e with apoC-I bound to either interface, the volume of the TO drop was oscillated sinusoidally at different periods ranging from 8 to 128 s and different amplitudes ranging from ±2 μL (~13%) to ±8 μL (50%). As the volume oscillated, the interfacial area (A) and surface tension (γ) were recorded continuously. From these measurements of γ and A, pressure−area (Π−A) isotherms were derived and plotted, as $\gamma = \Pi + 32.0$ mN/m. These measurements of γ and A also yielded the interfacial elasticity modulus, defined as $\varepsilon = d\gamma/d(\ln A)$, for each sinusoidal oscillation.[60] Via Fourier transform analysis, the real [$\varepsilon' = |\varepsilon| \cos(\varphi)$] and imaginary [$\varepsilon'' = |\varepsilon| \sin(\varphi)$] components of ε were determined, allowing for derivation of the phase angle (φ) between compression and expansion. Increasing degrees of

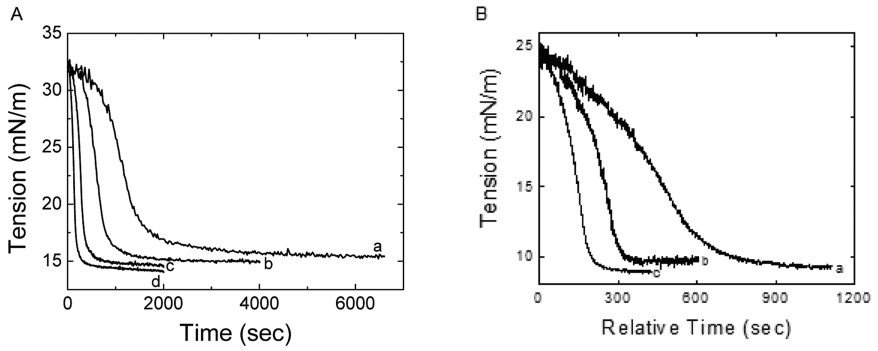

Figure 2. Plots of interfacial tension, γ, vs time of apoC-I at a TO/W interface (A) and a POPC/TO/W interface (B). A 16 μL TO drop was formed in 2 mM PB (pH 7.4). (A) ApoC-I was added at varying concentrations: (a) 7.5×10^{-8}, (b) 2.5×10^{-7}, (c) 5.0×10^{-7}, and (d) 1.0×10^{-6} M. (B) POPC was adsorbed to the surface, and γ reached ~24–25 mN/m after buffer exchange. ApoC-I was added at relative time zero at varying concentrations. (a) 1.0×10^{-7}, (b) 2.5×10^{-7}, and (c) 5.0×10^{-7} M.

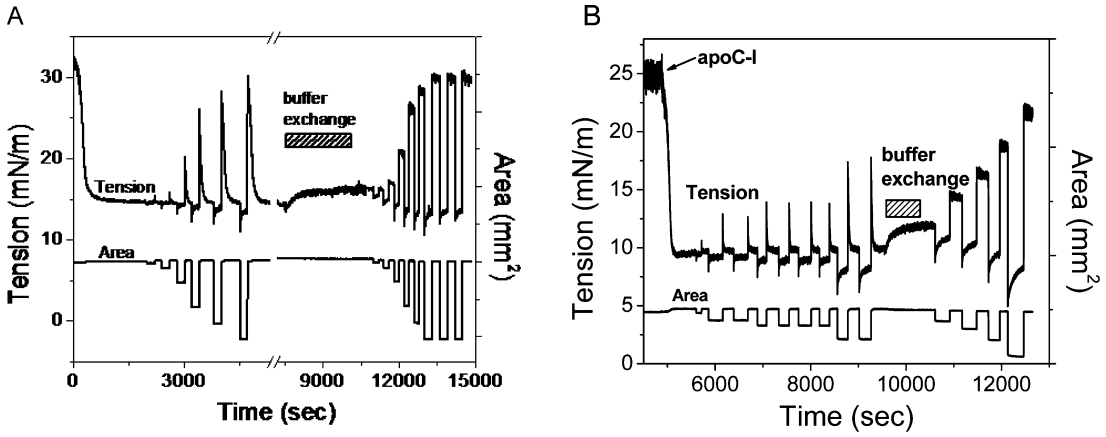

Figure 3. Desorption and readsorption curves of apoC-I at TO/W (A) and POPC/TO/W (B) interfaces. (A) A 16 μL TO drop was compressed and subsequently re-expanded in increments of ±1, ±2, ±4, ±6, ±8, and ±10 μL. Following a 150 mL buffer exchange (shown by the bar), the drop was compressed and re-expanded in increments of ±1, ±2, ±4, ±6, ±8, and ±10 μL (three times). (B) A 16 μL TO drop was compressed and re-expanded in increments of ±1, ±2 (two times), ±3 (two times), ±4 (two times), and ±6 μL (two times). Following a 150 mL buffer exchange (shown by the bar), the drop was compressed and re-expanded in increments of ±2, ±4, ±6, and ±8 μL. The apoC-I concentration in the aqueous phase was 5.0×10^{-7} M before and virtually zero after the buffer exchange for both panels A and B.

surface viscoelasticity are marked by decreasing ε and increasing φ values.

Ramping Protocol and Corresponding Π–*A* Isotherms. The goal of slowly expanding and sequentially compressing TO drops with apoC-I bound, but differing in POPC surface concentration (Γ_{POPC}), was to quantitatively compare the point of compression at which apoC-I begins to be expelled from the POPC/TO/W interface. After adsorption of POPC to a TO/W interface lowered γ to 23–25.0 mN/m, the bulk buffer was exchanged with 250 mL of 2 mM POPC-free PB (pH 7.4), and γ was increased to 24.5–25.0 mN/m. The volume of the drop was then increased or decreased slowly (±0.02 μL/s), causing increases or decreases in γ. This resulted in corresponding decreases or increases in Π, respectively. The pressure of the interface (Π_I) at which apoC-I was added could be correlated to Γ_{POPC}, and the equivalent percentage of POPC drop coverage, based on the work of Mitsche and Small,[57] Following adsorption of apoC-I to the POPC/TO/W interface lowering γ to a γ_e, the bulk buffer was exchanged with 150 mL of 2 mM PB (pH 7.4) devoid of apoC-I. The volume of the TO drop was then linearly increased at a rate of 0.02 μL/s until the volume reached 30 μL. After γ had been allowed to equilibrate for ≥200 s, the volume was linearly decreased at a rate of 0.02 μL/s with the lower surface area limit varying, based on the condition that γ remain above 5.0 mN/m. This protocol was repeated for several Π_I values, and pressure–area (Π–*A*) isotherms were calculated for each compression and expansion.

RESULTS

ApoC-I Is Surface Active and Binds to Triolein/Water (TO/W) and Palmitoyloleoylphosphatidylcholine/Triolein/Water (POPC/TO/W) Interfaces. Changes in interfacial tension (γ) upon injection of apoC-I into the aqueous phase reveal the peptide's surface activity at both TO/W and POPC/TO/W interfaces. Figure 2A shows a typical set of interfacial tension curves of the TO/W interface with varying concentrations of apoC-I in the aqueous phase. The initial γ of the TO/W interface was ~32 mN/m. When varying concentrations of apoC-I were added to the aqueous phase (7.5×10^{-8} to 1.0×10^{-6} M), the peptide adsorbed onto the TO/W interface, lowering γ and approaching a γ_e. At the lowest concentration (7.5×10^{-8} M), a lag time of ~800 s was observed before a sharp decrease in γ tapered into a gradual decrease approaching a γ_e between 1700 and 4000 s. As the apoC-I concentration increased, the lag period became shorter and the steepness of the rapid decrease in γ increased. Higher peptide concentrations also exhibited adsorption to lower γ_e

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values. The net changes in γ ranged from 16.5 mN/m ([apoC-I] = 7.5×10^{-8} M) to 18.0 mN/m ([apoC-I] = 1.0×10^{-6} M).

Figure 2B shows the set of interfacial tension curves of the POPC/TO/W interface with varying concentrations of apoC-I in the aqueous phase. The initial γ of the POPC/TO/W interface was ~24−25 mN/m. Addition of apoC-I to the aqueous phase was chosen as relative time zero. The adsorption curves of apoC-I at this interface also showed three regions: a lag period during which γ decreased slowly with time, a second period with a faster decrease in γ, and a final period characterized by a gradual decrease in γ upon its approach to a γ_e. Similar to apoC-I at a TO/W interface, as the concentration of apoC-I increased at a POPC/TO/W interface, the lag period became shorter and the steepness of the rapid decrease in γ increased. Higher peptide concentrations did not, however, exhibit adsorption to lower γ_e values. Net changes in γ ranged from 15.5 mN/m ([apoC-I] = 1.0×10^{-7} M) to 16 mN/m ([apoC-I] = 5.0×10^{-7} M). These values are comparable to those for apoC-I at a TO/W interface. Consequently, apoC-I is surface active at both TO/W and POPC/TO/W interfaces, lowering interfacial tension in a similar manner and amount.

ApoC-I Desorbs from TO/W and POPC/TO/W Interfaces on Compression. Following adsorption of apoC-I to TO/ or POPC/TO/W interfaces, changes in γ induced by instantaneous compressions and subsequent expansions of the TO drop allow for determination of the nature of peptide desorption and readsorption. Figure 3A shows γ and area changes during the sudden compression and re-expansion of the TO/W interface for apoC-I before and after buffer exchange. After apoC-I adsorption lowered γ to a γ_e of ~15.0 mN/m, the 16 μL TO drop was compressed by decreasing its volume in increments varying from 1 to 10 μL. This corresponded to area changes of 2−55%. Instantaneous decreases in γ increased with the size of compression. With a compression of 4 μL, γ decreased by ~2 mN/m, while a compression of 10 μL decreased γ by ~4 mN/m.

When the compressed volume was held for several minutes, γ rose toward a γ_e slightly lower than the initial γ_e. This rise in γ indicates that peptide desorbs from the surface after instant compression. Because the new γ_e values are lower than 15.0 mN/m, particularly for larger compressions (Figure 3A), this indicates that bound apoC-I is compressed and potentially undergoes conformational rearrangement at the surface as Π increases. When the drop was re-expanded back to 16 μL, γ increased above the initial γ_e. Within a relatively short time (<60s), however, peptide readsorbed and γ fell back to ~15.0 mN/m, the initial γ_e.

Buffer (150 mL) was exchanged (indicated by the bar in Figure 3A), and the apoC-I concentration was reduced to virtually zero. γ rose during the buffer exchange from ~15.0 to ~17.0 mN/m. This indicates that some bound apoC-I may desorb from the surface. The instant compression and expansion procedure was repeated with the TO drop (Figure 3A, right). A notable difference was that γ remained at or near heightened γ values following each re-expansion. If one AαH desorbed on compression and readily readsorbed on re-expansion, γ would decrease to a γ_e of ~17 mN/m. γ would remain high following each re-expansion only if the entire peptide desorbed from the surface, with virtually no peptide molecules in the aqueous phase available to readsorb and lower γ.

Figure 3B shows γ and area changes during the sudden compression and expansion of apoC-I at the POPC/TO/W interface before and after buffer exchange. Upon POPC absorption, γ fell to 23.2 ± 0.1 mN/m. A 250 mL buffer exchange caused γ to rise to 25.0 ± 0.3 mN/m, because of exchange of POPC with the bulk solution. ApoC-I was added in the aqueous phase, and γ approached a γ_e of 9.5 ± 0.1 mN/m. The TO drop was compressed by a decrease in its volume in increments ranging from 1 to 6 μL, corresponding to area changes of 2−22%.

Similar to γ responses at the TO/W interface, each instant compression caused an instantaneous decrease in γ, but γ rose toward a new γ_e when the compressed volume was held for several minutes. The new γ_e values were lower than the initial γ_e of the interface. This can be most noticeably seen in the γ_e reached (8.1 ± 0.2 mN/m) after the two 6 μL compressions immediately preceding the buffer exchange in Figure 3B. This again indicates that apoC-I could undergo conformational rearrangement as its AαHs are compressed as Π increases. After each re-expansion, apoC-I readsorption lowered γ to a γ_e similar to the initial γ_e (9.7 ± 0.2 mN/m in this case). This is preliminary evidence that apoC-I does not remove POPC when it desorbs from the POPC/TO/W interface. If apoC-I removed POPC as it desorbed upon compression, γ_e following re-expansion and readsorption of apoC-I would have risen to ~15.0 mN/m, that of a POPC-free surface.

ApoC-I was removed from the aqueous phase by buffer exchange (indicated by the bar in Figure 3B), and γ_e rose to 11.9 ± 0.1 mN/m, indicating that some apoC-I desorbs. An instant compression and expansion protocol similar to that before buffer exchange was repeated (Figure 3B, right). The POPC/TO/W interface, like the TO/W interface, did not relax back to γ_e levels after re-expansion but remained at or near the heightened γ. Consequently, at both interfaces, the entire apoC-I molecule is expelled upon compression and readsorbs when present in the bulk solution on re-expansion. Ejection of the entire peptide on compression is characteristic of exchangeable apolipoproteins.[52,53,55,57] The 44 N-terminal residues ([1−44]apoA-I) and 46 C-terminal residues ([198−243]apoA-I) of apoA-I are comprised of one and three AαHs, respectively, and completely desorb from and readsorb to TO/W[55] and POPC/TO/W[57] interfaces.

Π_{max} Values for ApoC-I at TO/W and POPC/TO/W Interfaces. Instant compression and re-expansion measurements were taken at different apoC-I concentrations to estimate the pressure (Π_{max}) at which the entire peptide, as shown in Figure 3, is ejected from each interface. Differences in Π_{max} between the TO/W and POPC/TO/W interfaces would elucidate the role of apoC-I−POPC interactions in retention of the peptide on the lipoprotein surface. Because the initial pressure (Π_I) at the POPC/TO/W interface prior to apoC-I adsorption was always ~7.0 mN/m (γ_I = 25.0 mN/m) for these measurements, the Π_{max} value corresponds to a surface with roughly 38% POPC coverage. The data points shown in Figure 4 represent compression and re-expansion measurements prior to buffer exchange at both interfaces. Figure 4 shows that the Π_{max} of apoC-I at a TO/W interface is 16.8 mN/m (●) and the Π_{max} of apoC-I at a POPC/O/W interface is 20.7 mN/m (○). The greater Π_{max} of apoC-I at a POPC/TO/W interface suggests that apoC-I binds more strongly to a POPC/TO/W interface than a TO/W interface. Interactions of the apoC-I AαH with POPC likely increase its affinity for the interface.

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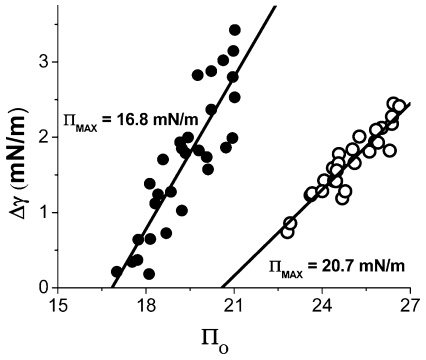

Figure 4. Π_{max} of apoC-I at the TO/W (●) and POPC/TO/W (○) interfaces. Each interface was instantly compressed to pressure Π_o. The $\Delta\gamma$ while the compressed volume was held for several minutes was plotted vs Π_o. Linear regression of the data at each interface yielded intercepts at $\Delta\gamma = 0$ equivalent to Π_{max}, the pressure at which peptides show no net desorption or adsorption. The data shown for both interfaces represent mixed compression and expansion experiments after adsorption of varying concentrations of apoC-I, but before buffer exchange.

Upon ApoC-I Adsorption, the POPC/TO/W Interface Shows Greater Elasticity Than the TO/W Interface. The viscoelasticity of apoC-I at a TO/W or POPC/TO/W interface can be determined by oscillating either interface. Viscoelasticity represents a surface's ability to reduce deviations of γ from its equilibrium value by allowing for relaxation processes.[60] Typical relaxation processes consist of periodic adsorption and desorption of surface-active material (here, apoC-I) from expanded and compressed surface elements. This causes a change in the surface being compressed versus that being expanded in an oscillation shown as a hysteresis between the respective pressure−area (Π−A) isotherms.

Derived from the measurements of γ and A during oscillations, the elasticity modulus (ε) is defined as the increase in γ corresponding to a small increase in the area of a surface element [$\varepsilon = d\gamma/d(\ln A)$]. The phase angle ($\varphi$) represents the difference between the real and imaginary components of ε. A higher ε and lower φ, therefore, indicate a surface more resistant to deformations or changes. If interactions of apoC-I with POPC retain the peptide at higher Π values, as indicated by a higher Π_{max} (Figure 4), apoC-I should be more elastic at a POPC/TO/W interface than at a TO/W interface. This would be marked by weaker hysteresis in oscillation Π−A isotherms, greater ε values, and lower φ values.

Figure 5 shows Π−A isotherms derived from equilibrium oscillations of apoC-I at TO/W (A) and POPC/TO/W (B) interfaces at different amplitudes and periods prior to buffer exchange. Two sets of Π−A isotherms for apoC-I at a TO/W interface, each representing two or three periods for the given amplitude, are shown in Figure 5A. The Π−A isotherms for apoC-I at a TO/W interface show significant hysteresis between compression and expansion when oscillated at larger amplitudes (±8 μL) and periods (32 and 128 s, represented by cyan and magenta lines, respectively). This indicates that sufficiently large changes in Π and sufficient time must be provided for observable desorption of apoC-I on compression.

The top half of Table 1 shows the ε and φ values calculated (as described in Materials and Methods) from the Π−A isotherms depicted in Figure 5A. Such analysis reveals ε and φ values at the TO/W interface ranging from 20.4 to 29.6 mN/m and from 10.6° to 23.5°, respectively, upon oscillations at amplitudes of ±2 and ±8 μL. With increasing periods at a small

Table 1. Dynamic Interfacial Properties of ApoC-I at Different Interfaces[a]

interface	[apoC-I] (M)	$V \pm \Delta V$ (μL)	period (s)	ε (mN/m)	φ (deg)
TO/W	5.0×10^{-7}	16 ± 2	8	29.6	10.6
		16 ± 2	32	25.6	15.1
		16 ± 2	128	20.4	23.5
		16 ± 8	32	27.7	10.7
		16 ± 8	128	25.6	12.3
POPC/TO/W	1.0×10^{-7}	18 ± 2	8	51.5	6.4
		18 ± 2	32	51.2	7.8
		18 ± 2	128	48.4	12.6
		18 ± 4	8	47.7	6.0
		18 ± 4	32	49.8	4.5
		18 ± 4	128	48.9	7.6

[a]Analysis of the Π−A oscillation isotherms sampled in Figure 5. Definitions: [apoC-I], concentration of apoC-I in the aqueous phase; V, initial drop volume; ΔV, oscillation amplitude; ε, elasticity modulus; φ, phase angle, a phase difference between $d\gamma$ and dA.

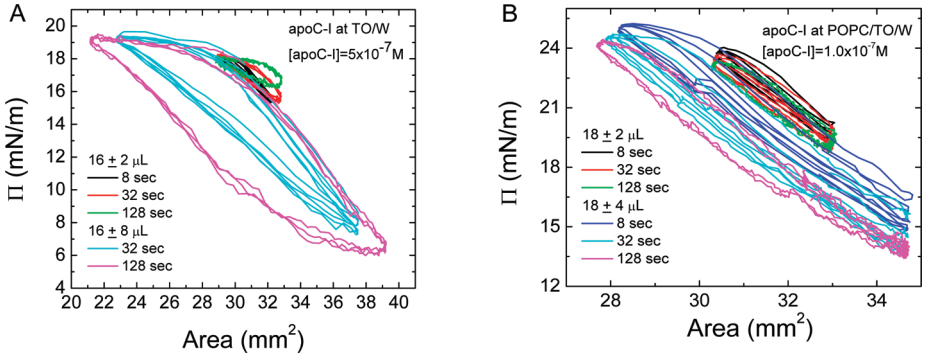

Figure 5. Plots of surface pressure (Π) vs area for apoC-I at TO/W (A) and POPC/TO/W (B) interfaces derived from oscillations prior to buffer exchange. (A) After the γ_e for apoC-I at the TO/W interface had been reached, the 16 μL TO drop was oscillated at 16 ± 2 μL (Π changes of approximately ±2 mN/m) and 16 ± 8 μL (Π changes of approximately ±6.5 mN/m) at periods of 8−128 s. The apoC-I concentration in the aqueous phase was 5.0×10^{-7} M. (B) After the γ_e for apoC-I at the POPC/TO/W interface had been reached, the volume of the TO drop was increased to 18 μL. The drop was oscillated at 18 ± 2 μL (Π changes of approximately ±2.5 mN/m) and 18 ± 4 μL (Π changes of approximately ±6 mN/m) and periods of 8, 32, and 128 s. The apoC-I concentration in the aqueous phase was 1.0×10^{-7} M.

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amplitude of ±2 μL, ε decreases by 9.2 mN/m and φ increases by 12.9°. These significant changes reveal more apoC-I desorbs from and readsorbs to the surface with a greater oscillatory period, such that the surface is more viscoelastic.

Two sets of Π−*A* isotherms for apoC-I at a POPC/TO/W interface, each representing three periods for the given amplitude, are shown in Figure 5B. The Π−*A* isotherms for apoC-I at a POPC/TO/W interface show significantly less hysteresis between compression and expansion than those at a TO/W interface (Figure 5A). The lower half of Table 1, representing analysis of Figure 5B, reveals ε and φ values for the POPC/TO/W interface ranging from 48.4 to 51.5 mN/m and from 4.5° to 12.6°, respectively, at oscillation amplitudes of ±2 and ±4 μL. Compared to those for a TO/W interface, these higher ε and lower φ values indicate apoC-I is more elastic at a POPC/TO/W interface. With increasing periods at a small amplitude of ±2 μL, the change in ε is insignificant (3.1 mN/m) and φ increases by 6.2°. Compared to those of a TO/W interface oscillated at the same amplitude, these changes are much smaller, indicating apoC-I binds with a higher affinity to a POPC/TO/W interface, creating a more elastic surface.

The small φ and slight hysteresis in oscillations of apoC-I at a POPC/TO/W interface are indicative of a very small fraction of apoC-I, not POPC, desorbing from the interface. A series of control oscillations of the POPC/TO/W interface at varying initial Π values were performed over a wide range of amplitudes and periods (data not shown), and no hysteresis was observed, even for large amplitudes. This suggests that the interface is nearly completely elastic, such that there is no surface relaxation in which POPC desorbs and the lag between area changes and tension responses (φ) is nearly zero.[60]

The Larger Percentage of POPC Coverage at the POPC/TO/W Interface Causes Greater Retention of ApoC-I upon Compression. To determine the effect of varying Γ_{POPC} on surface retention of apoC-I, TO drops with apoC-I bound and increasing Γ_{POPC} underwent slow (±0.02 μL/s) expansions and subsequent compressions. This allows for quantitative comparison of the point on compression (the envelope point) at which apoC-I begins to be expelled from the POPC/TO/W interface. Figure 6 shows compression Π−*A* isotherms of the POPC/TO/W interface when apoC-I adsorbs to the POPC/TO/W interface at various initial interfacial tension values (γ_I). Greater γ_I values correspond to a lower percentage of phospholipid coverage on the TO drop. Because Π = 32.0 mN/m − γ, lower Π_I values correspond to lower POPC surface concentrations (Γ_{POPC}) while higher Π_I values correspond to higher Γ_{POPC} values.[57] As listed in Table 2, Π_I values prior to apoC-I adsorption vary from 5.0 mN/m (31.8% of the drop is coated with POPC) in line a to 13.4 mN/m (51.8% of the drop is coated with POPC) in line d. Line e represents the Π−*A* compression isotherm for a POPC/TO/W interface devoid of apoC-I. As it does not overlap with the other compression isotherms, it shows that apoC-I adsorbed to and was being ejected from the POPC/TO/W interface in lines a−d.

In Figure 6, the envelope point (asterisk) represents the Π and *A* at which apoC-I begins to be ejected from the POPC/TO/W interface upon compression (direction indicated by an arrow). Visually, the envelope point is the point at which the slope of the compression isotherm dramatically decreases as area decreases. As Γ_{POPC} on the interface increases, the envelope point Π (Π_E) increases, while the envelope point area (A_E) decreases. As shown in Table 2, Π_E for apoC-I at the POPC/TO/W interface increases from 21.2 mN/m at the lowest Γ_{POPC} to 26.4 mN/m at the highest Γ_{POPC}. Conversely, A_E decreases by 15.1 mm^2 from 37.3 to 22.2 mm^2 over the same change in Γ_{POPC}. These data suggest that the higher the Γ_{POPC} on the POPC/TO/W interface, the greater the affinity of apoC-I for the interface and the more difficult it is to expel apoC-I from the surface. Consequently, apoC-I is retained up to higher pressures and smaller areas on the lipoprotein surface as Γ_{POPC} increases.

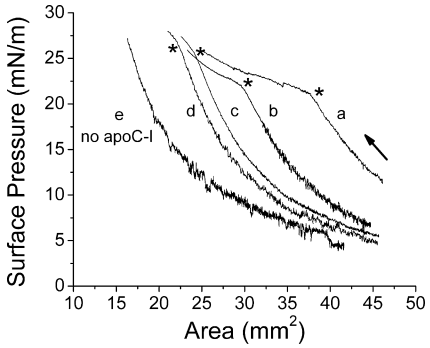

Figure 6. Pressure−area (Π−*A*) compression isotherms for apoC-I adsorbed to a POPC/TO/W interface at various initial Π values. ApoC-I, added at a concentration of 2.5 × 10^{-7} M in the aqueous phase, adsorbed to POPC/TO/W interfaces at various initial pressures (Π_I), listed for each curve in Table 2. After buffer exchange, each surface was slowly expanded and sequentially compressed. Shown here are compression isotherms, with the direction of compression marked by an arrow. Asterisks mark isotherm envelope points, where an abrupt change in slope indicates the beginning of ejection of apoC-I from the surface. Values of envelope pressure (Π_E) and area (A_E) for each curve are listed in Table 2. Line e represents the Π−*A* compression isotherm for the POPC/TO/W interface devoid of any apoC-I.

Table 2. Envelope Pressures and Areas for ApoC-I at POPC/TO/W Interfaces with Increasing Γ_{POPC} Values[a]

curve	Π_I (mN/m)	Γ_{POPC} (%)	Π_E (mN/m)	A_E (mm^2)
a	5.0 ± 0.2	31.8 ± 0.4	21.2 ± 0.2	37.3 ± 0.2
b	7.4 ± 0.2	38.2 ± 0.5	22.6 ± 0.2	28.6 ± 0.2
c	12.8 ± 0.2	50.6 ± 0.4	25.6 ± 0.3	24.2 ± 0.3
d	13.4 ± 0.2	51.8 ± 0.4	26.4 ± 0.3	22.2 ± 0.3

[a]Analysis of the Π−*A* compression isotherms shown in Figure 6. Definitions: Π_I, initial pressure of the POPC/TO/W interface before apoC-I adsorption; Γ_{POPC}, initial POPC surface concentration before apoC-I adsorption, expressed as a percentage of total TO drop area; Π_E and A_E, envelope pressure and area, respectively, or the pressure and corresponding area, respectively, at which there is an abrupt change in the slope of the Π−*A* isotherm signifying ejection of apoC-I from the surface.

DISCUSSION

On the surface of lipoproteins, it is reasonably assumed that apolipoproteins, including apoC-I, interact with phospholipids located on the surface.[55] It is also possible that apolipoproteins interact with core molecules, such as TAG or CE, notably when the number of surface lipid molecules is too small to adequately cover the core.[55] In this study, our goals were twofold. First, we sought to characterize the surface activity and reversible binding of human apoC-I at the TO/W interface and the more physiological POPC/TO/W interface. Second, we sought to understand the effects of interactions of apoC-I with POPC on

desorption of the peptide from and retention on the lipoprotein surface.

The first objective of this study was to demonstrate the surface activity of apoC-I at TO/W and POPC/TO/W interfaces. Figure 2 demonstrates the surface activity of apoC-I at both TO/W and POPC/TO/W interfaces. An increase in the rate of peptide adsorption was observed as the concentration of apoC-I in the aqueous phase increased. The decrease in γ (or increase in Π) upon binding to each interface was comparable, equal to 16.5−18.0 or 15.5−16.0 mN/m, respectively. At a concentration of 1.2×10^{-6} M, similar to that of apoC-I used at a TO/W interface, [1−44]apoA-I and [198−243]apoA-I could increase Π by ∼13.0 mN/m at a TO/W interface.[55] This suggests a higher surface affinity for apoC-I than the C- and N-termini of apoA-I.

The observed surface affinity of apoC-I for either interface is imparted by its structure. Apolipoproteins exhibit strong lipid binding properties because of their characteristic secondary structures, containing amphipathic α-helix (AαH) or amphipathic β-sheets (AβS).[29,49−53,61] The lipid-binding motifs of apoC-I lie in its predicted N- and C-terminal AαHs,[25,28] as shown in Figure 1. The hydrophobic face of each AαH (marked by a dotted line) is essential for interactions with apolar moieties on phospholipids (here, POPC) and neutral lipids (here, TO). According to the scale determined by Wimley and White,[62] the $\Delta G_{b\rightarrow w}$ of partitioning for the hydrophobic face of the N-terminal AαH is 3.76 kcal/mol, or 0.42 kcal/mol per residue. The $\Delta G_{b\rightarrow w}$ for the hydrophobic face of the C-terminal AαH is 4.13 kcal/mol, or 0.69 kcal/mol per residue. Preferential lipid binding is implicated in the unfavorable, positive $\Delta G_{b\rightarrow w}$ of transfer from a bilayer interface to the aqueous phase for the hydrophobic faces of both AαHs.

In association with its surface activity, we sought to determine the nature of binding of apoC-I to TO/W and POPC/TO/W interfaces. AαH structures typically show reversible binding to the TO/W surface, desorbing from and readsorbing to the surface upon compression and re-expansion.[49,52,55] Figure 3 reveals that apoC-I reversibly binds to both interfaces, desorbing when each interface is instantaneously compressed above Π_{max}. ApoC-I's complete peptide desorption is evident in the γ responses of both interfaces for compression−expansion protocols after buffer exchange (right side of Figure 3A,B). With peptide present in the aqueous phase (Figures 3A,B), apoC-I readsorbs to both interfaces on re-expansion, decreasing γ to its initial equilibrium value (15.0 and 9.5 mN/m, respectively).

The left halves of panels A and B of Figure 3 suggest conformational rearrangement on compression of the AαHs of apoC-I prior to complete peptide desorption at both interfaces, as the γ_e values reached after each compression are lower than the initial γ_e. The rise in γ at both interfaces due to the buffer exchange depicted in panels A and B of Figure 3 further suggests peptide conformational rearrangement at the surface, potentially followed by complete peptide desorption into the aqueous solution. To explain such conformational rearrangement, results of previous studies may suggest a two-step desorption model for apoC-I, with one AαH desorbing prior to complete peptide ejection.

Compression of apoC-I deposited on a DPPC monolayer at an A/W interface revealed two phase transitions.[46−48,73] The first occurs between Π values of 24 and 27 mN/m and the second at a Π of ∼49 mN/m. Brewster angle microscopy showed that only apoC-I was affected in each phase transition.[46−48] This led to the proposal that, at Π values from 24 to 27 mN/m, one AαH on apoC-I desorbs from the subphase, aligning itself with the air, in a direction similar to that of the hydrophobic DPPC acyl chains.[46,73] The entire peptide was ejected from the monolayer at a Π of ∼49 mN/m.

Indicative of the order of apoC-I AαH desorption, the amide proton resonance line widths and deuterium exchange rates of the NMR structure of apoC-I−sodium dodecyl sulfate complexes suggest that the N-terminal AαH binds less tightly to the detergent than the C-terminal AαH.[25] In agreement with these results, James et al.[36] used mass spectrometry to show that the C-terminal region was the portion of apoC-I most highly protected from proteolysis. DMPC clearance assays and electron microscopy further revealed that single or double mutations in the apolar face of the C-terminal AαH of F42 and F46 to G or A result in a 2−3.5-fold reduction in the affinity of apoC-I for DMPC vesicles and apoC-I−DMPC complexes with atypical diameters.[36] However, CD studies of apoC-I−DMPC complexes show that interruptions in the apolar face of either the N-terminal (G15P) or C-terminal (T41P and T45P) AαH greatly reduce the level of protein−lipid binding.[20,28] Of all explored mutations, these also are the least able to reconstitute apoC-I−DMPC disks on thermal renaturation.[20,28] This indicates an essential cooperativity in binding of the α-helices to the phospholipid surface.[20] The thermal denaturation and reconstitution of apoC-I−DMPC complexes, even at low ramp rates and small temperature changes, failed to show multiphase kinetics indicative of a two-step peptide desorption from the lipid−water interface.[20,28,34,35]

If apoC-I follows a two-step desorption from the lipoprotein surface, the NMR structure of apoC-I and greater $\Delta G_{b\rightarrow w}$ values for the hydrophobic face of the C-terminal AαH predict that the N-terminal AαH desorbs prior to complete peptide desorption. Consequently, in the seconds following each instantaneous compression in Figure 3, the N-terminal AαH desorbs from the surface. This conversion is followed by a longer period of desorption of the C-terminal AαH of apoC-I to reach a new peptide equilibrium with the bulk solution.

Having characterized the reversible binding of apoC-I to TO/W and POPC/TO/W interfaces, we then sought to determine the effect of POPC on apoC-I surface binding and retention. To that end, pressure Π_{max}, at which the entire peptide begins to be ejected from TO/W and POPC/TO/W interfaces, was determined. Figure 4 reveals a Π_{max} value of 16.8 mN/m for apoC-I at a TO/W interface. This Π_{max} is consistent with previous findings for AαHs at a TO/W interface, falling within the range of 13−19 mN/m.[49,52,55] Comparatively, the Π_{max} values for [198−243]apoA-I and [1−44]apoA-I are 16.2 and 13.2 mN/m, respectively.[55] Π_{max} for apoC-I at a POPC/TO/W interface depends on Γ_{POPC}. Figure 4 reveals that apoC-I has a Π_{max} of 20.7 mN/m when ∼38% of the TO drop is covered by POPC. This higher Π_{max} at a POPC/TO/W interface is likely caused by interactions of AαH with phospholipids and neutral lipids increasing the affinity of the peptide for the surface, keeping it bound at higher Π values.

The increase in the affinity for the interface induced by apoC-I−POPC interactions was further explored by sinusoidal oscillations of both interfaces following apoC-I adsorption. Figure 5 reveals the elastic properties of apoC-I at TO/W and POPC/TO/W interfaces. Previous studies have revealed that apoA-I, the major apolipoprotein constituent of HDL and primary activator of LCAT, binds TO/W interfaces via its AαH structural motifs and creates a highly flexible viscoelastic

interface.[52] Similarly, analysis of large oscillations or small oscillations with a slow period in Figure 5A reveals apoC-I forms a viscoelastic surface at the TO/W interface, marked by a large hysteresis in compression and expansion Π−*A* isotherms, a low ε, and a large phase angle (φ).

ApoC-I at a TO/W interface exhibits a marked hysteresis in its Π−*A* oscillation isotherms only at compressions of larger amplitudes and longer periods. This supports the two-step model of desorption of apoC-I from the interface, as ample time must be provided for the N-terminal AαH to desorb prior to complete peptide desorption. Oscillations with an amplitude of ±2 μL over a range of increasing periods yield Π−*A* isotherms for apoC-I at a TO/W interface with φ increasing by 12.9° from 10.6° to 23.5° and ε decreasing by 9.2 mN/m from 29.6 to 20.4 mN/m.

Comparatively, the Π−*A* isotherms for apoC-I at a POPC/TO/W interface, oscillated over a range of amplitudes and periods, exhibit a much smaller hysteresis, even at larger amplitudes and longer periods. This is indicative of a more elastic interface. Oscillations with an amplitude of ±2 μL over a range of increasing periods yield Π−*A* isotherms for apoC-I at a POPC/TO/W interface with φ increasing by only 6.2° from 6.4° to 12.6° and ε decreasing by only 3.1 mN/m from 51.5 to 48.4 mN/m. Compared to similar oscillations at a TO/W interface, these higher ε and lower φ values are more resistant to change at a POPC/TO/W interface, which demonstrates a more elastic interface. These data indicate that interactions of the apoC-I AαH with POPC increase its affinity for and retention on the surface, thereby increasing surface elasticity.

To elucidate the role of AαH−phospholipid interactions on peptide retention at the lipoprotein surface, TO drops with apoC-I bound and increasing Γ_{POPC} values underwent slow expansions and subsequent compressions. Figure 6 and Table 2 show the dependency of the expulsion of apoC-I from the POPC/TO/W interface on the Γ_{POPC} of the TO drop. Increases in the Γ_{POPC} of the interface yield increases in Π_E and decreases in A_E, that is, the envelope pressure and area, respectively, at which apoC-I begins to be ejected from the interface. When POPC covers 32.8% of the TO drop surface (line a in Figure 6), Π_E = 21.2 ± 0.2 mN/m and A_E = 37.3 ± 0.2 mm^2, while at a POPC coverage of 51.8% (line d), Π_E = 26.4 ± 0.3 mN/m and A_E = 22.2 ± 0.3 mm^2. These results reveal that larger POPC:apoC-I ratios cause retention of apoC-I at higher Π values and more compact areas.

On the basis of the findings of this study, we propose possible mechanisms by which apoC-I may contribute to steps of lipoprotein remodeling, including CETP inhibition and prevention of hepatic uptake of TAG-rich lipoproteins. To inhibit CETP activity, the N-terminus of apoC-I could desorb from the lipoprotein surface, interacting with CETP and thereby interfering with its function.[7,36] With no discovered CETP-binding domain on apoC-I, a more likely mechanism of inhibition[66] is one in which apoC-I has a higher affinity for the lipoprotein surface than CETP or its activators: apolipoproteins A-I, A-II, A-IV, and E.[63−67] ApoA-IV has the weakest lipid affinity of any apolipoprotein,[68,69] and human C-apolipoproteins have been shown to displace apoA-IV from TAG-rich lipoprotein surfaces.[69] The C-terminus of apoA-I is predicted to be the most lipophilic portion of apoA-I, with the N-terminal helix bundle providing stability at the lipoprotein surface,[70−72] yet as shown, apoC-I causes greater changes in Π and has a higher Π_{max} than the C-terminus of apoA-I.[55] In this model of inhibition, apoC-I, at a sufficiently high plasma concentration, could bind TAG-rich lipoproteins or compact HDL particles and drastically increase Π. These Π modifications could displace bound CETP and CETP activators or prevent them from binding.[66] Structurally similar to apoA-I, apoE could also be displaced from β-VLDL by apoC-I-induced Π modifications, thereby inhibiting lipoprotein uptake.[16−18,72]

In summary, our interfacial studies reveal that apoC-I is surface-active at both TO/W and POPC/TO/W interfaces, binding the interfaces via its two amphipathic α-helices and lowering interfacial tension, γ. ApoC-I exhibits properties of an exchangeable apolipoprotein, desorbing upon compression and readsorbing upon re-expansion of either interface. Interactions of the AαH with POPC increase the affinity of apoC-I for the interface, demonstrated by a higher Π_{max} when roughly 38% of the interface was covered with POPC. Interactions of the AαH with POPC also increase surface elasticity, demonstrated by a higher elasticity modulus (ε), a lower phase angle (φ), and reduced Π-A isotherm hysteresis during interface oscillation. Finally, the affinity of apoC-I for the POPC/TO/W interface increases as Γ_{POPC} increases, which is evident in higher envelope point Π values at lower envelope areas upon compression. We propose that the high-affinity binding of apoC-I to lipoprotein surfaces provides a possible model for inhibition of CETP activity by displacing or preventing the binding of its apolipoprotein activators. Similarly, the large changes in Π upon binding to triacylglycerol-rich lipoproteins could displace or prevent the binding of apoE to inhibit their receptor-mediated uptake.

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Notes

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ABBREVIATIONS

apoC-I, apolipoprotein C-I; HDL, high-density lipoprotein; VLDL, very-low-density lipoprotein; IDL, intermediate-density lipoprotein; LCAT, lecithin:cholesterol acyltransferase; CE, cholesterol ester; CETP, cholesterol ester transfer protein; LDL, low-density lipoprotein; TAG, triacylglycerol; HL, hepatic lipase; LPL, lipoprotein lipase; LRP, low-density lipoprotein receptor-related protein; AαH, amphipathic α-helix; A/W, air/water; DPPC, dipalmitoylphosphatidylcholine; POPC, 1-palmitoyl-2-oleoylphosphatidylcholine; TO/W, triolein/water; PBS, phosphate-buffered saline; SUV, small unilamellar vesicle; [1−44]apoA-I, 44 N-terminal residues of apoA-I; [198−243]apoA-I, 46 C-terminal residues of apoA-I; AβS, amphipathic β-sheet; DMPC, dimyristoylphosphatidylcholine; LDLR, LDL receptor; VLDLR, VLDL receptor.

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