Interfacial Properties of an Amphipathic α-Helix Consensus Peptide of Exchangeable Apolipoproteins at Air/Water and Oil/Water Interfaces*

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Amphipathic α-helices are the main structure and the major lipid binding motif of exchangeable apolipoproteins. To understand how these apolipoproteins behave at a hydrophobic lipoprotein interface, the interfacial properties of a consensus sequence peptide (CSP) derived from three exchangeable apolipoproteins (A-I, A-IV, and E) were studied using an oil drop tensiometer at air/water (A/W) and dodecane/water (DD/W) interfaces. CSP (PLAEELRALRAQLEELRERLG2-NH2) contains two 22-amino acid tandem repeat sequences that form amphipathic α-helices. CSP, when added into the aqueous phase, lowered the interfacial tension (γ) of A/W and DD/W in a concentration-dependent fashion. The γA/W was lowered −24 mN/m, and γDD/W −31 mN/m, indicating a greater affinity of CSP for DD/W. Using the Gibbs equation for surface, the surface area per CSP molecule was estimated at −702 Å2 (−16 Å2/aminio acid) on A/W and −622 Å2 on DD/W (−14 Å2/aminio acid) suggesting that adsorbed CSP lies flat with α-helices in the plane of both interfaces. At equilibrium γ, CSP desorbed from the interface when compressed and re-adsorbed when expanded. The adsorption rate was concentration-dependent, but the desorption rate was not. Less CSP desorbed from DD/W than A/W indicating that CSP has higher affinity for DD/W. Dynamic analysis of elasticity shows that the faster the oscillation period (4, 8 s) and the lower the oscillation amplitude the more elastic the surfaces. CSP can be compressed 6–12% while remaining on the surface, but large increases in pressure eject it from the surface. We suggest that surface pressure-mediated desorption and readesorption of amphipathic α-helices provide lipoprotein stability during remodeling reactions in plasma.

High density lipoproteins (HDLs) are small aggregates of exchangeable apolipoproteins (apoA-I, apoA-II, apoC’s, and apoE, etc.) and lipids that reside in the plasma and are important mediators in reverse cholesterol transport. They also play roles as cofactors for a number of plasma lipoprotein modifying enzymes (e.g. lechthin:cholesterol acetyltransferase, lipoprotein lipase). For many years it has been known (1) that apolipoproteins can exchange off of HDL onto other lipoproteins like very low density lipoprotein and move back and forth between different lipoprotein classes. Thus they are located at the lipoprotein/water interface. The predominant secondary structural feature of exchangeable apolipoproteins is the amphipathic α-helix (2), which has been described and studied by a number of investigators (1–3). These structural units are about 20 amino acids in length, fold into an α-helix, and present one face, sub-tending less than 180°, as a band of hydrophobic amino acids. It is this hydrophobic face along the amphipathic α-helix that allows it to bind to the lipoprotein surfaces. A variety of different kinds of amphipathic α-helices have been described (4), but the specific function of the different classes is not clear. The class A amphipathic helix (3, 4) is a major lipid binding motif of the exchangeable apolipoproteins and is also found in the α2 and α3 domains of apoB (6). It consists of a narrow band of hydrophobic amino acids on one face. At the hydrophilic/hydrophobic boundaries lysines and arginines give a positive charge, and acidic residues are located on the face opposite the hydrophobic side.

The interaction of soluble, exchangeable apolipoproteins with phospholipids and combinations of phospholipid together with hydrophobic core lipids (triglyceride, cholesterol ester) has been widely studied (1, 2, 5–13). Many soluble apolipoproteins can spontaneously penetrate and solubilize multilamellar liposomes of dimerstoyl phosphatidylinoline or dipalmityl phosphatidylcholine to form discrete small bilayered discoidal lipoproteins (5, 7–9). To form quasi-spherical HDL-like particles with apoA-I, phospholipids, and cholesterol ester, energy in the form of sonication or chaotropic agents is required (10–13). Apolipoprotein surface behavior and interaction with phospholipids spread at the air/water interface have been studied extensively, primarily by Phillips and others (14–22). In these experiments the surface pressure of the phospholipid monolayer was varied, and the increase in pressure upon injection of apolipoprotein into the sub-phase was recorded. The increase in pressure is because of the fact that the apolipoprotein penetrates the interface and compresses the lipid monolayer. When the increase in surface pressure is plotted against the initial pressure Πo of the monolayer a straight line is generated that extrapolates to the pressure where the apolipoprotein can no longer penetrate into the monolayer, i.e. no increase in pressure is recorded. This is called the exclusion pressure Πe. For instance, apoA-I has an exclusion pressure of about 32 mN/m (14).

A number of investigators have studied consensus sequences of amphipathic α-helices modeling different sequences of apolipoproteins (8, 9) using the Πe technique. Some of these peptides, as short as 18 amino acids, have high Πe in phospholipid monolayers, and others exhibit lower Πe, depending upon the
specific amino acid arrangement of the amphipathic helical segment. For instance, by substituting phenylalanine (F) for other hydrophobic amino acids in an A-type 18-amino acid α-helix (18A) \( \Pi \) rose from 30 mN/m for 18A – 1F to 46 mN/m for 18A – 6F (23).

A hypothesis suggested by \( \Pi \) studies is that if the interfacial pressure in the phospholipid monolayer on a lipoprotein surface is greater than \( \Pi \) for a given apolipoprotein, then the apoprotein cannot penetrate and bind to the lipoprotein. Conversely, if an apoprotein was present in the lipoprotein surface when the surface pressure increased above \( \Pi \), then that apoprotein would be desorbed from the surface into the aqueous media. This hypothesis suggests that the surface pressure and the kinetics of adsorption/desorption from lipoprotein surfaces governs which soluble apolipoproteins will be present on the lipoprotein. For instance, it has been suggested that LDL has a high surface pressure, because very little apoA-I is present in LDL isolated from plasma (24).

A second hypothesis is that in a complex apolipoprotein such as apoA-I or apoE where a number of different amphipathic helices are predicted to exist (25), an increase surface pressure might partially desorb those helices with a low \( \Pi \) from the interface, whereas those with a high \( \Pi \) would remain attached. This would allow for varying surface coverage by complex soluble apolipoproteins driven by small changes in the interfacial pressure.

Finally, the non-exchangeable apolipoprotein B appears to change its surface area coverage on different sized LDL particles (24, 26). If true, some domains must come off of the surface whereas other parts remain. It has been implied that certain amphipathic α-helices present in apolipoprotein B have the characteristics similar to those in soluble apolipoproteins and that these might be able to pop off the lipoprotein if surface pressure were increased (6). We have reported that consensus peptides modeling amphipathic β-strands in the first amphipathic β-sheet region of apoB (residues 960–1892) binds strongly to hydrophobic surfaces (dodecane/water and triolein/water) and are not readily ejected from the surface by increasing the surface pressure (27). It is our hypothesis that in apoB the amphipathic β-strands and -sheets remain attached as the surface pressure increases, but that amphipathic α-helices can be first compressed and then desorbed from the surface. If the surface pressure falls again, then the amphipathic α-helices can rapidly readsorb. This would allow apoB to cover varying areas of surface depending on surface pressure.

It is generally believed that apolipoproteins interact primarily with the phospholipids hydrocarbon chains on HDL. However, it is possible that they also interact with the hydrophobic core of the HDL particles, and it is probable that they interact with the hydrophobic core of very low density lipoprotein and LDL. Weinberg et al. (22) showed that apoA-I and apoA-IV could lower the triolein/water interface \( \gamma \), but little more is known about the interaction of the apolipoproteins with the hydrocarbon core. Especially, how do amphipathic α-helices lie at the interface, how much do they lower the interfacial free energy, how rapidly do they adsorb or desorb, and are they elastic, and if so under what conditions? Such information will be helpful not only to understand the structure of apolipoproteins on lipoproteins but also to understand the structural changes that occur during remodeling of lipoproteins in plasma and on assembly of apoB containing lipoproteins during synthesis in cells.

This paper compares the interaction of an amphipathic α-helical peptide with a hydrocarbon/water interface and an air/water interface. The peptide consists of two 22-residue, type A amphipathic helices designed from consensus sequences of apoA-I, A-IV, and E3 linked together, making a 44-amino acid peptide called consensus sequence peptide, CSP (25, 28). The results indicate that CSP binds strongly to the hydrocarbon/water interface and the air/water interface to lower the interfacial tension. Thus, this peptide is surface active and lowers the interfacial free energy at the air/water and oil/water interface.

The interaction at the air/water interface differs significantly from that at the oil/water interface. The peptide binds more tightly to the oil/water interface and is displaced less readily by compression of the surface. At equilibrium surface coverage CSP has a very limited compressibility (<12%) on both surfaces, and above a critical surface pressure (\( \Pi_{\text{max}} \)) it is ejected from the surface.

**EXPERIMENTAL PROCEDURES**

**Materials**—The CSP peptide (PLAEELRALKLEELERLRLG2-NH\(_{2}\)), which contains two 22-amino acid tandem repeats derived from the consensus sequence of the tandem repeats of human apoA-I, apoA-IV, and apoE (25), was synthesized at Quality Controlled Biochemical Inc. (Hopkinton, MA) using a Biosynthesis Plus continuous flow synthesizer, and purified to 97% purity. Stock solution of CSP (9–10 mg/ml) was prepared in ultra-filtered water obtained from a Hydro Picosystem (Research Triangle Park, NC). The peptide has been shown to exhibit ~90° α-helical conformation in solution (25, 28) and interact with dimyristoyl phosphatidylcholine to form well defined phospholipid bilayer discoidal complexes. To do interfacial tension measurements, varied amounts of peptide stocks were added to the aqueous phase to obtain different peptide concentrations (from 1.5 × 10\(^{-6}\) to 5 × 10\(^{-6}\) M).

Two interfacial systems were used, air/water and dodecane/water. The pH of the aqueous phase was kept at pH 7.4 with phosphate buffer (2 mM). Dodecane (>99% pure) was purchased from EM SCIENCE (Gibbstown, NJ). All other reagents were of analytical grade.

**Interfacial Tension Measurement**—The interfacial tension of air/water and dodecane/water interfaces in the presence of different amounts of CSP was measured with an I. T. CONCEPT (Longessagne, France) Tracker oil-drop tensiometer (29). Air bubbles (~4 μl), or dodecane drops (~8 μl) were formed in gently stirred pH 7.4 phosphate buffer (6.0 ml) containing a given amount of CSP peptide. The interfacial tension was then recorded continuously until it approached an equilibrium value. All experiments were carried out at 25 ± 0.1°C in a thermostated system.

**Estimation of the Surface Area per Molecule of Peptide**—From the interfacial tension measurements, the equilibrium interfacial tension \( \gamma \) was obtained for each concentration, c, of peptide in the aqueous phase. A plot of \( \gamma \) versus In c was fitted to a straight line, and the slope of the fitted line \( d\gamma/d\ln c \) was obtained. According to the Gibbs equation for the surface (30), the surface concentration of an adsorbed molecule, \( \Gamma \) (mol/cm\(^2\)) = \( -(1/RT)(d\gamma/d\ln c)_T \), was calculated. The surface area per molecule of peptide \( S \) (Å\(^2\)/molecule) = \( 14T \times N \), where \( N \) = Avogadro number, was obtained for different interfaces.

**Compression and Expansion of the Interfaces**—Once the interfacial tension curve approached equilibrium, the air bubble or oil drop was compressed by rapidly decreasing the volume by about 25, 12, or 6%, \( t \), then \( t \) change, then no net desorption or adsorption occurs. To expand the surface, the volume of the oil drop was rapidly increased by about 25, 12, or 6%, which suddenly increases the area and as a result \( \gamma \) abruptly increases. If molecules adsorb from the bulk phase to adhere to the newly formed extra surface, then \( \gamma \) will drop back toward equilibrium. This is called the desorption curve and results from the expansion of the interfacial area to produce new area to which molecules from the bulk can adsorb. These curves can be fitted by a bi-exponential equation: \( \gamma = \gamma_1 + \gamma_2(1 - \exp(-x_{1}t)) + \exp(-x_{2}t) \). The time constants \( t_{1} \) and \( t_{2} \) are related to the desorption and adsorption processes.

**Oscillation of the Interface and the Elasticity Analysis**

**Oscillations in Volume around the Equilibrium Surface Tension**—After the tension curve has reached a near equilibrium value, oscillations of the volume can be made at different amplitudes and periods (i.e., frequencies). The standard protocol for oscillations was carried out...
The same general features were present; however, the starting surface tension (and surface pressure), reached equilibrium. This gave a continuous measurement of the mean interfacial elasticity modulus, $\gamma$, from the slope of the curve plotted against the molar concentration of CSP. Linear regression was made to the plot, $r = -0.99$, $p = 0.00062$, and the slope is $-5.8 \times 10^5$. All experiments were carried out at $25 \pm 0.1 ^\circ C$.

Fig. 1 shows similar data collected at the DD/W interface. An 8-µl dodecane drop was formed in 2 mM phosphate buffer, pH 7.4, with different amounts of CSP, a, no CSP; b, $1.5 \times 10^{-7}$ M; c, $3.0 \times 10^{-7}$ M; d, $6.1 \times 10^{-7}$ M; e, $1.2 \times 10^{-6}$ M; f, $2.4 \times 10^{-6}$ M. The inset shows that the slope, $d\gamma/dt$, of the steep part of the curve plotted against the molar concentration of CSP. Linear regression was made to the plot, $r = -0.98$, $p = 0.0001$, and the slope is $-3.8 \times 10^7$. All experiments were carried out at $25 \pm 0.1 ^\circ C$.

To calculate the elasticity modulus as a function of surface pressure generated by the peptide monolayer at the surface, continuous oscillations were carried out 10 s after a bubble or oil drop was formed in the peptide solution. Allowing 10 s to form the drop allowed the system to stabilize, and then the volume was oscillated by about $\pm 25$, $\pm 12$, or $\pm 6$%. As the volume $V$ was oscillated in a sinusoidal fashion, interfacial area $A$ and surface tension $\gamma$ were recorded continuously, and the phase angle $\phi$ between compression and expansion was computed. The interfacial elasticity modulus $\gamma$ was derived ($\epsilon = d\gamma/dt$). The elasticity real part $\epsilon'$ and the elasticity imaginary part $\epsilon''$ were obtained ($\epsilon' = \epsilon \cos \phi$, $\epsilon'' = \epsilon \sin \phi$) (31, 32).

Continuous Oscillation Starting with Drop Formation—To calculate the elasticity modulus as a function of surface pressure generated by the peptide monolayer at the surface, continuous oscillations were carried out 10 s after an air bubble or oil drop was formed in the peptide solution. Allowing 10 s to form the drop allowed the system to stabilize, and then the volume was oscillated by about $\pm 25$, $\pm 12$, or $\pm 6$%. As the volume $V$ was oscillated in a sinusoidal fashion, interfacial area $A$ and surface tension $\gamma$ were recorded continuously, and the phase angle $\phi$ between compression and expansion was computed. The interfacial elasticity modulus $\gamma$ was derived ($\epsilon = d\gamma/dt$). The elasticity real part $\epsilon'$ and the elasticity imaginary part $\epsilon''$ were obtained ($\epsilon' = \epsilon \cos \phi$, $\epsilon'' = \epsilon \sin \phi$) (31, 32).

RESULTS

The Adsorption of CSP to Air/Water (A/W) and Hydrocarbon (Dodecane)/Water (DD/W) Surfaces—Fig. 1 shows a typical set of curves illustrating the effect of concentration on the adsorption isotherms of CSP at the A/W interface. Without CSP the surface tension $\gamma$ remained constant at about 71 mN/m. At the most dilute concentration ($1.5 \times 10^{-7}$ M) there was a short lag period of about 150 s and then a rapid fall in the surface tension. After 500 s the surface tension fell more slowly toward an equilibrium value. As the concentration increased, the lag period shortened, and the steepness of the curve increased. The slope of the rapid part of the descent of $\gamma$ versus time ($d\gamma/dt$) appears to be concentration-dependent (Fig. 1, inset). The final surface tension was very close to equilibrium by 1200 s (20 min). The equilibrium $\gamma_{A/W}$ at the highest concentration ($4.8 \times 10^{-6}$ M) was about 46.5 mN/m. Thus CSP lowered $\gamma_{A/W}$ by about 24.5 mN/m. Because $\Pi = \gamma_0 - \gamma_{CSP}$ the CSP produced about 24.5 mN/m surface pressure at the A/W interface.

Fig. 2 shows similar data collected at the DD/W interface. The same general features were present; however, the starting interfacial tension for DD/W was about 52 mN/m and fell to about 21 mN/m on approaching equilibrium at $1.2 \times 10^{-6}$ M. Thus CSP produced a $\Pi$ of about 31 mN/m on the DD/W interface. A lag period similar to that on the A/W interface occurred with the low concentrations of CSP. The lag period was shortened as the concentration increased. The most dilute concentration studied ($1.5 \times 10^{-5}$ M), $\gamma$, failed to fall below 42 mN/m even after 4 h. At $3.1 \times 10^{-5}$ M equilibrium was approached by 2.5 h (data is not shown). The surface tension approached equilibrium by 3600 s (1 h) at concentrations at and above $6.2 \times 10^{-6}$ M (Fig. 2). The most rapid fall in surface tension ($d\gamma/dt$) shows (Fig. 2, inset) that the rate decrease in the surface tension is a function of the concentration, being fastest at the highest concentration and slower at lower concentrations.

Estimation of the Surface Area at Saturation Using the Gibbs Adsorption Isotherm Equation—The equilibrium values of $\gamma$ for each peptide concentration were plotted against the natural log (ln) of the aqueous peptide concentration (Fig. 3), and the slope, $d\gamma/d\ln C$, was used to calculate the excess surface concentration, $\Gamma$, at the interface using the equation $\Gamma = -(1/RT)(d\gamma/d\ln C)$. The estimated surface concentration $\Gamma$ and the area per molecule (A) are given in Table I. These values suggest that the surface concentration is similar at the two interfaces. The area per residue of about 14 and 16 A$^2$ per amino acid at the DD/W and A/W interfaces are consistent with the peptide lying flat on the surface with both helices contacting the water along the hydrophilic face (13).

Desorption and Adsorption of CSP from A/W and DD/W Interfaces—After the surface tension fell to the apparent equilibrium value, the volume of the drop was decreased or increased $\sim 6, 12$, or 25% depending upon the protocol. To produce compression, the drop volume $V$ was decreased instantly, which results in a decreased surface area $A$. $\gamma$, $A$, and $V$ were followed with time (Fig. 4). At the A/W interface (Fig. 4A), immediately after compression, $A$ and $\gamma$ fell instantly. Because at the instant the $V$ and $A$ were reduced all the original molecules were at the interface, the surface concentration $\Gamma$ was increased. $\gamma$ then rose with time indicating the loss of molecules from the interface and a return toward the equilibrium surface concentration $\Gamma_{eq}$. Conversely when the surface was expanded
by increasing the volume instantaneously, the surface tension rose immediately and then fell as new molecules adsorbed to the surface to take the vacant space made by increasing A. An example of the changes that occurred at the A/W interface with an ~25% change in volume are shown in Fig. 4A. After decreasing the V from 5.4 to 4.4 μl, γ plummeted from about 48 to 37 mN/m, corresponding to an 11 mN/m increase in II and then rapidly moved back to the equilibrium value. The volume was then increased to about 6.4 μl, and expanding A and γ jumped to almost 60 mN/m and then fell back toward the equilibrium value as molecules from the aqueous phase adsorbed. These curves indicate that the adsorption and desorption phenomena around the equilibrium surface tension are the result of the surface attempting to maintain a constant surface concentration, Γeq. Data obtained at three different degrees of compression or expansion (~6, ~12, or ~25%) were similar although more extreme with the larger compression (i.e., ~25%).

An example of desorption and adsorption of CSP from the DD/W interface is shown in Fig. 4B. Starting from a γ of ~15.6 mN/m the volume was instantly decreased from 8.6 to 6.4 μl (~25% decrease). The γ instantly fell to about 9.8 mN/m. At this point the total number of molecules on the surface was the same as before the volume decrease, and therefore γ was increased proportionally to the decrease in surface area. Following compression, γ very gradually rose but did not approach the equilibrium value even after 300 s. This was quite different from the A/W interface where the equilibrium was approached in less than 100 s (see Fig. 4A). However, when the surface was expanded by increasing the volume to 9.8 μl, the surface tension instantly rose and then fell back rapidly to approach the starting value (~15.6 mN/m) within about 100 s. This indicates that the desorption and adsorption processes are quite different on the decane surface. Adsorption appears to be driven by movement of molecules from the aqueous phase to the new surface formed when it is expanded, but desorption seems to be retarded suggesting that a kinetic barrier to desorption from the DD/W surface exists.

The adsorption curves (obtained on expansion of the volume) were well fitted by a bi-exponential equation (χ² < 0.03), and a fast time constant (t₁) and a slower one (t₂) were derived. The t₁ constants were consistent with the concept that the adsorption should be more rapid the higher the concentration, and this is shown in the plots of t₁ for both the A/W and the DD/W interfaces as a function of peptide concentration (Fig. 5A). The curves were similar although the A/W interface was displaced to somewhat higher concentrations suggesting a higher affinity of the CSP for the hydrocarbon interface. The meaning of the second slower time constant t₂ is not clear but shows similar concentration-dependent trends (data is not shown). When CSP concentration was varied from 3 × 10⁻⁷ to 5 × 10⁻⁶ M, t₁ for the DD/W interface decreased from 172 to 10 s, whereas t₂ for the A/W interface decreased from 94 to 12 s.

The desorption curves were also analyzed by the bi-exponential equation and compared with adsorption (Fig. 5B). At the A/W interface the values for the rapid time constant t₁ probably are related to the peptide beginning to desorb from the surface as shown in Fig. 4A. As shown in Fig. 5, the t₁ of desorption is concentration-dependent at both DD/W and A/W interfaces. However the desorption t₁ is basically unchanged throughout a large concentration range from 3 × 10⁻⁷ to 5 × 10⁻⁶ M. The t₂ for desorption from the A/W interface is significantly more rapid (~1 s) than from the DD/W interface (~2 s). Thus desorption is a function of the rapid increase in surface pressure that occurs when the volume is decreased and is not dependent on the aqueous concentration of peptide. Similarly, the desorption t₂ of both interfaces is not concentration-dependent but slower than t₁. The average t₂ for the A/W interface is ~16 s, whereas the average t₂ for the DD/W interface is ~20 s.

To estimate the maximum pressure (Πmax) that the peptide could withstand without being ejected from the surface, a series of experiments were carried out in which the volume was decreased abruptly to increase the surface pressure to a given value, Πeq, and then γ was followed for 3 min. The change in surface tension over 3 min (Δγ) was then plotted against Πeq. A positive Δγ indicated that the peptide was desorbing from the surface. If there was no change, it indicated that peptide concentration on the surface remained unchanged, and if the Δγ was negative, it indicated that peptide was still able to adsorb to the surface. A plot of the Πeq versus the Δγ gave a straight line. The intercept at Δγ = 0 gives the Πeq at which peptide shows no net adsorption or desorption (Πmax). This is similar to Πex, the exclusion pressure determined at the air/phospholipids interface, except Πmax is at the peptide/hydrophobic interface. Sixty-nine measurements varying Πeq from 21 to 36 mN/m on the A/W interface gave a Πmax of 21.3 mN/M, and of 43 measurements varying Πeq from 29 to 46 mN/m at the DD/W interface gave a Πmax of 31.7 mN/M. These experiments show that it takes at least 10 mN/m more pressure to push CSP off the hydrocarbon surface.

Studies of the Elastic Behavior on the Interface at or Near the Equilibrium Surface Tension—In these studies the interfacial tension γ was allowed to approach equilibrium as indicated in Fig. 1 and then the drop volume was oscillated in a standard protocol varying the oscillation period between 4 and 128 s. The same protocol was repeated at each of three different amplitudes of about ±6, ±12, or ±25%. The data were analyzed for the change in V, A, and γ. From the simultaneous changes in A and γ the following parameters were calculated: the surface dilution modulus dγ/dlnA, the phase angle φ between compression and expansion, the elastic component or real component of the modulus, ε, and the imaginary or viscous component of the modulus, ε’, where ε = ε cosφ and ε’ = ε sinφ. The imaginary component is reflected in the phase difference between the stress, dγ, and the strain, dlnA. If the phase angle approaches zero then the surfaces can be considered completely elastic, and ε = ε’.

CSP at the A/W Interface—A typical experiment is given in Fig. 6 that is for oscillations of approximately ±12% (± 0.5 μl) volume. Five compression and expansion oscillations repeated
Interfacial Properties of an Exchangeable Apolipoprotein CSP

**Table I**

| Interfaces | Equation of the fit line | $R^2$ | $\Gamma$ | Area | Area/ amino acid |
|------------|--------------------------|-------|---------|-------|-----------------|
| A/W        | $Y = -0.5853 X + 39.285$ | 0.9631 | $2.36 \times 10^{-13}$ | 702   | 16              |
| DD/W       | $Y = -0.6696 X + 12.904$  | 0.6906 | $2.67 \times 10^{-13}$ | 622   | 14              |

**Fig. 4. Examples of desorption and adsorption curves.** Desorption (left side) occurs if the surface tension increases after compression. Adsorption (right side) occurs if surface tension falls after expansion. A 4-µl air bubble was allowed to reach equilibrium and compressed and expanded by ~25% (±1 µl) from 4 µl. The concentration of CSP in aqueous phase was 2.4 × 10^{-6} M. After compression (left) or expansion (right) the interfacial tension $\gamma$ moved back to equilibrium values. b, DD/W interface. An 8-µl dodecane drop was compressed and expanded by ~25% (±2 µl). The concentration of CSP in aqueous phase was 1.2 × 10^{-6} M. After compression $\gamma$ increased but did not go to equilibrium (~20 mN/m) in the 5-min period. Experiments were carried out at 25 ± 0.1°C in 2 mM phosphate buffer, pH 7.4.

Two times for each period are shown over a period of approximately 1 h. The experiment was repeated at 4 ± 1 µl. For the more rapid periods from 4 to 16 s, the volume curves were quite sinusoidal (data not shown), but as the periods become longer, the volume curves became truncated at the top and the bottom, and no longer were truly sinusoidal (see Fig. 6, bottom). This results in some distortion in the surface tension curve. For instance, when the period is long then the volume will rise and level off before falling. During the rise of the volume, $\gamma$ rises. When the volume levels off $\gamma$ begins to fall as peptide from the surrounding aqueous phase adsorbs. Then as the volume begins to decrease again, there is a rapid fall in $\gamma$ largely because of compression. Conversely at the bottom of the volume curve when the volume remains stationary for a short period of time, desorption occurs from the surface, distorting the surface tension curve (Fig. 6, bottom). Thus, strictly speaking, because the volume changes at long periods were not sinusoidal, the interpretation of both $\phi$ and $\epsilon$ are complicated by the desorption and adsorption occurring at the low and high volume parts of the oscillations.

A plot of the surface tension versus area (data not shown) showed that at rapid periods (4 and 8 s) of compression/expansion very little hysteresis was present, and thus the $\phi$ was negligible (Table II). At 16 s there was some separation between compression and expansion that became larger as the period increased. This gives rise to an increase in the $\phi$ (Table II). The phase angle $\phi$ derived from $\gamma$ versus $A$ (see Table II and Fig. 7) can be interpreted as follows: with oscillation of $\sim25\%$ at the shorter periods (4, 8 s), where the volume changes in a sinusoidal way, $\phi$ is very small, and the elastic modulus, $\epsilon$, and the real part of the elastic modulus, $\epsilon'$, are almost the same (Table II). This shows that with rapid oscillations of $\sim25\%$ the surface is almost entirely elastic. However, as the period increases, and the sinusoidal curve becomes truncated at high and low volume, adsorption and desorption of peptide occur, respectively, and account for the positive $\phi$ in the longer cycle periods. For a perfectly elastic surface $\epsilon$ must have a limiting value $\epsilon_o$ given by the surface equation of state (31): $\epsilon_o = (d^2y/dln\Gamma)$, where $\Gamma$ is the surface excess concentration. The limiting value $\epsilon_o$ is reached only if there is no exchange of molecules on the surface with those in the bulk aqueous phase. That means that the product of $A \times \Gamma$ is constant. Deviations from $\epsilon_o$ occur when relaxation processes at or proximal to the surface effect either $\gamma$ or $\Gamma$ within the time frame of the experiment. When this occurs, the phase angle $\phi$ will be positive, and $\epsilon$ becomes viscoelastic. The elastic part, $\epsilon'$, accounts for the reusable energy stored in the surface, and the viscous part, $\epsilon''$, reflects energy lost through any relaxation at the surface. In the present case $\Gamma \times A$ must remain constant for pure elasticity. If, during expansion when $\Gamma$ falls, molecules diffuse from...
the bulk and adsorb, then $\Gamma \times A$ is not constant, and the surface becomes viscoelastic. This does not necessarily mean that there is a conformational change in the peptide at the surface, but rather that as the surface expands the molecules adsorb, and as it contracts, molecules desorb but at relatively slow rates. Thus when the period is rapid (4–8 sec) molecules do not have adequate time to effectively desorb or adsorb to the surface, and the surface appears to be elastic, i.e. $\Gamma \times A$ is nearly constant. At longer periods both desorption and adsorption take place as the surface is compressed and expanded, respectively. This behavior is to be expected when one considers the static adsorption/desorption curves shown earlier in Fig. 4A. Data obtained ($\epsilon, \epsilon', \epsilon'', \phi$, and mean $\gamma$) for CSP at the A/W interface at a concentration of $6.1 \times 10^{-7}$ M are given in Table II. The mean surface tension for each oscillation remains relatively constant and near equilibrium values throughout these experiments. Phase angles $\phi$, $\epsilon$, and $\epsilon'$ are presented in Fig. 7. When the amplitude is high (±25%) then the phase angle is always positive, indicating that the surface is not totally elastic. This can be explained by the adsorption/desorption phenomena being more pronounced when the compression and expansion are greater. When the compression or expansion is smaller (±12%) the peptide can remain largely bound to the surface as long as the period is short (8 sec or less), but when the compression and expansion is of greater amplitude (±25%) then the peptide can be pushed off and re-adsorbed on expansion even when the period is short. This would indicate that the flexibility of the peptide structure at the interface cannot exceed 12% of its area. Compression beyond that forces it off the surface and leads to non-elastic behavior. Also, if the period is long (>16 sec), peptide desorbs, and $\epsilon$ diverges from $\epsilon'$ (Fig. 7B). $\epsilon$ is greater when the compression is small. However, it decreases at large compression (±25%), because molecules are pushed off the surface, and this relaxes the stress.
The initial volume of air bubble was ~4 µl, and the volume was oscillated at ±0.5-µl amplitude with different periods. The period is given above the oscillations. The concentration of CSP in aqueous phase was 6.1 × 10⁻⁷ M. The lower figure shows the tension and volume changes when the air bubble was oscillated at the 128-s period. The volume was truncated at the peaks of the oscillations. For further discussion see text.

**Table II**

*Dynamic interfacial properties of CSP at A/W interface*

All oscillation experiments were carried out on A/W interface in pH 7.4 phosphate buffer (2 mM) at 25 ± 0.1°C. The concentration of CSP in the aqueous phase was 6.1 × 10⁻⁷ M. V, initial drop volume; ΔV, oscillation amplitude; mean γ, mean interfacial tension of near equilibrium oscillation; ε, viscoelastic modulus; φ, viscous phase angle, a phase difference between dγ and dA; ε'', elastic component, the real part of modulus; ε'', viscous elastic component, the imaginary part of modulus.

| V (µl) | ΔV | Period | Mean γ | ε | φ | ε'' |
|-------|----|--------|--------|---|---|-----|
| 4     | ±1 | 8      | 52.7   | 27.8 | 72.2 | 38.1 |
| 4     | ±1 | 16     | 51.9   | 27.2 | 58.8 | 40.5 |
| 4     | ±1 | 32     | 51.4   | 26.3 | 55.2 | 43.4 |
| 4     | ±1 | 64     | 50.8   | 26.8 | 51.4 | 45.4 |
| 4     | ±1 | 128    | 50.1   | 26.3 | 45.2 | 45.5 |
| 4     | ±0.5| 8      | 50.9   | 26.3 | 42.6 | 13.6 |
| 4     | ±0.5| 16     | 50.1   | 26.0 | 40.9 | 16.3 |
| 4     | ±0.5| 32     | 49.9   | 25.6 | 42.0 | 26.9 |
| 4     | ±0.5| 64     | 49.6   | 25.2 | 40.8 | 40.0 |
| 4     | ±0.5| 128    | 49.1   | 24.7 | 36.8 | 46.0 |

**CSP at the DD/W Interface**—Similar sets of experiments were carried out with CSP at the DD/W interface, oscillated at about ±25, ±12, and ±6% (Table III). A typical oscillination experiment with CSP (6.1 × 10⁻⁷ M) at the DD/W interface at 8 ± 2 µl (±25%) is shown in Fig. 8. The volume changes were nearly sinusoidal even at the longest period (128 s), but desorption and adsorption also occurred when the volume reached its lowest point or highest point, respectively, at the longer periods. The out of phase behavior between volume or area and surface tension curves are obvious in the longer periods (Fig. 8, bottom). The fact that desorption is occurring during the slow part of the high volume cycle allows the surface tension to fall to a lower level at long periods compared with short. Thus, at 8- and 16-s periods the peak surface tension during expansion was about 31 mN/m, whereas at 128 s it fell to about 25 mN/m (Fig. 8, top). A plot of the γ tension versus A (data not shown) shows marked hysteresis in 25% compression/expansion and a positive φ at all periods (Table III) except the shortest (4 s). When the frequency is fast (4 s), the surface is elastic. A 4-s period only has 1 s of compression from equilibrium to the minimum volume, and this is not adequate time for desorption of significant CSP from the surface. The short term time constant, τ₁, for desorption from this surface is about 2 s (Fig. 5B). However, at longer periods, for instance 8 s, there is a clear hysteresis and a positive φ indicating that peptide is partly desorbed during compression and re-adsorbs during expansion. The elasticity analysis is shown in Table III and plotted in Fig. 9. When the amplitude was only ~6% (8 ± 0.5 µl), there is virtually no hysteresis, and the φ was virtually zero (Fig. 9, Table III). This indicates that when the peptide is on the DD/W interface and compressed only around 6%, it acts as a completely elastic system, even at very slow frequency (long periods). This suggests that the peptide is not readily pushed off the surface by small reductions in the surface area and that it acts as an elastic interface under these circumstances. That is, CSP seems to act like a spring, compressible by about 6–7%. Because molecules stay on the surface, ε is high (Fig. 9). However, as the amplitude of the compression increases then the phase angle φ becomes positive, and the ε has a viscous component that is related to the desorption at low surface areas and the adsorption at high surface areas. The desorption of
molecules relaxes the stress, and $\epsilon$ is reduced (see Table III and Fig. 9).

**Continuous Oscillation during Adsorption of the Peptide**

CSP at the A/W Interface—Continuous oscillation starting a few seconds after forming the drop allows a continuous plot of the elastic modulus $\epsilon$ and phase angle $\phi$ as a function of time, that is as a function of the decreasing mean surface tension during the adsorption process (Fig. 10). The phase angles $\phi$ and $\epsilon$ are plotted against time in seconds in Fig. 10 and show that during the early time points there is a low signal/noise ratio but that after about 50 s the phase angle moves from roughly zero with a lot of noise to slightly positive phase angle in the case of the 12% amplitude change ($4 \pm 0.5 \mu l$) and definitely positive angles at 25% change ($4 \pm 1 \mu l$) of compression and expansion. $\epsilon$ shows a uniform increase with time and then levels as equilibrium $\gamma$ is approached. When the compression amplitude is high, $\epsilon$ is less than when it is low. These data suggest that CSP behaves as an elastic peptide when the compression is small ($\pm 6\%$). However, when the amplitude is greater there is a small viscous component to the elasticity because of an increase in compression causing desorption. A plot of the mean surface pressure II against the modulus $\epsilon$ (Fig. 11) shows a nearly monotonous rise from low pressure to $\sim 10$ mN/m. The slope of this is about 4 (modulus to pressure), which is about double what one would expect for an ideal surface of C12-E6, a nonionic detergent at 2% less than at 6 mN/m (33). However, the ratio of 4 or 5 ($\epsilon$II) is very similar to several proteins at $\Pi < 10$ mN/m (31, 32, 34). Above 10 mN/m II, all the curves, except the 25% compression/expansion at the 8-s period, diverge from the line, and the slope increases. However, at higher pressures the $\epsilon$ increases more steeply than II, which indicates some increased strain in the surface, probably related to molecule/molecule interaction. Note that there is no third phase to these curves at high pressures as seen in several proteins at oil/water interfaces (31, 32) and as seen with CSP at the DD/W interface (see below).

CSP at the DD/W Interface—The phase angle $\phi$ and elastic modulus $\epsilon$ are given as a function of time after forming the drops in Fig. 12. Note that $\phi$ at a low amplitude and rapid period (4 s) does not deviate much from zero, and therefore the system is elastic throughout the adsorption and into the equilibrium phase of the experiment. The small amplitude and rapid frequency do not permit the peptide to appreciably desorb from the surface nor undergo conformational changes that would alter purely elastic behavior. Furthermore, $\epsilon$ is high and remains relatively constant after the first 50 s. Under all other conditions, $\phi$ becomes positive, and the $\epsilon$ decreases. This indicates that even if the amplitude is small, if the period is long enough, the system is elastic throughout the adsorption process.
enough to allow peptide to desorb and re-adsorb then the system has a viscous component to the modulus, and the surface is not perfectly elastic. Finally, a plot of the surface pressure, $\gamma$, against the modulus (Fig. 13) shows that at a low pressure up to about 7 mN/m the ratio $\gamma/\Pi$ is about 5 rather like that at the A/W interface. However, at higher pressures the curves deviate sharply upward and then after they reach a $\Pi$ of about 25 to 28 mN/m, there is a sharp decrease in $\gamma$ which is characteristic of many proteins at the oil/water interface (31, 32) and suggests marked changes in the behavior of the surface. Some of this behavior at high $\Pi$ is possibly because of desorption and re-adsorption occurring the cycling process. However, this could also indicate changes in the nature of the interface itself, including conformational changes in the peptide or different packing arrangements at the interface. This part of the curve is not seen when CSP adsorbs to the A/W interface (see Fig. 11), probably because the mean surface $\Pi$ at the A/W interface does not increase beyond 20 mN/m. In fact the curves in Fig. 13 up to a $\Pi$ of 20 mN/m look rather similar to those in Fig. 11.

**DISCUSSION**

It is unclear exactly how apolipoproteins interact with the lipid in HDL, LDL, or triacylglycerol-rich particles. There are a number of possibilities. One perhaps more relevant to HDL is that they simply wedge in between the phospholipids and do not contact the core. A second is that they contact the core of triacylglycerol and/or cholesterol ester interacting with the ester groups. A third is that they interact with the core but primarily with the hydrocarbon chains in the core. Therefore, as an initial study we have chosen to use a hydrocarbon as a potential interface for the interaction of an amphipathic $\alpha$-helical peptide and to compare the DD/W with A/W interface.

A number of groups have studied the penetration of soluble apolipoproteins and peptides into phospholipid monolayers (14–23). Phillips and Krebs (14) compared human apoA-I ($H_\Pi = 32$ mN/m) to apoA-II ($H_\Pi = 34$ mN/m). Weinberg et al. (21, 22) showed that apoA-I and apoA-IV have exclusion pressure, $H_\Pi$, of 33 and 28.5 mN/m, respectively. Using a pulsating air bubble technique apoA-I and A-IV exerted a maximum surface pressure of 32 and 29 mN/m, respectively, quite similar to their exclusion pressures (22). However, when spread on a surface balance, A-I cannot be compressed beyond about 20–25 mN/m (area $\sim 15 \text{ Å}^2$ per amino acid) considerably below 32–33 mN/m noted above (14, 22). A-IV in fact produces a higher surface pressure when spread and compressed on a surface balance, rather similar to that found in the pulsating bubble experiment (22). Very little information exists on the absorption of soluble apolipoproteins to hydrocarbon or oil surfaces. Weinberg et al. (21) studied the adsorption of 10 $\mu$g/ml solutions of apoA-I and A-IV to triolein droplets using a drop volume technique. By expressing the droplets at different rates they were able to show that apoA-I approached an equilibrium interfacial value of about 16 mN/m by about 20 min. That amounts to lowering the triolein/water interfacial tension ($\gamma$) by 15 mN/m. On the other hand, apoA-IV failed to reach an equilibrium value by 20 min. At 20 min apoA-IV reduced the triolein/water
Thus the affinity for the oil/water surface is greater than for the DD/W interface, the interfacial tension is lowered in a concentration- and time-dependent way. At the A/W interface, on the rates of adsorption and desorption of the peptide adsorbs to both the A/W interface and the DD/W interface, to lower the interfacial tension and interfacial free energy, peptide adsorbs to a triolein/water interface consistent with the two helices lying flat on the surface. At the A/W interface, on the effects of protein concentration, the rates of adsorption or desorption, or the viscoelastic behavior of these proteins at an oil/water interface.

To understand in more depth the interaction of exchangeable amphipathic α-helices with hydrophobic or core-like interfaces, we have compared the behavior of a 44-amino acid consensus sequence peptide CSP at the DD/W interface with the A/W interface. These studies provide new data on several important physical parameters of CSP at these two interfaces, which can be compared in the future to other peptides and to native and mutant apolipoproteins. The new data include studies on the concentration- and time-dependent changes of interfacial tension, on the surface area of the peptide at the A/W and DD/W interface, on the rates of adsorption and desorption of the peptide when the surface is expanded or compressed, and on the elastic properties of the peptide under varying amplitudes and frequencies of compression. These data enhance our understanding of the interaction of an amphipathic α-helical peptide with a hydrophobic interface that potentially models the core of a lipoprotein.

We have shown that this consensus amphipathic α-helical peptide adsorbs to both the A/W interface and the DD/W interface to lower the interfacial tension and interfacial free energy in a concentration- and time-dependent way. At the A/W interface, the interfacial tension is lowered ~24 mN/m, and at the DD/W interface the interfacial tension is lowered ~31 mN/m. Thus the affinity for the oil/water surface is greater than for the A/W surface. The peptide lowers γ with a rate that is increasingly fast depending on the external concentration of the peptide consistent with the rate of adsorption to the surface being concentration-dependant (Fig. 5A). The surface area estimated from the Gibbs adsorption isotherm equation was about 16 Å²/amino acid on the A/W interface and 14 Å²/amino acid on the DD/W interface consistent with the two helices lying flat on the surface. At the A/W interface, CSP readily desorbs when the surface is compressed and re-adsorbs when the surface is expanded to return to a near equilibrium pressure. By plotting the initial pressure after sudden compression, Π(o), versus the change in γ during a 3-min recovery period (Δγ) the maximum II beyond which CSP is ejected (II(max)) was estimated. As a concept, this pressure II(max) is similar to Π(o) for...
apolipoproteins placed in the aqueous phase under a phospholipid monolayer. However, \( \Pi_{\text{max}} \) is the maximum II allowed in a compressed peptide monolayer at a hydrophobic interface. It will be important to compare \( \Pi_{\text{max}} \) with the \( \Pi_{\text{c}} \) for the same peptide. The \( \Pi_{\text{max}} \) on A/W surface is 21.3 mN/m. In contrast, CSP at the DD/W interface desorbs more slowly than at the A/W interface perhaps reflecting its greater affinity for dodecane. The \( \Pi_{\text{max}} \) at DD/W interface is 31.7 mN/m, 10.4 mN/m greater than at A/W. In compression and expansion of the A/W interface CSP can desorb provided the period is long enough or the increase in pressure is high enough. Thus when the amplitude is low (\( \pm 12\% \)) and the period is rapid (\( \pm 8 \) s), peptide stays at the interface and behaves as an elastic molecule able to breathe within a \( \pm 12\% \) space. When the period is lengthened, even at low compression, the peptide desorbs indicating that even a small compression can displace CSP if given adequate time. However, when the compression/expansion amplitude is greater than \( \pm 12\% \) even during rapid oscillations, CSP is pushed off into the aqueous phase and re-adsorbs when the surface is expanded. This leads to a non-ideal viscoelasticity with a positive phase angle and some part of the elastic modulus being due to desorption and adsorption from the interface, i.e. \( \Gamma \times A \) is not constant during the compression/expansion cycle. As a result of desorption, some of the stress is relaxed, and \( \epsilon \) falls. When the frequency of oscillation is rapid, the desorption is decreased. When the amplitude is small and the frequency rapid, the surface is elastic and shows the highest \( \epsilon \).

The DD/W interface behaves in a rather similar way in terms of its elasticity. When the period is fast the peptide has more elastic behavior, because it does not have adequate time to come off the surface. However, when the period increases, peptide can desorb from the surface, albeit more slowly than the adsorption, to give hysteresis on compression and expansion and a non-elastic component to the modulus. However, when the amplitude is low (\( \pm 6\% \)) no matter what the period, the peptide appears to behave as an elastic surface rather like a spring compressible up to 6% (Table III). The major differences in CSP behavior at these two interfaces are as follows: 1) a greater lowering of the surface \( \gamma \) and free energy on the DD/W surface, 2) a higher \( \Pi_{\text{max}} \) on DD/W surface 31.7 mN/m versus 21.3 mN/m on A/W, and 3) failure of desorption of the peptide from the DD/W interface allowing it to act as an elastic peptide when the surface is oscillated at \( \pm 6\% \).

Several metabolic reactions can lead to expansion of core lipid and/or depletion of the surface phospholipids of lipoproteins or vice versa. We suggest that exchangeable apolipoproteins and perhaps the amphipathic \( \alpha \)-helices of apoB can bind to the hydrophobic core surface to provide part of the cover of this surface protecting it from exposure to water and thus lowering the free energy of the hydrocarbon/water surface to...
promote stability. It is clear from studies of LDL that contain a hydrophobic core primarily of cholesterol ester that the protein component must interact with core lipids (24, 26). There is simply not enough phospholipid and cholesterol to cover the surface. On different sized LDLs it has been estimated that apoB covers 26–53% of the core lipids (24). Because apoB also contains at least two major domains (αI and αII) of amphipathic α-helices (6, 35, 36), the present study may suggest a model of hydrocarbon/amphipathic α-helix interaction that should be present in the LDL particle. We suggest that when the surface pressure in the interface increases to a certain high level (above $\Pi_{\text{max}}$), minimally flexible helices are forced off the interface into the surrounding aqueous media whereas the amphipathic $\beta$-sheets remain attached to the LDL core. When the surface pressure decreases, the α-helices snap back on. This process is relatively fast, i.e. seconds to minutes. Even in a large soluble apoprotein consisting of many amphipathic helices such as apoA-I, apoE, or apoA-IV, perhaps only some of the amphipathic helices (with lower $\Pi_{\text{max}}$) are forced off when the surface pressure is moderately increased. High $\Pi_{\text{max}}$ helices could keep the apolipoprotein bound. When the surface pressure is again lowered, then these amphipathic helices can rapidly snap back on to the surface providing a mechanism for rapidly adjusting stability during metabolic alterations in lipid core/surface ratios. Any plasma enzymatic reaction that reduces the core lipids much more than the surface, for instance the action of lipoprotein lipase, should increase the surface pressure in the interfacial region and force helices with a low $\Pi_{\text{max}}$ off. Anything that expands the core relative to the surface, such as the lecithin:cholesterol acyltransferase reaction or the cholesterol ester transfer protein catalyzed transfer of triglyceride in exchange for cholesterol ester (triglyceride has a larger volume than cholesterol ester), should decrease the surface pressure and allow the expelled helices of the apolipoprotein to snap back on the surface. In contrast to the rapid expulsion or reabsorption of some of the helices of an apolipoprotein with low $\Pi_{\text{max}}$ within a complex apolipoprotein, large increases in pressure might force the entire apolipoprotein into the aqueous phase and then reabsorption would be slower and concentration-dependant. The present hypothesis allows for very rapid adjustment in the surface to maintain lipoprotein stability.

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REFERENCES
1. Atkinson, D., and Small, D. M. (1986) Annu. Rev. Biophys. Biophys. Chem. 15, 403–456
2. Segrest, J. P., Jackson, R. L., Morrisett, J. D., and Gotto, A. M., Jr. (1974) FASEB J. 15, 247–253
3. Segrest, J. P., Jones, M. K., DeLoof, H., Brouilette, C. G., Venkatachalapathi, Y. V., and Anantharamaiah, G. M. (1992) J. Lipid Res. 33, 141–166
4. Segrest, J. P., DeLoof, H., Dohlman, J. G., Brouilette, C. G., and Anantharamaiah, G. M. (1990) Proteins 8, 103–117
5. Tall, A. R., Small, D. M., Deckelbaum, R. J., and Shipley, G. G. (1977) J. Biol. Chem. 252, 4701–4717
6. Segrest, Jere P., Jones, M. K., DeLoof, H., and Dashti, H. (2001) J. Lipid Res., 1346–1367
7. Pownall, H. J., Massey, J. B., Kaserow, S. K., and Gotto, A. M., Jr. (1979) Biochemistry 18, 574–579
8. Brouilette, C. G., and Anantharamaiah, G. M. (1995) Biochim. Biophys. Acta 1256, 103–129
9. Brouilette, C. G., Anantharamaiah, G. M., Engler, J. A., and Borhani, D. W. (2001) Biochim. Biophys. Acta 1511, 4–16
10. Scanna, A. M. (1972) Biochim. Biophys. Acta 265, 471–508
11. Ritter, M. C., and Scanna, A. M. (1977) J. Biol. Chem. 252, 1200–1216
12. Ritter, M. C., and Scanna, A. M. (1980) J. Biol. Chem. 254, 2517–2525
13. Pittman, R. C., Glass, C. A., Atkinson, D. A., and Small, D. M. (1987) J. Biol. Chem. 262, 2435–2442
14. Phillips, M. C., and Krebs, K. E. (1986) Methods Enzymol. 128, 387–403
15. Krebs, K. E., Phillips, M. C., and Sparkes, C. E. (1985) Biochim. Biophys. Acta 751, 470–473
16. Shen, B. W., and Scanna, A. M. (1986) Biochemistry 26, 3653–3656
17. Krebs, K. E., Ibahah, J. A., and Phillips, M. C. (1988) Biochim. Biophys. Acta 959, 229–237
18. Ibahah, J. A., and Phillips, M. C. (1988) Biochim. Biophys. Acta 27, 7155–7162
19. Ibahah, J. A., Lund-Katz, S., and Phillips, M. C. (1989) Biochim. Biophys. Acta 1004, 300–308
20. Weinberg, R. B., Ibahah, J. A., and Phillips, M. C. (1992) J. Biol. Chem. 267, 8977–8983
21. Weinberg, R. B., Cook, V. R., DeLozier, J. A., and Shelneth, G. S. (2000) J. Lipid Research 41, 1419–1427
22. Datta, G., Chaddha, M., Hama, S., Navab, M., Fogelman, A. M., Garber, D. W., Misfris, V. K., Epand, R. F., Epand, R. F., Lund-Katz, S., Phillips, M. C., Segrest, J. P., and Anantharamaiah, G. M. (2001) J. Lipid Res. 42, 1096–1104
23. McNamara, J. R., Small, D. M., Li, Z., and Schaefer, E. J. (1996) J. Lipid Res. 37, 1924–1935
24. Nolte, R. T., and Atkinson, D. (1992) Biophys. J. 63, 1221–1239
25. Hurt-Camejo, K., Camejo, G., Rosengren, B., Lopez, F., Willund, O., and Bondjers, G. (1990) J. Lipid Res. 31, 1387–1396
26. Wieser, G., and Small, D. M. (2002) Biochim. Biophys. Acta 1531, 384–387, Elsevier Science Publishers B.V., Amsterdam
27. Labourdenne, S., Gaudry-Rolland, N., Letellier, S., Lin, M., Cagna, A., Esposito, G., Verger, R., and Riviere, C. (1994) Chem. Phys. Lipids 71, 163–173
28. Adam, N. K. (1941) The Physics and Chemistry of Surfaces, 3rd Ed., pp. 167–115, Oxford University Press, London
29. Benjamin, J., and Lucassen-Reynders, E. H. (1998) in Proteins in Liquid Interfaces (Mobius, D., and Miller, R., eds) pp. 341–384, Elsevier Science Publishers B.V., Amsterdam
30. Benjamin, J., Cagna, A., and Lucassen-Reynders, E. H. (1996) Colloids Surf. A 114, 245–254
31. Lucassen-Reynders, E. H., Cagna, A., Lucassen, J. (2001) Colloids Surf. B 186, 63–72
32. Segrest, J. P., Jones, M. K., Misfris, V. K., Anantharamaiah, G. M., and Garber, D. W. (1994) Arterioscler. Thromb. 14, 1674–1685