Surface pressure-dependent conformation change of apolipoprotein-derived amphipathic α-helices

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Abstract  Amphilic α-helices (AαH) are the primary structural motif of exchangeable apolipoproteins. AαHs in exchangeable apolipoproteins adsorb, remodel, and desorb at the surface of plasma lipoproteins in response to changes in their size or composition. A triolein/water (TO/W) interface was used as a model surface to study adsorption and desorption of AαHs at a lipoprotein-like interface. We previously reported that AαH peptides spontaneously adsorb to a TO/W interface, but they only partially desorb from the surface when the excess peptide was removed from the system. This finding suggests that “exchangeable” apolipoproteins are in fact partially exchangeable and only desorb from a surface in response to compression or change in composition. Here, we develop a thermodynamic and kinetic model to describe this phenomenon based on the change in the interfacial pressure (surface tension) of the C-terminal 46 amino acids of apolipoprotein A-I (C46) at a TO/W interface. This model suggests that apolipoproteins have at least two interfacial conformations that are in a surface concentration and II-dependent equilibrium. This two-state surface equilibrium model, which is based on experimental data and is consistent with dynamic changes in II(ζ), provides insights into the selective metabolism and clearance of plasma lipoproteins and the process of lipoprotein remodeling. —Mitsche, M. M., and D. M. Small. Surface pressure-dependent conformation change of apolipoprotein-derived amphipathic α-helices. J. Lipid Res. 2013. 54: 1578–1588.

Supplementary key words  apolipoproteins • protein-lipid interactions • lipoprotein remodeling

High-density lipoproteins (HDL) are protein-lipid assemblies that circulate in blood plasma and have a role in reverse cholesterol transport, detoxification, and inflammation response. HDL is commonly known as “good cholesterol” because it prevents the development of atherosclerotic plaques. HDL removes cholesterol and oxysterols from foam cell macrophages, preventing the progression of fatty streak development. Fatty streak development is an initial step in plaque formation. Therefore by removing sterols from macrophages, atherosclerotic plaque development is impeded. High plasma atherosclerotic plaque concentration is generally associated with decreased risk of heart attack and stroke.

Mature plasma lipoproteins, such as mature-HDL, as well as chylomicrons, very low density lipoproteins (VLDL), and low-density lipoproteins (LDL), are emulsion-like particles that consist of a two distinct phases: a core phase and a surface phase (1). The core phase is primarily composed of hydrophobic lipids, such as triacylglycerides and esterified cholesterol. To stabilize the particles and prevent coalescence, the core phase is covered with a surface phase. The surface phase is composed of amphipathic molecules, mainly polar lipids, such as phospholipids, cholesterol, and proteins. The proteins on the surface of lipoprotein particles constitute a family of proteins called “apolipoproteins.” Apolipoproteins are surface-active molecules that function on a lipoprotein surface to both stabilize the emulsion particle and act as cofactors and ligands for numerous biological processes required for proper assembly, metabolism, and clearance of lipoprotein particles. A subclass of this family called “exchangeable apolipoproteins” includes apolipoproteins A, C, and E. Exchangeable apolipoproteins transfer between different types of lipoproteins in response to changes in size and lipid composition of the particles.

Ampipathic α-helices (AαH) are an ubiquitous structural feature of exchangeable apolipoproteins. AαHs are distinguished from generic α-helices because between 90° and 180° of the lateral cross-section of the helix is composed of hydrophobic residues. This portion of the helix is called the “hydrophobic face.” The remainder of the helix, called the “hydrophilic face,” is composed of hydrophilic and charged residues (2, 3). The hydrophobic face of the helix binds to the hydrophobic core of the lipoprotein, and the hydrophilic face interacts with water. This stabilizes a lipoprotein surface.

Abbreviations: AαH, amphipathic α-helix; APM, area per molecule; C46, C-terminal 46 amino acids of apolipoprotein A-I; Ex, exchangeable; H, helix; NE, nonexchangeable; TO, triolein; TO/W, triolein/water; II, surface pressure γ, surface tension.

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Apolipoprotein (apo)A-I is the principal protein component of HDL and is present in the surface of chylomicrons and VLDL. The structure of apoA-I is a series of AαHs (3). The core of apoA-I (aa 44–185) is a series of homologous 11/22 mer tandem repeats of AαHs (called A- and B-type helices), which are separated by prolines to encourage bend, turn, or loop formation (2, 4). The N-terminus (aa 1–43) and C-terminus (aa 186–243) of apoA-I also contain some AαHs but do not have the repeated sequence of the core helices (5, 6). By forming a series of AαHs with flexible loops between each helix, apoA-I can form soluble monomers in water at low concentration by forming a hydrophobic protein core. It can also bind to a lipoprotein with its amphipathic helices interacting with the lipids (2, 7, 8). The conformation of apoA-I at a lipid interface likely depends on the lipid composition and protein surface concentration. For example, at near-saturated protein conditions (when apoA-I was in excess), only the C-terminus of apoA-I bound to egg phosphatidylcholine (EPC) vesicles, and the remaining N-terminus (aa 1–198) did not interact with lipid (9). When EPC surface was in excess, the entire protein appeared to interact (9). This suggests a bulk concentration dependence on the apoA-I interface structure in which only the C-terminus of apoA-I directly interacts with lipid at a high protein surface concentration and presumably lipoprotein surface pressure (II).

The C-terminal 46 residues (aa 198–243) of apoA-I (C46) are predicted to be the most lipophilic portion of the peptide and have the highest lipid affinity within the apoA-I sequence (10). C46 is studied in this article and serves as a model of other AαHs in apolipoproteins. Below a concentration of less than 0.02 mg/ml, C46 forms an unstructured monomer (5). Upon lipid (or lipid mimic) binding, the helical content of C46 increased from ~16% to ~60%. At higher concentrations, C46 self-associates, forming tetramers or pentamers with ~50% helical content (5). When bound to lipid, C46 is predicted to form a helix-loop-helix structure (11–13). The N-terminal part of C46 (198–209 or 218) is predicted to have a lower lipid affinity than the C-terminal helix (220–238) (2, 14–16).

An unanswered question in lipoprotein physiology is how exchangeable apolipoproteins transfer between lipoprotein particles. One possibility is that lipid-bound apolipoproteins are in a concentration-dependent equilibrium with soluble plasma apolipoproteins. In other words, the exchangeable apolipoproteins are constantly adsorbing and desorbing from a lipoprotein surface. To test this hypothesis, Small et al. adsorbed small AαH peptides to a pendant triolein (TO) drop and examined their compressibility and exchangeability (17–20). A TO drop is an adsorbed triolein (TO) drop and examined their compressibility and exchangeability (17–20). The N-terminus of AαH derived peptides we have tested, including apoC-I, short apoA-I-derived peptides (N44 and C46), and apolipoprotein mimetic peptides (Refs. 17, 24, and unpublished observations). Partial exchangeability also occurred when the N-terminus of apoA-I (N44), C46, and full-length apoA-I were adsorbed to a TO/POPC/W interface and washed out (24, 25). When partial exchangeability was originally reported in 2009, we did not have an explanation (17). In this article, we present a two-surface state thermodynamic and kinetic model that adequately describes the change in II during a washout, using C46 as a model AαH-forming peptide. This model is used to speculate on the interfacial conformation and remodeling of C46 at a lipoprotein-forming interface, and it may describe how exchangeable apolipoproteins remodel and exchange in response to changes in lipoprotein composition and size.

**MATERIALS AND METHODS**

**Methods**

All experiments were performed with an IT Concepts Oil-Drop Tensiometer (Longessaigne, France) (26). See supplementary Fig. I for more detail about the instrument. A 16 μl drop of TO was formed at the tip of a J-tube submerged in 6 ml of bulk buffer. The bulk buffer was 2 mM sodium phosphate buffer (PB) at a pH of 7.4 ± 0.1. The drop was >99.7% pure TO (Nu-Chek Prep, Elysian MN). A 0.5–250 μl of the C46 stock solution (see below) was added to the 6 ml of bulk solution. For most experiments, 25 μl of 1 mg/ml C46 stock was added for a final concentration of ~4.2 μg/ml C46 spontaneous adsorbs to the triolein/water interface, and raises the II (18). The peptide was removed from the bulk solution by flowing 220 ml of peptide-free bulk buffer through the cuvette. This effectively depleted the bulk peptide by >99.9% (17, 21–23). The process is referred to as a “washout.” In general, after the washout, the drop either i) expanded linearly and then linearly compressed. In some experiments, peptide was readded to the bulk buffer after a washout. During the experiments, drop volume, area, and surface tension (γ) were recorded. II was calculated by subtracting the γ of a TO/C46/W interface from the γ of a clean TO/W interface (II = 32 − γ). The specific details of individual experiments are given in the Results section.
RESULTS

Characterization of partial exchangeability

C46 is partially exchangeable at a TO/W interface. Partial exchangeability was demonstrated by washing the peptide out of the solution surrounding a TO drop after adsorption (Fig. 1A, B). Partial exchangeability is characterized by a change in Π during a washout, without Π returning to the Π of a peptide-free TO/W interface (i.e., Π = 0 mN/m). Partial exchangeability is a thermodynamic phenomenon; thus, by developing a thermodynamic model of the process, we can better understand and potentially prove the mechanism. One requirement for using thermodynamic analysis is that the change must be reversible. To prove reversibility, we readded the peptide to the surrounding solution at the prewashout concentration (Fig. 1A). Readding the peptide caused Π to return to the prewashout value; therefore, the change in Π caused by the washout is reversible. Washing out and readding the peptide was repeated up to four times with a similar result (data not shown).

In 2007, Wang et al. demonstrated that the prewashout equilibrium Π was dependent on the bulk concentration of C46 adsorbed at a TO/W interface (18). In this article, we confirm those results (Fig. 1A) and show that the postwashout Π was independent of the prewashout bulk concentration. Four examples of the washout intervals at different initial concentrations are shown in Fig. 1B. The relationship between prewashout and postwashout equilibrium surface tension (γ) is shown in Fig. 1C, D. The prewashout γ did not change above a concentration of ~10 μg/ml, which was used to define the saturated pressure (ΠSAT = 32 - γSAT). The Π after a washout was independent of prewashout bulk concentration and was defined as the washout pressure (Πw = 32 - γw). This indicates that the exchangeable state is in equilibrium with the solution, whereas the nonexchangeable state is not.

Wang et al. showed that prewashout compression of a TO/C46/W interface caused the peptide to be expelled from the surface but that the peptide reabsorbs when the surface was reexpanded (18). Postwashout compressions and reexpansions of C46, also known as a stress-response experiment, are shown in Fig. 2. When a TO/C46/W interface was rapidly compressed and then reexpanded to the original area, Π returned to a lower value than before the compression. The difference in Π means that the peptide desorbed from the surface after a compression. Compression of the surface caused the peptide surface concentration (Γ) to increase. The increase in Γ caused some of the nonexchangeable peptide on the surface to become exchangeable and desorb from the surface. Therefore, compression of C46 caused the peptide to desorb from the surface. However, C46 does not fully desorb spontaneously without a compression.

Model of partial exchangeability

Using this data, a two-surface state model of C46 or N44 was developed (Fig. 3). The most reasonable conclusion from this data is that the soluble state (S) of the peptide is in bulk concentration-dependent adsorption-desorption equilibrium with the exchangeable (Ex) surface state of the peptide. The Ex and nonexchangeable (NE) states are in a surface concentration (Γ)- and/or Π-dependent equilibrium. The Ex conformation of the peptide can exert more Π on the surface, but the NE conformation occupies a larger area. The peptide maximizes Π by adopting both the Ex and NE conformations when there is not an excess of surface peptides. A quantitative thermodynamic model would better describe this system and allow testing of these hypotheses about helix exchangeability.

We make the assumption that the soluble state of the peptide is a random unfolded coil. Zhu and Atkinson (5) found that at concentrations of 5 μg/ml, a random coil circular dichroism spectrum was obtained, and they concluded that protein was unfolded and only folded at higher concentrations, reaching a fully folded state only about concentration of 100 μg/ml. This is well above the concentration of this study. While it is possible that the soluble peptide at this concentration might exist in many partly folded states that only exist for a very short time (<1 msec), we have chosen to assume that the "soluble state" of the peptide is a single state.

The state variables of the system can be defined from first principles. A mass balance can be used to define the number of moles in each state:

\[ N = N_{\text{Ex}} + N_{\text{NE}} + N_s \]  \hspace{1cm} (Eq. 1)

Where N is the number of molecules, and the subscripts Ex, NE, and S represent the exchangeable, nonexchangeable, and soluble states of the peptide. A useful definition is the number of surface molecules (Nsurf):

\[ N_{\text{Sur}} = N_{\text{Ex}} + N_{\text{NE}} \]  \hspace{1cm} (Eq. 2)

The area is defined as:

\[ A = APM_{\text{Ex}}N_{\text{Ex}} + APM_{\text{NE}}N_{\text{NE}} + C_sA_B \]  \hspace{1cm} (Eq. 3)

Where APM is the area per molecule in each state, C_s is the number of vacant binding site, and A_B is the area per binding site. At equilibrium, C_sA_B will make a small contribution to A, so the first assumption is C_sA_B = 0, simplifying equation 3 to:

\[ A = APM_{\text{Ex}}N_{\text{Ex}} + APM_{\text{NE}}N_{\text{NE}} \]
Exchangeability of ActHs

A

0

5

10

15

20

25

30

35

40

45

50

Time [min]

Pressure [mN/m]

Re-Added C46

Washout

Fig. 1. (A) When C46 was adsorbed to a TO/W interface at a bulk concentration of 4.2 μg/ml, the surface pressure (Π) rose to ~16 mN/m. After reaching an equilibrium Π, C46 was washed out of the bulk (indicated by the black bar). The washout caused Π to decrease to a new steady state Π. When C46 was readded after the washout at a concentration of ~4.2 μg/ml (indicated by the arrow on the far right), Π returned to the prewashout Π. Note that the γ axis is inverted. The area was kept constant throughout this experiment. (B) The prewashout surface tension (γ) of C46 was dependent on the bulk concentration (18), but the postwashout γ was independent of the prewashout bulk concentration. The prewashout γ was higher at a higher bulk C46 concentration, e.g., at 16.6 μg/ml (blue) the equilibrium γ was 1.5 mN/m lower than that at 0.16 μg/ml (purple). (C and D) The prewashout II was dependent on bulk concentration (black diamonds, top). Before the washout, II was related to [C46] by the relation II = 1.12log([C46])+15.162 (R² = 0.946). II was saturated at a bulk concentration >10 μg/ml. The Π at the saturated concentration is defined as Π\text{SAT}. The postwashout γ was not dependent on bulk concentration (gray squares, bottom). The Π after a washout is defined as Π\text{WO}. Π was calculated by subtracting the γ of a TO/C46/W interface from the γ of a clean TO/W interface (Π = 32 – γ).

\[ A = APM_{Ex}N_{Es} + APM_{NE}N_{NE} \]  
(Eq. 4)

Surface pressure (Π) can be defined by the law of partial pressures adapted for two dimensions:

\[ \Pi = \pi_{Es}x_{Es} + \pi_{NE}x_{NE} \]  
(Eq. 5)

Where \( \pi \) is the partial surface pressure of the Ex or NE state and \( x \) is the fraction of the area occupied by each state, defined as:

\[ x_{Es} = \frac{APM_{Es}N_{Es}}{A} \]  
(Eq. 6)

\[ x_{NE} = \frac{APM_{NE}N_{NE}}{A} \]  
(Eq. 7)

Partial surface pressure (\( \pi \)) is thermodynamically defined as:

\[ \pi_{Es} = \frac{\Delta G_{\text{Eq},s+1}}{APM_{Es}} \]  
(Eq. 8)

\[ \pi_{NE} = \frac{\Delta G_{\text{Eq},s+1}}{APM_{NE}} \]  
(Eq. 9)

By combining equations 4–7, the surface equilibrium constant is defined as:

\[ K_{s+1}^{\text{Sur}} = \frac{N_{NE}}{N_{Es}} \frac{APM_{Es}(\Pi - \pi_{Es})}{APM_{NE}(\pi_{NE} - \Pi)} \]  
(Eq. 10)
Postwashout compressions caused \( \Pi \) to rapidly rise and then recovered to a new equilibrium value near the precompression \( \Pi \). When the surface was reexpanded, \( \Pi \) was lower than the precompression \( \Pi \). This indicates that some of the peptide was expelled from the surface by compression. In other words, the non-exchangeable state can convert to the exchangeable state of the peptide by a compression.

A derivation of \( K_{\text{sur}}^{\text{eq}} \) is included in the supplemental materials. Equation 10 can be used to define the surface Gibbs free energy between the two surface states as:

\[
\Delta G_{\text{Ex-NE}} = -RT \ln \left( \frac{\text{APM}_{\text{Ex}}}{\text{APM}_{\text{NE}}} \right) \left( \frac{\Pi_{\text{NE}} - \Pi_{\text{Ex}}}{\Pi_{\text{Ex}} - \Pi_{\text{NE}}} \right) \quad (\text{Eq. 11})
\]

Where \( R \) is the universal gas constant, and \( T \) is the temperature.

Equation 10 is an insightful formula, which requires some thought to appreciate. It defines the relative number of molecules in the two surface states as a function of an experimentally measureable parameter (\( \Pi \)). Based on this equation, \( K_{\text{sur}}^{\text{eq}} \) is dependent on three factors: a structural constant \( \frac{\text{APM}_{\text{Ex}}}{\text{APM}_{\text{NE}}} \), which is based on the difference in conformation of the two states, and two energy constants (\( \Pi_{\text{NE}}, \Pi_{\text{Ex}} \)), which are related to the interfacial activity of the two surface states. These parameters define \( K_{\text{sur}}^{\text{eq}} \) (\( \Pi \)). The constants in this equation \( (\Pi_{\text{NE}}, \Pi_{\text{Ex}}) \) and \( \frac{\text{APM}_{\text{Ex}}}{\text{APM}_{\text{NE}}} \) can be calculated from drop tensiometry data. With these constants known, the surface equilibrium of NE and Ex can be quantitatively defined.

**Two-state surface equilibrium model constants**

Calculating the value of \( \Pi_{\text{NE}} \) and \( \Pi_{\text{Ex}} \) is reasonably straightforward by analyzing two cases: (a) after a washout and (b) before a washout at saturating bulk peptide concentration (>10 \( \mu \)g/ml; see Fig. 1B). In the first case, we assume that, after a washout, the number of soluble and exchangeable peptide molecules is effectively zero:

\[
N_{\text{S}} = N_{\text{Ex}} = 0
\]

\( \Pi \) after a washout is \( \Pi_{\text{WO}} \) (defined in Fig. 1C), which according to equations 1 and 5 is equal to \( \pi_{\text{NE}} \). Therefore:

\[
\pi_{\text{NE}} = \Pi_{\text{WO}}
\]

In the second case, after a washout, there is an excess of surface molecules in the system. When there is an excess of bulk peptide to saturate \( \Gamma \), most of the peptide will be in the smaller APM conformation. The Ex form has a smaller APM than the NE form because compressing the surface postwashout (Fig. 3) causes the NE to convert to the Ex state. If the NE had a smaller APM, expanding the surface postwashout would cause NE to convert to Ex, which is not the case. Therefore, we can assume that when \( \Pi \) is saturated by having a high bulk concentration of peptide, the number of NE molecules is negligible because there will be a much larger population of Ex than NE molecules. Thus, we have assumed:

\[
N_{\text{NE}} = 0
\]

The \( \Pi \) at saturated concentration equals \( \Pi_{\text{SAT}} \) (defined in Fig. 1D), which according to equations 2 and 5, is equal to \( \pi_{\text{Ex}} \):

\[
\pi_{\text{Ex}} = \Pi_{\text{SAT}}
\]

Calculating the structural factor \( \frac{\text{APM}_{\text{Ex}}}{\text{APM}_{\text{NE}}} \) first requires consideration of how a TO/C46/W interface responds to a rapid compression (shown in Fig. 2). When the surface was compressed, \( \Pi \) rapidly rose and then slowly fell over the next 3–5 min. The change in \( \Pi \) was accompanied by a decrease in the number of peptide molecules on the surface. There are two steps to the conversion of NE to S molecules. The first step is a conformational arrangement of the protein on the surface to go from NE to Ex, presumably through protein remodeling and two-dimensional diffusion. In the second step, Ex molecules desorb from the surface. In general, protein remodeling events occur on the timescale of nanoseconds to microseconds. On the other hand, protein desorption occurs on the timescale of milliseconds to hours. Therefore, it is reasonable to assume that NE and Ex reach an equilibrium much faster than the Ex and S forms. After a rapid compression, \( \Pi \) rapidly decreases. Since NE and Ex reach an equilibrium quickly (meaning faster than the sampling rate of \( \sim 2 \) points/sec), \( \Delta G_{\text{Ex-NE}} \) will equilibrate before a significant amount of peptide has desorbed from the surface. The larger the compression, the more favorable the Ex form will be. If a compression is large enough, such that \( \Pi \) immediately after a compression is very close to \( \Pi_{\text{SAT}} \), the ratio of the area before and after a compression will be equal to the ratio of the APM of the NE and Ex forms. Mathematically stated:

![Fig. 2](image-url) Postwashout compressions caused \( \Pi \) to rapidly rise and then recovered to a new equilibrium value near the precompression \( \Pi \). When the surface was reexpanded, \( \Pi \) was lower than the precompression \( \Pi \). This indicates that some of the peptide was expelled from the surface by compression. In other words, the non-exchangeable state can convert to the exchangeable state of the peptide by a compression.

![Fig. 3](image-url) Two-surface state thermodynamic model to explain the partial exchangeability of C46 before washout (left) and after washout (right). The soluble form (S) is in an adsorption-desorption equilibrium with the exchangeable form (Ex). On the surface, the Ex form and a nonexchangeable form (NE) are in a \( \Pi \)-dependent equilibrium. When the S form is removed by a washout (right), some of the Ex form of C46 desorbs from the surface and \( \Pi \) fall. The fall in \( \Pi \) encourages the NE conformation of C46.
Where $\Pi^C$ is the $\Pi$ immediately after a compression, $A^C$ is the area after a compression, and $A^{\text{INT}}$ is the area before a compression. Equation 12 is rigorously defined in supplementary proof IV. The relationship between $\frac{\Delta G_{\text{Ex}}}{}$ and $\Pi_{\text{SAT}}$ is $\frac{A^{\text{INT}}}{A^C}$ is equal to the x-intercept, which is equal to 1.45 ± 0.07 for C46.

With $\pi_{\text{NE}}$, $\pi_{\text{Ex}}$, and $\frac{\Delta P_{\text{NE}}}{\Delta P_{\text{Ex}}}$ determined, the surface equilibrium constant (equation 10) is fully defined. Equation 11 can be used to calculate $\Delta G_{\text{Ex} \rightarrow \text{NE}}$ during desorption, during a washout, and after a postwashout rapid compression. The value of $\Delta G_{\text{Ex} \rightarrow \text{NE}}$ during these events is shown in Fig. 4. During the adsorption, $\Delta G_{\text{Ex} \rightarrow \text{NE}}$ increased until a new equilibrium value near 7 kJ/mol. During a washout, $\Delta G_{\text{Ex} \rightarrow \text{NE}}$ decreased sigmoidally to a value of $\sim$7 kJ/mol. When the surface was compressed, $\Delta G_{\text{Ex} \rightarrow \text{NE}}$ immediately increased, then slowly decreased to a new equilibrium value near the postwashout value.

### Kinetic model of desorption

Although this is a fully defined surface equilibrium model, the validity of the model would be strengthened by demonstrating its ability to predict a change in $\Pi(t)$ either during a washout or after a postwashout compression. Unfortunately, the thermodynamic data has been exhausted in determining $K_{\text{eq}}^m$. To test the validity of the two-state equilibrium model of $K_{\text{eq}}^m$, a kinetic model must be invoked to predict either a washout or the compression response over 3–5 min. Without any information about the order of the kinetics, a first-order adsorption and desorption model is the best possible assumption. By again assuming that NE and Ex reach equilibrium much faster than Ex and S, the rate equations can be simplified to the rate of adsorption ($r_A$) and the rate of desorption ($r_D$):

$$ r_A = \frac{dN_{\text{NE}}}{dt} = k_A N_C \pi_{\text{NE}} \quad \text{(Eq. 13)} $$

$$ r_D = \frac{dN_{\text{Ex}}}{dt} = -k_D N_{\text{Ex}} \quad \text{(Eq. 14)} $$

Fig. 4. The change in $\Pi$ immediately after a compression was dependent on the size of the compression. This relationship can be used to calculate the value of $\frac{\Delta P_{\text{NE}}}{\Delta P_{\text{Ex}}}$, which is equal to the value of $\frac{A^{\text{INT}}}{A^C}$ as $\Pi_{\text{SAT}} \Pi^C$ approaches zero. The value of $\frac{\Delta P_{\text{NE}}}{\Delta P_{\text{Ex}}}$ = 1.46 for C46.

Where $k_A$ is the adsorption constant, $C_v$ is the number of vacated binding sites, and $k_D$ is the desorption constant.

This kinetic model now introduces a new assumption and three new unknowns to the system of equations. Fortunately, after a washout $N_S$ will be equal to zero, therefore $r_A$ will also be equal to zero (see equation 13). With this in mind, equation 14 can be combined with the two state thermodynamic model to predict the $\Pi(t)$ during a washout:

$$ \Pi = \Pi_{\text{Sat}} + (\Pi_{\text{Sat}} - \Pi_{\text{WO}}) \left(1 - \frac{\Pi_{\text{Sat}}}{\Pi_{\text{WO}}}\right) \left(1 - \frac{\pi_{\text{Ex}}}{\pi_{\text{NE}}} \frac{A_{\text{Ex}}}{A_{\text{Ne}}} (1 + k_D)\right) + \Pi_{\text{WO}} \quad \text{(Eq. 15)} $$

after a rapid compression from $A^{\text{INT}}$ to $A^C$:

$$ \Pi = \frac{\Pi_{\text{Sat}}}{\frac{A_{\text{Ex}}}{A_{\text{Ne}}} (1 - k_D)} \left(1 - \frac{A_{\text{Ex}}}{A_{\text{Ne}}} \frac{A_{\text{Ex}}}{A_{\text{Ne}}} - 1\right) + \Pi_{\text{WO}} \quad \text{(Eq. 16)} $$

See the supplementary material for the derivation of these two kinetic equations in proof V.

Although these are complex equations, all the parameters in these equations are known from experimental data except $k_D$. One set of experiments can be used to correlate $k_D$ using the known values of $\pi_{\text{NE}}$, $\pi_{\text{Ex}}$, and $\frac{\Delta P_{\text{NE}}}{\Delta P_{\text{Ex}}}$. Then the other experiment can be used to predict the $\Pi(t)$. The resulting correlations using both models are shown in Fig. 6. The predicted correlated models are in good agreement with the experimental data. The value of $k_D$ is either 0.24 or 0.3 min$^{-1}$ depending on which experiment is used for the correlation. Additional correlating methods are used in

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**Fig. 5.** Surface Gibbs free energy as a function of time during the adsorption of C46 to the surface (far right), a washout (black bar), and after a rapid postwashout compression (arrow on the far right). The area was kept constant for the first 47 min of the experiment at 30 mm$^2$. At 47 min (arrow), the surface was rapidly reduced by $\sim$30% to 20.6 mm$^2$. After the compression, peptide slowly desorbed from the surface. Gibbs free energy is defined as: $\Delta G = -\Delta H + T \Delta S$. Pressure is traced in black and Gibbs free energy is traced in gray.

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supplementary Figs. II and III. The good agreement between the model and experimental data indicates that the two-surface state thermodynamic model and first-order kinetics adequately describe partial exchangeability of C46. In addition, the constants derived from this model are physically reasonable.

DISCUSSION

In circulation, plasma lipoproteins remodel as their composition and size change due to enzyme-mediated flux of hydrophobic core lipids. For example, lecithin-cholesterol acyltransferase (LCAT) esterifies amphipathic cholesterol with a fatty acid-forming hydrophobic cholesterol ester on the surface of HDL. Lipid-bound apoA-I is a co-factor for LCAT. The esterification causes the cholesterol to transfer from the surface to the core, thus increasing the number of core lipids. Due to LCAT and other plasma factors, HDL particles can grow as they accumulate cholesterol ester. Presumably as the particle grows, $\Pi_{\text{WO}}$ decreases due to less surface crowding. In other words, as the surface area increases, the surface pressure decreases. Lipoprotein particles can also shrink in circulation due to the enzymatic activity of lipoprotein lipase (LPL). LPL requires apoC-II on the surface of chylomicrons or VLDL as a co-factor (27). On the surface of lipoproteins, LPL hydrolyzes hydrophobic triacylglyceride to two free fatty acids and a monoacylglyceride. The products of this reaction are amphipathic and surface active. Therefore, LPL-mediated hydrolysis results in a depletion of the lipoprotein core and an increased number of molecules on the surface, causing surface crowding. The surface crowding and decrease in surface area result in an increase in $\Pi_{\text{A}}$ of a lipoprotein.

Alternate methods of exchange have been employed by others. The presence of exchange and rate of exchange depend on the system employed and the methods. In an in vitro system, after incubating chylomicrons with increasing larger compressions (see supplementary Fig. IV). When $\Pi$ remained below $\Pi_{\text{WO}}$, the compressions were reversible, and when $\Pi$ exceeded $\Pi_{\text{WO}}$, there was a loss of area during the next compression (supplementary Fig. IV).
amounts of HDL for 1 h, the apoA-I (but not apoA-II) mass moved from HDL to nascent chylomicrons. However, when apoA-I-labeled chylomicrons were injected into live monkeys in vivo, the labeled chylomicron apoA-I was transferred to HDL for the first ~3 h, showing that, unlike the in vitro system, chylomicron apoA-I moves to HDL. The difference in these systems shows that apoA-I movement off of chylomicrons to HDL is driven by a number of factors in plasma, especially LPL activity and possibly a host of other plasma enzymes, coeffectors, and transfer proteins. We demonstrated that compression is sufficient to exchange amphipathic α-helices independent of other plasma factors.

As lipoproteins remodel, the exchangeable apolipoprotein profile changes. In general, larger, low II lipoproteins, such as VLDL and chylomicrons, have more exchangeable apolipoproteins than smaller, high II lipoproteins, such as LDL. Exchangeable apolipoproteins are unable to bind a high II surface (28, 29) and desorb when compressed (17–20). The II where a lipoprotein is incapable of binding a lipoprotein surface is defined as the exclusion pressure (28, 29). Apolipoproteins remodel their conformation in response to changes in II. The two-state thermodynamic model implies that the adsorption and remodeling are independent events. The binding of an exchangeable apolipoprotein is likely initiated by the most hydrophobic portion of the protein interacting with a lipoprotein surface with a II below its exclusion pressure. After interacting, the apolipoprotein can remodel to a NE surface conformation in which the apolipoprotein spreads on the surface. The II-dependent equilibrium between the interfacial conformations of an exchangeable apolipoprotein is defined by the surface equilibrium constant (equation 10):

\[
K_{\text{sat}}^{\text{NE}}(\Pi) = \frac{N_{\text{NE}}}{N_{\text{II}}} = \frac{\text{APM}_{\text{II}}}{\text{APM}_{\text{NE}}} \left( \frac{\Pi - \pi_{\text{NE}}}{\pi_{\text{NE}} - \Pi} \right)
\]

The Ex conformation of an apolipoprotein has a smaller APM and higher π than the NE conformation. As a lipoprotein shrinks, II increases, which prefers the compact Ex conformation. As a lipoprotein grows, II decreases, encouraging an apolipoprotein to spread and convert to the NE conformation.

According to the two-state thermodynamic model, the interfacial structure affects both APM and partial pressure (π). Without any information about the absolute number of molecules on the surface, there is no way to calculate the APM of each surface state. π was inversely related to APM, and thus, was also unknown. However, the relative areas and π’s of surface transition states were directly calculated from experimental data. This can be related to the structure by making an educated guess about all the possible interfacial structures of each transition state. The possible interfacial structure combinations of high and low II states are related to the structure and energy factors, which can be used to relate structure and thermodynamics. To address this question, we can consider each AαH as an independent lipid-binding unit. In this model, when a helix adsorbs to a hydrophobic interface, the entire hydrophobic face binds. The helix also desorbs as a single unit. Between each helix is a flexible loop or proline. Aside from the peptide backbone constraints between the two helices, the AαHs were independent of one another in regard to their adsorption/desorption behavior. The desorption of the exchangeable form of C46 was quite slow (k_θ = 0.2 – 0.35 min^{-1}), suggesting that helix desorption was a rare event on a molecular scale that occurs stochastically. If only one helix was bound, the rare desorption events still allowed C46 to diffuse away from the surface. When a second helix binds, the peptide cannot diffuse from the surface because it was anchored by the lipid-bound helix. Both helices desorbing simultaneously is unlikely. The protein can only adsorb when all helices are detached. The NE form became exchangeable upon compression because at least one helix was pushed off by reduced area and the remaining helix desorbed stochastically.

There are three potential helices in C46, which are punctuated with prolines at aa 209 and 220 to disrupt
an independent lipid-binding unit, there are only seven potential interfacial structures: only one helix on the surface (H1, H2, or H3); two helices on the surface (H1 + H2, H2 + H3, or H1 + H3); or all three helices on the surface (H1 + H2 + H3).

Treating A/H9251 Hs as independent binding units allows thermodynamics to be related to lipid-bound structure. To establish this relationship, we must make two assumptions: i) the APAA was similar for in all A/H9251 Hs (i.e., AEXNE = AEXW), and ii) ΔGW -O (calculated from the White-Wimley hydrophobicity scale) was roughly proportional to ΔGW -O.

These assumptions led to the following feasibility criteria (see the supplementary material for a derivation): i) \( \frac{\Delta \text{GW}_{\text{N} \to \text{E}}}{\Delta \text{GW}_{\text{E} \to \text{N}}} > 1 \) and ii) \( \frac{\Delta \text{GW}_{\text{N} \to \text{E}}}{\Delta \text{GW}_{\text{E} \to \text{N}}} > 1 \).

Both of these criteria are directly related to experimental data and were used to determine the feasible lipid-bound structures of C46 as a function of H9016.

There are seven potential structures in either the Ex or NE form (supplementary Table I) for a total of 49 possible Ex-NE combinations. To evaluate the first feasibility criterion, the value of \( \Delta \text{GW}_{\text{N} \to \text{E}} \) for each of these models is shown in Table 1. Twenty Ex-NE structure combinations are feasible according to the first criterion (bolded in Table 1). The feasible structure combinations require that an additional helix binds when converting from the Ex to NE forms. The value of each combination of the second feasibility criterion \( \Delta \text{GW}_{\text{N} \to \text{E}} \) is shown in supplementary Table III. There were 22 structural combinations that were valid according to the second feasibility criterion. According to this criterion,

continuous helix formation (2, 4–6, 12, 16, 30). A rough structure of the potential α-helices can be identified by making a helical wheel diagram. See Fig. 8 for a summary of the predicted helices of C46. The helical wheel predicts the amino acids in the hydrophobic face of an AαH and thus the potential protein in contact with the lipid. Two parameters were deduced from the helical wheel diagram: i) the number of amino acids in contact with the surface in that helix (\( \#_{\text{AA}} \)) and ii) the Gibbs free energy of transfer from water to oil of that helix (\( \Delta \text{GW}_{\text{N} \to \text{E}} \)). The \( \#_{\text{AA}} \) was determined by simply counting the number of amino acids in the hydrophobic face. The \( \Delta \text{GW}_{\text{N} \to \text{E}} \) was the sum of the \( \Delta \text{GW}_{\text{N} \to \text{E}} \) of all the amino acids in the hydrophobic face. The \( \Delta \text{GW}_{\text{N} \to \text{E}} \) was determined using the White-Wimley hydrophobicity scale (31–33). The \( \#_{\text{AA}} \) and \( \Delta \text{GW}_{\text{N} \to \text{E}} \) of each potential structure (where different sets of helices were on the surface) was calculated by summing the values of each individual helix in supplementary Table II.

The N-terminal helix is aa 198–208. In most models of full-length apoA-I, this helix extends to aa 189 (2, 5, 10–13, 16). There are three amino acids in the hydrophobic face of the AαH (LAL), with a total \( \Delta \text{GW}_{\text{N} \to \text{E}} = -8.4 \text{ kJ/mol} \), based on the White/Wimley hydrophobicity scale. The second helix, which spans aa 210–219, has four amino acids in the hydrophobic face (ALLL) and a \( \Delta \text{GW}_{\text{N} \to \text{E}} = -13.6 \text{ kJ/mol} \). The third helix, aa 219–243, is distinguished from the other two helices because it is longer and has three aromatic amino acids in the face. For helix 3, \( \#_{\text{AA}} = 7 \) (VAFYFLL) and \( \Delta \text{GW}_{\text{N} \to \text{E}} = -31.8 \text{ kJ/mol} \). If each helix is considered

![Fig. 8. Lipid binding of C46 induces the formation of AαHs. C46 is predicted to form three potential helices separated by prolines at aa 209 and 220. In full-length apoA-I, the first helix (198–209) likely extends to aa 189. The C-terminal helix, helix 3 aa 220–238, is the longest and most hydrophobic in C46. The number of amino acids in the hydrophobic face (\( \#_{\text{AA}} \)) and the Gibbs free energy of transfer from water to oil (\( \Delta \text{GW}_{\text{N} \to \text{E}} \)) of each helix were determined based on the helical wheel diagrams based on the White/Wimley (W/W) and GES hydrophobicity scales.](image-url)
the Ex form has a higher average amino acid hydrophobicity (i.e., more aromatics or leucines) than the NE form.

Only six structural combinations of the NE-Ex forms meet both criteria (bolded in supplementary Table III). The feasible structures are listed in Table 1. All six structure combinations have an additional helix interacting with the surface to make C46 the NE form. The helix that binds must have a lower mean hydrophobicity than the helix (or helices) interacting in the Ex form. Helix 3 has the highest mean hydrophobicity (because of the aromatics), helix 1 has the lowest, and helix 2 is in the middle. So if helix 3 interacts in the Ex form, then helices 1 and/or 2 bind to convert it to NE. If helix 2 interacts in the Ex form, helix 1 binding converts it to the NE form. The values of $\frac{\sigma_{AA}}{\Sigma_{AA}}$ were in reasonably good agreement with the experimentally measured value of the structural factor ($\frac{\sigma_{PM}}{\Sigma_{PM}} = 1.45 \pm 0.07$). This agreement suggests that assuming the APAA was similar for all AaHs was a reasonably good assumption. Two structural models (3, 6) are within the standard deviation of the experimental value. In model 3, helix 3 was bound in the Ex form, and H1 binds to convert to the NE form. In model 6, both helices 1 and 3 are bound in the Ex form, and helix 2 binds to convert to the NE form. Both models suggest helix 2 had a unique role as a conformational switch in converting from the Ex to NE form (34). The first feasibility criterion could be made more stringent by restricting the value of $\frac{\sigma_{AA}}{\Sigma_{AA}}$ to within the standard deviation of $\frac{\sigma_{PM}}{\Sigma_{PM}}$, or at least within double the standard deviation ($\sigma^2$). This changes first feasibility criterion to:

$$\frac{APM_{Ex}}{APM_{Ex}} - 2\sigma^2 < \frac{\#_{AA}}{\#_{AA}} < \frac{APM_{Ex}}{APM_{Ex}} + 2\sigma^2 \quad (Eq. 19)$$

With this criterion, there are only three feasible structure combinations: models 2, 3, and 6. In each of these models, helix 3 is bound in the exchangeable form. To convert to the NE form, helix 1 or 2 binds and prevents exchange. Model 2 is depicted in Fig. 9.

Conclusions

This explanation of partial exchangeability suggests that “exchangeable” apolipoproteins have at least two distinct surface conformations. When an apolipoprotein adsorbs to a lipoprotein particle, it has a compressed conformation at first, in which only the most hydrophobic helix (or helices) interact with the surface. Once the apolipoprotein binds, it may remain associated with the surface in the Ex form for an extended period due to the slow off-rate ($k_d$). When $\Pi$ is between $\Pi_{SAT}$ and $\Pi_{WO}$, the two structural states of the protein are in a $\Pi$-dependent equilibrium. If $\Pi$ is below $\Pi_{WO}$, the apolipoprotein will spread by binding more AaHs and occupying a larger APM. The surface remodeling of apolipoprotein physically buffers changes in $\Pi$ during metabolism by rapidly adopting a new conformation, providing stability and flexibility to dynamic lipoprotein particles.

The metabolic state of plasma lipoproteins (composition, type, size, etc.) is reflected in the composition and conformation of the associated apolipoproteins. It follows that only one of the surface conformations would be the “active form” for a particular enzyme. A hypothesis can be developed about the $\Pi$-dependent surface equilibrium of apolipoproteins to determine the active form. Speculative examples would be apoC-II and apoE. ApoC-II, the cofactor for lipoprotein lipase (27), would be active in the low $\Pi$ NE form but inactive in the Ex form, although it could remain on the surface. The low $\Pi$ NE form of apoC-II would be the cofactor of lipoprotein lipase. If this hypothesis were true, triacylglyceride hydrolysis could only occur on lipoproteins with a sufficiently large hydrophobic core (e.g., chylomicrons and VLDL as opposed to their remnant particles). The equilibrium between these two conformations is dependent on the surface equilibrium constant.

Experimental values of the criteria were similar to the structural factors for models 2, 3, and 6 (bolded).

**Table 1.** Summary of the six models of Ex and NE structures of C46 that met the original feasibility criteria

| Model of C46 | Ex Binding Helices | NE Binding Helices | $\frac{\#_{AA}}{\Sigma_{AA}}$ | $\frac{\#_{PM}}{\Sigma_{PM}}$ |
|-------------|-------------------|-------------------|-----------------|-----------------|
| 1           | H2                | H1 + H2           | 1.75            | 1.08            |
| 2           | H3                | H1 + H2           | 1.57            | 1.05            |
| 3           | H3                | H1 + H3           | 1.43            | 1.10            |
| 4           | H3                | H1 + H2 + H3      | 2.0             | 1.11            |
| 5           | H2 + H3           | H1 + H2 + H3      | 1.27            | 1.06            |
| 6           | H1 + H3           | H1 + H2 + H3      | 1.4             | 1.02            |

Experimental values: $1.45 \pm 0.07$. 1.23
forms dictates the rate of lipoprotein lipase and thus how triacylglyceride is distributed. Conversely, apoE only binds to LDLr (or lipoprotein receptor-related protein) when it is associated to high II remnant particles (35). This model suggests that apoE must be in the compressed form (Ex) to clearly chylomicron remnants, and although apoE is associated with low II chylomicrons, it only binds LDLr when it is compressed. These hypotheses are only speculation. ApoA-I is multifunctional and likely has multiple II-dependent surface conformations, each of which dictates the metabolic state of the lipoprotein. The remodeling and clearance of lipoproteins is dependent on both the apolipoproteins associated with the particle and their conformation.

REFERENCES

1. Miller, K. W., and D. M. Small. 1987. Structure of triglyceride-rich lipoproteins: an analysis of core and surface phase. New Comprehensive Biochemistry 16:143–166.

2. Brouillette, C. G., G. M. Anantharamaiah, J. A. Engler, and D. W. Borhani. 2001. Structural models of human apolipoprotein A-I: a critical analysis and review. Biochim. Biophys. Acta 1531:4–46.

3. Segrest, J. P., M. K. Jones, H. Deloof, C. G. Brouillette, Y. V. Venkatachalanpathi, and G. M. Anantharamaiah. 1992. The amphipathic helix in the exchangeable apolipoproteins - a review of secondary structure and function. J. Lipid Res. 33:141–166.

4. Silva, R. A. G. D., R. Huang, J. Morris, J. Fang, E. O. Gracheva, G. Ren, A. Kontush, W. G. Jerome, K. A. Rye, and W. S. Davidson. 2008. Structure of apolipoprotein A-I in spherical high density lipoproteins of different sizes. Proc. Natl. Acad. Sci. USA. 105:12176–12181.

5. Zhu, H. L. and D. Atkinson. 2004. Conformation and lipid binding of the C-terminal (198–243) peptide of human apolipoprotein A-I. Biochemistry. 43:13156–13164.

6. Zhu, H. L., and D. Atkinson. 2004. Conformation and lipid binding of the N-terminal (1–44) domain of human apolipoprotein A-I. Biochemistry. 43:13165–13164.

7. Thomas, M. J., S. Bhat, and M. G. Sorci-Thomas. 2008. Three-dimensional models of HDL apoA-I: implications for its assembly and function. J. Lipid Res. 49:1875–1883.

8. Tali, A. R., L. Van-Charvet, N. Terasaka, T. Pagler, and N. Wang. 2008. HDL, ABC transporters, and cholesterol efflux: implications for the treatment of atherosclerosis. Cell Metab. 7:365–375.

9. Komo, M. Y., O. Okumura, M. Tanaka, D. Nguyen, P. Dhanasekaran, S. Lund-Katz, M. C. Phillips, and H. Saito. 2008. Conformational flexibility of the N-terminal domain of apolipoprotein A-I bound to spherical lipid particles. Biochemistry. 47:11340–11347.

10. Lacroix, M., S. Makrides, A. Jonas, and V. I. Zannis. 1997. The carboxyl-terminal hydrophobic residues of apolipoprotein A-I affect its rate of phospholipid binding and its association with high density lipoprotein. J. Biol. Chem. 272:17511–17522.

11. Silva, R. A. G. M. Hilliard, L. Li, J. P. Segrest, and W. S. Davidson. 2005. A mass spectrometric determination of the conformation of dimeric apolipoprotein A-I in discoidal high density lipoprotein. Biochemistry. 44:8600–8607.

12. Borhani, D. W., D. P. Rogers, J. A. Engler, and C. G. Brouillette. 1997. Crystallization of truncated human apolipoprotein A-I suggests a lipid-bound conformation. Proc. Natl. Acad. Sci. USA. 94:12291–12296.

13. Okon, M., P. G. Frank, Y. L. Marcel, and R. J. Cushey. 2002. Heteronuclear NMR studies of human serum apoprotein A-I. Part I. Secondary structure in lipid-mimetic solution. FEBS Lett. 517:130–134.

14. Mishra, V. K., M. N. Palgunachari, G. Datta, M. C. Phillips, S. Lund-Katz, S. O. Adeeye, J. P. Segrest, and G. M. Anantharamaiah. 1998. Studies of synthetic peptides of human apoprotein A-I containing tandem amphipathic alpha-helices. Biochemistry. 37:10313–10324.

15. Tanaka, M., P. Dhanasekaran, D. Nguyen, S. Ohta, S. Lund-Katz, M. C. Phillips, and H. Saito. 2006. Contributions of the N- and C-terminal helical segments to the lipid-free structure and lipid interaction of apoprotein A-I. Biochemistry. 45:10351–10358.

16. Silva, R. A. G. D., G. M. Hilliard, J. W. Fang, S. Macha, and W. S. Davidson. 2005. A three-dimensional molecular model of lipid-free apoprotein A-I determined by cross-linking/mass spectrometry and secondary structure predictions. J. Lipid Res. 44:2759–2769.

17. Small, D. M., L. B. Wang, and M. A. Mitsche. 2009. The adsorption of biological peptides and proteins at the oil/water interface. A potentially important but largely unexplored field. J. Lipid Res. 50:5329–5334.

18. Wang, L., N. Hua, D. Atkinson, and D. M. Small. 2007. The N-terminal (1–44) and C-terminal (198–243) peptides of apolipoprotein A-I behave differently at the triolein/water interface. Biochemistry. 46:12140–12151.

19. Wang, L., D. Atkinson, and D. M. Small. 2005. The interfacial properties of ApoA-I and an amphipathic alpha-helix consensus peptide of exchangeable apolipoproteins at the triolein/water interface. J. Biol. Chem. 280:4154–4165.

20. Wang, L., D. Atkinson, and D. M. Small. 2003. Interfacial properties of an amphipathic alpha-helix consensus peptide of exchangeable apolipoproteins at air/water and oil/water interfaces. J. Biol. Chem. 278:37480–37491.