Enthalpy-Driven Apolipoprotein A-I and Lipid Bilayer Interaction Indicating Protein Penetration upon Lipid Binding†

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ABSTRACT: The interaction of lipid-free apolipoprotein A-I (apoA-I) with small unilamellar vesicles (SUVs) of 1-palmitoyl-2-oleoylphosphatidylcholine (POPC) with and without free cholesterol (FC) was studied by isothermal titration calorimetry and circular dichroism spectroscopy. Parameters reported are the affinity constant (K_a), the number of protein molecules bound per vesicle (n), enthalpy change ($\Delta H°$), entropy change ($\Delta S°$), and the heat capacity change ($\Delta C_p°$). The binding process of apoA-I to SUVs of POPC plus 0−20% (mole) FC was exothermic between 15 and 37 °C studied, accompanied by a small negative entropy change, making enthalpy the main driving force of the interaction. The presence of cholesterol in the vesicles increased the binding affinity and the α-helix content of apoA-I but lowered the number of apoA-I bound per vesicle and the enthalpy and entropy changes per bound apoA-I. Binding affinity and stoichiometry were essentially invariant of temperature for binding to SUVs of POPC/FC at a molar ratio of 6/1 at $(2.8-4) \times 10^6$ M^{-1} and 2.4 apoA-I molecules bound per vesicle or 1.4×10^2 phospholipids per bound apoA-I. A plot of $\Delta H°$ against temperature displayed a linear behavior, from which the $\Delta C_p°$ per mole of bound apoA-I was calculated to be −2.73 kcal/(mol·K). These results suggested that binding of apoA-I to POPC vesicles is characterized by nonclassical hydrophobic interactions, with α-helix formation as the main driving force for the binding to cholesterol-containing vesicles. In addition, comparison to literature data on peptides suggested a cooperativity of the helices in apoA-I in lipid interaction.

Protein and lipid interactions are known to define structure and function of apolipoproteins and thus modulate the metabolism of lipoproteins (*1*). One class of lipoproteins, the human high-density lipoproteins (HDL),[1] participates in the early steps of cholesterol efflux from cells and plays a central role in the subsequent steps of the reverse cholesterol transport (RCT) process that removes excess cholesterol from peripheral tissues for excretion or recycling (*2*). Apolipoprotein A-I (apoA-I) is the main protein component of HDL. The polypeptide chain is arranged in a globular N-terminal domain (residues 1−43) and a C-terminal lipid-binding domain (residues 44−243). There are eight 22-mer and two 11-mer tandem amino acid sequence repeats, each with the periodicity of an amphipathic α-helix (*3*). ApoA-I binds to the periphery of lipid bilayer disks and to the surface of spherical lipoproteins and synthetic lipid microemulsions (*1*). On the basis of thermodynamic and circular dichroism measurements, lipid-free apoA-I has been proposed to exhibit a molten globule-like state under physiological conditions (*4*). Upon lipid binding, the protein α-helix content increases from ∼50% to ∼78% depending on the nature of the lipid bilayer. The roles that different amphipathic α-helices play in the interaction of apoA-I with lipids have been reviewed by Frank and Marcel (*5*). While the N-terminal helix 44−65 and the C-terminal helix 210−241 are important for the initial association with lipids, helices 100−121 and 122−143 are important for lipid binding and HDL maturation. The two helices between residues 144 and 186 contribute less to lipid binding. The central region (residues 87−112) was also found to interact specifically with phosphatidylcholine (PC) liposomes (*6*). The structural arrangement of apoA-I in discoidal HDL has been modeled, depicting the protein as having eight amphipathic α-helices connected by β-turns (*7*, *8*).

The aim of this work is to understand how the interaction of apoA-I with cell membranes is influenced by the presence of cholesterol. In a previous work we examined, using gel electrophoresis and two-photon fluorescence microscopy, the effect of three distinct conformations of apoA-I on its ability to bind and extract lipids from POPC membrane vesicles (*9*). We found that apoA-I binds reversibly to the vesicles with high affinity but does not extract significant amounts of lipid nor perturb the vesicle structure under the experimental conditions used. In the present work, we studied the interaction of lipid-free apoA-I with vesicles of POPC containing varying amounts of cholesterol using two different approaches, isothermal titration calorimetry (ITC) and cir-

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[1] Abbreviations: apoA-I, apolipoprotein A-I; POPC, 1-palmitoyl-2-oleoylphosphatidylcholine; FC, free cholesterol; SUVs, small unilamellar vesicles; HDL, high-density lipoproteins; PC, phosphatidylcholine; ITC, isothermal titration calorimetry; CD, circular dichroism spectroscopy; RCT, reverse cholesterol transport.

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cular dichroism (CD) spectroscopy. ITC yields thermodynamic parameters of interaction and thus provides critical information about the nature of the interaction. CD provides structural information on apoA-I in lipid-free and lipid-bound states. We found that binding exhibited enthalpy−entropy compensation and was driven by enthalpy; thus nonclassical hydrophobic interaction was the driving force of the interaction.

MATERIALS AND METHODS

Materials

Human apoA-I was purified as described previously (*10*) from blood plasma purchased from the Champaign County Blood Bank of the Regional Health Center, Champaign, IL. 1-Palmitoyl-2-oleoylphosphatidylcholine (POPC) was purchased from Avanti Polar Lipids, Inc. (Alabaster, AL); crystalline free cholesterol (FC) and Trizma-HCl [tris-(hydroxymethyl)aminomethane hydrochloride] were from Sigma Biochemicals (St. Louis, MO); [^{3}H]dipalmitoylphosphatidylcholine (DPPC, 92.3 Ci/mmol) was from Du Pont NEN (Boston, MA), and dichloromethane was from Fisher Scientific (Fairlawn, NY).

Methods

Small Unilamellar Vesicle (SUV) Preparation. To prepare small unilamellar vesicles 250−300 Å in diameter, a given amount of POPC with or without FC was combined in $CHCl_3$ with a trace amount of [^{3}H]DPPC to a specific radioactivity of about 90 cpm/μg of phospholipid. Lipids were dried under N_2 at ∼50 °C and then redissolved in dichloromethane in order to remove trace amounts of ethanol, which is frequently used to stabilize $CHCl_3$ (*11*). Samples were then dried again under a N_2 stream at ∼50 °C followed by overnight drying under high vacuum. Dried lipids were dispersed in Tris salt buffer (TSB) (10 mM Tris-HCl, pH 8.0, 0.15 M NaCl, 1 mM NaN_3, and 0.1 mM EDTA) and vortexed extensively to obtain a milky dispersion of multilamellar liposomes (MLV). The suspension was placed in an ice bath under a flow of N_2 and sonicated at 35% amplitude using a 4 s on/4 s off pulse on a VCX-400 Vibra cell with a microprobe (Sonics & Materials Inc.) until the solution became translucent. Titanium debris was removed by subsequent centrifugation for 10 min at 12000 rpm. SUV particles were separated from larger liposomes on a 2.5 × 60 cm Sepharose CL-4B column (Pharmacia) equilibrated with TSB buffer. Radioactivity in the eluted fractions was detected and quantified by liquid scintillation counting. Typically, a small amount of larger liposomes was eluted in the excluded volume, and SUVs were eluted as a single symmetrical peak well separated from the larger liposome peak. The SUV fractions corresponding to half of the peak width centered on the peak were pooled and concentrated to ∼4 mg/mL of phospholipid and used within 2 weeks.

Polyacrylamide Gel Gradient Electrophoresis. To detect possible lipid solubilization mediated by apoA-I under at the concentration used for ITC, 10 μL of apoA-I at 4 mg/mL was mixed with 10 μL of 10 mg/mL liposomes in TSB for 2 h at 25 °C. As a control, apoA-I with buffer was also incubated at the same conditions. After incubation, samples were analyzed on native polyacrylamide gradient (8−25%) gels (PAGGE) (Miniprotean II System; Pharmacia), and protein bands were visualized by Coomassie Blue stain (Bio-Rad).

Isothermal Titration Calorimetry. The VP-ITC high-sensitivity titration calorimeter [MicroCal, Northampton, MA; for a description, see Wiseman et al. (*12*)] was used to study the interaction of lipid-free apoA-I with POPC SUVs containing varying amounts of cholesterol. Typically, 20 consecutive injections of 10 μL aliquots of the protein at a concentration of 30−110 μM were injected from the syringe into the cell of 1.43 mL filled with 3−30 mM SUV. To minimize the contribution to binding heat from dilution, the protein solution and the SUV preparation were dialyzed against the same buffer prior to the ITC experiments. Both lipid and protein solutions were degassed under vacuum immediately before use. Injections were made at intervals of 10 min, and the duration of each injection was 2 s/μL of injected protein. To ensure proper mixing after each injection, a constant stirring speed of 300 rpm was maintained during the experiment. Dilution heats of protein into the lipid solution were determined from the last few injections of the titration series and were subtracted from measured heats of binding. The values of these heats of dilution agreed with those obtained from the corresponding protein−buffer titration. Data were analyzed using the Origin software provided by MicroCal Inc.

The equilibrium association constant (K_a), the enthalpy change ($\Delta H°$), and the stoichiometry parameter (N, the number of protein molecules bound per lipid molecule) were obtained from curve fitting of the experimental data to a Langmuir adsorption model of a single class of binding site. The areas under the negative peaks corresponded to the heat released during the reaction after the addition of the protein into the solution containing the SUVs. The values of K_a and $\Delta H°$ obtained from curve fitting were used to calculate the standard free energy change ($\Delta G°$) and the standard entropy change ($\Delta S°$) for the binding using the equation:

$$\Delta G° = -RT \ln K = \Delta H° - T\Delta S° \qquad (1)$$

where R is the molar gas constant. In addition, the stoichiometry in terms of the number of phospholipid molecules per bound apoA-I (r) was calculated from the N values ($r = 0.6/N$) by assuming that only the lipids located on the outer leaflet of the vesicles, 60% of total lipids, are available for binding. From the r values, the number of apoA-I molecules bound per vesicle (n) was calculated assuming each vesicle contained 3000 phospholipid molecules (*13*). Additionally, heat capacity change ($\Delta C_p°$) for the binding was also determined from enthalpy changes at different temperatures.

Circular Dichroism (CD) Spectroscopy. All circular dichroism (CD) measurements were carried out on a Jasco-720 CD spectrophotometer.

Typically, 5 μL of apoA-I at 1.5 mg/mL was incubated with 100 μL of POPC or POPC/FC SUVs at 3 mg/mL. Tris salt buffer was added to a final volume of 200 μL. The sample was mixed and vortexed immediately before each experiment, placed in a 0.1 cm path length cuvette, and scanned from 250 to 190 nm with a 0.1 nm resolution and a 1.0 nm bandwidth. The temperature of the sample was kept constant with an exterior circulating water bath. The α-helix

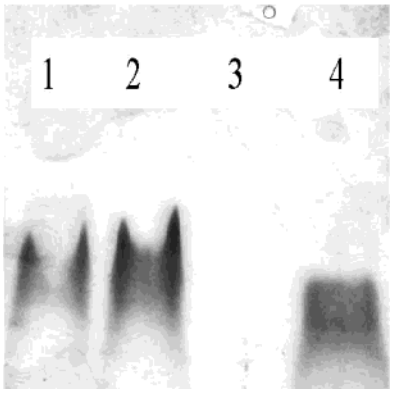

FIGURE 1: Native 8−25% polyacrylamide stained with Coomassie Blue. Lanes 1 and 2 show the product of apoA-I interaction with SUVs made of POPC and POPC/FC (6/1 molar ratio), respectively. Lane 3 corresponds to POPC SUVs; as a control, lane 4 shows apoA-I incubated in the same conditions but in the absence of liposomes.

contents of lipid-free apoA-I and apoA-I bound with lipid vesicles were calculated using the empirical relation:

$$f_\Theta = [\Theta]_{222} + 2340/(-30300) \qquad (2)$$

where $[\Theta]_{222} = (\mathrm{MRW})\theta_{222}/10lc$, θ_{222} is the observed negative ellipticity at 222 nm in degrees, l is the optical path length of the cuvette in centimeters, and c is the protein concentration in grams per milliliter. A mean residue weight (MRW) of 115.4 was used for apoA-I (MW 28000 Da).

RESULTS

Lipid Solubilization Assay. To evaluate the effect of apoA-I interaction on protein and liposome structure, we incubated lipid-free apoA-I for 2 h at room temperature with SUVs of different lipid compositions. The products were analyzed by polyacrylamide gel gradient electrophoresis (PAGGE) under nondenaturing conditions, and proteins were stained with Coomassie Blue. In Figure 1, lanes 1 and 2 represent apoA-I migration after incubation with liposomes of pure POPC and POPC plus FC (6/1 molar ratio). Lanes 3 and 4 are POPC SUVs and apoA-I, respectively. No significant change in protein migration was detected in any case, indicating that there is no lipid solubilization from these vesicles. These results are in total agreement with previous experiments performed by our group analyzing the binding of different apoA-I conformations to POPC SUVs and giant unilamellar vesicles (*9*). Lipid-free apoA-I was neither able to solubilize lipids, as indicated by PAGGE, nor able to change the morphology of the giant vesicles, in clear contrast with the ability of apoA-I to rearrange when reconstituted in small lipid complexes (*9*).

Effect of Cholesterol Content on Thermodynamic Parameters. Isothermal titration calorimetry (ITC) was used to obtain thermodynamic parameters for the interaction of lipid-free apoA-I with small unilamellar vesicles of phospholipids with varying cholesterol content. Figure 2 shows typical calorimetric tracings. The thermodynamic parameters obtained at 30 °C for apoA-I interacting with POPC SUVs are listed in Table 1.

For all three cholesterol levels studied, binding of apoA-I to POPC SUVs was an exothermic process ($\Delta H° < 0$) with a negative entropy change ($\Delta S° < 0$). In the absence of cholesterol, K_a, $\Delta H°$, and $T\Delta S°$ were found to be $(4.1 \pm 0.9) \times 10^6$ M^{-1}, -101 ± 2 kcal/mol, and -89 ± 3 kcal/mol, respectively. In the presence of cholesterol at a molar ratio of 4/1 POPC/FC, K_a, $\Delta H°$, and $T\Delta S°$ values were $(9 \pm 2) \times 10^6$ M^{-1}, -73 ± 4 kcal/mol, and -63 ± 2 kcal/mol, respectively. Thus the presence of 20% (mol) cholesterol resulted in significantly less negative $\Delta H°$ and $T\Delta S°$ values. This effect of cholesterol on $\Delta H°$ and $T\Delta S°$ offset each other, resulting in only a slight (2-fold) increase in binding affinity (K_a). The number of lipid molecules in the complex corresponding to each bound apoA-I (r) was 937 ± 95 in the absence of cholesterol but increased by 2.5-fold in the presence of 20% (mol) cholesterol. The increase in r corresponded to a decrease in the number of protein molecules bound per vesicle (n) from 3.2 to 1.2. A similar 2-fold increase in r was also observed by Yokoyama et al. from a gel permeation chromatography study (*14*).

Effect of Temperature on Enthalpy Change. ITC experiments were performed at four different temperatures between 15 and 37 °C for lipid-free apoA-I interacting with SUV of 6/1 (mol) POPC/FC. The binding constant (K_a) and the number of lipid molecules corresponding to each bound apoA-I (r) are shown in Figure 3. Over the experimental temperature range investigated, r varies between 900 and 3000 lipid molecules bound per protein and K_a between 2.8 and 4.1×10^6 M^{-1}.

Figure 4 shows the enthalpy change per mole of lipid as a function of temperature. The slope of the curve yields the heat capacity change ($\Delta C_p°$) of -2.07 ± 0.28 cal/(mol·K). The enthalpy change of the interaction can be reported in terms of per mole of lipid ($\Delta H°_L$) or protein ($\Delta H°_P$), and they are related by $\Delta H°_L = \Delta H°_P N$. The change in $\Delta H°_L$ or $\Delta H°_P$ yields the heat capacity change. The heat capacity change per mole of protein ($\Delta C_p°$) was -2.73 kcal/(mol·K). A negative heat capacity change suggests the transfer of hydrophobic groups from an aqueous into a nonpolar environment (*15*).

Effect of Lipid Binding on the Secondary Structural Content. Figure 5 shows the circular dichroism spectra of apoA-I in the three different media studied. The corresponding ellipticity values of apoA-I measured at 222 nm are summarized in Table 2. The increased α-helix content due to lipid−membrane binding is comparable to those that have been measured by other authors (*16*).

CD spectra showed increased α-helix content of apoA-I upon lipid binding, in particular to cholesterol-containing vesicles. Calculated α-helical fractions were 0.47 for lipid-free apoA-I, 0.73 for apoA-I bound to POPC SUVs, and 0.83 for apoA-I bound to POPC/FC (6/1) SUVs. The increase in α-helix content of apoA-I upon binding to POPC SUV corresponded to 62 out of the total 243 residues of the protein. Surely, the property of apoA-I is not a simple summation of the properties of its constituent peptides. However, we could compare the thermodynamic binding parameters of apoA-I to SUVs with that obtained by Wieprecht et al. (*17*) for the interaction of the amphipathic peptide magainin-2 amide to similar membrane surfaces. According to that information, the contribution of α-helix formation (per mole residue) to the enthalpy and entropy change is -0.7 kcal/mol and -1.9 cal/(mol·K), respectively. The observed gain of helix content thus translated to a $\Delta H°$ of -44 kcal/mol and a $T\Delta S°$ of -36 kcal/mol at 30 °C. Helix

Table 1: Thermodynamic Parameters for Lipid-Free ApoA-I Binding to POPC SUVs Containing Varying Amounts of Cholesterol[a]

membrane lipid (mol/mol)	K_a (M^{-1})	r (=0.6/N)	n (=3000/r)	$\Delta H°$ (kcal/mol)	$T\Delta S°$ (kcal/mol)
POPC	$(4.1 \pm 0.9) \times 10^6$	$(9.4 \pm 1.0) \times 10^2$	3.2 ± 0.7	-101 ± 2	-89 ± 3
POPC/FC (6/1)	$(3.9 \pm 0.2) \times 10^6$	$(1.3 \pm 0.1) \times 10^3$	2.2 ± 0.2	-87 ± 4	-78 ± 2
POPC/FC (4/1)	$(9.2 \pm 2.3) \times 10^6$	$(2.4 \pm 0.1) \times 10^3$	1.2 ± 0.1	-73 ± 4	-63 ± 2

[a] ITC binding parameters of lipid-free apoA-I to POPC SUVs at 30 °C based on protein concentration. The values are an average of at least three experiments. The $\Delta H°$ and $\Delta S°$ values are per mole of apoA-I.

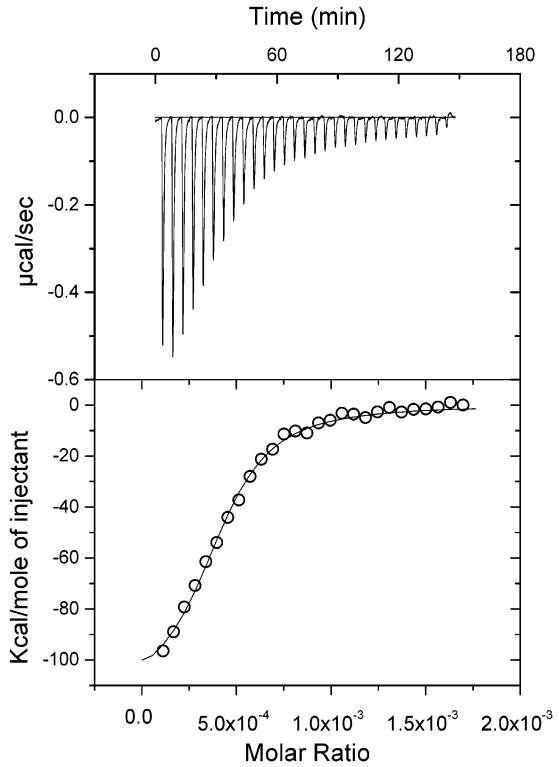

FIGURE 2: Isothermal titration calorimetric data of lipid-free apoA-I binding to POPC/FC (6/1 mol/mol) SUVs at 30 °C. Upper panel: Calorimetric tracings for injections of 10 μL of protein (1.2 mg/mL) into 4.85 mg/mL lipid solution at 5 min intervals. Lower panel: Heat of each injection in kcal/mol of apoA-I injected, calculated from the area underneath each injection peak. The last few injections were taken as heat of dilution, which was subtracted from the entire run. The *X*-axis is the protein to lipid molar ratio. The first injection peak was smaller than expected, a common phenomenon due partly to sample dilution in the syringe during baseline establishment.

formation thus accounted for ~44% and 46% of the observed $\Delta H°$ and $T\Delta S°$, respectively, for apoA-I binding to POPC SUVs. After subtraction of this contribution from the measured enthalpy and entropy change (Table 1), the remaining $\Delta H°$ and $T\Delta S°$ (−57 kcal/mol and −53 kcal/mol, respectively) can be attributed to additional interactions including lipid−lipid and lipid−protein interactions.

A similar calculation revealed that 87 residues acquired α-helical conformation upon binding to POPC/FC (6/1 mol:mol) SUVs, i.e., 25 residues more than in the absence of cholesterol. This conformational change contributes −61 kcal/mol to $\Delta H°$ and −47 kcal/mol to $T\Delta S°$. These accounted for 83% and 75% of the observed $\Delta H°$ and $T\Delta S°$, respectively. Clearly, α-helix formation is the dominant driving force for apoA-I binding to vesicles containing cholesterol but plays a less leading although still significant role for the binding to POPC vesicles without cholesterol.

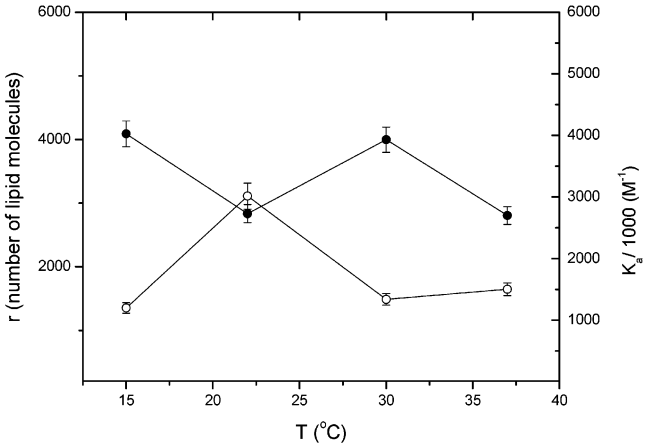

FIGURE 3: Effect of temperature on the thermodynamic parameters of lipid-free apoA-I binding to POPC/FC (6/1 mol/mol) SUVs. Both the equilibrium binding constant (●) and the number of lipid molecules corresponding to each bound protein molecule (○) are rather constant over the temperature range studied.

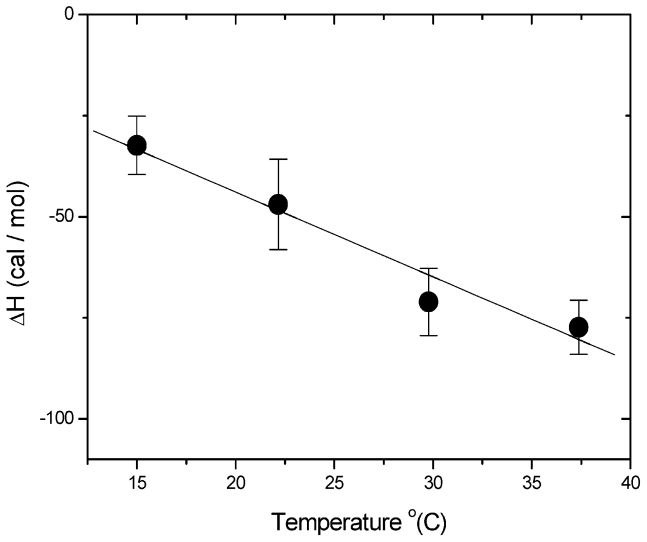

FIGURE 4: Enthalpy change per molar lipid as a function of temperature for apoA-I binding to POPC/FC (6/1 mol/mol) SUVs. The slope of the curve yields the heat capacity change, $\Delta C_p° = -2.07$ cal/(mol of lipid·K).

DISCUSSION

The negative values of $\Delta H°$ and $\Delta S°$ obtained for the binding of lipid-free apoA-I to SUV of POPC with or without cholesterol indicate that binding of lipid-free apoA-I to SUV is driven by enthalpy rather than entropy. As the hydrophobic interaction has traditionally been viewed exclusively as an entropically driven process, the enthalpy-driven hydrophobic interaction has thus been attributed to nonclassical hydrophobic interaction (*18*, *19*). ApoA-I binding to POPC SUV

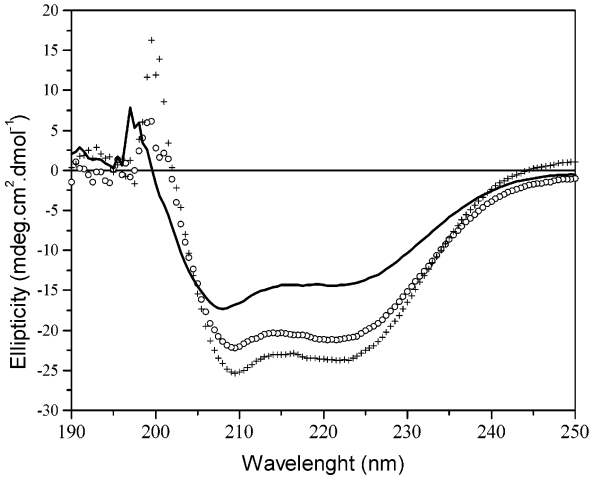

FIGURE 5: Circular dichroism of apoA-I in three different media at 37 °C: Tris salt buffer (—), POPC SUVs (○), and POPC/FC (6/1 mol/mol) SUVs (+). Experimental conditions as described in the text.

Table 2: Circular Dichroism Parameters Obtained at 37 °C for Lipid-Free ApoA-I[a]

	ellipticity (mdeg)	f_Θ	no. of residues
TSB buffer	-14.4 ± 0.3	0.47 ± 0.08	114 ± 2
POPC	-21.1 ± 0.4	0.73 ± 0.08	176 ± 3
POPC/FC (6/1 mol/mol)	-23.8 ± 0.7	0.83 ± 0.09	201 ± 4

[a] Ellipticity values of apoA-I were measured at 222 nm. The corresponding fractions of α-helical structure were determined from eq 2.

with or without cholesterol was clearly driven by the nonclassical hydrophobic interaction.

The negative $\Delta H°$ values obtained for the binding of apoA-I to lipid vesicles indicate that there is a net increase in bonding upon lipid binding by apoA-I. Bonding contributing to the enthalpy change when proteins partition between the membrane and the aqueous phase includes protein−lipid interactions, intermolecular interactions among the peptide chains, van der Waals interactions among the fatty acyl chains of the lipid bilayer, water−lipid bilayer, and water−protein interactions (*20*). The net increase in bonding observed here likely included increased van der Waals interactions among the lipids and between hydrophobic residues of the protein and the fatty acyl chains. Our results showed that this favorable enthalpic contribution dominated over the unfavorable entropic contribution, which likely resulted from conformational changes of the protein and from the decrease in protein dynamics.

Increased van der Waals interaction among the lipids could result if apoA-I penetrated into the lipid bilayer. This penetration would compress the spaces between the lipids, enhancing the distance-dependent van der Waals interactions and yielding an overall increase of bonding and thus a negative $\Delta H°$. The compression of lipids due to apoA-I binding could lead to partial crystallization of the adjacent lipids as was observed by Derksen and Small for the binding of apoA-I to triolein-rich emulsions with increasing cholesterol content (*20*). This partial crystallization resembles the liquid-crystalline to gel-phase transition in lipid bilayers. The resulting reduced lipid movement could also contribute negatively to the overall entropy change. Although the water molecules released to the bulk solvent as a result of protein penetration should contribute positively to the entropy change, the overall entropy change for the binding process is clearly negative, due essentially to the significantly reduced dynamics of the lipid and the protein upon binding.

Heat capacity changes ($\Delta C_p°$) provide information on the nature of the forces responsible for molecular interaction. $\Delta C_p°$ values control how $\Delta H°$ and $\Delta S°$ and hence $\Delta G°$ change with temperature. A negative heat capacity change means that enthalpy change becomes more negative or less positive with increasing temperature, thus indicating that bond formation is increased (or breaking of bonds is reduced) as temperature is increased (*20*, *21*). The change of $\Delta H°$ for apoA-I binding to POPC/FC (6/1) SUVs as a function of temperature, which yielded a $\Delta C_p°$ of -2.73 kcal/(mol of protein·K), suggests burial of nonpolar surface area and is consistent with the penetration of the protein into the bilayer. As apoA-I penetrates the membrane, the nonpolar amino acid side chains insert into and interact with the hydrophobic interior of the lipid bilayer while the polar face of the helices are exposed to the polar headgroups of the membrane bilayer. At the same time apoA-I shifts conformation from a highly dynamic and marginally stable solution conformation to the lipid-bound state that has more nonpolar interaction, thus giving rise to a net increase in nonpolar interaction. $\Delta C_p°$ for binding of apoA-I to pure POPC vesicles was determined to be -4.37 kcal/(mol of protein·K) (J. Seelig, personal communication). The less negative heat capacity change associated with the binding of apoA-I to cholesterol-PC membranes is consistent with cholesterol reducing penetration of the protein into lipid bilayers. Adsorption and penetration of apolipoprotein A-I to phospholipid monolayers were observed by Lecompte et al. using alternative current polarography (*22*). The authors also reported that penetration disappeared when cholesterol was present.

The equilibrium binding constant (K_a) of lipid-free apoA-I to POPC SUVs at $(4.05-9.22) \times 10^6$ M^{-1} corresponds to a $\Delta G°$ of about -10 kcal/mol, a small value considering the large negative $\Delta H°$, a result of the compensating effect of a large negative $\Delta H°$ and a large negative $\Delta S°$. Thus, binding of lipid-free apoA-I to POPC SUVs exhibits enthalpy−entropy compensation, a general feature seen for many chemical reactions and biological processes (*23*).

We compared the values of enthalpy and entropy changes of the reaction of apoA-I binding to POPC SUVs with those obtained for the partitioning of model peptide 18A, an amphipathic class A helix of 18 amino acids, into POPC SUVs where the peptide undergoes folding (*24*). The thermodynamic parameters reported by Gazzara and coworkers at 25 °C were $\Delta H° = -11.3 \pm 0.4$ kcal/mol, $\Delta S° = -12 \pm 2$ cal/(mol·K), and $T\Delta S° = -3.6 \pm 2$ kcal/mol. The total enthalpy and entropy changes measured in this work at 30 °C for apoA-I binding to POPC SUVs are -101 ± 2 kcal/mol ($\Delta H°$) and -89 ± 3 kcal/mol ($T\Delta S°$). The $\Delta H°$ values for the entire protein could be comparable to that of the peptide, if we considered that apoA-I contains eight 22-mer amphipathic helical domains. However, the same is not true for the entropy change, since the value of $T\Delta S°$ per residue is much more negative for apoA-I than for peptide 18A. Clearly, the intact protein is not a simple addition of its constituent helices in binding to the vesicles. A more negative entropy change suggests more loss of

freedom in apoA-I than the sum of individual helices upon vesicle binding. It could be indicative of a cooperative effect of the whole protein on the lack of mobility of the system, more than the contribution of the separate α-helices.

Our data showed that cholesterol in the lipid bilayer increased affinity of apoA-I for the vesicles but reduced the enthalpy and entropy changes as well as the number of apoA-I molecules bound per vesicle. Our rationale is that the POPC molecules ($T_m = -2$ °C) were already more ordered in the presence of cholesterol than in their absence, as was shown by Brown and Seelig (*25*), by ^{31}P NMR and ^{2}H NMR above the gel- to liquid-crystalline phase transition temperature of dipalmitoyl phosphatidylethanolamine (DPPE) and dipalmitoylphosphatidylcholine (DPPC). They demonstrated that the addition of cholesterol has opposite effects on the hydrocarbon and polar regions of the membrane. Cholesterol makes the hydrocarbon region gel-like, whereas the conformation of the polar headgroups is very similar to that found in the liquid-crystaline phase of pure phospholipid bilayer. Therefore, the van der Waals interactions among the lipids are already stronger in the presence than in the absence of cholesterol before apoA-I binding. Upon apoA-I binding, the lipids are further compressed, further strengthening the van der Waals interactions. The increase in the interactions upon binding is less in the presence of cholesterol, resulting in a less exothermic enthalpy change. The less exothermic $\Delta H°$ could also be due to the additional work that apoA-I must do to insert and compress the surface monolayer in the presence of cholesteol. Similarly, as phospholipid molecules were already less dynamic in the presence of cholesterol, upon apoA-I binding, the loss of entropy was less in the presence of cholesterol.

Using the helicity data from the CD measurements, we calculated the favorable contribution of the increased α-helix content to the total enthalpy change of the binding. The data show that helix formation is the major driving force for the interaction of apoA-I to cholesterol-containing vesicles, whereas in pure POPC vesicles helix formation is a significant but not dominant driving force. Others, including lipid−lipid and lipid−protein interactions, also play a significant role in the binding.

ApoA-I penetration into the amphipathic lipid bilayer have been reported by other researchers. The thermodynamic results of this study, specifically that $\Delta H°$ and $\Delta C_p°$ are both negative, are consistent with apoA-I penetrating into the lipid bilayer and thus contribute to the better understanding of apoA-I lipid bilayer interactions.

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REFERENCES

1. Jonas, A. (1992) in *Structure and function of apolipoproteins* (Rosseneu, M., Ed.) pp 217−250, CRC Press, Boca Raton, FL.
2. Fielding, C. J., and Fielding, P. E. (1995) Molecular physiology of reverse cholesterol transport, *J. Lipid Res. 36*, 211−228.
3. Segrest, J. P., Jones, M. K., De Loof, H., Brouillette, C. G., Venkatachalapathi, Y. V., and Anantharamaiah, G. M. (1992) The amphipathic helix in the exchangeable apolipoproteins: a review of secondary structure and function, *J. Lipid Res. 33*, 141−166.
4. Gursky, O., and Atkinson, D. (1996) Thermal unfolding of human high-density apolipoprotein A-1. Implications for a lipid-free molten globule state, *Proc. Natl. Acad. Sci. U.S.A. 93*, 2991−2995.
5. Frank, P. G., and Marcel, Y. L. (2000) Apolipoprotein A-I: structure−function relationship, *J. Lipid Res. 41*, 853−872.
6. Córsico, B., Toledo, J. D., and Garda, H. A. (2001) Evidence for a central apolipoprotein A-I domain loosely bound to lipids in discoidal lipoproteins that is capable of penetrating the bilayer of phospholipid vesicles, *J. Biol. Chem. 276*, 16978−16985.
7. Sparks, D. L., Lund-Katz, S., and Phillips, M. C. (1992) The charge and structural stability of apolipoprotein A-I in discoidal and spherical recombinant high-density lipoprotein particles, *J. Biol. Chem. 267*, 25839−25847.
8. Nolte, R. T., and Atkinson, D. (1992) Conformational analysis of apolipoprotein A-I and E-3 based on primary sequence and circular dichroism, *Biophys. J. 63*, 1221−1239.
9. Tricerri, M. A., Sanchez, S. A., Arnulphi, C., Durbin, D. M., Gratton, E., and Jonas, A. (2002) Interaction of apolipoprotein A-I in three different conformations with palmitoyl oleoyl phosphatidylcholine vesicles, *J. Lipid Res. 43*, 187−197.
10. Leroy, A., and Jonas, A. (1994) Native-like structure and self-association behavior of apolipoprotein A-I in a water/*n*-propanol solution, *Biochim. Biophys. Acta 1212*, 285−294.
11. Breukink, E., Ganz, P., De Kruijff, B., and Seelig, J. (2000) Binding of nisin Z to bilayer vesicles as determined with isothermal titriation calorimetry, *Biochemistry 39*, 10247−10254.
12. Wiseman, T., Williston, S., Brandst, J. F., and Lin, L.-N. (1989) Rapid measurement of binding constants and heats of binding using a new titration calorimeter, *Anal. Biochem. 179*, 131−137.
13. Newman, G. C., and Huang, C. (1975) Structural studies on phophatidylcholine-cholesterol mixed vesicles, *Biochemistry 14*, 3363−3370.
14. Yokoyama, S., Fukushima, D., Kupferberg, J. P., Kezdy, F. J., and Kaiser, E. T. (1980) The mechanism of activation of lecithin: cholesterol acyltransferase by apolipoprotein A-I and an amphiphilic peptide, *J. Biol. Chem. 255*, 7333−7339.
15. Makhatadze, G. I., and Privalov, P. L. (1990) Heat capacity of proteins. I. Partial molar heat capacity of individual amino acid residues in aqueous solution: hydration effect, *J. Mol. Biol. 213*, 375−384.
16. Pownall, H. J., Hsu, F. J., Rosseneu, M., Peeters, H., Gotto, A. M., and Jackson, R. L. (1977) Thermodynamics of lipid protein associations. Thermodynamics of helix formation in the association of high density apolipoprotein A-I (apoA-I) to dimyristoyl phosphatidylcholine, *Biochim. Biophys. Acta 482*, 190−197.
17. Wieprecht, T., Beyermann, M., and Seelig, J. (2002) Thermodynamics of the coil-α-helix transition of amphipatic peptides in a membrane environment: the role of vesicle curvature, *Biophys. Chem. 96*, 191−201.
18. Huang, C. H., and Charlton, J. P. (1972) Interactions of phosphatidylcholine vesicles with 2-*p*-toluidinylnaphthalene-6-sulfonate, *Biochemistry 11*, 735−740.
19. Beschiaschvili, G., and Seelig, J. (1992) Peptide binding to lipid bilayers. Nonclassical hydrophobic effect and membrane-induced p*K* shifts, *Biochemistry 31*, 10044−10053.
20. White, S. H., and Wimley, W. C. (1999) Membrane protein folding and stability: physical principles, *Annu. Rev. Biophys. Biomol. Struct. 28*, 319−365.
21. Privalov, P. L., and Makhatazde, G. I. (1990) Heat capacity of proteins. II. Partial molar heat capacity of the unfolded polypeptide chain of proteins: protein unfolding effects, *J. Mol. Biol. 213*, 385−391.
22. Lecompte, M. F., Bras, A. C., Dousset, N., Portas, I., Salvayre, R., and Ayrault-Jarrier, M. (1998) Binding steps of apolipoprotein A-I with phospholipid monolayers: adsorption and penetration, *Biochemistry 37*, 16165−16171.
23. Dunitz, J. D. (1995) Win some, lose some: enthalpy−entropy compensation in weak intermolecular interactions, *Chem. Biol. 2*, 709−712.

24. Gazzara, J. A., Phillips, M. C., Lund-Katz, S., Palgunachari, M. N., Segrest, J. P., Anantharamaiah, G. M., Rodrigueza, W. V., and Snow, J. W. (1997) Effect of vesicle size on their interaction with class A amphipathic helical peptides, *J. Lipid Res. 38*, 2147−2154.

25. Brown, M. F., and Seelig, J. (1978) Influence of cholesterol on the polar region of phosphatidylcholine and phosphatidylethanolamine bilayers, *Biochemistry 17*, 381.

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