Effects of Lipid Composition and Packing on the Adsorption of Apolipoprotein A-I to Lipid Monolayers†

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ABSTRACT: To better understand the factors controlling the binding of apolipoprotein molecules at the surfaces of serum lipoprotein particles, the adsorption of human apolipoprotein A-I to phospholipid monolayers has been studied. The influence of lipid packing was investigated by spreading the monolayers at various initial surface pressures (π_i) and by using various types of lipid. The adsorption of ^{14}C-methylated apolipoprotein A-I was monitored by simultaneously following the surface radioactivity (which could be converted to the surface concentration of protein, Γ) and the change in surface pressure ($\Delta\pi$). In general, increasing the π_i of lipid monolayers reduces the adsorption of apolipoprotein A-I; for expanded egg phosphatidylcholine (PC) monolayers at $\pi_i \geq 32$ dyn/cm, Γ and $\Delta\pi$ are zero. The degree of adsorption of the apolipoprotein is also influenced by the physical state of the lipid monolayers. Thus, at a given π_i, apolipoprotein A-I adsorbs more to expanded monolayers than to condensed monolayers so that, at a given subphase concentration of protein, Γ of apolipoprotein A-I with various phospholipid monolayers decreases in the order egg PC > egg sphingomyelin > distearoyl-PC. The plot of Γ against π_i for adsorption of apolipoprotein A-I to dipalmitoylphosphatidylcholine (DPPC) monolayers shows an inflection at $\pi_i = 8$ dyn/cm; at this π, the DPPC monolayer undergoes a phase transition from liquid (expanded) to solid (condensed) state. Addition of cholesterol generally decreases the adsorption of apolipoprotein A-I to egg PC monolayers. Analysis of the adsorption data suggests that the lateral compressibility of a lipid monolayer is a major determinant of the extent to which apolipoprotein A-I adsorbs. The protein penetrates into the interface to occupy space made available by the concomitant compression of phospholipid molecules so Γ is higher for relatively compressible lipid monolayers. Lipid–protein interactions appear to influence the degree of adsorption to only a minor degree.

Apolipoproteins differ in their affinities for various lipoprotein classes in vivo. Particular apolipoproteins are associated preferentially with certain lipoprotein classes; for example, apolipoprotein A-I (apo A-I)[1] is absent from the surface of low-density lipoprotein (LDL) but present in high-density lipoprotein (HDL). This occurs even though, during the metabolism of lipoproteins, there is movement of apolipoproteins between lipoprotein classes (Schaefer et al., 1978a; Eisenberg, 1984). For instance, hydrolysis of very low density lipoprotein (VLDL) triglycerides by lipoprotein lipase results in the transfer of apolipoprotein C from VLDL to HDL, while during the action of lecithin–cholesterol acyltransferase (LCAT) on HDL, apolipoprotein E is transferred from HDL to VLDL (Eisenberg et al., 1972; Eisenberg, 1975; Glangeaud et al., 1977). There is also evidence for the transfer of apo A-I from triglyceride-rich particles to HDL during lipolysis (Schaefer et al., 1978b; Tall et al., 1979, 1982; Daerr et al., 1986). Moreover, apolipoproteins A-I and A-II have been demonstrated to exchange to some extent between HDL_2 and HDL_3 subclasses (Grow & Fried, 1978; Grow, 1983).

Given the important metabolic functions of apolipoproteins, it is essential to understand the physicochemical parameters underlying the distribution and transfer of apolipoproteins among lipoprotein classes. There is evidence that both the size and core lipid composition of lipoprotein particles are important factors to be considered in the association of apolipoproteins with lipoprotein particles (Connelly & Kuksis, 1981; Tajima et al., 1983). However, little is known about the effect of surface lipid composition on the binding of apolipoproteins. The surface lipid compositions of the various circulating lipoprotein classes are different. For instance, LDL has greater acyl chain saturation of its phosphatidylcholines, a higher sphingomyelin:phosphatidylcholine ratio, and a higher unesterified cholesterol:phospholipid molar ratio compared to HDL (Skipski, 1972; Scanu, 1979). There is evidence that surface lipid composition affects the physical state and lipid packing at the surface. Nuclear magnetic resonance (NMR) studies have suggested that the LDL surface cholesterol/phospholipid monolayer has a tighter packing compared to the HDL surface; the cholesterol oscillatory motions are slower and/or more restricted in the surface of LDL compared to the surface of HDL (Lund-Katz & Phillips, 1984, 1986). The differences in the surface lipid packing are most probably partially a reflection of differences in the surface lipid composition although variations in apolipoproteins may also contribute.

The effects of surface lipid composition and packing on the affinity of apolipoproteins for the surfaces of lipoprotein particles can be modeled by using an insoluble lipid monolayer spread at the air–water interface. The advantages of this system are that the surface area, lipid packing density, lipid composition, and apolipoprotein composition can be controlled. There is only limited data in the literature on the effects of lipid composition on the adsorption of apolipoproteins to lipid

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[1] Abbreviations: apo, apolipoprotein; DBPC, dibehenoylphosphatidylcholine; DPPC, dipalmitoylphosphatidylcholine; DSPC, distearoylphosphatidylcholine; Gdn-HCl, guanidine hydrochloride; HDL, high-density lipoprotein; LDL, low-density lipoprotein; NMR, nuclear magnetic resonance; PAGE, polyacrylamide gel electrophoresis; PC, phosphatidylcholine; POPC, palmitoyloleoylphosphatidylcholine; SDS, sodium dodecyl sulfate; VLDL, very low density lipoprotein.

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monolayers. Earlier experiments demonstrated that apolipoproteins from HDL are highly surface active; they adsorb strongly to the air–water interface and exert high surface pressures relative to other classes of proteins [for a review, see Camejo and Munoz (1981)]. Different steady-state $\Delta\pi$ values were obtained when apo HDL was allowed to adsorb to lipid monolayers which varied in their composition (Camejo et al., 1968; Phillips et al., 1975c). However, it is difficult to draw definite conclusions from $\Delta\pi$ measurements when the protein adsorbs to lipid monolayers which differ in compressibility. A direct method for evaluating the adsorption of proteins to lipid monolayers is to measure the interfacial concentration of the adsorbed proteins (Phillips & Krebs, 1986). Here, the adsorption of reductively methylated ^{14}C-labeled apo A-I to lipid monolayers which differ in composition and physical state is evaluated. The interfacial conformation of apo A-I adsorbed to various lipid monolayers and the underlying mechanisms that control the adsorption of the protein are discussed in terms of a model in which the lateral compressibility of the lipid monolayer is a critical parameter.

Experimental Procedures

Preparation of Lipid Solutions. Egg yolk phosphatidylcholine (egg PC), distearoyl-PC (DSPC), dibehenoyl-PC (DBPC), and cholesterol were purchased from Sigma Chemical Co. (St. Louis, MO). Egg sphingomyelin and dipalmitoyl-PC (DPPC) were obtained from Calbiochem-Behring Corp. (La Jolla, CA). [^{14}C]DPPC (115 Ci/mol) and palmitoyl[^{14}C]oleoylphosphatidylcholine (POPC) (57 Ci/mol) were purchased from Amersham Co. (Arlington Heights, IL). To form monolayers at the air–water interface, spreading solutions were prepared by dissolving known amounts of cholesterol and phospholipid in hexane/ethanol (9:1 v/v). Because surface chemistry techniques are particularly sensitive to impurities, 99 mol % chromatographically pure hexane was purchased from Fisher Scientific Co. (Malvern, PA) and further purified on an activated alumina column prior to its use. The purities of lipid samples were assayed by thin-layer chromatography on silica gel G plates (Analtech, Newark, DE) using two solvent systems: (1) petroleum ether/diethyl ether/acetic acid (75:24:1 v/v) and (2) chloroform/methanol/water (65:25:4 v/v). All samples were >99% pure. Lipid solutions were stored in a desiccator saturated with hexane vapor at 4 °C to minimize evaporation of the solvent. The concentrations of phospholipid and cholesterol in solution were determined by phosphorus assay and gas–liquid chromatography, respectively (Sokoloff & Rothblat, 1974).

Human Apolipoprotein Isolation. Human apolipoprotein A-I was isolated from the total HDL fraction, taken at a density of $1.0163 < d < 1.21$ g/mL. The total HDL fraction was delipidated with 3:2 (v/v) ethanol/diethyl ether at 0 °C (Scanu & Edelstein, 1971), and the protein was chromatographed on a Sephacryl S-300 column (200 × 2.5 cm) using 6 M urea, 1 M NaCl, 10 mM Tris-HCl (pH 8.6), 1 mM EDTA, and 0.02% NaN_3 as the elution buffer (Scanu et al., 1969). The column fractions which corresponded to the elution positions of apolipoproteins A-I and A-II were pooled and rechromatographed under reducing conditions using the above elution buffer with 10 mM dithiothreitol on Sephacryl S-200 (140 × 1.6 cm). The fractions which corresponded to the elution position of apo A-I were dialyzed against 50 mM ammonium bicarbonate solution, lyophilized, and stored at −70 °C. The apo A-I preparation gave a single band on sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) when 50 μg of protein was applied. Protein concentrations of stock solutions were determined by the SDS procedure of Markwell et al. (1978), adapted from that of Lowry et al. (1951). Combination of the Lowry determination with an absorbance value for the protein solution at 280 nm yielded an extinction coefficient which permitted rapid measurement of the protein concentration during later use. A 1 mg/mL solution of the human apolipoprotein A-I in phosphate buffer (1-cm path length) gave an absorbance at 280 nm of 1.23.

Radiolabeling of Apolipoproteins. Labeling of apo A-I was necessary to measure quantitatively its adsorption to spread lipid monolayers by the use of the surface radioactivity method. Lysine residues were reductively methylated using [^{14}C]-formaldehyde and sodium cyanoborohydride reducing agent as described by Jentoft and Dearborn (1979, 1983). Prior to the labeling procedure, lyophilized apolipoprotein A-I was dissolved in 3 M guanidine hydrochloride (Gdn-HCl) and desalted with a Bio-Gel P_2 desalting column (5.0 × 1.0 cm) to keep the apolipoprotein in monomeric form. In a typical reaction, 1 mg of freshly desalted apolipoprotein in a reaction volume of 0.5 mL of phosphate buffer (5.65 mM Na_2HPO_4, 3.05 mM NaH_2PO_4, and 0.08 M NaCl, pH 7) was incubated with 2.5 μCi of [^{14}C]formaldehyde of high specific activity (52 Ci/mol; New England Nuclear, Boston, MA) and 25 μL of 0.1 M sodium cyanoborohydride (Aldrich Chemical Co., Milwaukee, WI). The mixture was held at 4 °C for 18 h, and then the reaction was stopped by dialysis against several changes of 0.1 M NaCl (pH 7). According to this method, the specific activity of [^{14}C]apo A-I was about 2 μCi/mg which corresponds to single methylation of ~5% of the lysine residues in the apolipoprotein molecule (i.e., approximately one lysine residue per apo A-I molecule was modified). Amino acid analysis showed that apolipoprotein preparations, in which up to three lysine residues per apo A-I molecule were reductively methylated, contain primarily monomethyllysine residues (Krebs et al., 1988).

Surface Pressure–Molecular Area Isotherms. A Langmuir–Adam surface balance comprised of a hydrophobic Teflon rectangular trough was used to measure the surface pressure–molecular area (π–A) isotherms for spread lipid films (Phillips & Krebs, 1986). The Langmuir trough was filled with phosphate buffer (5.65 mM Na_2HPO_4, 3.05 mM NaH_2PO_4, and 0.08 M NaCl, pH 7) at 25 ± 1 °C. The chromatographically pure lipids were spread over the clean air–water interface by applying the solution dropwise from a Hamilton syringe (Camejo et al., 1968). The lipid film was spread between a movable Teflon barrier which was used for the film compression, and a Teflon float was used to monitor changes in π following changes in the surface area. The lateral pressure exerted by the lipid monolayer in the surface displaced the float, thereby rotating a torsion wire connected to the float. The torsion wire was interfaced, in turn, to a force detection device consisting of a light lever which was calibrated with known weights. Ten minutes after the lipid film was spread, the surface area was reduced with a movable Teflon barrier in 1–3 Å^2/molecule decrements and 20-s intervals between successive compressions until the collpase (π) was attained. The π value was recorded and plotted as a function of the molecular area of the lipid molecules. The estimated error in π was ±1 dyn/cm for a given amount of lipid spread onto a constant surface area.

Adsorption of Apolipoproteins. The absorption of ^{14}C-labeled apo A-I to spread lipid monolayers was studied by using a Teflon dish, 10.8 cm in diameter, containing 80 mL of phosphate buffer (pH 7.0) at 25 ± 1 °C. A lipid monolayer was spread to a certain initial surface pressure (π_i) at the clean air–water interface using a Hamilton syringe as described

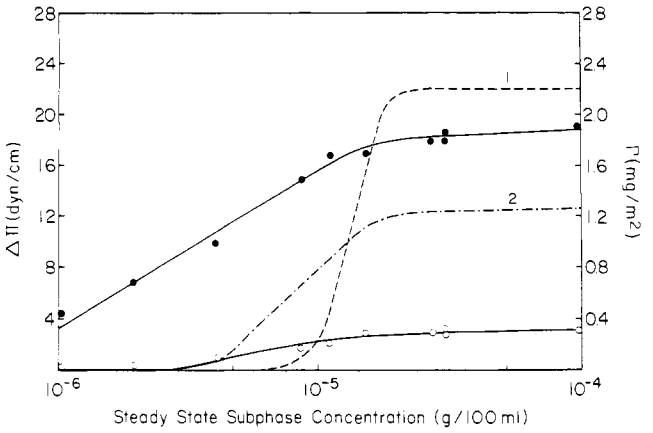

FIGURE 1: Steady-state adsorption isotherms for ^{14}C-labeled apolipoprotein A-I adsorbed to the clean air–water interface and to an egg PC monolayer spread at an initial surface pressure (π_i) of 10 dyn/cm. The experimental conditions are the same as those described under Experimental Procedures. The increases in surface pressure [$\Delta\pi$ (●)] and the surface concentration of apo A-I [Γ (○)] at the egg PC monolayer are plotted against the steady-state subphase apolipoprotein concentration. The data for adsorption of apo A-I to the clean air–water interface are taken from Krebs et al. (1988) and are shown as the dashed line (1) for $\Delta\pi$ and the dot–dashed line (2) for Γ.

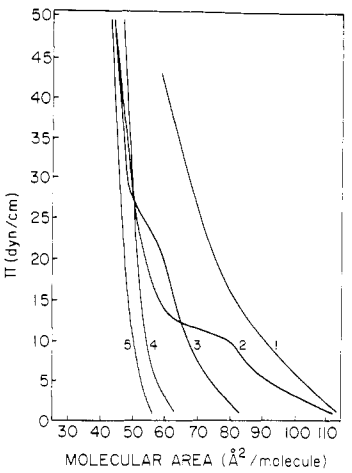

FIGURE 2: Surface pressure (π)–molecular area (A) isotherms for (1) egg phosphatidylcholine (PC), (2) dipalmitoyl-PC, (3) egg sphingomyelin, (4) distearoyl-PC, and (5) dibehenoyl-PC monolayers spread on a subphase of phosphate buffer (5.65 mM Na_2HPO_4, 3.05 mM NaH_2PO_4, and 0.08 M NaCl, pH 7) at 25 ± 1 °C; see Experimental Procedures for details. The depicted π–A isotherms were obtained by drawing smooth curves through at least 10 data points for condensed isotherms and 20 data points for expanded isotherms (the data points are omitted for clarity).

above; the ^{14}C-labeled apolipoprotein was then injected beneath the lipid monolayer through a polypropylene tube to give the desired subphase concentration. The apolipoprotein at a concentration of ~0.5 mg/mL was injected either in phosphate buffer solution from which 3 M Gdn-HCl had just been removed on a desalting column or in a 2 M Gdn-HCl solution. In the latter procedure, the final subphase concentration of Gdn-HCl was <1 mM, which was too low to cause any denaturation of the apolipoprotein (Reigngoud & Phillips, 1982). Both methods of avoiding injection of solutions containing aggregated apolipoprotein gave identical results. The adsorption rate was increased by constant stirring of the substrate following protein injection. Adsorption of apo A-I molecules to the interface was monitored over time by measuring the increase in surface pressure of the film ($\Delta\pi$) and the surface concentration (Γ) of the labeled apolipoprotein.

π_i and $\Delta\pi$ were measured by using the Wilhelmy plate technique [for a review, see Phillips and Krebs (1986)]. The reproducibility of π measurements using this technique was ±1 dyn/cm. Γ of the ^{14}C-labeled apo A-I at the lipid–water interface was monitored by the use of the surface radioactivity technique [for a review, see Phillips and Krebs (1986)]. A gas flow counter (98% argon with 2% propane as carrier gas) was positioned 3 mm above the surface to detect the radioactivity of the adsorbed apolipoprotein. Calibration curves relating counts to protein mass per unit surface area were generated by spreading known quantities of the radioactive protein (Trurnit, 1960) onto different areas of the air–water interface using a 3.5 M KCl substrate solution to avoid protein loss to the subphase. The error in surface radioactivity measurements was approximately ±5%.

RESULTS

Adsorption of Apo A-I to PC Monolayers. Figure 1 compares the steady-state adsorption isotherm of ^{14}C-labeled apo A-I injected beneath an egg PC monolayer spread at π_i = 10 dyn/cm to the equivalent adsorption isotherm for the clean air–water interface which was reported in an earlier study (Krebs et al., 1988). It is apparent from Γ measurements that significant adsorption of apo A-I to the egg PC monolayer and to the air–water interface starts at a final subphase concentration of approximately 4×10^{-6} g/100 mL. At low subphase concentrations, adsorption of apo A-I to the lipid–water interface is minimal and cannot be detected by the gas flow counter but still can result in $\Delta\pi$ values of several dynes per centimeter due to compression of lipid molecules at the interface. Maximum adsorption of apo A-I to either the air–water interface or the lipid monolayer–water interface occurs at a final subphase concentration of $\geq 2 \times 10^{-5}$ g/100 mL. The steady-state surface pressures of the mixed apolipoprotein–lipid monolayer ($\pi_i + \Delta\pi$) and of the adsorbed apolipoprotein monolayer at the air–water interface are 28 and 22 dyn/cm, respectively, at saturating subphase concentrations of apo A-I. The higher surface pressure of the mixed lipid–apolipoprotein monolayer is probably due to a stabilizing effect of the lipid molecules. The corresponding maximum Γ values of adsorbed apo A-I at the lipid monolayer and the air–water interface are 0.32 and 1.26 mg/m^2, respectively. The lower surface concentration of apo A-I at the lipid monolayer is due to the presence of lipid molecules occupying interfacial space, thereby limiting the adsorption of apo A-I to the interface.

Figure 2 depicts the π–A isotherms for egg PC, DSPC, DBPC, and DPPC monolayers. These isotherms for PC molecules which differ in their acyl chain length and saturation indicate that the egg PC monolayer is in a liquid (expanded) state whereas the DSPC and DBPC monolayers are in a solid (fully condensed) state. The π–A isotherm for the DPPC monolayer shows a transition from liquid to solid state at π = 8 dyn/cm, at 25 °C. These π–A isotherms are in agreement with equivalent isotherms published in the literature (Phillips & Chapman, 1968).

The adsorption of ^{14}C-labeled apo A-I to these monolayers was studied at a subphase concentration of 3×10^{-5} g/100 mL which results in maximum protein adsorption (see Figure 1); Γ/π_i and $\Delta\pi/\pi_i$ plots were constructed and are shown in Figures 3 and 4. It is apparent that for a given monolayer physical state, there is a linear decrease in the Γ of apo A-I with increasing π_i of the lipid monolayer. The Γ/π_i plots in Figure 3 indicate that the adsorption of apo A-I to the lipid monolayers is affected by the lipid physical state. At any given π_i, the adsorption of apo A-I to the liquid egg PC monolayer is greater than the adsorption to the solid DSPC and DBPC

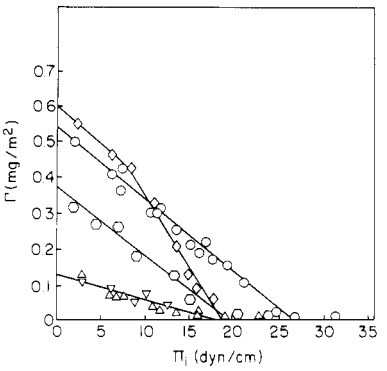

FIGURE 3: Surface concentration (Γ)–initial surface pressure (π_i) plots for the adsorption of apolipoprotein A-I to monolayers of egg phosphatidylcholine (PC) (○), dipalmitoyl-PC (◆), egg sphingomyelin (○), distearoyl-PC (△), and dibehenoyl-PC (▽). The experimental conditions are described under Experimental Procedures. The subphase concentration of apolipoprotein A-I was 3×10^{-5} g/100 mL. The steady-state Γ is plotted against the π_i of the lipid monolayer. Linear regression coefficients for the lines through the egg PC and egg sphingomyelin data are 0.98 and 0.97, respectively. The Γ values for the distearoyl and dibehenoyl monolayers are similar and therefore fitted with one regression line (the linear regression coefficient is 0.93). The estimated error in Γ measurements is ± 0.03 mg/m^2.

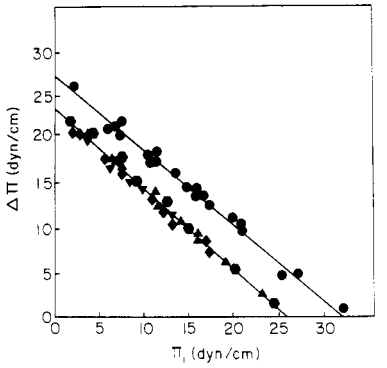

FIGURE 4: Change in surface pressure ($\Delta\pi$)–initial surface pressure (π_i) plots for the adsorption of apolipoprotein A-I to monolayers of egg phosphatidylcholine (PC) (●), dipalmitoyl-PC (◆), egg sphingomyelin (●), distearoyl-PC (▲), and dibehenoyl-PC (▼). The experimental conditions are described under Experimental Procedures. The steady-state $\Delta\pi$ is plotted against the π_i of the lipid monolayer. The linear regression coefficient for the egg PC data is 0.99. The $\Delta\pi$ values for the dipalmitoyl-PC, egg sphingomyelin, and distearoyl-PC and dibehenoyl-PC monolayers are similar and therefore fitted with one regression line (the linear regression coefficient is 0.99). The estimated error in $\Delta\pi$ measurements is ± 1 dyn/cm.

monolayers. This effect of the physical state of the lipid monolayer on the adsorption of apo A-I was confirmed by studying the adsorption behavior of apo A-I to a DPPC monolayer. The Γ/π_i plot shows a dependence of the Γ values on the physical state of the DPPC monolayer (Figure 3). The Γ/π_i plot has an inflection at $\pi_i = 8$ dyn/cm, where a change in the slope of the regression line occurs. The inflection in the Γ/π_i plot occurs at a similar π to the transition of a DPPC monolayer from liquid to solid physical state (cf. Figures 2 and 3). At $\pi_i > 12$ dyn/cm, there is a marked decrease of Γ values compared to the egg PC monolayer; at $\pi \geq 12$ dyn/cm, the DPPC monolayer is in a fully solid state.

In agreement with previous studies (Camejo et al., 1968; Colacicco, 1970; Phillips et al., 1975c), plots of $\Delta\pi/\pi_i$ show a linear decrease in $\Delta\pi$ with increasing π_i of the lipid monolayers (Figure 4). The $\Delta\pi$ values for an egg PC monolayer are higher than those for DPPC, DSPC, and DBPC monolayers. When Γ/π_i and $\Delta\pi/\pi_i$ plots are compared (cf. Figures 3 and 4), it is obvious that the same surface concentration of apo A-I results in a greater increase in surface pressure in the solid monolayers than in the liquid monolayers. It is interesting to note that the adsorption of apo A-I to the DPPC, DSPC, and DBPC monolayers results in a common final surface pressure ($\pi_i + \Delta\pi$) of 25 ± 1 dyn/cm (Figure 4); at this surface pressure, the above pure lipid monolayers occupy approximately the same molecular area of 50 ± 3 Å^2/molecule (Figure 2). In contrast, the adsorption of apo A-I to the egg PC monolayer results in a greater final surface pressure (29 ± 1 dyn/cm); at this surface pressure, the pure egg PC monolayer occupies a higher molecular area of 70 ± 1 Å^2/molecule.

Extrapolation of $\Delta\pi$ to zero in Figure 4 reveals that apo A-I fails to penetrate the egg PC monolayer at $\pi_i \geq 32$ dyn/cm and the DPPC, DSPC, and DBPC monolayers at $\pi_i \geq 25$ dyn/cm. By extrapolation of the data in Figure 3, zero surface concentrations of apo A-I are expected at π_i values of 27, 18, and 17 dyn/cm for egg PC, DPPC, and DSPC monolayers, respectively. At π_i values above the π_i intercepts in the Γ/π_i plots, the surface concentration of apo A-I is minimal and cannot be detected by the gas flow counter; these minimal surface concentrations can still result in a $\Delta\pi$ values of several dynes per centimeter.

In the studies described above, about one lysine residue per apo A-I molecule was methylated. To check the effect of this modification on the affinity of apolipoprotein molecules for the lipid–water interface, the adsorption of ^{14}C-labeled and native apo A-I to an egg PC monolayer was compared. The data (not shown) showed that both ^{14}C-labeled and native apo A-I yield identical values for $\Delta\pi$ as a function of π_i. This indicates that at the degree of labeling mentioned above, reductive methylation of apo A-I does not alter its affinity for lipid monolayers. In a previous study (Krebs et al., 1988), we reported that reductive methylation of up to four lysine residues per molecule does not alter the surface properties of apo A-I at the air–water interface. This probably occurs because the net charge of the protein molecule is not altered and the positively charged methylamino group remains hydrated whether the protein molecule is present in the subphase or adsorbed at the lipid–water interface.

It should be noted that adsorption of apo A-I to phospholipid monolayers does not result in desorption of lipid molecules from the interface. Adsorption of unlabeled apo A-I at a subphase concentration of 3×10^{-5} g/100 mL to ^{14}C-labeled DPPC and POPC monolayers spread at various π_i values was studied. The results (not shown) indicated that there was no detectable desorption of [^{14}C]PC molecules from the interface.

Adsorption of Apo A-I to Sphingomyelin Monolayers. The π–A isotherm for egg sphingomyelin (see Figure 2) indicates that the monolayer is relatively condensed when compared to an egg PC monolayer or to a DPPC monolayer at $\pi < 12$ dyn/cm. The egg sphingomyelin isotherm shows a phase transition from liquid to solid state at $\pi \sim 22$ dyn/cm whereas the equivalent value for DPPC is 8 dyn/cm. The adsorption of ^{14}C-labeled apo A-I to an egg sphingomyelin monolayer spread at various π_i values is compared to the adsorption of the protein to various PC monolayers in Figures 3 and 4. Both Γ/π_i and $\Delta\pi/\pi_i$ plots show a linear decrease in Γ and $\Delta\pi$ with increasing π_i of the egg sphingomyelin monolayer. The surface concentrations of apo A-I associated with an egg sphingomyelin monolayer are intermediate to values measured with liquid- and solid-state PC monolayers; this correlates with the physical state of the sphingomyelin monolayer which is relatively condensed (cf. Figures 2 and 3). The Γ values of apo A-I with the egg sphingomyelin monolayer are lower than

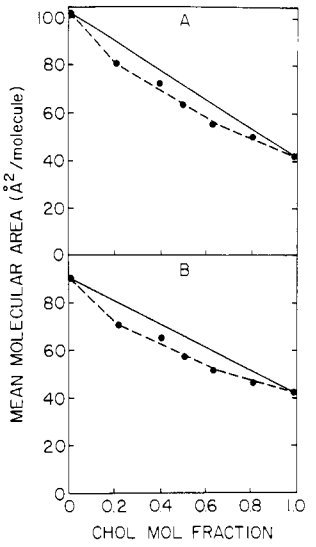

FIGURE 5: Dependence on cholesterol content of the mean molecular area of cholesterol/egg phosphatidylcholine mixed monolayers spread at an initial surface pressure (π_i) of 5 dyn/cm (panel A) and 10 dyn/cm (panel B). The solid lines (—) represent ideal mixing where the mean molecular area is described by eq 1. The dashed lines (●---●) represent the measured mean molecular areas obtained from π–A isotherms of the mixed cholesterol/egg phosphatidylcholine monolayers.

those for an egg PC monolayer. Similarly, the Γ values of apo A-I with egg sphingomyelin are lower than those for the DPPC monolayer at $\pi_i \leq 12$ dyn/cm, whereas there is no significant difference in Γ values at $\pi_i > 12$ dyn/cm. The surface concentrations of apo A-I with the egg sphingomyelin monolayer are higher than those for DSPC and DBPC monolayers.

The Γ/π_i plot does not show an inflection corresponding to that observed in the π–A isotherm of the egg sphingomyelin monolayer because the phase transition occurs at a surface pressure of ~22 dyn/cm (Figure 2); at this π_i, there is minimal adsorption of apo A-I to the egg sphingomyelin monolayer (Figure 3). The $\Delta\pi/\pi_i$ plot for the adsorption of apo A-I to the egg sphingomyelin monolayer shows that the $\Delta\pi$ values are significantly lower than those for the egg PC monolayer. The final surface pressure value of the egg sphingomyelin monolayer after adsorption of apo A-I is ~26 dyn/cm; at this surface pressure, the egg sphingomyelin monolayer is in a solid state (Figure 2). Consequently, $\Delta\pi$ values are similar to those observed with solid phospholipid monolayers. Extrapolation of the Γ/π_i data for the egg sphingomyelin monolayer shows that a zero surface concentration of apo A-I occurs at π_i = 19 dyn/cm, whereas $\Delta\pi$ extrapolates to zero at π_i = 26 dyn/cm (cf. Figures 3 and 4).

Effects of Cholesterol on Apo A-I Adsorption to Phospholipid Monolayers. The π–A isotherms for egg PC/cholesterol mixed monolayers containing various mole fractions of cholesterol were obtained (data not shown). The condensation of the egg PC by cholesterol is shown by plotting the mean molecular area against the mole fraction of cholesterol (Figure 5). Ideally, if there is no interaction between cholesterol and PC, the mean area is calculated according to an equation which describes the average area of two immiscible components (Chapman et al., 1969):

$$A_{12} = nA_1 + (1 - n)A_2 \quad (1)$$

A_{12} is the average area for the mixed components at a certain π, n is the mole fraction of component 1, and A_1 and A_2 are the molecular areas of components 1 and 2 obtained from their π–A isotherms at the same π. The deviation of the mean molecular area/mole fraction plot from ideality is a measure

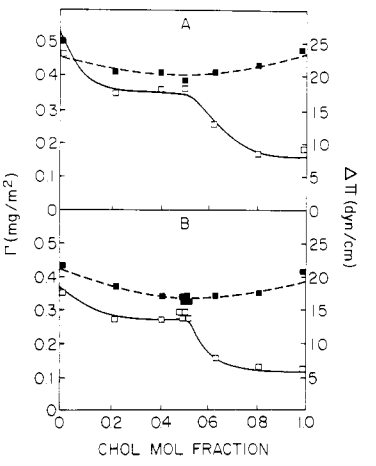

FIGURE 6: Plots of the increase in surface pressure [$\Delta\pi$ (■)] and the surface concentration of apolipoprotein A-I [Γ (□)] against cholesterol mole fraction for the adsorption of [^{14}C]apolipoprotein A-I to cholesterol/egg phosphatidylcholine mixed monolayers spread at a π_i of 5 dyn/cm (panel A) and 10 dyn/cm (panel B). The experimental conditions are described under Experimental Procedures. The subphase concentration of apolipoprotein A-I was 3×10^{-5} g/100 mL. The estimated errors in Γ and $\Delta\pi$ measurements are approximately ±0.02 mg/m² and ±1 dyn/cm, respectively.

of the condensation by cholesterol of the PC monolayer; this effect is due to interaction between cholesterol and phospholipid acyl chains (Chapman et al., 1969; Phillips, 1972).

The adsorption of ^{14}C-labeled apo A-I to mixed egg PC/cholesterol monolayers was studied at π_i values of 5 and 10 dyn/cm in order to explore the effects of cholesterol content on the adsorption behavior of apo A-I. Plots of Γ and $\Delta\pi$ against cholesterol mole fraction for adsorption of apo A-I to the mixed egg PC/cholesterol monolayers are shown in Figure 6. The Γ/mole fraction plot shows that generally there is a decrease in the adsorption of apo A-I to the mixed egg PC/cholesterol monolayers with increasing cholesterol content. It is interesting to note that there appears to be no change in Γ for cholesterol mole fractions ranging from 0.2 to 0.5. Unlike the Γ/mole fraction plot, the $\Delta\pi$/mole fraction plot shows that $\Delta\pi$ due to adsorption of apo A-I is not very sensitive to the exact mole fraction of cholesterol present in the mixed monolayer.

Discussion

The data on the adsorption of ^{14}C-labeled apo A-I to various lipid monolayers suggest that the effect of lipid composition on the adsorption of apo A-I correlates with the lipid physical state. This agrees with previous monolayer studies which suggested that adsorption of proteins to lipid monolayers is generally affected by the physical state of the lipid monolayer (Camejo et al., 1968; Colacicco, 1970; Phillips et al., 1975c). The lateral compressibility of a lipid monolayer, $-1/A(\delta A/\delta\pi)_T$ where A is molecular area and T is temperature, is the parameter which best reflects the effect of the physical state on protein penetration (Phillips et al., 1975a–c). The effect of lateral compressibility of lipid monolayers on protein penetration can be described by the equation (Phillips et al., 1975b):

$$\Gamma = \Delta\pi C/A_P \quad (2)$$

where Γ is the protein surface concentration, $\Delta\pi$ is the increase in the surface pressure, C is the lateral compressibility of the monolayer, and A_P is the average area per protein molecule. The above equation shows that, for a given surface concentration of protein, $\Delta\pi$ is inversely proportional to the lateral compressibility of the lipid monolayer. In agreement with this, larger $\Delta\pi$ values were recorded for relatively more condensed

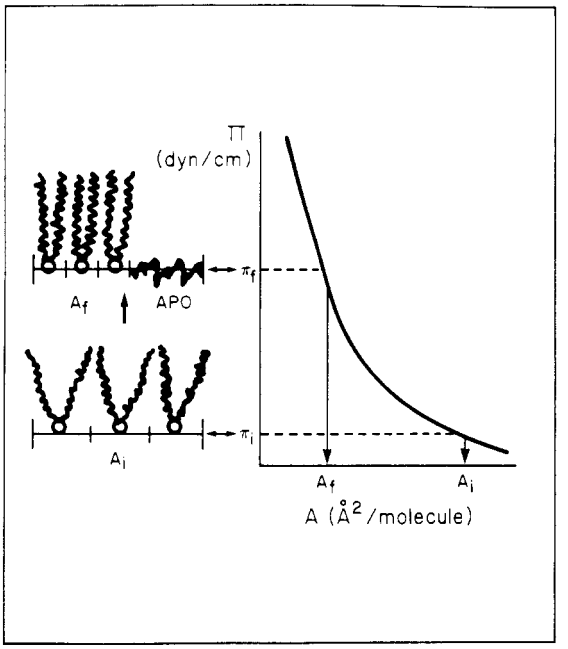

FIGURE 7: Illustration of a proposed model to calculate the available space (at the air–water interface) created in a lipid monolayer by apolipoprotein adsorption. If ideal mixing of apolipoprotein and lipid molecules occurs, the total space available in the lipid monolayer that can be created by compression of lipid molecules by the adsorbing apolipoprotein can be calculated from eq 3. In this treatment, $A_t = (A_i - A_f)n$ where A_t is the total space created in a lipid monolayer containing n lipid molecules. A_i is the initial lipid molecular area before adsorption of apoprotein; A_i at the initial surface pressure (π_i) can be obtained from the π–A isotherm of the lipid monolayer. A_f is the final lipid molecular area after adsorption of the apolipoprotein; A_f can be obtained from the π–A isotherm at the final surface pressure (π_f) of the monolayer after adsorption of the apolipoprotein, as illustrated in the diagram. See text for further details.

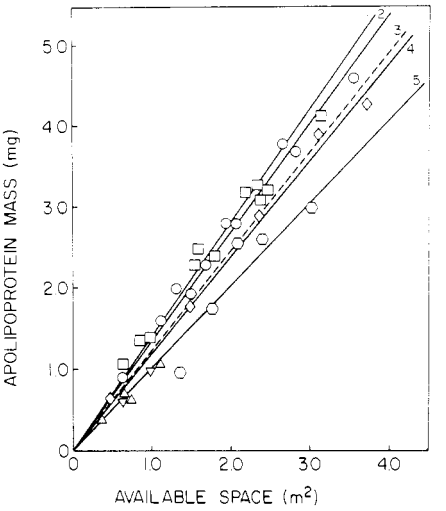

FIGURE 8: Plot of the mass of adsorbed apolipoprotein A-I against the space available (at the air–water interface, see Figure 7) for adsorption of apolipoprotein A-I to monolayers of egg phosphatidylcholine (PC) (○), egg PC/cholesterol at various molar ratios (□), egg sphingomyelin (○), dipalmitoyl-PC (◇), distearoyl-PC (△), dibehenoyl-PC (▽) monolayers, and to the air–water interface (---). The available space in the lipid monolayers is calculated as described in Figure 7 using the data points in Figures 3 and 6. In the interest of clarity, not all the data points in Figure 3 and 6 are plotted in Figure 8; data points with Γ values <0.1 mg/m² are omitted because of the large error in the calculated available space using small values of Γ. The regression lines were drawn through the origin by using the Apple Pharmacologic Calculations program. Lines 1, 2, 3, and 4 are for egg PC/cholesterol, egg PC, air–water interface, and dipalmitoyl-PC data, respectively. The data for egg sphingomyelin, distearoyl-PC, and dibehenoyl-PC are fitted with one regression line (5).

Table I: Available Space Model and Adsorption of Apolipoprotein A-I to Phospholipid Monolayers

monolayer system	slope[a] (mg of apo A-I/m² of available space)	molecular area[a] (Å² of available space/residue)
air–water[b]	1.26	15.0[d]
egg PC	1.39 ± 0.08	13.7 ± 0.8 (n = 10)[e]
cholesterol/egg PC[c]	1.44 ± 0.1	13.3 ± 1.0 (n = 11)[e]
dipalmitoyl-PC	1.22 ± 0.06	15.6 ± 0.8 (n = 5)[d]
distearoyl-PC or dibehenoyl-PC	1.02 ± 0.07	18.6 ± 1.3 (n = 6)[f]
egg sphingomyelin	1.03 ± 0.23	18.4 ± 4.0 (n = 5)[f]

[a] These values are the means (±SD) calculated from the slopes of the lines drawn between the origin and individual data points in Figure 8. [b] Taken from the adsorption isotherm of apo A-I to the air–water interface at a subphase protein concentration of 3 × 10^{-5} g/100 mL (Figure 1; Krebs et al., 1988). [c] At various molar ratios. [d,e,f] These groups of molecular areas are statistically different from each other at the level $p < 0.05$ when compared by the Student's t test for unpaired data.

lipid monolayers due to their lower lateral compressibility. For instance, the same Γ of apo A-I results in a larger $\Delta\pi$ for the condensed DSPC monolayer compared to the expanded egg PC monolayer (cf. Figures 3 and 4). To determine whether apo A-I simply interacts with lipid monolayers by penetrating and laterally compressing the lipid molecules, it is necessary to relate the mass of adsorbed apo A-I to the space (at the air–water interface) available in the lipid monolayer that can be created by compression of lipid molecules. To calculate the "available space" in the lipid monolayer, a model is proposed which assumes that the lipid molecules are compressed by adsorbing apolipoprotein molecules and that ideal mixing of protein and lipid molecules occurs in the resulting monolayer. The proposed model is illustrated in Figure 7 which shows that the space available for apolipoproteins can be calculated according to the equation:

$$A_t = (A_i - A_f)n \qquad (3)$$

where A_t is the total space available for apolipoprotein molecules, A_i is the molecular area of lipid molecules at the π_i, A_f is the molecular area of lipid molecules after compression by adsorbed apolipoprotein molecules, and n is the total number of lipid molecules in the monolayer.

Plots of the mass of apo A-I against the available space in various lipid monolayers spread at different π_i values are shown in Figure 8. The slopes of regression lines forced through the origin are calculated for the various lipid monolayers and shown in Table I. It is important to point out that the slopes in Table I represent the calculated surface concentrations of apo A-I adsorbed at the air–water interface available within the mixed lipid–protein monolayers (i.e., excluding the area occupied by lipid molecules). This parameter should be differentiated from the Γ measured by the gas flow counter which is milligrams of apolipoprotein per unit of total surface area. For a given lipid monolayer, the constant slope seems to indicate that apo A-I adopts the same conformation at the air–water interface when adsorbed to the lipid monolayer at various π_i values.

The plots in Figure 8 indicate that generally the amount of adsorbed apo A-I is proportional to the space that can be created within a lipid monolayer. This suggests that the compressibility of the lipids is the major factor controlling the extent of adsorption of apo A-I to various lipid monolayers. However, Figure 8 and Table I show that there are minor, but statistically significant, differences in the slopes of apo A-I mass/available space plots for egg PC, DPPC, and DSPC

monolayers. In addition, the slope of the line for the egg sphingomyelin monolayer is statistically different from those for egg PC and DPPC monolayers but not different from that of the DSPC monolayer. These results suggest that adsorption of apo A-I to various lipid monolayers is also affected to some extent by the type of lipid monolayer. In the proposed available space model, it is assumed that there is ideal mixing between lipid and protein molecules; consequently, the available space after compression of lipid molecules by apolipoprotein molecules is calculated from the π–A isotherms of pure lipid monolayers. The observed differences in mass/available space plots suggest that the assumption of ideal mixing is not valid due to lipid–protein interaction. To confirm this, the various plots are compared with the plot of mass/space for the adsorption of apo A-I to the air–water interface (Figure 8). The slope of the ideal plot is taken as the surface concentration value of 1.26 mg/m^2 of adsorbed apo A-I at the air–water interface for a subphase concentration of 3×10^{-5} g/100 mL (Figure 1), which is the same subphase concentration of apo A-I used for adsorption to lipid monolayers. A negative deviation of the plots for egg sphingomyelin and DSPC monolayers, and a positive deviation for egg PC and mixed cholesterol/PC monolayers, is observed. The deviations from ideality indicate lipid–protein interactions, so that adsorption of apo A-I to a lipid monolayer results in a compression of lipid molecules in a more complex fashion than the simple lateral compression proposed by the available space model.

The nature of this lipid–protein interaction and the significance of the observed negative and positive deviations from ideality are not entirely clear at this point. It is possible that apo A-I penetrates into the lipid monolayer and alters the packing of the lipid molecules, presumably by an interaction at the interface between the amphipathic α-helical segments of apo A-I (Segrest et al., 1974) and the lipid molecules. Since the adsorbed apolipoprotein molecules are surrounded by a boundary layer of lipid molecules (Jost et al., 1973; Phillips et al., 1975b), it is possible that this interaction occurs only within this region of the mixed monolayer. Therefore, at the final surface pressure (π_f) of the mixed lipid/apo A-I monolayer, the molecular area of the boundary lipid layer could be different from the molecular area of the remaining lipid molecules. Lipid molecules not in the boundary region are presumed to occupy an area at π_f consistent with the π–A isotherm of the pure lipid monolayer. Consequently, the average lipid molecule area in a mixed lipid–protein monolayer can be described by the equation:

$$A_1 = XA_b + (1 - X)A_f \quad (4)$$

where A_1 is the average lipid molecular area, A_b is the boundary lipid molecular area, A_f is the lipid molecular area at π_f calculated from the π–A isotherm of the pure lipid monolayer, and X is the fraction of lipid molecules in the boundary layer surrounding the protein molecules. The apo A-I molecules may exert either an expanding or a condensing effect on the boundary lipid molecules depending on the nature of the lipid monolayer. In the case of the expanded egg PC monolayer, apo A-I probably exerts a condensing effect which could explain the positive deviation of the mass/available space plot from ideality. Condensation of the boundary layer of egg PC molecules by apo A-I results in a larger actual available space than that calculated from the original model (Figure 7) so that more apo A-I can adsorb to the lipid monolayer than in the ideal situation. Contrary to the effect on the egg PC monolayer, apo A-I apparently expands the lipid boundary layer in the condensed egg sphingomyelin and DSPC monolayers. At π_f, the lipid molecular areas for the boundary regions in the egg sphingomyelin/apo A-I and DSPC/apo A-I mixed monolayers are probably larger due to an expanding effect of the adjacent apo A-I molecules. This effect probably occurs because apo A-I prevents the complete condensation of the egg sphingomyelin and DSPC molecules in the boundary region. This explanation is supported by previous calorimetric studies on recombinant HDL particles which demonstrated that the association of apo A-I with phospholipid results in a boundary lipid layer that is perturbed by the apolipoprotein (Tall & Lange, 1978a; Pownall et al., 1979).

As an alternative to the above explanation based solely on variations in lipid packing, it is possible that the physical properties of lipid molecules are not perturbed but rather the conformation of apo A-I varies from one lipid monolayer to another. The conformation of apo A-I may depend upon the type of lipid monolayer and the nature of the lipid–protein interaction. The space occupied by apo A-I in various lipid monolayers can be calculated as illustrated in Figure 7 with the assumption that apo A-I does not affect the compressibility of the lipid molecules. The calculated molecular areas for apo A-I in various lipid monolayers are shown in Table I. The molecular area of apo A-I adsorbed at the air–water interface is 0.79 m^2/mg of apo A-I which corresponds to 15 Å^2/residue; this is consistent with the limiting area per amino acid residue in a close-packed film of α-helices (Malcolm, 1973; Jones, 1975). The calculated values indicate that the conformation of adsorbed apo A-I in the egg sphingomyelin and DSPC monolayers is more expanded than at the air–water interface and could be less helical. In contrast, the conformation of apo A-I in the egg PC and mixed cholesterol/egg PC monolayers is more condensed. Since the molecular area of apo A-I in these monolayers is lower than the limiting area per amino acid residue, it is possible that not all the amino acid residues in apo A-I are located in the lipid–water interface. Apo A-I may adopt a conformation with some residues in loops and/or trains out of the plane of the egg PC–water interface. Such conformations have been suggested for β-casein molecules adsorbed to an egg PC monolayer (Phillips et al., 1975a).

The adsorption studies with apo A-I have shown that cholesterol decreases the adsorption of the apolipoprotein to a phospholipid monolayer. Similarly, studies of the association of apolipoproteins with microemulsions have shown that there is a negative correlation between free cholesterol content and binding of apolipoproteins (Erkelens et al., 1981; Maranhao et al., 1986). The effect of cholesterol on the surface concentration of apo A-I adsorbed to the mixed cholesterol/egg PC monolayer is presumably due to the condensing effect of cholesterol on the egg PC monolayer. This condensing effect of cholesterol is demonstrated in Figure 5 and is consistent with previous studies which indicated that cholesterol condenses phospholipid monolayers (Chapman et al., 1969; Joos & Demel, 1969) and bilayers (Phillips, 1972). Analysis in terms of the available space model (Figure 7) of the data for the adsorption of apo A-I to mixed cholesterol/egg PC monolayers at various cholesterol contents is shown in Figure 8. It is interesting to note that the mass/available space slopes for the egg PC and the mixed cholesterol/egg PC monolayers are similar. This suggests that apo A-I adopts the same conformation in the egg PC and the mixed cholesterol/egg PC monolayers (Table I) and apo A-I has the same condensing effect on the boundary lipid monolayer. This analysis suggests that cholesterol and the adsorbed apo A-I molecules do not interact in the phospholipid monolayer. This may imply that cholesterol and apo A-I molecules occupy separate regions in

the mixed apolipoprotein–lipid monolayer and do not interact directly. This conclusion is in good agreement with previous studies on HDL recombinants which suggested that cholesterol is excluded from the boundary layer of phospholipid in contact with apo A-I (Tall & Lange, 1978a,b; Pownall et al., 1979). Interpretation of the constant Γ for apo A-I at a cholesterol mole fraction of 0.2–0.5 (Figure 6) in terms of the available space model suggests that the lateral compressibilities of the mixed cholesterol/egg PC monolayers containing these mole fractions of cholesterol are similar. The π–A isotherms for these mixed monolayers (data not shown) are consistent with the idea that the available space for adsorbing apo A-I in monolayers containing 0.2–0.5 mole fraction of cholesterol is constant.

In conclusion, the present studies suggest that the differences in the surface lipid composition and packing that exist among various types of lipoprotein particles could be crucial in controlling the distribution and the transfer of apolipoproteins among various lipoprotein classes in vivo. Further studies using lipid monolayers prepared from the surface lipids of lipoprotein particles should provide further insight into this.

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