Decreased binding of apoA-I to phosphatidylcholine monolayers containing 22:6 n-3 in the *sn*-2 position

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Abstract Previous studies have shown that dietary fish oil can modify the distribution and fatty acyl composition of plasma phospholipids. Although it is known that the type of phospholipid can affect the binding of apolipoprotein A-I (apoA-I), little is known about the effect of n-3 fatty acid enrichment in phospholipids on apoA-I binding. We hypothesize that phosphatidylcholine (PC) surfaces containing n-3 fatty acids at the *sn*-2 position bind apoA-I less avidly than those containing *sn*-2 18:1. PC species containing *sn*-1 16:0 and *sn*-2 18:1 (POPC), *sn*-2 20:5 n-3 (PEPC), or *sn*-2 22:6 n-3 (PDPC) were used in apoA-I monolayer binding studies. The molecular surface area at any given surface pressure (π) was ordered: PDPC > PEPC > POPC and at π = 25 mN/m the molecular surface areas were 86.2, 78.8 and 72 Å²/molecule, respectively. Binding of [^{14}C]apoA-I (radiolabeled by reductive methylation) to PDPC at πi = 15 mN/m was less than that for POPC whether expressed as nmol A-I/m² surface or molecules A-I/1000 PC. The apparent K_d for steady state apoA-I binding to PEPC (2.1 nM) and PDPC (2.2 nM) was greater than that for POPC (1.2 nM); the maximum binding capacity (nmol/m²) was ordered PEPC (9.4) > POPC (8.1) > PDPC (6.7). Similar results were found when a fixed amount of apoA-I was injected beneath the PC monolayers equilibrated at different initial surface pressures. The calculated surface area available for bound apoA-I was 15, 17, and 23 Å²/amino acid for POPC, PEPC, and PDPC at πi = 15 mN/m, respectively. We conclude that the binding affinity of apoA-I for PDPC and PEPC is less than that for POPC and that apoA-I bound to PDPC is more loosely folded than that to POPC. These studies suggest that the type of *sn*-2 fatty acid can influence apoA-I binding to PC.—**Parks, J. S., and T. Y. Thuren.** Decreased binding of apoA-I to phosphatidylcholine monolayers containing 22:6 n-3 in the *sn*-2 position. *J. Lipid Res.* 1993. **34:** 779–788.

Supplementary key words molecular surface curves • surface pressure • 1-palmitoyl-2-eicosapentaenoyl-*sn*-glycerol-3-phosphocholine • 1-palmitoyl-2-linoleoyl-*sn*-glycero-3-phosphocholine • 1-palmitoyl-2-docosahexaenoyl-*sn*-glycero-3-phosphocholine

Apolipoprotein A-I (apoA-I) is the major apolipoprotein of high density lipoproteins. Its primary sequence consists of eight amphipathic α-helical repeats of 22 amino acids and two repeats of 11 amino acids. Segrest et al. (1) first suggested that the amphipathic α-helix was important in determining the lipid binding properties of apolipoproteins. When apoA-I binds to phospholipid, the hydrophobic face of the amphipathic α-helix inserts into the phospholipid monolayer, leaving the hydrophilic face of the helix exposed to the aqueous environment. The α-helices of apoA-I contain charged amino acid residues at the interface between the hydrophilic and hydrophobic face which could potentially interact with the zwiterionic head groups of the phospholipid molecules and act to stabilize the binding of apoA-I to the phospholipid surface. Thus, insertion of apoA-I into the phospholipid monolayer is a critical step in the binding and stabilization of apoA-I at the lipoprotein particle surface.

Although apoA-I is an exchangeable apoprotein it is almost exclusively bound to HDL. A recent study by Ibdah, Lund-Katz, and Phillips (2) suggested that apoA-I preferentially binds to HDL rather than LDL because the surface of LDL has a different phospholipid composition (i.e., more sphingomyelin), a higher free cholesterol content, and a stronger interaction between cholesterol and phospholipid molecules, which effectively exclude apoA-I from binding to its surface. Other studies have shown that apoA-I can bind to the surface of vesicles, emulsions, and microemulsions that contain a single type of phospholipid such as egg yolk lecithin even though the size of the particles is similar to or larger than plasma LDL. These results taken together suggest that the type of phospholipid in the lipoprotein surface may modulate the binding of apoA-I.

Abbreviations: LCAT, lecithin:cholesterol acyltransferase; PC, phosphatidylcholine; apoA-I, apolipoprotein A-I; HDL, high density lipoprotein(s); LDL, low density lipoprotein(s); PL, phospholipid; TLC, thin-layer chromatography; POPC, 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine; PLPC, 1-palmitoyl-2-linoleoyl-*sn*-glycero-3-phosphocholine; PEPC, 1-palmitoyl-2-eicosapentaenoyl-*sn*-glycero-3-phosphocholine; PDPC, 1-palmitoyl-2-docosahexaenoyl-*sn*-glycero-3-phosphocholine; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; π, surface pressure; πi, initial surface pressure.

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Lipoprotein phospholipid type and fatty acid distribution are modified by the type of dietary fat fed to nonhuman primates (3, 4). Specifically, when fish oil was isocalorically substituted for lard in the diet of monkeys, the percentage of phosphatidylcholine (PC) in LDL was decreased and the percentage of sphingomyelin and lysoPC was increased (3). The PC species were also enriched in n-3 fatty acids (at the expense of oleic acid and linoleic acid). The effect of these diet-induced changes on apoA-I binding to PL surfaces is unknown. However, preliminary studies have shown that less apoA-I was associated with liver perfusate lipoproteins of monkeys fed the fish oil versus lard diet even though hepatic apoA-I production was similar for both diet groups (J. S. Parks, M. D. Wilson, F. L. Johnson and L. L. Rudel, unpublished observations). We hypothesized that PC surfaces containing *sn*-2 n-3 fatty acids bind apoA-I less avidly than those containing oleic acid. The purpose of this study was to determine the effect of substituting eicosapentaenoic acid (20:5 n-3) or docosahexaenoic acid (22:6 n-3) for oleic acid in the *sn*-2 position of PC on the binding of apoA-I to PC surfaces. We used a monolayer trough for the binding experiments so that the effect of apoA-I binding on the lateral packing of the phospholipid monolayer could be monitored. The results of the study suggest that apoA-I binding to PC monolayers is influenced by the type of fatty acid in the *sn*-2 position of the PC.

MATERIALS AND METHODS

Materials

The following reagents were purchased from Sigma Chemical Co. (St. Louis, MO): *cis*-5,8,11,14,17-eicosapentaenoic acid (EPA); *cis*-4,7,10,13,16,19-docosahexaenoic acid (DHA); 1-palmitoyl-*sn*-glycero-3-phosphocholine (LysoPC); 4-dimethylaminopyridine; 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC), and 1-palmitoyl-2-linoleoyl-*sn*-glycerol-3-phosphocholine (PLPC). N,N'-dicyclohexylcarbodiimide was purchased from Aldrich Fine Chemicals (Milwaukee, WI). Cholesterol was obtained from Nu-Chek Prep (Elysian, MN). All other reagents, chemicals and solvents were purchased from Fisher Scientific Co. (Pittsburgh, PA).

Phospholipid synthesis

1,2-Diacyl-*sn*-glycerol-3-phosphocholine (PC) species were synthesized from 1-palmitoyl lysoPC and the appropriate fatty acid as described previously (5). Synthesized PC species were purified by silicic acid chromatography (5). The purified PC samples were filtered through a 0.2 μm Prep-Disc Membrane (Bio-Rad, Richmond, CA) to remove any residual silicic acid and brought to a final volume of 10 ml with $CHCl_3$. Aliquots were taken for phosphorus analysis and TLC as described previously (5). The PC samples were flushed with Ar and stored at −20°C. At all steps during the isolation procedure, where feasible, the PC preparations were under an Ar atmosphere.

Characterization of PC preparations

The purified PC preparations were analyzed by TLC in neutral (hexane–diethyl ether–acetic acid, 70:30:1, v/v) and polar (chloroform–methanol–acetic acid–water, 65:45:12:6, v/v) solvent systems using silica gel H TLC plates (Analtech, Newark, DE). Detection of bands on TLC plates was accomplished with I_2 vapor, molybdenum spray, and primulin spray (5). Molecular species of the PC preparations were determined after hydrolysis with phospholipase C, benzoylation of the diacylglycerides, and HPLC separation of the benzoylated derivatives as described by Blank et al. (6). The position of the fatty acids in the PC species was also investigated by phospholipase A_2 digestion and subsequent fatty acid analysis as described previously (4). The thiobarbituric acid reactive substance assay was used to determine whether lipid peroxidation of the PC species had occurred during the studies (7).

Isolation and radiolabeling of apolipoprotein A-I

ApoA-I was isolated from HDL of fresh human plasma by gel filtration chromatography (8) and its purity was verified by SDS-polyacrylamide gel electrophoresis as described previously (9). The isolated apoA-I was stored at −20°C in 0.01% EDTA, 0.01% NaN_3, pH 7.4, at a concentration of 500 μg/ml, as determined by the published extinction coefficient (ϵ = 1.21 ml/mg • cm; ref. 10) and Lowry protein assay (11). ApoA-I was radiolabeled by reductive alkylation with [^{14}C]formaldehyde as described previously (12). The [^{14}C]apoA-I had a specific activity of 3.9 μCi/mg and was stored at 4°C in a solution of 0.9% NaCl, 0.01% EDTA, 0.01% NaN_3, pH 7.4, at a protein concentration of 200 μg/ml.

Monolayer studies

Force-area isotherms. Lateral compression isotherms and apoA-I binding studies were performed using a KSV LB5000 monolayer apparatus (KSV-Instruments USA, Inc., Stamford, CT). A Wilhelmy platinum plate was used for surface pressure measurements and a personal computer was used for data collection and analysis with the LB5000 software.

A rectangular Teflon trough (50 × 20 cm) was used to measure the lateral compression isotherms of POPC, PEPC, and PDPC alone or with increasing amounts of cholesterol up to 50 mole %. The PC species were mixed with an appropriate amount of a stock solution of cholesterol in $CHCl_3$ and were spread from $CHCl_3$ at a clean argon–water surface. At the time the lipids were spread on the monolayer surface, aliquots of the PC/cholesterol mixtures were taken in triplicate for quantification of lipid phosphorus (13) and cholesterol

(14). The assayed values for PC and cholesterol were used to calculate the mole percentage cholesterol in PC. The subphase buffer was 20 mM Tris-HCl, 150 mM NaCl, 1 mM EDTA, pH 7.4. The monolayer trough was maintained under an argon atmosphere throughout the studies. Monolayers were compressed laterally at a rate of 5 mm/min while surface pressure was continuously monitored.

ApoA-I binding to monolayers. ApoA-I binding was monitored by a surface radioactivity probe mounted 3 mm above the surface of the monolayer (15) and by monitoring the increase in surface pressure. The surface radioactivity probe was calibrated by measuring the radioactivity of monolayers containing a known amount of [1-^{14}C]palmitoyl-PC prepared from rabbit liver PC (16). Results of the calibration curve were the average of five separate experiments at eight different surface radioactivity concentrations varying from 250 cpm to 2080 cpm. After the measurement of the surface radioactivity, the monolayer was collected by suction and radioactivity was determined by liquid scintillation counting. These radioactivity measurements agreed within 5% of the predetermined radioactivity that was applied to the monolayer. The resulting calibration curve was then used to convert cpm measured by the probe into dpm. The efficiency of the surface radioactivity probe was approximately 26% for ^{14}C. The calibration procedure was also performed at different surface pressures (10, 15, 20, 25 mN/m) by diluting [1-^{14}C]palmitoyl-PC with rabbit liver PC in order to have equal amounts of radioactivity at different surface pressures. The efficiency of the surface radioactivity probe remained approximately constant over the surface pressure range studied.

Binding of [^{14}C]apoA-I to PC monolayers was determined in two ways. First, PC species were spread to an initial surface pressure of 15 mN/m as described above and increasing amounts of apoA-I (1–11 μg; 1–10 nM final subphase concentration) were injected into the subphase. The subphase was mixed with a magnetic stirring bar at a rate of $<$50 rpm during the experiment. Background radioactivity was measured after the monolayer was spread at the argon–water interface prior to injection of [^{14}C]apoA-I into the subphase and was subtracted from the total cpm after apoA-I binding to the surface had equilibrated. In a second set of experiments 2.9 μg [^{14}C]apoA-I (2.6 nM final subphase concentration) were injected into the subphase beneath individual PC species spread at initial surface pressures of 10, 15, 18, 20, and 23 mN/m and binding of apoA-I to the monolayer was determined by measurement of surface radioactivity and by the increase in surface pressure as described above. All apoA-I binding studies were performed at room temperature, in duplicate, and in almost all cases the duplicate results obtained varied $<$10% from one another.

Statistics

Analysis of covariance was used to test for significant differences in the double reciprocal plots of the apoA-I binding to the different PC surfaces, where steady state subphase apoA-I concentration was used as the covariant. A Fisher's least significant difference test was used to detect binding differences of individual PC surfaces among the three PC species.

RESULTS

All synthetic and purchased PC species used in these studies were characterized by HPLC, phospholipase A_2 digestion, TLC, and thiobarbituric acid assay. All PC species migrated with authentic egg yolk lecithin in both neutral and polar TLC solvent systems and contained no detectable contaminating species when 300 μg of PC was loaded on the plate. HPLC analysis of each PC benzoate derivative gave only one peak and the retention times for the peaks were similar to those reported by Blank et al. (6). Results from the phospholipase A_2 digestion demonstrated that 80–90% of the fatty acid in the *sn*-2 position was the expected fatty acid with 10–20% contamination from 16:0, which was likely the result of acyl migration from the *sn*-1 position during the phospholipase A_2 digestion. The amount of thiobarbituric acid reactive substance was $<$0.25 mole % with respect to phospholipid for all PC species.

Fig. 1 shows representative force–area isotherms for POPC, PLPC, PEPC, and PDPC. There was little detectable difference in the isotherms for POPC and PLPC. However, the isotherms for PEPC and PDPC were displaced to the right indicating that, at an equivalent surface pressure, the two PC species containing n-3 fatty acids occupied a greater area per molecule compared to POPC and PLPC. This was true regardless of the lateral surface pressure of the monolayer up to the collapse pressure of the monolayer ($\sim$45 mN/m). The isotherms were repeated at least twice with different preparations of PC and were found to be virtually superimposable.

Table 1 shows representative values of molecular surface area at selected surface pressures from 10 to 40 mN/m. At relatively low lateral surface pressure (i.e., 10 mN/m), the molecular area of PEPC and PDPC was 11.1 and 19.6 Å^2/molecule greater than that of POPC, respectively. Even at very high lateral surface pressures (40 mN/m) PEPC and PDPC occupied 5–10 Å^2/molecule more area than did POPC.

Fig. 2 shows the force–area isotherms for POPC, PEPC, and PDPC alone and with increasing amounts of cholesterol up to $\sim$50 mole %. For all three species of PC there was a decrease in surface area with increasing mole

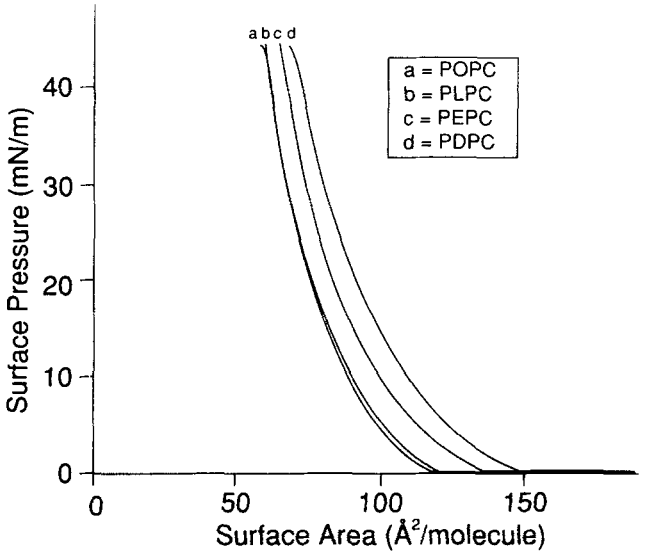

Fig. 1. Force-area isotherms for purified PC species containing different fatty acids in the *sn*-2 position. PC species were spread on a rectangular monolayer trough at an argon-buffer interface from a $CHCl_3$ solution, the $CHCl_3$ was allowed to evaporate, and the PC molecules were compressed laterally at a rate of 5 mm/min while the surface pressure was continuously monitored by means of a Wilhelmy plate. The subphase buffer was 20 mM Tris-HCl, 150 mM NaCl, 1 mM EDTA, pH 7.4.

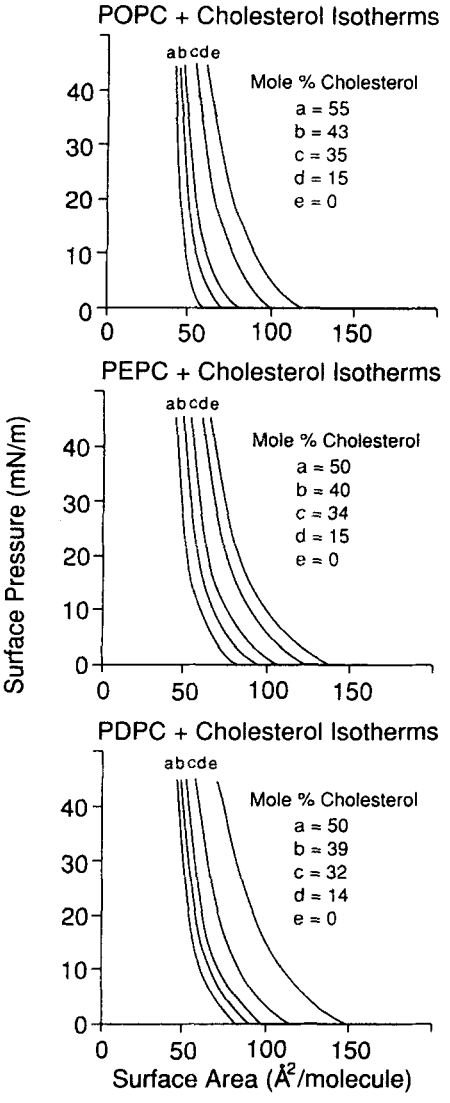

Fig. 2. Force-area isotherms of PC species containing different amounts of cholesterol. Experimental details for the monolayer apparatus are given in Fig. 1.

percentage of cholesterol at any given surface pressure. The "condensing" effect of cholesterol on PC monolayers is well documented for many species of PC (17-19). Close inspection of the isotherms revealed several interesting results. In spite of the condensing effect of cholesterol the relative difference in molecular surface area was maintained between PEPC and POPC at any given mole fraction of cholesterol. However, a different result was observed for the PDPC isotherms where addition of cholesterol resulted in a displacement of the curves to smaller molecular areas (i.e., displaced to the left) compared to PEPC at any given mole fraction of cholesterol. Thus, cholesterol appeared to have a greater condensation effect on PDPC compared to PEPC.

To examine these data more closely, a plot of mole percentage cholesterol versus surface area (**Fig. 3**; top) and change in surface area (Fig. 3; bottom) of the three PC species at a surface pressure of 25 mN/m was made. POPC and PEPC had a nearly linear decrease in surface area with increasing mole percentage cholesterol (−0.53 and −0.59 Å²/molecule per mole percentage cholesterol, respectively). However, increasing the mole percentage of cholesterol from 0 to 15% had a remarkable effect on the condensation of the PDPC monolayer, decreasing the molecular area from 86.2 to 67.0 Å²/molecule (Δ = −19.2 Å²) as compared to 72 to 62.6 Å²/molecule (Δ = −9.4 Å²) for PEPC. From 15 to 50 mole % cholesterol the decrease in surface area as a function of cholesterol was similar to that of POPC and PEPC (i.e., −0.48 Å²/molecule per mole percentage of cholesterol). Extrapolation of the line of best fit for the PDPC data from 15 to 50 mole % cholesterol back to 0% cholesterol

TABLE 1. Molecular surface area of phosphatidylcholine species at selected surface pressures[a]

	Molecular Surface Area			
π	POPC	PLPC	PEPC	PDPC
mN/m		*Å²/molecule*		
10	89.4	90.5	100.5	109.0
15	82.0	82.4	90.5	98.9
20	76.4	76.7	84.1	91.5
25	72.0	71.7	78.8	86.2
30	68.3	67.7	74.6	80.7
40	62.4	61.9	67.7	72.8

[a]Values derived from the force-area isotherms in Fig. 1.

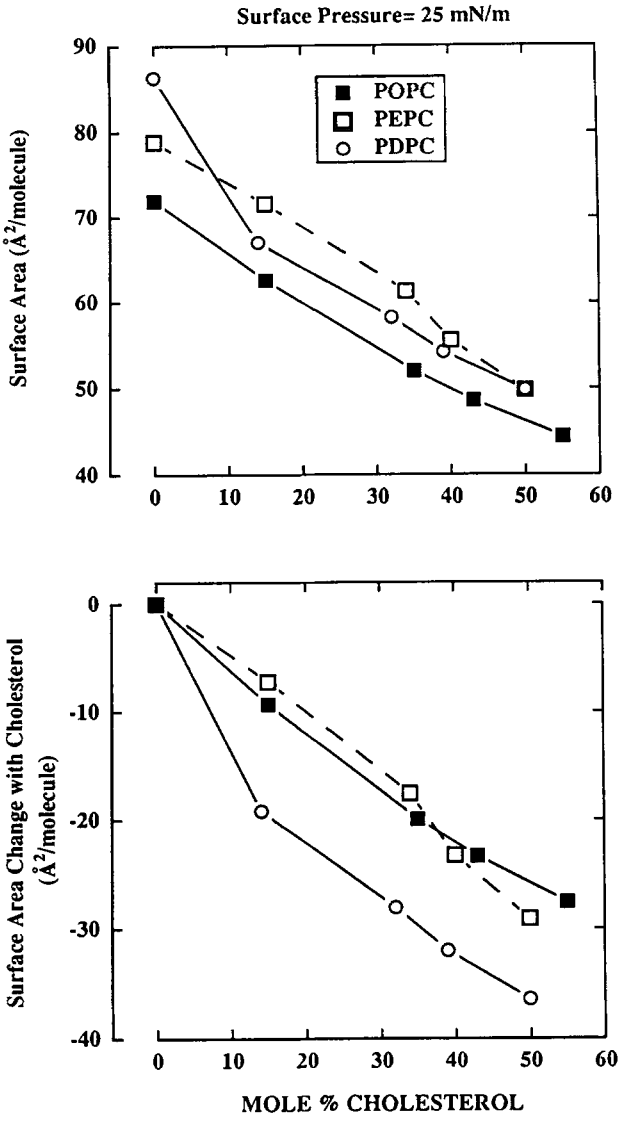

Fig. 3. Effect of mole percentage of cholesterol on the mean molecular surface area of POPC, PEPC, and PDPC (top panel) and on change in surface area (bottom panel) at a surface pressure of 25 mN/m. Data were taken from the isotherms in Fig. 2 where surface pressure = 25 mN/m.

predicted a molecular area of 73.6 Å²/molecule rather than the area of 86.2 Å²/molecule that was actually observed.

Results of apoA-I binding to POPC, PEPC, and PDPC monolayers as a function of the steady state subphase concentration of apoA-I at an initial surface pressure of 15 mN/m are shown in **Fig. 4.** The data in the top panel were normalized for the amount of total surface available for apoA-I binding while the results in the lower panel are normalized for the number of PC molecules in the surface monolayer. The data are presented in both ways because of the difference in molecular surface area among the three PC species (Fig. 1 and Table 1). Binding of apoA-I to all three monolayers appeared saturable above a subphase concentration of 2 nM. The binding of apoA-I to PDPC was less than that for POPC and PEPC when binding was expressed as nmol apoA-I/m² of surface (top panel). To determine whether the decreased binding of apoA-I was due to fewer PDPC molecules per available surface area, the results were normalized to molecules of apoA-I bound per 1000 PC molecules. However, apoA-I binding to PDPC remained less than that to POPC and the binding to PEPC appeared to be greater than that to POPC and PDPC.

The data in Fig. 4 were analyzed as double reciprocal plots and are shown in **Fig. 5.** The maximum binding ca-

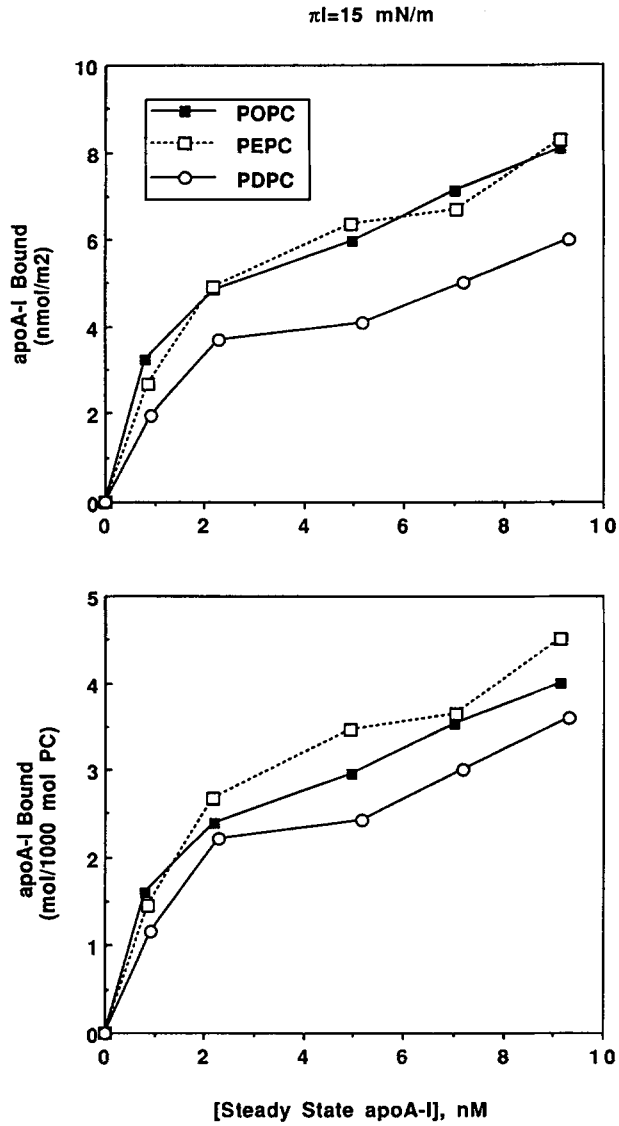

Fig. 4. ApoA-I binding to POPC, PEPC, and PDPC monolayers as a function of steady state subphase concentration of apoA-I at an initial surface pressure of 15 mN/m. [¹⁴C]apoA-I was injected into the subphase and binding to the monolayer was monitored with a radioactivity probe mounted 3 mm above the surface. Details of the measurement and calibration are given in the Methods section. Each point represents the mean of duplicate observations. Results are expressed as nmol of apoA-I bound/m² surface (top) or as molecules of apoA-I bound/1000 PC molecules (bottom).

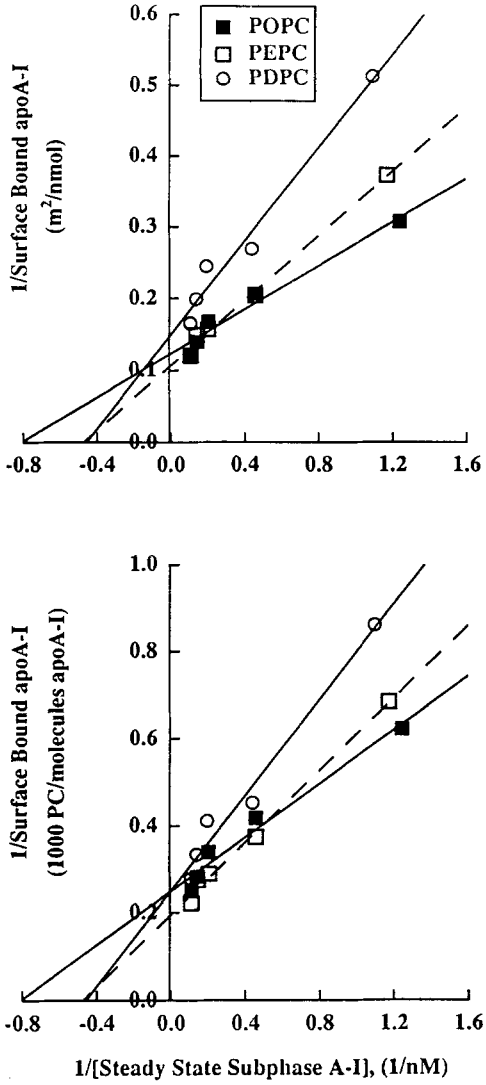

Fig. 5. Double reciprocal plots of apoA-I binding to POPC, PEPC, and PDPC monolayers. Data points were taken from Fig. 4 and represent duplicate determinations. The line of best fit, determined by linear regression analysis, is shown for each PC. Data are expressed as m^2/nmol apoA-I bound (top) or as 1000 PC/molecules apoA-I bound (bottom).

pacity (B_{max}) of apoA-I to the monolayers was calculated as the reciprocal of the y-intercept of the line of best fit for each double reciprocal plot. The apparent dissociation constant (K_d) was calculated from the slope of the line of best fit for each double reciprocal plot using the equation: slope = (K_d/B_{max}). The apparent K_d was defined as the concentration of apoA-I necessary to achieve half of the maximal apoA-I surface concentration; the term "apparent K_d" was used so as to make no assumption regarding reversibility of apoA-I binding to the monolayer surface. The results of the analysis are shown in **Table 2.** The B_{max} values are presented in terms of nmol/m^2 and molecules of apoA-I/1000 PC molecules. The apparent K_d for apoA-I binding was approximately 1.5-fold higher for PEPC and PDPC compared to POPC, suggesting that apoA-I had less affinity for the two PC species containing n-3 fatty acids. The B_{max} of apoA-I for PEPC was 1.15–1.30 times higher than that for POPC or PDPC regardless of whether capacity was expressed as nmol of apoA-I/m^2 surface or molecules of apoA-I/1000 molecules PC. The values for B_{max}/apparent K_d are also shown in Table 2. This number represents the maximum binding capacity normalized for any differences in the apparent K_d. The B_{max}/apparent K_d value was significantly less ($P < 0.01$) for apoA-I binding to PDPC compared to POPC or PEPC regardless of whether the data were expressed as total surface (m^2) or total number of PC molecules. Although the B_{max}/apparent K_d value was less, on average, for apoA-I binding to PEPC compared to POPC, the difference was not statistically significant ($P > 0.05$).

ApoA-I binding to PC monolayers was also measured by injecting a fixed amount of apoA-I (2.9 μg; 2.59 nM subphase concentration) beneath the surface of monolayers equilibrated at different initial surface pressures ranging from 10 to 23 mN/m. The results are shown in **Fig. 6,** normalized to the amount of total surface available for apoA-I binding (top panel) and to molecules of apoA-I bound per 1000 PC molecules (bottom panel). When

TABLE 2. Apparent binding constants of apoA-I to different phosphatidylcholine surfaces[a]

PC Type	App K_d (nM)	Maximum Binding Capacity (nmol/m^2)	Maximum Binding Capacity (Molecules ApoA-I/1000 PC)	Max. Binding Capacity/App K_d (nmol/m^2 / nM)	Max. Binding Capacity/App K_d (Molecules ApoA-I/1000 PC / nM)
POPC	1.24	8.10	4.00	6.53	3.23
PEPC	2.11	9.38	5.10	4.45	2.42
PDPC	2.19	6.70	3.99	3.06[b]	1.82[b]

[a] Binding constants were derived from the line of best fit for the double reciprocal plots of apoA-I subphase concentration vs. amount of surface bound apoA-I. Surface bound apoA-I was expressed in two ways: nmol/m^2 of surface and molecules of apoA-I/1000 PC molecules. Maximum binding capacity was obtained from the reciprocal of the y-intercept of the double reciprocal plots while apparent K_d (app K_d) was obtained using the equation: slope = K_d/maximum binding capacity. The data are mean values from two separate experiments.

[b] Significantly different ($P < 0.01$) from POPC and PEPC based on analysis of covariance and Fisher's least significant difference test of the double reciprocal plots.

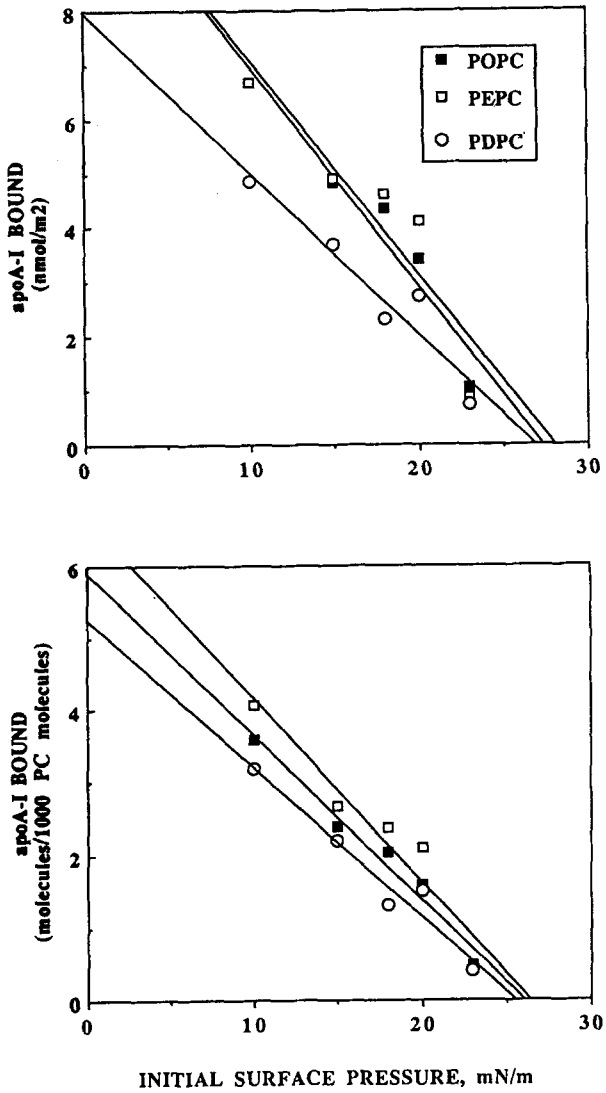

Fig. 6. ApoA-I binding to monolayers of POPC, PEPC, and PDPC equilibrated at different initial surface pressures from 10 to 23 mN/m. For all experiments the initial [^{14}C]apoA-I subphase concentration was 2.6 nM. ApoA-I binding was measured as described in Fig. 4 and the Methods section. Each point represents the mean of duplicate observations and the line of best fit, determined by linear regression analysis, is shown. Results are expressed as nmol apoA-I bound/m^2 surface (top) or as molecules apoA-I bound/1000 PC molecules (bottom).

represented as nmol apoA-I bound/m^2 surface, the binding to PDPC was significantly less ($P < 0.03$) than that for POPC and PEPC, similar to results shown in Fig. 4 (top panel). When the results were expressed as molecules of apoA-I bound/1000 PC molecules, the binding of apoA-I to PDPC was significantly less ($P < 0.02$) than that to PEPC but not that to POPC. This pattern was similar to that observed in Fig. 4 (bottom panel) when different amounts of apoA-I were injected into the subphase. Extrapolation of the lines of best fit to the x-intercept gave the critical penetration pressure of apoA-I for each monolayer. The critical penetration pressure is the surface pressure above which a protein can not penetrate the monolayer. The values of critical penetration pressure were similar for all three PC species and ranged from 26 to 28 mN/m, similar to previously reported values for apoA-I (18).

Using data from the force-area isotherms of the PC species (Fig. 1) and the apoA-I binding data (Fig. 6), it was possible to calculate the available surface area for bound apoA-I using the procedure described by Ibdah and Phillips (18). The calculation assumes that ideal mixing of apoA-I and phospholipid occurs and that the change in surface pressure of the monolayer is due to the adsorption of apolipoprotein. Thus, the final surface pressure of the monolayer after apoA-I binding can be converted to surface area based on the isotherms of the pure PC and used to calculate the amount of surface available for the apoA-I molecules bound to the monolayer. The results are shown in **Table 3** for two different initial surface pressures. ApoA-I bound to POPC had an available space of 14.5–15.1 Å^2/amino acid residue, a value that is very similar to that of apoA-I at the air-water interface (i.e., 15 Å^2/amino acid residue; ref. 20). The molecular area available to apoA-I bound to PEPC was slightly larger than that for POPC and ranged from 15.5 to 17.4 Å^2/amino acid). ApoA-I bound to PDPC occupied a much larger molecular area (22.9–23.2 Å^2/amino acid) compared to that of POPC or PEPC, suggesting that apoA-I bound to PDPC was not as tightly packed in the monolayer as apoA-I bound to POPC.

DISCUSSION

The purpose of this study was to determine the effect of substituting eicosapentaenoic acid or docosahexaenoic acid for oleic acid in the *sn*-2 position of PC on apoA-I binding using a monolayer system. Before initiating the apoA-I binding studies, we characterized the force-area isotherms of the PC species with and without cholesterol. Incorporation of eicosapentaenoic acid or docosahexaenoic acid into the *sn*-2 position of PC resulted in a remarkable

TABLE 3. Calculated space available for apoA-I bound to different monolayer surfaces[a]

Surface	πi = 10 mN/m	πi = 15 mN/m
	Å^2/amino acid	
POPC	14.5	15.1
PEPC	15.5	17.4
PDPC	22.9	23.2
Air-water interface		15.0[b]

[a] Space available for apoA-I binding was calculated as described by Ibdah and Phillips (18) assuming ideal mixing of lipid and apoprotein molecules and using the apoA-I binding data and the force-area isotherms for the pure PC species.

[b] Value taken from Krebs et al. (20).

increase in the molecular area of the PC molecules (Fig. 1 and Table 1). At most surface pressures the presence of an n-3 fatty acid increased the molecular area of the PC molecule by 7–20 Å^2, with greater molecular areas observed for docosahexaenoic acid relative to eicosapentaenoic acid. These results are in general agreement with previous studies (21–23), although in some instances the molecular areas for PDPC and PEPC were similar to one another. The reason for these discrepancies is unclear but may be related to the PC preparations. We carefully analyzed our PC preparations for the presence of lysoPC as well as oxidation products and found no detectable amounts in the case of the former and < 0.25 mole % in the case of the latter. The extent to which these potentially confounding variables were present in past studies is unknown. However, the bulk of the evidence suggests that *sn*-2 eicosapentaenoic acid and docosahexaenoic acid increase the molecular area of PC relative to oleic acid.

Cholesterol has been shown in several studies to have a condensing effect on monolayers (17–19). Presumably this results from the van der Waals attractive forces between the sterol ring of cholesterol and the fatty acyl chains of the phospholipid molecule. We also found that cholesterol reduced the mean molecular area of monolayers for all three PC species examined (Fig. 2). However, the force–area isotherms for PDPC containing cholesterol were quite distinct from those of POPC and PEPC. The addition of 15 mole % cholesterol resulted in a remarkable condensation of PDPC of 19 Å^2 per molecule at 25 mN/m compared to cholesterol-free monolayers of PDPC (Fig. 3). Such a marked reduction in molecular area probably results from a conformational change in the PDPC. Molecular modeling studies of Applegate and Glomset (24–26) have suggested that phospholipid species containing DHA in the *sn*-2 position can pack into an "angle iron" conformation where the *sn*-2 DHA chain is tightly associated with the *sn*-1 fatty acyl chain. Their modeling studies were based on the packing of diacylglycerol molecules that were constrained to contain an *sn*-1 stearic acid chain and to have conformations that resembled that of the diacylglyceryl moiety in crystalline dilaurylphosphatidylethanolamine oriented normal to the monolayer surface. Though it may be difficult to directly compare the results of the computer modeling of docosahexaenoic acid to the results of the present monolayer studies, we speculate that the results of the two studies are in agreement. When PDPC is spread at the monolayer interface it is in an expanded conformation and even lateral compression will not reduce its molecular area to that of POPC (Fig. 1). However, when sufficient cholesterol is present in the monolayer the PDPC undergoes a conformational change so that it occupies a molecular area more similar to that of POPC (Fig. 3). Thus, in the condensed conformation in the presence of cholesterol, PDPC may have a molecular packing similar to that proposed by Applegate and Glomset (24–26). Presumably, the more condensed conformation of docosahexaenoic acid is possible because of the regular spacing of double bonds throughout the length of the fatty acyl chain.

Our studies also indicated that the presence of an *sn*-2 n-3 fatty acid in PC influenced the binding of apoA-I to PC monolayers. ApoA-I binding to PDPC was less than that for POPC regardless of whether the data were normalized to total surface available for binding or to the total number of PL molecules in the monolayer (Fig. 4). The latter normalization was to account for the difference in molecular surface area among PC species (Fig. 1; Table 1). The decreased binding of apoA-I to PDPC relative to POPC or PEPC was also observed regardless of whether different amounts of apoA-I were injected into the subphase with monolayers equilibrated at an initial surface pressure of 15 mN/m (Fig. 4) or a constant amount of apoA-I was injected beneath monolayers equilibrated at different initial surface pressures (Fig. 6). Analysis of the binding data using double reciprocal plots suggested that the interaction of apoA-I with all three PC species was distinctive. Relative to POPC, apoA-I had a lower apparent binding affinity but higher maximal binding capacity to PEPC (Table 2) so overall binding of apoA-I was similar for POPC and PEPC. However, apoA-I apparent binding affinity and maximal binding capacity were lower for PDPC compared to POPC. The value for maximal binding capacity of apoA-I to POPC (4 mol/1000 mol) was similar to that of apoA-I bound to egg yolk lecithin vesicles or emulsions in other studies (27–29). Since the difference in apoA-I binding to PDPC versus POPC was apparent even when the data were normalized to the number of PC molecules on the monolayer, the binding difference may be related to the ability of apoA-I to induce a tight, regular acyl chain packing for PDPC because of the even distribution of double bonds throughout the docosahexaenoic acid chain. Presumably, PEPC could not form a similar tightly packed conformation because the double bonds are not as evenly distributed along the eicosapentaenoic acid chain as the case of docosahexaenoic acid.

Our current working hypothesis to explain the experimental observations is shown schematically in **Fig. 7.** When apoA-I binds to a POPC monolayer there is lateral compression of POPC molecules to accommodate the bound apoA-I. This lateral compression of PC molecules results in an increase in the surface pressure of the monolayer. If ideal mixing of PC and apoA-I is assumed (i.e., no interaction between the two) then the amount of space available in the monolayer for apoA-I can be calculated from the measured increase in surface pressure and the force–area isotherms of the pure POPC, as explained in detail by Ibdah and Phillips (18). When apoA-I binds to a POPC monolayer the limiting molecular area is 15 Å^2/amino acid, which is similar to the value for apoA-I bound to an air–water interface (Table 3). Thus, bound

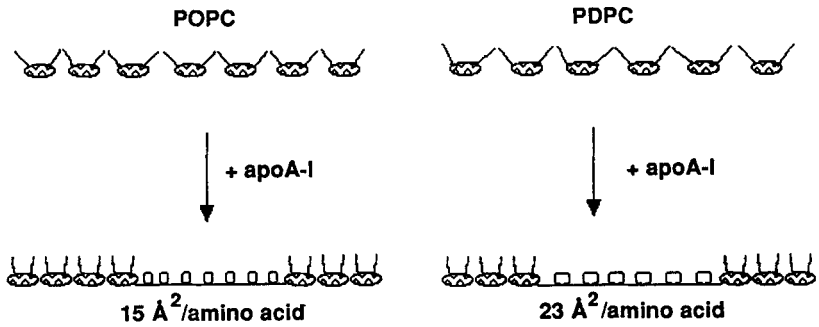

Fig. 7. Summary diagram of monolayer–apoA-I binding results. When apoA-I binds to POPC monolayers, the POPC is laterally displaced and apoA-I occupies 15 Å²/amino acid residue. However, when apoA-I binds to PDPC it may induce a conformational change, resulting in a tighter packing of PDPC molecules and more available space for apoA-I (23 Å²/amino acid). A more expanded conformation for apoA-I may prevent additional apoA-I molecules from binding to the PDPC monolayer.

apoA-I compresses the POPC, resulting in an increase in surface pressure, with little interaction between the apoA-I and POPC molecules. However, when apoA-I binds to monolayers of PDPC we hypothesize that the bound apoA-I induces a conformational change in the PDPC, resulting in a tighter molecular packing for PDPC molecules. Our isotherm data also support this hypothesis as relatively small amounts of cholesterol (< 15%) in the PDPC monolayer resulted in a decrease in surface area out of proportion to that observed for POPC or PEPC. The tightly packed molecular conformation of PDPC would occupy relatively less surface area than the more expanded conformations of phospholipid species containing polyunsaturated fatty acids in the *sn*-2 position. If this hypothesis were true, the tight packing of the PDPC molecules induced by apoA-I would result in more available space in the monolayer for apoA-I. Indeed, apoA-I bound to PDPC monolayers occupied 23 Å²/amino acid instead of the typical value of 15 Å²/amino acid observed for apoA-I bound to POPC, PEPC, or the air–water interface (Table 3). There must also be an interaction between the PDPC molecules and the expanded form of apoA-I in the monolayer that precludes additional molecules of apoA-I from binding to the monolayer. Taken together these data suggest that when apoA-I binds to PDPC monolayers, the PDPC undergoes a transition from a rather loose to a more compact conformation resulting in an interaction between PDPC and apoA-I molecules and a more expanded conformation for the bound apoA-I.

An alternative interpretation may also explain the experimental observations. When apoA-I binds to PDPC it may cause the PDPC molecules to occupy a more expanded area, resulting in less available surface for apoA-I binding. This would decrease the amount of apoA-I bound to the monolayer and the bound apoA-I would occupy 15 Å²/amino acid. We do not favor this interpretation because there is no known precedent for apolipoprotein binding to expand a PC monolayer and the PC-cholesterol force–area isotherm results do not support this interpretation. However, whether the PDPC molecules are contracted and the apoA-I molecules are expanded, as we favor, or vice versa cannot be distinguished from our studies.

Do our experimental findings in monolayer systems that model lipoprotein surfaces have any physiological relevance? We believe that they may. Although pure monolayers of PDPC are not likely to exist in plasma, regions of lipoprotein surface or cell membrane surface may be enriched in phospholipids that contain DHA in the *sn*-2 position. These regions in lipoprotein particles may be functionally distinct with regard to apoA-I (and perhaps other apolipoproteins) binding and conformation compared to regions that are less enriched with n-3 fatty acids. The interaction of apoA-I with PDPC and the resulting conformational change in apoA-I may explain, in part, the decreased LCAT reactivity of recombinant complexes containing phospholipids enriched in n-3 fatty acids observed in our previous studies (4, 5). ApoA-I is a cofactor for LCAT and studies by Jonas, Këzdy, and Wald (30) have shown that the conformation and molecular packing of apoA-I on recombinant particles dramatically influence LCAT activation. Additional studies are needed to determine whether the decreased LCAT reactivity of recombinant particles containing PC enriched in n-3 fatty acids is due to differences in apoA-I conformation, substrate reactivity, or both.

The authors gratefully acknowledge the technical assistance of Abraham K. Gebre and the assistance of Ms. Linda Odham with the manuscript preparation. This work was supported by grants from the National Institutes of Health, National Heart, Lung, and Blood Institute, HL-24736, HL-46805, and a Grant-in-Aid from the American Heart Association, 91008310.

Manuscript received 24 July 1992 and in revised form 9 December 1992.

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