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Lateral Interactions of Pig Apolipoprotein A-1 with Egg Yolk Phosphatidylcholine and with Cholesterol in Mixed Monolayers at the Triolein–Saline Interface†

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ABSTRACT: Interfacial tensions of egg yolk phosphatidylcholine (PC) and cholesterol monolayers adsorbed at the triolein–saline interface were measured in the presence and absence of pig apolipoprotein A-1 (apoA-1) in the saline phase. In the absence of apoA-1, the adsorptions of PC and cholesterol at the interface from the triolein phase are cooperative, showing large lateral attractive interactions between the PC molecules and the cholesterol molecules in the monolayer. In the presence of apoA-1, the PC adsorption is anti-cooperative, indicating strong lateral attractive interactions between the PC and the apoA-1 molecules, i.e., apparently, repulsive lateral interactions between the PC molecules. On the other hand, lateral interactions of very low magnitude are observed between the cholesterol and apoA-1 molecules in the monolayer. Values of the lateral interaction energy are evaluated from the adsorption data by the Defay–Prigogine–Flory theory of monolayers. The large difference in lateral interaction energy with apoA-1 between PC and cholesterol in a mixed monolayer is discussed in connection with current problems in lipoprotein catabolism: reverse cholesterol transport, alterations in affinity of lipid particles to apoA-1, and formation of high-density lipoproteins and abnormal lipoproteins.

Interactions among phosphatidylcholine (PC),[1] free cholesterol (Chol), and apolipoproteins at a lipoprotein surface are considered to be important in determining the metabolic fate of the lipoprotein in plasma. Plasma HDL's or reconstituted HDL particles act as efficient acceptors for Chol in reverse cholesterol transport (Brown et al., 1980; Pittman et al., 1987). Even bilayer vesicles of PC or sphingomyelin are capable of extracting Chol from cells in the culture without apolipoprotein of HDL's (Williams et al., 1984; Williams & Scanu, 1986). Apolipoprotein (apoA-1, apoA-2, or apoE)-containing vesicles are more effective in extracting Chol from cultured cells than either the proteins or PC alone (Oram et al., 1983) and mobilize intracellular deposits of esterified Chol from cells (Williams et al., 1984; Ho et al., 1980). Free apolipoproteins (apoA-1, apoA-2, or apoE) also interact with Chol-loaded macrophages to remove Chol. The products of the interaction are rich not only in Chol but also in PC (Hara & Yokoyama, 1991), and PC is assumed to play important roles in the extraction of Chol by apoA-1, apoA-2, or apoE.

Nascent chylomicrons and VLDL's usually contain a low content of Chol (Miller & Small, 1983). During the lipolysis of TG-rich lipoprotein particles in plasma, Chol is transferred from other lipoproteins and cellular elements of the blood (Atokinson & Small, 1986). The chol accumulation at the particle surface is assumed to affect the binding of apolipoproteins. The composition of the exchangeable apolipoprotein (apoA-1, apoA-2, apoC, and apoE, etc.) at the surface probably controls the metabolic fate of the particles (Gotto et al., 1986). Derksen and Small (1989) have shown that the binding capacities of apoA-1 and apoE to TG–PC emulsion particles decrease sharply when the Chol content is increased.

In vitro, single-bilayer vesicles acquire apoA-1 from HDL and are converted into small protein–phospholipid discs (Jonas, 1986) and serve as substrates for lecithin–cholesterol acyltransferase (LCAT) (Albers et al., 1986). These processes can occur, however, only if the mole ratio of Chol/PC is not larger than 0.5 (Fielding et al., 1972; Tall & Lange, 1978). An abnormal lipoproteins, known as lipoprotein X's (LP-X's), are Chol-rich single-bilayer vesicles containig albumin (30% of the protein), apoC (50% of the protein), and a trace of apoA-1 (LCAT activator) (Hauser et al., 1977; Kostner & Laggner, 1989). LP-X's are poor substrates for LCAT (Patsch, 1977) and cannot undergo the above transformations, thus accumulating in plasma (Untracht, 1982).

The mechanism of redistribution of soluble apolipoproteins among lipoprotein particles during the catabolism in plasma has not been completley defined. A more rigorous understanding of the lipoprotein metabolism requires a quantitative evaluation on interactions among PC, Chol, and soluble apolipoproteins. In this work, we measured the interfacial tensions of mixed monolayers of apolipoproteins from pig HDL with PC and with Chol at the TG [triolein (TO) in this work]–saline interface. The PC monolayer at the TO–saline interface studied is a proper model for the surface of TG-rich lipoproteins: chylomicrons, VLDL's, and TG-rich HDL_2. The

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[1] Abbreviations: PC, egg yolk phosphatidylcholine or phosphatidylcholine; Chol, (free) cholesterol; apoA-1, pig or human apolipoprotein A-1; TG, triglyceride; TO, triolein; VLDL's, very low density lipoproteins; HDL's, high-density lipoproteins; LP-X's, abnormal lipoprotein-X's; LCAT, lecithin–cholesterol acyl transferase.

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majority of pig apoHDL has been identified as apoA-1, which gives adsorption and CD spectra similar to those of human apoA-1 and activates human LCAT (Jackson et al., 1973). The magnitudes of the lateral interaction between the lipid and apoA-1 in the monolayer are estimated by an interfacial physicochemical method, and the physiological relevance of the results is discussed.

Experimental Procedures

Materials. Pig HDL's with density in the range of 1.063–1.21 g/mL were isolated from the plasma by the standard ultracentrifugal techniques (Schumaker & Puppione, 1986). A gel filtration in the saline (0.15 M NaCl, 0.01 M Tris-HCl, pH 7.0) was performed to purify the HDL's on Sepharose CL-6B (Pharmacia). ApoHDL was separated by the delipidation of the HDL's with a mixture of ethanol and diethyl ether 3:2 (v/v) at −20 °C (Osborne, 1986). The purity of the resultant residues was analyzed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis. The apoHDL from pig plasma has been identified as apoA-1 (Jackson et al., 1973). Egg yolk phosphatidylcholine (PC) was kindly provided by Asahi Kasei Co. The purity (over 99.5%) was determined by thin-layer chromatography. The small amount of impurity (0.5%) was identified as sphingomyelin by HPLC. Triolein (TO) obtained from Taiyo Chemical Co. was purified by silicate (Wakogel C-200, Wako Pure Chemicals) column chromatography to remove fatty acid, diglyceride, and monoglyceride, using chloroform/methanol (99:1) as an eluent. The purity of TO thus obtained was over 99%. Water was doubly distilled with a quartz still.

Interfacial Tension Measurement. We measured the interfacial tension of the triolein–saline (0.01 M Tris-HCl/0.15 M NaCl, pH 7.0) interface as a function of either PC or Chol concentration in the triolein phase by the drop weight method. During the measurements, the concentration of apoA-1 in the saline phase was kept constant, and the apoA-1 concentration used in this work was in the range between 1×10^{-6} and 2.5×10^{-5} g/mL. If the protein adsorption from the dilute solution is large enough to significantly lower the concentration in the drop, serious time lags in equilibrium may be involved (Krebs et al., 1988; Mukerjee & Handa, 1981). We avoided these problems by use of the TO solution of PC or Chol as the drop and by use of a protein concentration higher than 1×10^{-6} g/mL. The mixed adsorption of apoA-1 with PC or Chol reduces the equilibrium adsorption amount of the protein. The time lags in adsorption equilibrium of apoA-1 for 10–20 min were observed when the lipid concentration was less than 10^{-5} M. Such data were not used in our further analysis. The inverted TO drops (TO was less dense than the surrounding saline) were formed by the use of an all-glass micrometer syringe in a double-walled jacket equipped with a Teflon stopper. The drops were formed at different intervals and the proper drop-forming rate was determined in order to obtain reproducible interfacial tensions (±0.4 mN/m). More than 10 drops were formed for an experimental point of certain lipid and apoA-1 concentrations, and the average value of interfacial tension was obtained. The temperature was maintained at 25 °C by circulation of water through the outer jacket. The particulars in the interfacial tension measurement have been described elsewhere (Mukerjee & Handa, 1981; Handa et al., 1990a).

Results

Interfacial Tension and Adsorption Isotherms. Figure 1A,B shows the interfacial tensions at the TO–saline interface (γ) as a function of the PC and Chol concentrations in the TO

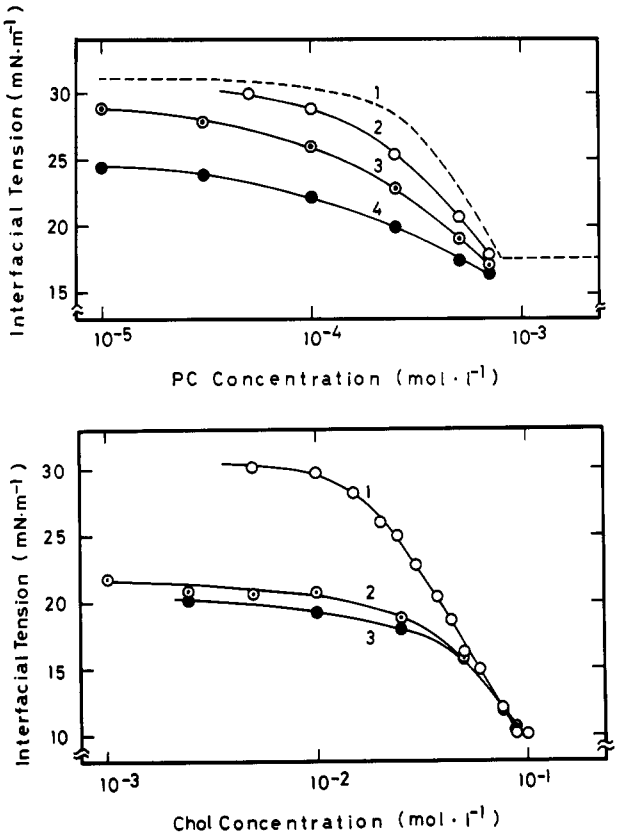

FIGURE 1: (A, top) Interfacial tension of the TO–saline (10 mM Tris-HCl/150 mM NaCl, pH 7.0) interface as a function of PC concentration in the TO phase. The apoA-1 concentration in the saline phase (g/mL) was as follows: curve 1, 0; curve 2, 1×10^{-6}; curve 3, 5×10^{-6}; curve 4, 1×10^{-5}. The solubility of PC in TO at 25 °C was 8×10^{-4} M. (B, bottom) Interfacial tension of the TO–saline interface as a function of Chol concentration in the TO phase. The apoA-1 concentration in the saline phase (g/mL) was as follows: curve 1, 0; curve 2, 1×10^{-5}; curve 3, 2.5×10^{-5}. The solubility of Chol in TO at 25 °C was 9×10^{-2} M.

phase (c_L), respectively, at constant apoA-1 concentrations (c_P). The value at $c_L = c_P = 0$ (γ_0) was 31.08 mN/m (Handa et al., 1990a). The solubilities of PC and Chol in TO at 25 °C were 8×10^{-4} and 9×10^{-2} M (mol/L), respectively. The addition of apoA-1 in the saline phase lowers the interfacial tension.

The adsorption amount of PC (or Chol) at the interface (Γ) in mol/cm^2 is correlated with the change of interfacial tension by the Gibbs adsorption equation:

$$\Gamma = -\frac{1}{RT}\left(\frac{\partial \gamma}{\partial \ln c_L}\right)_{T,P,c_P} \quad (1)$$

Where, R is the gas constant. The subscripts (T, P, c_P) indicate that the measurement is performed at constant temperature, pressure, and apoA-1 concentration.

Figure 2A,B shows the adsorption amounts of lipid (Γ) calculated by the use of eq 1 as a function of the lipid concentration. When apoA-1 is not added in the saline phase, large cooperativities are observed in the adsorptions of PC and Chol at the interface. The cooperativity in the PC adsorption has been ascribed to the lateral repulsive interaction between PC and TO molecules (or the apparent attractive interaction between PC molecules) in the mixed lipid monolayer at the interface (Handa et al., 1990a). The Chol molecule also interacts repulsively with the TO molecule in the monolayer. The saturated adsorption amounts of PC and Chol (Γ_{so}) were 4.10×10^{-10} and 4.70×10^{-10} mol/cm^2, respectively. The corresponding cross-sectional molecular areas are 40.5 Å^2/PC

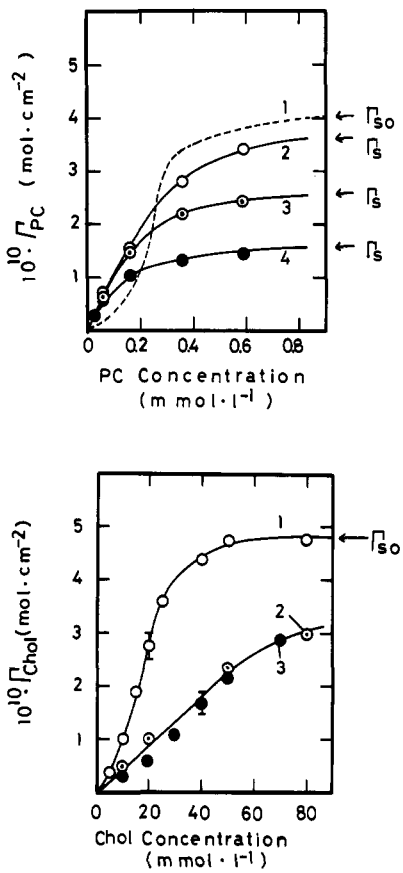

FIGURE 2: (A, top) Adsorption isotherms of PC at the TO–saline interface. The apoA-1 concentration in the saline phase (g/mL) was as follows: curve 1, 0; curve 2, 1×10^{-6}; curve 3, 5×10^{-6}; curve 4, 1×10^{-5}. (Γ_{so}) Saturated adsorption amount of PC (= 4.10×10^{-10} mol/cm^2). (Γ_s) Apparent saturated adsorption amount of PC. The experimental uncertainty in evaluating the adsorption amount with eq 1 was ±10%. (B, bottom) Adsorption isotherms of Chol at the TO–saline interface. The apoA-1 concentration in the saline phase (g/mL) was as follows: curve 1, 0; curve 2, 1×10^{-5}; curve 3, 2.5×10^{-5}. (Γ_{so}) Saturated adsorption amount of Chol (= 4.70×10^{-10} mol/cm^2). The experimental uncertainties in evaluating the adsorption amount are shown by error bars (±10%).

molecule and 35.3 Å^2/Chol molecule. When the apoA-1 is added and is adsorbed at the interface, the apparent saturated adsorption amount of PC, Γ_s, decreases. The occupation of interface by the protein molecules is responsible for the decline. The fraction of the interface occupied by apoA-1 is calculated as $\theta_{apoA\text{-}1} = (\Gamma_{so} - \Gamma_s)/\Gamma_{so}$.

Figure 3 show the correlation between $\theta_{apoA\text{-}1}$ and apoA-1 concentration (c_P) in the most closely packed PC monolayers at the TO–saline interface ($c_L = 8 \times 10^{-4}$ M). The dissociation coefficient of pig apoA-1 at the PC monolayers obtained in Figure 3 is 6×10^{-6} g/mL, which is comparable to the value of human apoA-1 at the surface of PC/TO emulsion particles in the 10 mM phosphate buffer containing 0.15 M NaCl, pH 7.4 [$(4.6 \pm 2.0) \times 10^{-6}$ g/mL (Yokoyama et al., 1985)].

Lateral Interactions between Lipid and ApoA-1 at the Interface. With the addition of apoA-1 in the saline phase, the adsorption amount of PC increases in the lower concentration region and decreases in the higher concentration region (Figure 2A). These results suggest the attractive lateral interaction between PC and apoA-1 and, therefore, the apparent repulsive interaction among PC molecules. In the Chol monolayers, no increase in the adsorption amount of Chol with the addition of apoA-1 is observed (Figure 2B).

Defay et al. (1966) have described chemical potentials in a mixed monolayer formed by lipid and (polymer) macro-

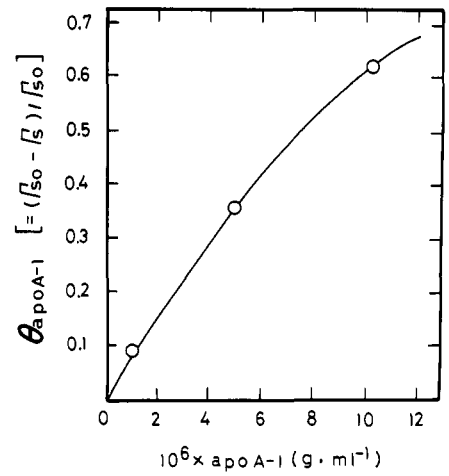

FIGURE 3: Adsorption isotherm of apoA-1 in the PC monolayer of the most closely packed state. ($\theta_{apoA\text{-}1}$) The fraction of the interface occupied by apoA-1. The PC concentration in TO is 8×10^{-4} M.

Table I: Experimental Values for the Saturated Adsorption Amount Γ_{SO}, Magnitude of Adsorption K, and Lateral Interaction Parameter ω of Phosphatidylcholine and Cholesterol at the Triolein–Saline Interface

	Γ_{SO} (mol/cm^2)	K (L/mol)	ω (RT)
PC–TO[a]	4.10×10^{-10}	7.0×10^2	1.8
Chol–TO	4.70×10^{-10}	1.32×10^{-1}	1.6

[a] Handa et al. (1990a).

molecules using the Flory equation. When apoA-1 is absent, the adsorption isotherm of PC (or Chol) at the TO–saline interface is given by (see Appendix, eq A11)

$$\ln\left[\left(\frac{\theta}{1-\theta}\right)\left(\frac{1}{c_L}\right)\right] = 2\omega\theta + \ln K \qquad (2)$$

where L = PC or Chol. The fraction of the interface (θ) occupied by either PC or Chol is equal to Γ/Γ_{so}. K and ω are parameters expressing the magnitudes of adsorption and of the lateral interaction between PC (or Chol) and TO in the monolayer, respectively. The attractive and repulsive interactions are represented by negative and positive values of the ω parameter, respectively. The monolayer is composed of PC (or Chol) and TO, and the fraction of the interface occupied by TO is equal to $(1 - \theta)$.

When apoA-1 is added to the saline phase, the mixed monolayer at the interface consists of apoA-1, PC (or Chol), and TO. If the fraction of the interface occupied by TO is negligibly small, the following equation (see Appendix, eq A13) should hold:

$$\ln(\theta/c_L) = 2\epsilon\theta + (\omega - \epsilon - \delta) + \ln K \qquad (3)$$

Here, ϵ and δ are parameters for the lateral interactions between PC (or Chol) and apoA-1 and between TO and apoA-1 in the mixed monolayer, respectively. The saturated adsorption of human apoA-1 at the air–saline interface (the complete occupation of interface by the protein) is attained when the concentration in the saline phase increases above 4×10^{-7} g/mL (Krebs et al., 1988; Shen & Scanu, 1980). We applied eq 3 to the adsorption data at $c_P = 1$ and 2.5×10^{-5} g/mL.

Figure 4A, illustrates the $\ln\{\theta/[(1-\theta)c_L]\}$ vs θ and $\ln(\theta/c_L)$ vs θ plots for the cases of PC and Chol, respectively. According to eq 2, the slope and the intercept to the ordinate of the straight line give the lateral interaction parameter ω and the magnitude of adsorption K, respectively. The ω and K values for the PC and Chol monolayers, obtained in Figure 4A,B, are summarized in Table I. The positive values of ω indicate

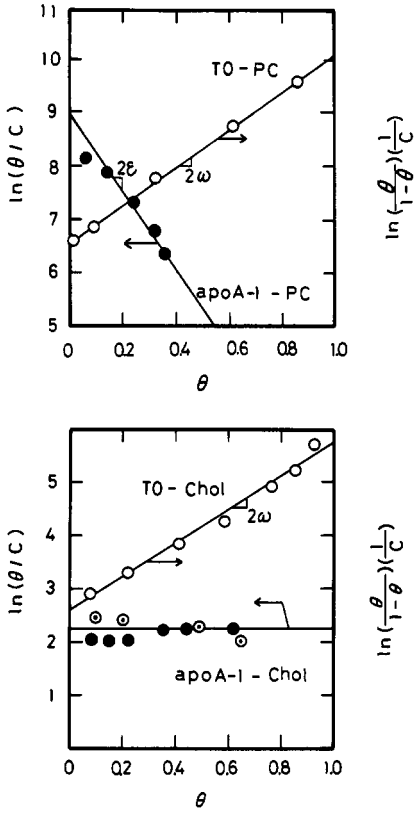

FIGURE 4: (A, top) Evaluation of lateral interaction parameters of PC in mixed monolayer at the interface. See eqs 2 and 3. (B, bottom) Evaluation of lateral interaction parameters of Chol in a mixed monolayer at the interface. See eqs 2 and 3.

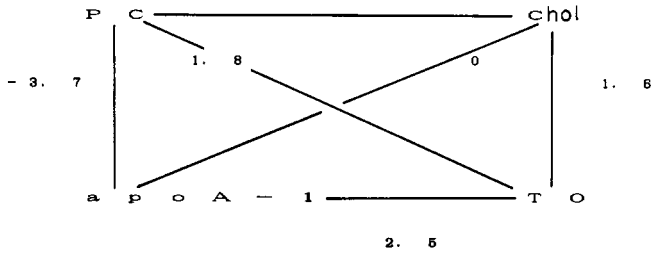

FIGURE 5: Lateral interaction parameters in mixed monolayers at the TO–saline interface (unit: RT).

the repulsive lateral interactions between PC and TO and between Chol and TO.

The negative value of ϵ for the PC–apoA-1 mixed monolayers (Figure 4A) indicates a large attractive interaction energy between the lipid and the protein (ϵRT = −2.2 kcal/mol). This value is comparable to that of hydrogen-bonding interaction. On the other hand, a negligible value of ϵ was obtained for the mixed monolayers of Chol and apoA-1 (Figure 4B). According to eq 3, the lateral interaction parameter between apoA-1 and TO (δ) can be calculated from the experimental values of ω, ϵ, ln K, and the intercept to the ordinate (see Figures 4). The PC–apoA-1 and Chol–apoA-1 mixed monolayers give δ values of 3.0 and 2.0, respectively (i.e., δ = 2.5 ± 0.5). The lateral interaction energies obtained are summarized in Figure 5. Here, the large attractive interaction between PC and apoA-1 distinctly contrasts with the negligible interaction between Chol and apoA-1.

Discussion

Mixed Monolayers of ApoA-1 and Lipid. The apoA-1 molecules at the air–saline interface maintain a substantial amount of secondary structure and are in equilibrium with those in solution (Shen & Scanu, 1980; Krebs et al., 1988; Ibdah et al., 1989). The equilibrium can occur only when the surface concentration is higher than 5×10^{-4} amino acid/cm^2 [20 Å^2/amino acid (Krebs et al., 1988)]. Upon dilution below this surface concentration, the protein denatures, like other proteins at the interface (Shen & Scanu, 1980). The surface density of the equilibrium monolayer (15–20 Å^2/amino acid) is consistent with the monolayer composed of α-helical protein molecules lying with the long axes of the helical segments in the plane of the interface. The capability of apoA-1 to fold and unfold readily at the interface (the capability to maintain the adsorption equilibrium) is dictated by its primary sequence (Ibdah et al., 1989; Krebs et al., 1988). The chemical potential of apoA-1 in saline is not represented in a simple form as PC and Chol, since the interaction among the apoA-1 molecules in saline is strong enough to oligomerize in the concentration range above 5×10^{-5} g/mL (Ritter & Scanu, 1977). In this study, we measured the interfacial tension as a function of either the PC or Chol concentration in TO, c_L, keeping the concentration (i.e., chemical potential and activity) of apoA-1 constant (c_P = 1×10^{-6}–2.5×10^{-5} g/mL).

The adsorption isotherms in Figure 2A,B clearly show the strong attractive interaction between apoA-1 and PC and the weak interaction between apoA-1 and Chol, respectively, in the mixed monolayer. The adsorption isotherm of apoA-1 in the PC monolayer of the most closely packed state (Figure 3) gives a dissociation coefficient of pig apoA-1 comparable to the value of human apoA-1 at the surface of emulsion particle consisting of PC and TO (Yokoyama et al., 1985). To discuss the lipid–protein interactions in detail, we employ an interfacial chemical method based on the Defay–Prigogine–Flory theories (Defay et al., 1966; see Appendix). We have used a similar method to investigate the phospholipid–TO interactions in the monolayer at the interface (Handa et al., 1990a).

Equation 3 holds when the fraction of interface occupied by TO is negligible (see Appendix, eq A12). The saturation of human apoA-1 adsorption is attained at 4×10^{-6} g/mL at the air–phosphate buffer solution interface (Shen & Scanu, 1980) and at 1×10^{-7} g/mL in the PC monolayer with an initial surface pressure of 10 mN/m (10 dyn/cm) (Ibdah et al., 1989). The repulsive lateral interaction of apoA-1 with TO (δRT = 1.5 ± 0.3 kcal/mol) will lead to about 10 times lower affinity of the protein with the TO–saline interface than with the air–water interface. The conditions, the absence of TO and the saturation of apoA-1 adsorption at the interface, may be satisfied when the pig apoA-1 concentration in the saline is higher than 1×10^{-5} g/mL. The lateral interaction parameters between apoA-1 and Chol are equal to zero at c_P = 1 and 2.5×10^{-5} g/mL (Figure 4B), which seems to suggest a constant and saturated occupation of the interface by apoA-1 at these concentrations.

Interactions of ApoA-1 with PC and Chol. Free apoA-1 interacts with cholesterol-loaded macrophages to remove the free cholesterol and PC from the cell and forms HDL-like particles in the medium (Hara & Yokoyama, 1991). Bilayer vesicles of PC also are capable of extracting Chol from cells in the culture without apoA-1 (Williams et al., 1984; Williams & Scanu, 1986). The results in Figure 5 show that apoA-1 has high and low affinities with PC and Chol, respectively. The very weak lateral interaction between apoA-1 and Chol suggests a complete absence or some low frequency of direct contact of apoA-1 with Chol at the HDL surface. A simple calculation shows the ratio of apoA-1 peripheral sites occupied by PC to those occupied by Chol as $\exp(-\epsilon_{\text{apoA-1-PC}})/\exp$-

($-\epsilon_{apoA\text{-}1\text{-}Chol}$) = 40. The Chol molecules at the surfaces of HDL's or reconstituted HDL's are intercalated mainly between PC molecules in the PC–apoA-1 mixed monolayer. When free apoA-1 interacts with cells, first the protein extracts PC from the cells, and then the Chol molecules are taken up from the cells and accommodated in the array of the PC molecules in the apoA-1–PC complexes. In similar manners, the PC molecules in bilayer vesicles and in PC–apoA-1 complexes can extract Chol molecules from cells and accommodate them.

Human and also pig apoA-1's contain a large amount of amphipathic α-helical structure (Andrews et al., 1976; Handa et al., 1990b). The amphipathic helices are substantially hydrophilic, and the proteins are soluble in the aqueous phase. On the other hand, PC shows balanced amphipathic characters (Kunieda & Ohyama, 1990). Israelachivili et al. (1976) have shown that the volume ratio of hydrophobic and hydrophilic residues in an amphipathic molecule plays important roles in determining the shape of the molecular assembly. The strong interaction of apoA-1 with PC leads to the formation of an amphipathic complex of rather hydrophilic nature. The complexes are, therefore, capable of constituting a very small particle with diameters less than 10 nm (i.e., an HDL or HDL-like particle). If the Chol content of such a small particle increases, the reduction in the lateral interaction among apoA-1 and lipids at the particle surface will result in the collapse of the particle. Jonas and Krajnovich (1978), Tall and Lange (1978), and Pownall et al. (1979) have shown inhibition of human apoA-1/Chol–PC interaction above a Chol/PC mole ratio of 0.25 in their reconstitution works.

In plasma, Chol is transferred to chylomicrons (Atokinson & Small, 1986). The Chol accumulation at the particle surface decreases the affinity with apoA-1, and the protein is transferred to the HDL or the surface remnant fractions (Tall et al., 1979; Schaefer et al., 1978; Tall & Small, 1980; Patsch et al., 1977). The increased amount of Chol in artificial chylomicrons (TG–PC emulsions) changes the catabolism of the particles in vivo so that they behave like chylomicron remnants with rapid uptake by liver but without significant lipolysis (Maranhao et al., 1986).

When TG of chylomicrons is removed from circulation, new phospholipids appear in the HDL fraction. Apolipoproteins are transferred simultaneously with phospholipids from the chylomicrons to the HDL's (Tall et al., 1979; Schaefer et al., 1978). It has been suggested that single-bilayer vesicles (surface remnants of chylomicrons) are intermediates through which phospholipids are transferred from chylomicron to HDL (Tall & Small, 1980). Such transformation or conversion of phospholipids has been discussed on the basis of the evaluation of the monolayer–bilayer equilibrium at the TG–saline interface (Handa et al., 1990a). The Chol content of the surface remnants affects the lipid–protein interactions and determines their catabolic fate: conversions to either HDL's (Tall & Small, 1980) or abnormal lipoproteins, LP-X's (Hauser et al., 1977; Untracht, 1982; Kostner & Laggner, 1989). The Chol-rich single-bilayer vesicles, LP-X's, are presumed to have low affinity with apoA-1, circulating and accumulating in plasma. The fate in vivo of exogenous Chol-rich PC vesicles is similar to that of LP-X (Gregoriadis & Davis, 1979; Tall, 1980; Roerdink et al., 1989).

Lipid–protein complexes of various sizes (9–30 nm) were reconstituted from PC, TO, Chol, and apoA-1. The free energy of the complex was calculated from the composition, interfacial tension, and lateral interaction parameters estimated in this work and was represented as a function of the diameter. The free energy profile indicates that stable complexes are obtained in a certain range of the diameter. These results may be correlated with lipoprotein catabolism and lipid–transfer between lipoproteins in plasma. Further studies on the stability of lipid–apolipoprotein complexes and the effect of cholesterol are in progress and will be published in the near future.

Appendix

Defay et al. (1966) introduced lateral interaction parameters to Butler's chemical potentials of lipid in a monolayer. For a mixed monolayer of lipids of similar size, we consider TO (component 1), either PC or Chol (component 2), and a macromolecule, apoA-1 (component 3). Their chemical potentials may be expressed by the application of the Flory equation of macromolecular solution:

$$\mu_1^{m} = \mu_1^{mo} + RT[\ln \theta_1 + (r-1)\theta_3/r + (\omega\theta_2 + \delta\theta_3)(1-\theta_1) - \epsilon\theta_2\theta_3] - Na\gamma \quad \text{(A1)}$$

$$\mu_2^{m} = \mu_2^{mo} + RT[\ln \theta_2 + (r-1)\theta_3/r + (\epsilon\theta_3 + \omega\theta_1)(1-\theta_2) - \delta\theta_3\theta_1] - Na\gamma \quad \text{(A2)}$$

$$\mu_3^{m} = \mu_3^{mo} + RT[\ln \theta_3 + (1-r)(1-\theta_3) + r[(\delta\theta_1 + \epsilon\theta_2)(1-\theta_3) - \omega\theta_1\theta_2]] - rNa\gamma \quad \text{(A3)}$$

Here, μ^{mo} are standard potentials for pure lipids and protein and are constant under constant temperature and pressure. ω, δ, and ϵ are the lateral interaction parameters between PC (or Chol) and TO, TO and apoA-1, and apoA-1 and PC (or Chol), respectively. θ are the fractions of the interface occupied by the lipids and protein ($\theta_1 + \theta_2 + \theta_3 = 1$). N, a, and γ are the Avogadro's number, the cross-sectional area of lipid molecule ($Na = 1/\Gamma_{so}$), and the interfacial tension, respectively. r is the ratio of the cross-sectional area of apoA-1 to that of lipid.

For dilute solutions of PC (or Chol) in TO and apoA-1 in saline, the chemical potentials are expressed by

$$\mu_1^{b} = \mu_1^{bo} + RT \ln (1 - X_2) \quad \text{(A4)}$$

$$\mu_2^{b} = \mu_2^{b*} + RT \ln X_2 \quad \text{(A5)}$$

$$\mu_3^{b} = \mu_3^{b*} + RT \ln a_3 \quad \text{(A6)}$$

Here, X_2 is the mole fraction of PC (or Chol) in the TO solution and is very small since $1 - X_2 \doteq 1$. μ^{b*} are standard chemical potentials based on the asymmetric choice, and a_3 is the activity of apoA-1 in the saline phase.

In the equilibrium of TO between the monolayer and the solution, eqs A1 and A4 are equated with each other to give the relation

$$\gamma_0 - \gamma = -(RT/Na)[\ln \theta_1 + (r-1)\theta_3/r + (\omega\theta_2 + \delta\theta_3)(1-\theta_1) - \epsilon\theta_2\theta_3] \quad \text{(A7)}$$

Where, $Na\gamma_0 = \mu_1^{mo} - \mu_1^{bo}$. In the equilibrium of PC (or Chol) between the monolayer and the solution, eqs A2 and A5 give the relation

$$\gamma_0 - \gamma = -(RT/Na)[\ln \theta_2 + (r-1)\theta_3/r + \omega\theta_1(1-\theta_2) - \omega + \epsilon\theta_3(1-\theta_2) - \delta\theta_1\theta_3 - \ln Kc_2] \quad \text{(A8)}$$

Here,

$$K = (v_1 M_1/1000)[\lim_{X_2 \to 0} (\theta_2/X_2)]_{c_3=0} = \text{constant} \quad \text{(A9)}$$

v_1 and M_1 are the specific volume and molecular weight of TO, respectively. At 25 °C, $(v_1 M_1/1000) = 0.967\ M^{-1}$. In the equilibrium of apoA-1 between the monolayer and the saline solution, eqs A3 and A6 give the relation

$$\gamma_0 - \gamma = -(RT/rNa)[\ln \theta_3 + (r-1)\theta_3 + r(\delta\theta_1 + \epsilon\theta_2)(1-\theta_3) - r(\delta + \omega\theta_1\theta_2) - \ln K'a_3] \quad \text{(A10)}$$

Here,

$$K' = [\lim_{a \to 0} (\theta_3/a_3)]_{c_2=0} = \text{constant}$$

When $c_3 = \theta_3 = 0$, eqs A7 and A8 give

$$\ln \left[\left(\frac{\theta_2}{1 - \theta_2} \right) \left(\frac{1}{c_2} \right) \right] = 2\omega\theta_2 + \ln K \qquad \text{(A11)}$$

When $\theta_1 = 0$, i.e., $\theta_2 + \theta_3 = 1$, eqs A8 and A10 give

$$\ln (\theta_2/c_2) = 2\epsilon\theta_2 + (\omega - \epsilon - \delta) + \ln K + (1/r)[\ln \theta_3/K'a_3] \qquad \text{(A12)}$$

The r value falls in the range between 40 and 50, and the last term is negligible in as much as

$$\ln (\theta_2/c_2) = 2\epsilon\theta_2 + (\omega - \epsilon - \delta) + \ln K \qquad \text{(A13)}$$

In the text, components 1, 2, and 3 are TO, either PC or Chol, and apoA-1, respectively.

Registry No. Cholesterol, 57-88-5; triolein, 122-32-7.

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