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Apolipoproteins C-I, C-II, and C-III: Kinetics of Association with Model Membranes and Intermembrane Transfer†

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ABSTRACT: The apoproteins (apo) C-I, C-II, and C-III are low molecular weight amphiphilic proteins that are associated with the lipid surface of the plasma chylomicron, very low density lipoprotein (VLDL), and high-density lipoprotein (HDL) subfractions. Purified apoC-I spontaneously reassociates with VLDL, HDL, and single-bilayer vesicles (SBV) of 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine. ApoC-I also transfers reversibly from VLDL to HDL and from VLDL and HDL to SBV. The kinetics of association of the individual apoC proteins with SBV are second order overall and first order with respect to lipid and protein concentrations. At 37 °C, the rates of association were 2.5×10^{10}, 4.0×10^{10} and 3.8×10^{10} M^{-1} s^{-1} for apoC-I, apoC-II, and apoC-III, respectively. Arrhenius plots of association rate vs temperature were linear and yielded activation energies of 11.0 (apoC-I), 9.0 (apoC-II), and 10.6 kcal/mol (apoC-III). The kinetics of vesicle to vesicle apoprotein transfer are biexponential for intermembrane transfer, indicating two concurrent transfer processes. Rate constants at 37 °C for the fast component of dissociation were 11.7, 9.5, and 9.9 s^{-1}, while rate constants for the slow component were 1.3, 0.6, and 0.9 s^{-1} for apoC-I, apoC-II, and apoC-III, respectively. The dissociation constants, K_d, of apoC-I, apoC-II, and apoC-III bound to the surface monolayer of phospholipid-coated latex beads were 0.5, 1.4, and 0.5 μM, respectively. These studies show that the apoC proteins are in dynamic equilibrium among phospholipid surfaces on a time scale that is rapid compared to lipolysis, lipid transfer, and lipoprotein turnover.

The C apolipoproteins, apoC-I, apoC-II, and apoC-III, are low molecular weight proteins of known sequence derived from the high-density lipoprotein (HDL),[1] very low density lipoprotein (VLDL), and chylomicron subfractions of the plasma lipoproteins [for a review, see Mahley et al. (1984)]. The main source of apoC proteins in plasma appears to be the liver, although some synthesis may occur in the intestine (Krause et al., 1981). The apoC proteins stabilize lipoprotein structure (Morrisett et al., 1977) and in part regulate their catabolism. ApoC-I activates the enzyme lecithin:cholesterol acyltransferase in vitro (Soutar et al., 1975); apoC-II has been shown to activate extrahepatic lipoprotein lipase (Breckenridge et al., 1978). Though its role is less clear, apoC-III may regulate uptake of chylomicron remnants by the liver (Shelburne et al., 1980). The apoC proteins are in dynamic equilibrium among the lipoprotein classes and subclasses (Eisenberg et al., 1979). In the lipolytic cascade that begins with VLDL, the apoC proteins transfer spontaneously from VLDL to HDL (Berman et al., 1978). Newly secreted VLDL subsequently acquires its complement of apoC proteins from circulating HDL (Wu & Windmueller, 1979). The size, surface properties, and chemical composition of the lipoproteins have a role in determining the distribution of the apoC proteins between density classes (Patsch et al., 1978). To quantitatively define the regulatory factors that control distribution of apoC proteins and their respective roles in lipoprotein catabolism, it is important to understand the underlying mechanisms of protein–lipid association. Herein, we report on the thermodynamics and mechanisms by which the apoC proteins distribute among model lipoproteins.

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[1] Abbreviations: PC, phosphatidylcholine; POPE, 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphoethanolamine; POPC, 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine; VLDL, very low density lipoprotein; HDL, high-density lipoprotein; dansyl-PE or DPE, dansyl [8-(dimethylamino)-1-naphthalenesulfonate] derivative of POPE; SBV, single-bilayer vesicle(s); Tris-HCl, tris(hydroxymethyl)aminomethane hydrochloride; EDTA, ethylenediaminetetraacetic acid.

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Experimental Procedures

Materials

ApoC Proteins were isolated essentially by the method of Brown et al. (1969, 1970). Purity of the apoC proteins was determined by gradient gel electrophoresis and amino acid analysis. ApoC-I was radiolabeled at Met-38 using C^3H_3I according to Chen et al. (1980). POPC and POPE were obtained from Avanti Polar Lipids (Birmingham, AL) and the dansyl derivative of POPE was prepared according to Waggoner and Stryer (1970). Phospholipid SBV were prepared according to Huang (1969).

Methods

Gel Filtration Chromatography. ApoC-I association with lipid particles was evaluated by incubating 5.6 μg of ^{3}H-labeled apoC-I (1.1 mCi/mmol) with 0.2 mg of HDL, VLDL, or POPC SBV phospholipid for 1 h at room temperature followed by chromatography on a 1.6 × 30 cm Sepharose CL4B column which was eluted with a standard buffer of 10 mM Tris-HCl, 100 mM NaCl, 1 mM EDTA, and 1 mM NaN_3. Fractions (1 mL) were collected, and elution volumes of VLDL and HDL were determined by their absorbance at 280 nm, while apoC-I was measured by liquid scintillation counting. Elution of POPC SBV was determined by the inorganic phosphorus assay of Bartlett (1959). For assessment of intermembrane transfer, the apoC-I donor species (0.2 mg) was added to a 10-fold excess of acceptor phospholipid (2 mg) and incubated for 5 min. The mixtures were chromatographed, and VLDL, HDL, and apoC-I elution volumes were determined as above. Elution of POPC SBV was determined by liquid scintillation counting of [^{14}C]POPC.

ApoC–SBV Fluorescence Spectral Studies. Fluorescence emission spectra for the apoproteins alone and associated with SBV of POPC with and without 5 mol % dansyl-PE were obtained by mixing the apoC proteins with either standard buffer or solutions of the SBV to produce a 2.5 μM solution of the apoC proteins alone or associated with 0.625 mM phospholipid with a protein to lipid ratio of 1/250. Emission spectra were measured on an SLM 8000 spectrofluorometer at 25 °C with excitation at 280 nm.

ApoC–SBV Association Kinetic Studies. The kinetics of association of apoproteins with SBV of POPC were evaluated by the initial rate method (Laidler, 1965) according to the equation:

$$P_{free} + SBV \underset{k_{off}}{\overset{k_{on}}{\rightleftharpoons}} P\text{–}SBV \qquad (1)$$

where P_{free} represents the unbound apoprotein, k_{on} was the rate constant for association, and P–SBV was the resulting protein–SBV complex. The overall rate of association was given by

$$d(P\text{–}SBV)/dt = k_{on}[P_{free}]^{\alpha}[SBV]^{\beta} \qquad (2)$$

where α and β represented the reaction orders with respect to P_{free} and SBV, respectively. Under conditions where the concentrations of SBV or apoprotein, respectively, were held constant, eq 2 was rewritten as

$$d(P\text{–}SBV)/dt = k'[P_{free}]^{\alpha} \qquad (3a)$$

$$d(P\text{–}SBV)/dt = k''[SBV]^{\beta} \qquad (3b)$$

Equations 3a and 3b were then put into linear form by taking the natural logarithm, giving

$$\ln [d(P\text{–}SBV)/dt] = \ln k' + \alpha \ln [P_{free}] \qquad (4a)$$

$$\ln [d(P\text{–}SBV)/dt] = \ln k'' + \beta \ln [SBV] \qquad (4b)$$

k' and k'' were constants, and ln [d(P–SBV)/dt] was the natural logarithm measured initial velocity which, when plotted against ln [P_{free}] and ln [SBV], respectively, yielded α and β as the slopes of a linear regression through the points.

ApoC–SBV association rates were determined by observing the rate of increase in fluorescence intensity above 450 nm following mixing of the apoprotein and the labeled SBV (Lakowicz, 1983). Stopped-flow kinetic studies were performed on a Durrum D-100 stopped-flow fluorometer (Palo Alto, CA) connected to a Biomation waveform recorder (Cupertino, CA) interfaced to an Apple computer (Cupertino, CA). Excitation was at 280 nm, and emission was measured through a Corning 3-72 cutoff filter (Corning, NY). Data analysis was performed by using a nonlinear least-squares fitting algorithm for a single-exponential fluorescence intensity increase.

ApoC Intermembrane Transfer Kinetics. The expression for the rate of apoprotein transfer between two particles is given by eq 5 It was assumed that the mechanism of transfer

$$d(transfer)/dt = k_{off}[P_{bound}] \qquad (5)$$

was by the dissociation of monomeric peptides from the surface of the lipid particle rather than transfer of the protein via a complex formed by the collision of two lipid particles.

Intermembrane transfer of apoC proteins from donor SBV containing 5 mol % dansyl-PE and 95 mol % POPC to unlabeled POPC acceptor SBV was determined by following fluorescence recovery at 345 nm. Stopped-flow kinetic studies were conducted by using a Hi-tech SFA-11 mixer (Wiltshire, England) in an SLM 8000 (Urbana, IL) fluorometer with excitation at 280 nm. Data acquisition was performed by using a Data Translation (Marlboro, MA) analog to digital converter interfaced to a Sperry (Salt Lake City, UT) microcomputer, and analysis was performed by using the algorithm of Marquardt (1963) for biexponential nonlinear fit of the data.

Equilibrium Determination of ApoC Dissociation Constant. ApoC dissociation constants were determined by the method of Retzinger et al. (1985). Briefly, varying amounts of apoprotein dissolved in 100 mM NaCl, 10 mM $NaHPO_4$, and 1 mM NaN_3, pH 7.4 (0.75 mL), were mixed with (30 mg/mL) divinylbenzene/styrene latex beads (Seragen, Indianapolis, IN) coated with a monolayer of ^{14}C-labeled POPC suspended in the same buffer (0.75 mL) and centrifuged for 5 min to precipitate the beads. The amount of protein bound to the beads was determined from the difference between the initial amount added to the tubes and the unbound protein after centrifugation using the fluorescamine protein assay (Udenfriend, 1972). The results were plotted as bound protein vs initial concentration of apoC proteins and were fitted to a nonlinear regression:

$$[P_{bound}] = n[P_{added}]/(K_d + [P_{added}]) \qquad (6)$$

Results

Gel Filtration Chromatography. The coelution of apoC-I with both HDL and VLDL (Figure 1A,B) indicated that the affinity of the apoprotein for these lipoproteins was not altered by delipidation and column chromatography. ApoC-I (Figure 1C) also associates with phospholipid single-bilayer vesicles. ApoC-I transferred from VLDL to HDL (Figure 2A) when the donor was added to a 10-fold molar excess of HDL phospholipid. The converse was true (Figure 2B) when the acceptor was a 10-fold molar excess of VLDL phospholipid and the donor was HDL. ApoC-I also transferred reversibly from VLDL to POPC SBV (Figure 2C) and from POPC SBV to VLDL (Figure 2E) when the acceptor species was added

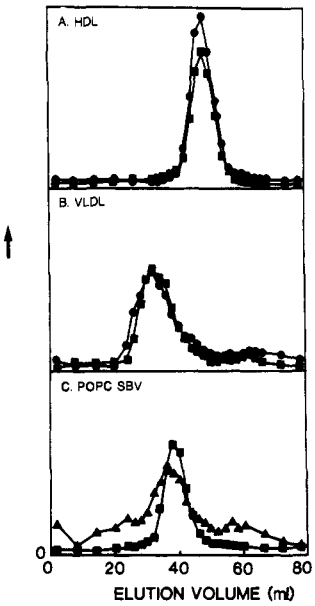

FIGURE 1: Coelution of delipidated apoC-I with HDL (panel A), VLDL (panel B), and POPC SBV (panel C). HDL, VLDL, and POPC SBV (0.2 mg of phospholipid) were incubated with 5.6 μg of ^{3}H-labeled apoC-I (1.1 mCi/mmol) for 1 h and chromatographed on a 1.6 × 30 cm Sepharose CL4B column. HDL and VLDL elution was measured by absorption at 280 nm (●), while apoC-I elution was determined by liquid scintillation counting (■) and POPC SBV elution was measured by inorganic phosphorus assay (▲).

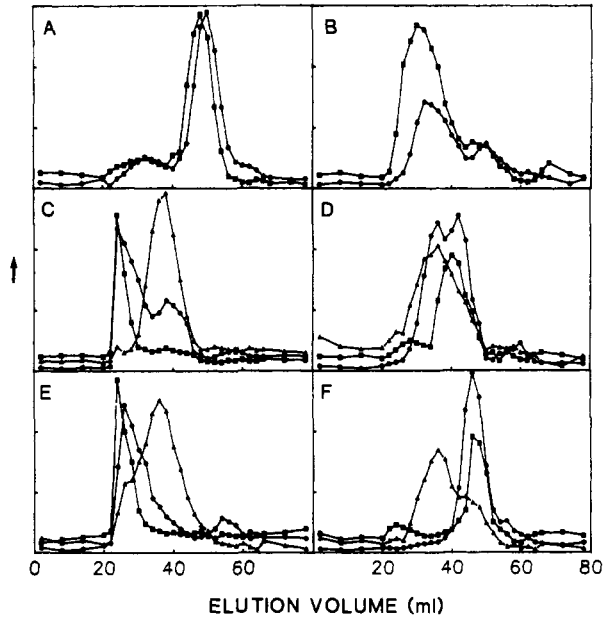

FIGURE 2: Intermembrane transfer of apoC-I between lipid particles. Elution profiles of 0.2 mg of HDL, VLDL, or POPC phospholipid plus 5.6 μg of apoC-I donor species were mixed with 2.0 mg of HDL, VLDL, or POPC acceptor phospholipid. The donor and acceptor were mixed together for 5 min and then chromatographed as in Figure 1. HDL and VLDL elution was measured by absorption at 280 nm (■), while [^{3}H]apoC-I (●) and [^{14}C]POPC SBV (▲) elution volumes were determined by liquid scintillation counting. The transfer of apoC-I counts from donor VLDL to a 10-fold excess of HDL acceptor phospholipid was demonstrated (panel A) along with reverse transfer from HDL to VLDL (panel B). Reversible apoC-I transfer from VLDL to POPC SBV was shown (panel C) and from POPC SBV to VLDL (panel E). Finally, the reversible transfer of apoC-I from HDL to POPC SBV was demonstrated (panel D) and from POPC SBV to HDL (panel F).

as a 10-fold molar excess of phospholipid. Transfer from HDL to POPC SBV (Figure 2D) and from POPC SBV to HDL (Figure 2F) was also reversible.

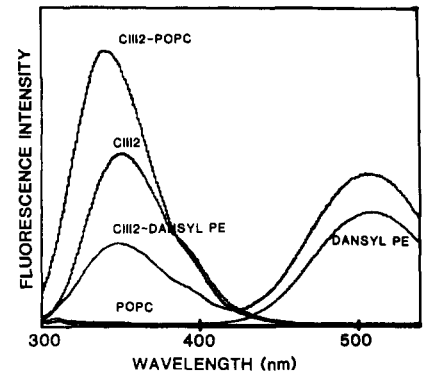

FIGURE 3: Fluorescence emission spectra for apoC-III (0.625 μM) dissolved in standard buffer and in association with POPC SBV or POPC SBV containing 5 mol % dansyl-PE at a protein to lipid ratio of 1 to 250.

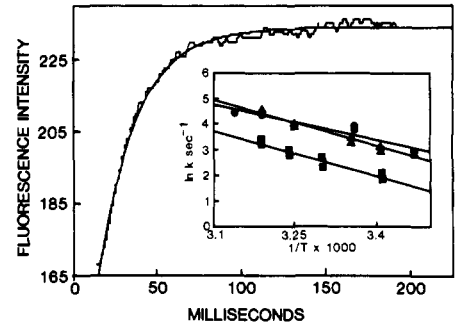

FIGURE 4: Kinetics of apoC protein association with 5 mol % dansyl-PE/95 mol % POPC SBV. A representative kinetic trace of 5 μM apoC-III plus 1.25 mM SBV, after mixing, at 25 °C displays the raw data plus the line fitted to the data from the equation $I = I_0 + Ae^{-kt}$. In the insert are Arrhenius plots of the natural logarithm of the association rate vs the inverse of the absolute temperature for apoC-I (■), apoC-II (●), and apoC-III (▲). Activation energies for apoC protein association were determined from the slope of the Arrhenius plots.

Fluorescence Studies of ApoC–SBV Association. Association of the C apoproteins with single-bilayer vesicles produced changes in the intrinsic fluorescence of the protein tryptophan residues that could be used to distinguish bound and free protein. ApoC-III in standard buffer (Figure 3) exhibited a tryptophan fluorescence emission maximum at 349 nm. Upon association with POPC SBV, there was an increase in relative quantum efficiency of the fluorescence emission and a blue shift in the wavelength of the emission maximum to 339 nm. When apoC-III was added to POPC SBV containing 5 mol % dansyl-PE, the tryptophan fluorescence intensity decreased while a second peak appeared at 510 nm. We assigned the concomitant decrease in fluorescence at 339 nm and the appearance of a peak at 510 nm to resonance energy transfer from tryptophan to the dansyl group. ApoC-I and apoC-II displayed similar changes in fluorescence intensity and emission maximum, although apoC-I produced a smaller intensity increase at 510 nm upon association with POPC SBV. These experiments demonstrated (1) that the different spectral properties exhibited by an apoC protein in water and bound to phospholipid could be used to determine whether it is bound or free and (2) that both the disappearance of tryptophan fluorescence and the appearance of sensitized fluorescence of dansyl-PE could be used to follow the binding of the apoC proteins to dansyl-labeled SBV.

ApoC–SBV Association Kinetic Studies. Upon mixing the apoC proteins with 5% dansyl-PE/95% POPC SBV, an increase in fluorescence intensity at 510 nm due to nonradiative transfer of energy from the tryptophan residues of the protein

Table I: Rate Constants (37 °C) and Activation Energies (E_a) for the Transfer of ApoC Proteins between POPC–SBV

		association			dissociation			
apoprotein	order	E_a[a]	k_{on}[b]		E_a[a]	k_{off}[c]	$t_{1/2}$[d]	K_d (μM)[e]
apoC-I	α = 0.83	11.0	2.5	fast	21.2	11.7	60	0.5
	β = 1.3			slow	19.3	1.3	540	
apoC-II	α = 0.56	9.0	4.0	fast	7.1	9.5	74	1.4
	β = 0.84			slow	5.0	0.6	1180	
apoC-III	α = 0.55	10.6	3.8	fast	15.4	9.9	71	0.5
	β = 0.73			slow	14.4	0.86	810	

[a] Kilocalories per mole. [b] In $M^{-1}/s \times 10^{-10}$. [c] In s^{-1}. [d] In milliseconds. [e] At 25 °C.

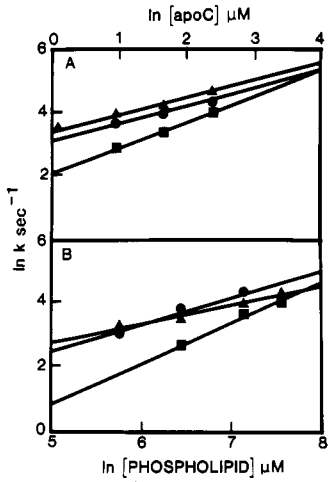

FIGURE 5: Determination of reaction order and overall rate constant. Reaction order was determined from the slope of the natural logarithm of the rate of apoprotein association vs the natural logarithm of either apoprotein concentration (panel A), while the phospholipid concentration of 5 mol % dansyl-PE/95% mol % POPC SBV was held constant at 5 mM, or phospholipid concentration (panel B), while apoprotein concentration was kept constant at 10 μM, before mixing for apoC-I (■), apoC-II (●), or apoC-III (▲). Overall rate constants were calculated by assuming that the reaction was first order for both lipid and protein concentration.

was observed (Figure 4). The smooth curve represents a single-exponential nonlinear fit of the data. An Arrhenius plot of the natural logarithm of the apoC–SBV association rate vs the reciprocal of the absolute temperature (Figure 4, insert) produced a linear increase in the rate of association with temperature. Values for the activation energy of apoC–SBV association obtained from the data in Figure 4 are shown in Table I.

The initial velocity for the association of the apoC proteins with SBV was measured and plotted according to eq 4a,b. From the slope of the linear least-squares fit of the data, the reaction orders with respect to protein (Figure 5A) and lipid (Figure 5B) concentration were determined (Table I). For the association of the apoC proteins with a lipid surface, the reaction appeared to be first order with respect to both lipid and protein concentration. The overall rate constant (Table I) for association at 37 °C was determined assuming that the reaction was second order overall.

ApoC Intermembrane Transfer Studies. ApoC transfer kinetic traces were characterized by an increase in fluorescence intensity at 345 nm (Figure 6A) as the apoprotein transferred from the quenched donor DPE SBV to an excess of acceptors which were SBV of POPC. The line through the curve represents a nonlinear biexponential fit of the data. The rate of dissociation was much slower than the rate of association; thus, the kinetic traces only represent protein transfer. Generally, a 10-fold excess of acceptor vesicles was used to minimize back-transfer to the donor species. Table II gives rate data for the fast and slow components where the donor to acceptor

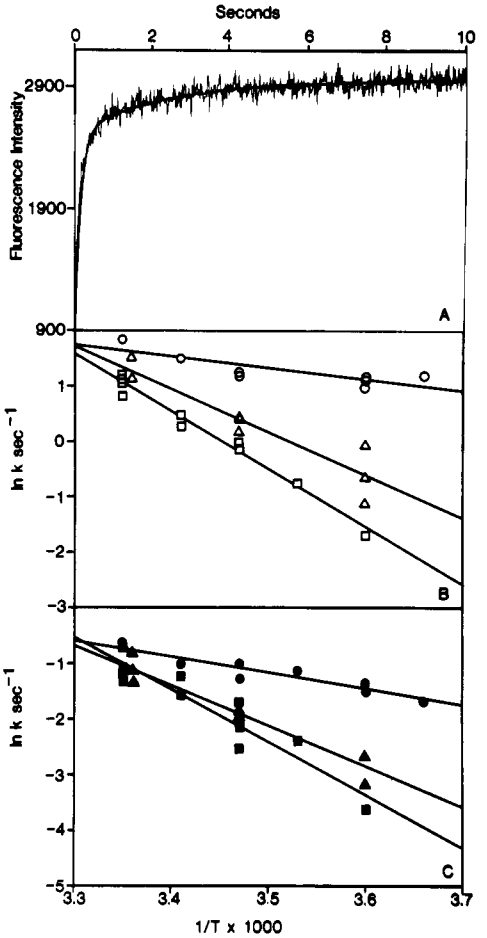

FIGURE 6: Kinetic traces of apoC-III transfer between SBV and Arrhenius plots of the fast component and the slow component of apoC protein transfer. Fluorescence recovery at 345 nm (panel A) of apoC-III (15 μM) at 25 °C upon mixing 5% dansyl-PE/95% POPC SBV (1.25 mM) with POPC SBV (12.5 mM). The raw data were fitted to the equation $I = I_0 + Ae^{-k_1 t} + Be^{-k_2 t}$. Arrhenius plots of the fast component of the biexponential fluorescence recovery (panel B) for apoC-I (□), apoC-II (○), and apoC-III (△) and the slow component (panel C) for apoC-I (■), apoC-II (●), and apoC-III (▲). All concentrations given were after dilution due to mixing.

Table II: Effect of the Number of Peptides per Vesicle upon Transfer Rate[a]

	[PC] (mM)	fast component (s^{-1})	slow component (s^{-1})
10/1	3.2	4.00	0.22
1/1	32	0.91	0.06

[a] If transfer occurs via the collision of vesicles, then an increase in vesicle number should increase the rate of transfer. ApoC-III transfer at 25 °C was measured by using 10 μM apoC–III and either 3.2 mM DPE SBV/16 mM POPC or 32 mM DPE SBV/160 mM POPC as donor–acceptor pairs. Vesicle molecular weight was assumed to be 2.5 $\times 10^6$. The first donor–acceptor pair gave a protein to donor vesicle ratio of 10/1, while the second gave a protein to donor vesicle ratio of 1/1.

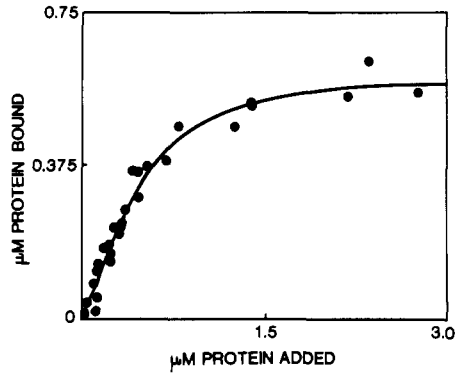

FIGURE 7: Adsorption isotherm of apoC-I association with latex beads coated with a monolayer of POPC. A typical plot of bound protein vs protein added to 30 mg/mL latex beads displayed saturable association with the lipid surface. The data points are represented by (●) while the line through the points represents a computer fitting of the data to $[P_{bound}] = n[P_{added}]/(K_d + [P_{added}])$.

ratio was kept constant at 1/5 while the total amount of lipid was increased 10-fold. If transfer occurs via the formation of a collisional complex between donor and acceptor particles, then the increased probability of collision at the higher lipid concentration should give a faster rate of transfer; on the other hand, if the rate remains the same, desorption of the protein from the particle surface is more likely to be the mechanism of transfer (Doody et al., 1980; Lund-Katz et al., 1982). Assuming a vesicle molecular weight of 2.5×10^6, the rates at 1 and 10 proteins per vesicle were determined (Table II). The slower rate of transfer at the lower peptide to vesicle ratio indicated that desorption of monomeric proteins was the more likely mechanism. Steric crowding of the vesicle surface may account for the faster rate of transfer at 10 peptides per vesicle.

Arrhenius plots of the fast component (Figure 6B) and the slow component (Figure 6C) showed a similar temperature dependence for both components. ApoC-II transfer rates displayed a much smaller temperature dependence than those of apoC-I and apoC-III. Activation energies for the fast and slow components of the fluorescence recovery are listed in Table I.

ApoC Dissociation Constant Determination. The dissociation constant was determined by equilibrium measurement. Equilibrium measurement of the dissociation constant was determined from the association of the apoC proteins with POPC-coated latex beads which produced an adsorption isotherm (Figure 7) that demonstrated saturable binding of the apoprotein to the lipid surface. The values of the dissociation constants (K_d) for the equilibrium constant determination are displayed in Table I.

Discussion

Apoprotein–SBV Association. Apoproteins are believed to form a part of the polar monolayer of molecules that surrounds a core of neutral lipids in plasma lipoproteins. Our discussion will focus upon the transport dynamics for the apoC proteins, which transfer between lipid surfaces. The rate constant for a diffusion-controlled reaction, k_{diff}, between the apoC proteins and SBV may be calculated from a modified form of the Debye equation (Backstrom & Sandros, 1960):

$$k_{diff} = \frac{1}{4}\left(2 + \frac{d_1}{d_2} + \frac{d_2}{d_1}\right)\frac{8RT}{3000\eta} \qquad (7)$$

where η is the viscosity of the solvent in poise units, $R = 8.31 \times 10^7$ erg/(mol·K), and d_1 and d_2 are the respective diameters of the reacting species. If one assumes molecular weights of 2.5×10^6 and 10^4 for SBV and the C proteins, respectively, one obtains $k_{diff} \sim 5 \times 10^{10}$ M^{-1} s^{-1}. This value is very close to the measured value for the rate of association binding of the apoC proteins with SBV. We conclude that nearly every collision between an apoC protein and a vesicle results in binding. This result is in sharp contrast to the much slower association of apoproteins with multilamellar liposomes of dimyristoylphosphatidylcholine, which exhibits a phase transition near 24 °C (Mabrey & Sturtevant, 1976). We attribute the difference to several factors. First, association of apoproteins with multilayered liposomes was followed by the attendant decrease in light scattering, which reflected the conversion of the large liposomes into small lipid–protein complexes. Light-scattering measurements detect only the change in the size of the liposomes and not the primary process of apoprotein penetration of the lipid surface. Second, the surface structures of an SBV and a multilayered liposome are distinctly different. Amphiphiles such as apoproteins (Pownall et al., 1978, 1981) and certain phospholipases (Op den Camp et al., 1975) do not readily penetrate multilayers except at the phase transition of the lipid. In contrast, SBV have a more permeable surface that readily associates with apoproteins, even above the transition temperature (Jonas et al., 1977).

ApoC Transfer between Lipid Surfaces. Movement of lipids between plasma lipid compartments occurs either by desorption and diffusion of monomeric lipid molecules through the aqueous media or with the aid of lipid transfer proteins (Pownall et al., 1983; Zilversmit, 1984). Protein transfer also occurs via the desorption of monomeric peptides from the lipid surface. The faster rate of protein transfer that was observed when there were 10 peptides per vesicle indicated that apoprotein transfer was negatively cooperative, probably due to steric crowding of the protein and lipid molecules on the vesicle surface. We speculate that the biexponential recovery of fluorescence intensity represents two processes; the fast component may be due to desorption of peptides that are partially associated with the vesicle surface and partially self-associated with other peptides on the vesicle surface, while the slower component may be desorption of individual peptides associated with the lipid bilayer (Pownall et al., unpublished experiments). Arrhenius plots for the fast and slow components of transfer produced similar values for the activation energy (Table I) for the two components. The values obtained for apoC-I and apoC-III are comparable to those obtained for protein desorption of approximately 20 kcal/mol (Reijngoud & Phillips, 1984). ApoC-II had lower association and transfer activation energies than apoC-I or apoC-III; however, the association of apoC-II with lipid particles may be aided by interaction with lipoprotein lipase (Voyta et al., 1983).

Protein–lipid association and transfer do not occur exclusively in amphipathic proteins; it has also been demonstrated for cytochrome b_5, which contains a hydrophobic membrane-spanning domain (Leto et al., 1980), and blood coagulation factor V, which associates with acidic bilayers via an electrostatic interaction (Pusey & Nelsestuen, 1984). In both cases, transfer kinetics were not the simple single-exponential kinetics seen for the transfer of lipids between particles. Thus, negative cooperativity in the binding of large, complex molecules to lipid surfaces may be more common than originally thought.

ApoC Dissociation Constants. The equilibrium dissociation constants for the apoC proteins (Table I) were approximately an order of magnitude lower than the values obtained by Cardin et al. (1982). This was not surprising, however, as Retzinger et al. (1985) reported that, in general, K_d values obtained using POPC-coated latex beads were an order of

magnitude lower than those obtained by titration of unilamellar vesicles with protein due to a higher affinity for the hydrophobic surface of the latex beads. However, latex beads coated with phospholipid represent an improvement over unilamellar vesicles, because they do not fuse into multilamellar vesicles (McKeone et al., 1986) or form discoidal particles upon apoC association (Morrisett et al., 1977).

The rapid rate of apoprotein–lipid particle association and transfer between particles in plasma suggests that most of the apoC proteins are associated with lipid surfaces but can respond to rapid changes in the distribution of lipid particles that would be expected if the surface structure of the lipid is altered by other processes. This rapid distribution of apoproteins between lipid surfaces permits reutilization of apoproteins that are activators of lipolytic enzymes. This may account, in part, for the much longer lifetime of apoproteins compared with other components of lipoproteins. Presumably, when sufficient depletion of the surface coat of triglyceride-rich lipoproteins is achieved, additional apoproteins and phospholipids are transferred to the HDL fraction. Our current evidence favors a mechanism in which phospholipids and proteins are transferred monomerically through the surrounding aqueous phase to a preformed acceptor. These processes occur on a time scale that is rapid compared to the turnover of lipoproteins.

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