Surface Properties of Native Human Plasma Lipoproteins and Lipoprotein Models

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ABSTRACT Plasma lipoprotein surface properties are important but poorly understood determinants of lipoprotein catabolism. To elucidate the relation between surface properties and surface reactivity, the physical properties of surface monolayers of native lipoproteins and lipoprotein models were investigated by fluorescent probes of surface lipid fluidity, surface lateral diffusion, and interfacial polarity, and by their reactivity to *Naja melanoleuca* phospholipase A_2 (PLA_2). Native lipoproteins were human very low, low-, and subclass 3 high-density lipoproteins (VLDL, LDL, and HDL_3); models were 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC) or its ether analog in single-bilayer vesicles, large and small microemulsions of POPC and triolein, and reassembled HDL (apolipoprotein A-I plus phospholipid). Among lipoproteins, surface lipid fluidity increased in the order $HDL_3 < LDL < VLDL$, varying inversely with their (protein + cholesterol)/phospholipid ratios. Models resembled VLDL in fluidity. Both lateral mobility in the surface monolayer and polarity of the interfacial region were lower in native lipoproteins than in models. Among native lipoproteins and models, increased fluidity in the surface monolayer was associated with increased reactivity to PLA_2. Addition of cholesterol (up to 20 mol%) to models had little effect on PLA_2 activity, whereas the addition of apolipoprotein C-III stimulated it. Single-bilayer vesicles, phospholipid-triolein microemulsions, and VLDL have surface monolayers that are quantitatively similar, and distinct from those of LDL and HDL_3. Surface property and enzymatic reactivity differences between lipoproteins and models were associated with differences in surface monolayer protein and cholesterol contents. Thus differences in the surface properties that regulate lipolytic reactivity are a predictable function of surface composition.

INTRODUCTION

Native human plasma lipoproteins are composed of a surface monolayer of phospholipid, cholesterol, and apolipoproteins surrounding a core of triglyceride and cholesteryl ester (Shen et al., 1977; Atkinson and Small, 1986). Lipolytic enzymes, lipid transfer proteins, and the exchangeable apolipoproteins associate with the surface monolayer. Investigators have frequently used model systems when the structures and compositions of native lipoproteins were too complex for direct study. Models include phospholipid single-bilayer vesicles (SBVs) (McLean and Jackson, 1985; McKeone et al., 1988; Mims and Morrisett, 1988), microemulsions of phospholipid and neutral lipids (Tajima et al., 1983; Yokoyama et al., 1985; Mims and Morrisett, 1988; Derksen and Small, 1989; Small et al., 1991; Ohnishi and Yokoyama, 1993), discoidal particles including reassembled POPC–apoA-I particle (R-HDL) prepared from apolipoproteins and phospholipids (Reijngoud and Phillips, 1982; Massey et al., 1985a,b; Jonas, 1986, 1991, 1992; Pownall et al., 1987; Parks et al., 1992; Sparks et al., 1993), and phospholipid monolayers (Ibdah et al., 1989, 1990; Handa et al., 1992). These studies indicate that the lipids in the surface monolayer determine its physical properties, which in turn determine apolipoprotein composition, conformation, and binding. Plasma lecithin:cholesterol acyl transferase activity is a function of the surface properties of its substrates. Some of these properties are macromolecular, including apolipoprotein composition and physical properties of the bulk lipid matrix. Others are strictly molecular and depend on the covalent structure of substrate lipids (Massey et al., 1985a; Pownall et al., 1985; Jonas et al., 1987; Jonas, 1991; Parks et al., 1992; Sparks et al., 1993). Similarly, cholesteryl ester transfer protein (CETP) activity depends on the surface composition of the substrate lipoproteins that are the lipid donors and acceptors (Ohnishi and Yokoyama, 1993).

To determine how the properties of surface lipids regulate apolipoprotein distribution and function, we compared the physicochemical properties of the surface monolayers of native lipoproteins and lipoprotein models. In the studies reported here, we performed parallel assessments of the surface monolayer properties of the major human lipoproteins; very low density lipoproteins (VLDL), low-density lipoproteins (LDL), subclass 3 high-density lipoproteins (HDL_3), and several well-characterized lipoprotein models. We used the fluorescent probes 1-(4-trimethylammoniumphenyl)-1,3,5-hexatriene (TMA-DPH) and 4-heptadecyl-7-hydroxycouramin (HC), which are sensitive to differences in the microviscosity of the acyl chain and the headgroup regions of surface phospholipids (Prendergast et al., 1981; Massey et al., 1985b; Pal et al., 1985); 1-pyreneundecyltrimethylammonium iodide (PUTA), the lateral diffusion of which can be followed spectroscopically (Massey et al., 1985b); and Prodan (6-propionyl-2-dimethylaminonaphthalene) and Patman (6-palmitoyl-2-(((trimethyl)ammonium)-

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ethyl)methyl)amino)naphthalene chloride), which are spectroscopic probes of interfacial polarity (Massey et al., 1985c; Jonas et al., 1987; Parasassi et al., 1994). Because phosphatidylcholine (PC) is the major phospholipid in plasma lipoproteins, accounting for 70–80% of the phospholipid, phospholipase A_2 (*Naja melanoleuca*; EC 3.1.1.4), it was used as an enzymatic probe to correlate the physicochemical properties of the surface monolayer with the activity of a lipolytic enzyme that associates with phospholipid surfaces (Mims and Morrisett, 1988; Cunningham et al., 1989). Validation of methods for the characterization of the interfacial physical properties of membranes and lipoproteins should lay the groundwork for understanding how the catalytic efficiencies of proteins that associate with the phospholipid-water interface of lipoproteins are regulated.

EXPERIMENTAL PROCEDURES

Materials

1-Palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC) was purchased from Avanti Polar Lipids (Birmingham, AL). Triolein and fatty acid-free albumin were from Sigma Chemical Co. (St. Louis, MO). TMA-DPH, HC, PUTA, Prodan, and Patman were purchased from Molecular Probes (Grand Junction, OR). *Naja melanoleuca* venom was obtained from Miami Serpentarium (Miami, FL), and its fraction III phospholipase A_2 was purified by a previously described method (Joubert and Van der Walt, 1975; Massey et al., 1985a). Apolipoprotein A-I (apoA-I), apolipoprotein C-III (apoC-III), 1-palmityl-2-oleyl-*sn*-glycero-3-phosphocholine (POPC-ether), 1,2-tetradecyl-*sn*-glycero-3-phosphocholine (DMPC-ether), and 1-myristoyl-2–9-(1-pyrene)nonanoyl-*sn*-glycero-3-phosphocholine (MPNPC) were prepared as previously described (Massey et al., 1985b; Pownall et al., 1985; McKeone et al., 1988).

Isolation of native lipoproteins and preparation of lipoprotein models

VLDL ($d < 1.006$ g/ml), LDL ($d = 1.019–1.063$ g/ml), and HDL_3 ($d = 1.125–1.210$ g/ml) were isolated from pooled normolipidemic plasma by density ultracentrifugation (Havel et al., 1955). Microemulsions of POPC and triolein were prepared by sonication of the POPC-triolein mixture. The large microemulsion (LME) and small microemulsion (SME), which were separated by chromatography on Sepharose CL-4B, exhibited compositions and hydrodynamic properties similar to those previously described (Tajima et al., 1983); rechromatography of the pooled fractions duplicated the profile obtained in the first chromatography. Except where noted, the phospholipid in LME and SME is POPC.

SBVs of POPC or POPC-ether were prepared by sonication and isolated by chromatography on Sepharose CL-4B (Barenholz et al., 1977; McKeone et al., 1988). Typical elution profiles of LDL, POPC SBV, LME, and SME are shown in Fig. 1. R-HDL, comprising POPC and apoA-I, prepared by a cholate dialysis procedure, had a lipid-to-protein stoichiometry of 100:1 (Massey et al., 1985b; Pownall et al., 1985). Lipid particles containing ether and fluorescent analogs of phospholipids were prepared similarly (Massey et al., 1985a). The lipids were mixed in chloroform, and the organic solvent was removed under vacuum before incorporation into lipoprotein models. ApoC-III was incorporated into preformed vesicles and microemulsions by simple mixing. The phospholipid concentrations of lipoproteins and lipoprotein models were determined as phosphorus, using a molecular weight of 750 for the phospholipid (Bartlett, 1959). A standard buffer composed of 100 mM NaCl, 1 mM NaN_3, 1 mM EDTA, and 10 mM Tris (pH 7.4) was used throughout, except as noted.

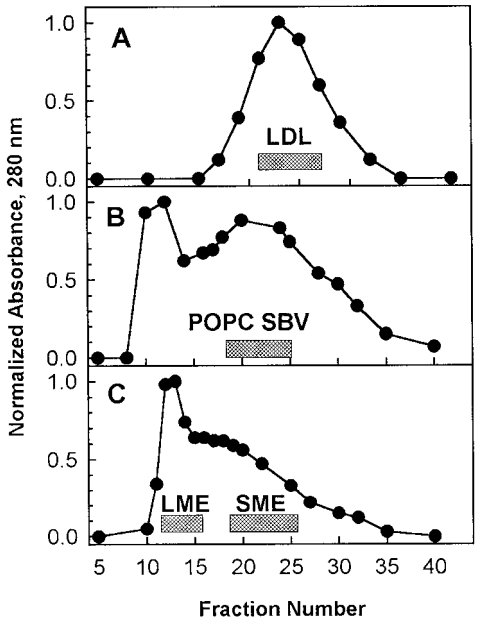

FIGURE 1 Native lipoproteins and lipoprotein models were isolated by chromatography on Sepharose CL-4B (1.6 cm × 40 cm column). For each type of particle, fractions indicated by the cross-hatched bars were pooled. (*A*) LDL. (*B*) POPC SBV. (*C*) LME and SME. The SBVs, LMEs, and SMEs were prepared by sonication followed by low-speed centrifugation to remove titanium fragments. For the microemulsions, the two pooled fractions used were one eluting in the void volume (LME) and one eluting in the included volume (SME). In the void volume, the apparent absorbance was higher because of light scattering by the large particles.

Fluorescence methods

A variety of fluorescence methods and probes were used to evaluate molecular motion, lateral mobility, and microviscosity within native lipoproteins and lipoprotein models. For this report, lateral mobility is defined as the ability of molecules to move within and parallel to the surface of the particles; microviscosity is viscosity measured at the molecular or microscopic scale; molecular motion is the movement of probe molecules laterally or rotationally within lipoprotein particles. The fluorescence polarization of TMA-DPH and HC and the fluorescence spectra of PUTA, Prodan, and Patman in native lipoproteins and lipoprotein models were recorded on an SLM 8000 spectrofluorimeter equipped with Glan-Thompson prisms (Small et al., 1991). The polarization of TMA-DPH, which is a cationic membrane probe that does not partition into the lipoprotein core, is sensitive to the fluidity of the acyl chains of the phospholipids in the surface monolayer (Prendergast et al., 1981). HC polarization is sensitive to the motion at the interfacial headgroup region of a phospholipid bilayer (Pal et al., 1985). PUTA is a single-chain cationic amphiphile whose excimer fluorescence intensity increases with surface lateral mobility. The fluorescence properties of Prodan and Patman depend on the polarities of their microenvironments.

The fluorescent probes were introduced into the samples by injection of microliter aliquots of a solution (1 mM) of the probe in ethanol. The samples were incubated at 37°C for 1 h to ensure probe equilibration. Final concentrations were less than 0.1% ethanol and contained less than 1 mol of probe per 500 mol of phospholipid. The sample chamber of the fluorimeter was maintained at a constant temperature with a thermostat-controlled water bath, and the temperature in the cuvette was recorded with a Bailey Instruments digital thermometer (model Bat 8). The excitation wavelength was 350 nm for TMA-DPH and 330 nm for HC; the emission was monitored with a Corning 3–144 cutoff filter.

The concentration dependence of the fluorescence emission of the pyrenyl probes MPNPC and PUTA is described by the equation

$$E/M = ([\mathrm{P}] \cdot T \cdot k)/\eta$$

where E is the excimer fluorescence intensity, M is the monomer fluorescence intensity, [P] is the concentration of the pyrene analogue, T is the absolute temperature, k is a constant incorporating both experimental variables and the lateral diffusion coefficient of the lipid molecule, and η is the viscosity of the medium surrounding the pyrene. Under defined conditions, the E/M ratio can provide a relative measure of the lateral diffusion of the probe in the surface monolayer (Mantulin et al., 1981; Massey et al., 1985b; Vauhkonen et al., 1989; Sassaroli et al., 1990). The excimer and monomer fluorescence intensities were measured at 470 nm and 397 nm, respectively. The excitation wavelength was 327 nm.

Partition coefficients for Prodan were determined so that more than 99% of the probe was associated with the phospholipid matrix for the fluorescence measurements. The fluorescence spectra of Prodan, which is a hydrophobic membrane probe, were analyzed for wavelength of maximum fluorescence intensity and $\nu_{1/2}$, fluorescence spectral width at half-maximum intensity (Massey et al., 1985c). Patman, which is a cationic membrane probe that does not partition into the lipoprotein core, was analyzed for the fluorescence wavelength maxima. Patman was added at a ratio of 1 mol of probe to 500 mol of phospholipid.

Phospholipase A_2 measurements

PC hydrolysis was followed by pH-stat titration at pH 8.0 (Brinkman Metrohm). Solutions of native lipoproteins or lipoprotein models (2 ml) containing 10 mg of fatty acid-free albumin (0.5 mM Tris, 50 mM $CaCl_2$, and 100 mM NaCl, pH 8.0) and 40 ng phospholipase A_2 were combined at 25°C to start the reaction; initial rates were determined over an interval in which less than 10% of the total phospholipid was hydrolyzed.

Alternatively, hydrolysis of a pyrene-labeled phospholipid in a PC-ether matrix by phospholipase A_2 was followed by a fluorescence assay (Massey et al., 1985a; Rosseneu et al., 1985). The basis of this assay was that catalysis is much slower than the transfer of pyrene nonanoic acid to albumin, which can be followed fluorimetrically. Thus the rapid transfer of the product to albumin allowed the continuous monitoring of the enzymatic reaction. The rate of hydrolysis, dP/dt, was calculated as $dP/dt = -k[\mathrm{P}]$, where k is the first-order rate constant and [P] is the phospholipid concentration. A typical assay contained 10 mg/ml bovine serum albumin, substrate, 0.2 μg phospholipase A_2, and buffer (10 mM Tris, 25 mM $CaCl_2$, 100 mM NaCl, 1 mM EDTA, 1 mM NaN_3, pH 7.4) to a final volume of 1.5 ml.

RESULTS

Fluorescence polarization

The polarization of fluorescence of TMA-DPH and HC was used to investigate the relative fluidity of the surface monolayers of native lipoproteins, POPC SBV, POPC-ether SBV, POPC-triolein microemulsions, and R-HDL. For TMA-DPH, the polarization values were linear with respect to temperature (Fig. 2, *A* and *B*). For the native lipoproteins, the magnitude of the polarization at 37°C decreased in the order $HDL_3 > LDL > VLDL$. Although the slopes of the plots were similar, surface fluidity was significantly higher for VLDL than for HDL_3 or LDL. For the lipoprotein models containing POPC, the fluorescence polarization of TMA-DPH decreased in the order R-HDL > SBV ≈ SME > LME (Fig. 2 *B*). According to TMA-DPH fluorescence polarization, SBV and SME, which have similar diameters as determined by Sepharose chromatography (Fig. 1), also have similar surface fluidities. The fluorescence polarization of TMA-DPH in POPC-ether SBV was greater than that in POPC SBV (Fig. 2 *B*). Differences in the quantum yields of lipophilic fluorescence probes in PC and PC-ether have been reported previously (Massey et al., 1985b), and underlying differences in fluorescence lifetimes may contribute to the different polarizations. On average, the fluorescence polarization of TMA-DPH was greater in native lipoproteins than in most lipoprotein models. At 37°C, VLDL surface fluidity was similar to values for R-HDL and POPC-ether SBV. For HC, fluorescence polarization also varied linearly with temperature; at 37°C, the order was $HDL_3 \approx LDL >$ POPC-ether SBV ≈ R-HDL > POPC SBV > SME > VLDL ≈ LME (Fig. 2, *C* and *D*). The HC fluorescence polarization values were much lower in VLDL, LME, and SME, all of which have large, hydrophobic lipid cores. They were also much lower than TMA-DPH values for VLDL, LME, and SME; for the other native lipoproteins and models, TMA-DPH and HC values were similar.

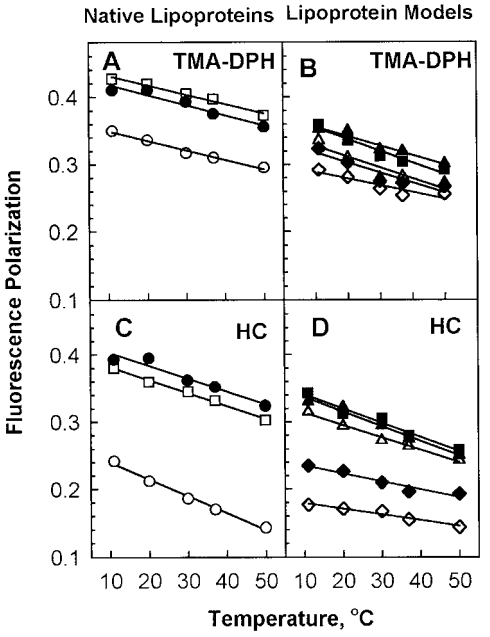

FIGURE 2 Fluorescence polarization in native lipoproteins (*A* and *C*) and models (*B* and *D*) labeled with TMA-DPH (*A* and *B*) and HC (*C* and *D*) as a function of temperature. ○, VLDL; ●, LDL; □, HDL_3; △, POPC SBV; ▲, POPC-ether SBV; ◇, LME; ◆, SME; ■, R-HDL.

Excimer fluorescence

The charged cationic excimer probe PUTA was used to determine the relative lateral mobility within the surface monolayer of native lipoproteins and lipoprotein models. In both groups, the E/M ratio at 37°C varied directly with probe concentration (Fig. 3 *A*). According to Eq. 1, a relative lateral diffusion coefficient for PUTA was calculated from the slope (m) of a plot of E/M against mol% PUTA.

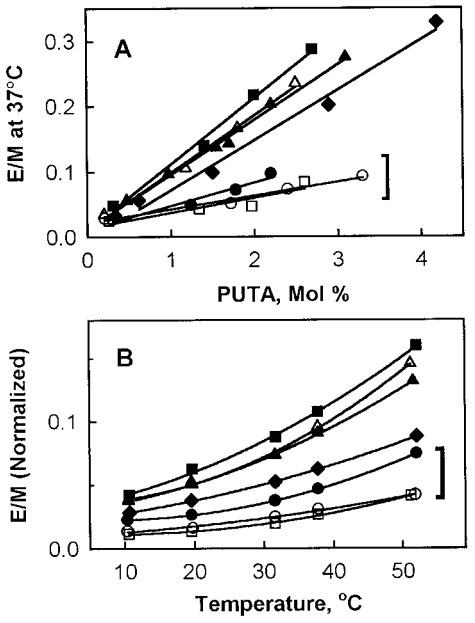

FIGURE 3 Lateral diffusion of PUTA in surface monolayers as determined from excimer/monomer (E/M) ratios. (*A*) The slopes of the plots of E/M versus mol% PUTA were used to calculate a normalized lateral diffusion coefficient (*D*) for the probe in different lipid environments at 37°C. LDL (●, $D = 35$), HDL_3 (□, $D = 23$), VLDL (○, $D = 21$), R-HDL (■, $D = 100$), POPC SBV (△, $D = 87$), POPC-ether SBV (▲, $D = 84$), SME (♦, $D = 76$). For POPC SBV and POPC-ether SBV, the mol% PUTA was determined, assuming that PUTA distributed into only the two-thirds of the total phospholipid that was in the outer monolayer. (*B*) E/M ratio as a function of temperature. The PUTA concentrations were 2.6–3.3 mol%. The E/M values were normalized by division by the mol% of PUTA in the sample. Symbols are as in *A*. Brackets show the clustering of the behavior of native lipoproteins.

TABLE 1 Partition coefficients and quantum yields for Prodan in native lipoproteins and lipoprotein models

Lipoprotein or lipoprotein model	$K_p^* \times 10^{-5}$	Relative quantum yield[#]
VLDL	12.7	1.6
LDL	10.2	1.0
HDL_3	5.2	2.4
POPC SBV	3.4	4.9
POPC-ether SBV	3.0	4.6
LME	5.0	2.0
SME	5.3	1.2
R-HDL	2.3	3.7

*K_p (partition coefficient) = (mol bound probe/mol phospholipid)/(mol free probe/mol H_2O).

[#]The relative quantum yield was calculated as the integrated fluorescence intensity of the bound probe divided by the intensity of the same concentration of Prodan in buffer. The concentration of Prodan was 1 μM. For VLDL, LDL, HDL_3, POPC SBV, POPC-ether SBV, and R-HDL, the concentration was 1.3 mg/ml phospholipid. For LME and SME, the phospholipid concentrations were 0.4 and 0.5 mg/ml, respectively.

For each particle *p*, the slopes were used to calculate a normalized lateral diffusion coefficient *D* according to $D_p = m_p/m_{R\text{-}HDL} \times 100$. The lateral diffusion of the probe increased in the order $HDL_3 \approx$ VLDL $<$ LDL $<$ SME $<$ POPC-ether SBV $\approx$ POPC SBV $<$ R-HDL. The *E*/*M* ratio at one probe concentration was measured as a function of temperature (Fig. 3 *B*). At all temperatures, the lateral mobility followed the same order among native lipoproteins and lipoprotein models. The most striking difference was that the lateral mobility of the probe was much higher in pure phospholipid matrices of lipoprotein models than in the surface monolayers of native lipoproteins, which contain apolipoproteins and cholesterol.

Prodan and Patman fluorescence

As noted above, the fluorescence spectra of Prodan and Patman are a sensitive function of the probe microenvironment (Weber and Farris, 1979; Massey et al., 1985c; Jonas et al., 1987; Parasassi et al., 1994). To verify the binding of Prodan to the native lipoproteins and lipoprotein models, lipid-water partition coefficients were determined. The partition coefficients were similar except for those of VLDL and LDL, which were at least twofold higher (Table 1) (Jonas et al., 1987). Because the partition coefficients were determined on the basis of number of moles of phospholipid, data for particles with neutral lipid cores cannot be meaningfully compared with particles without cores. By using the partitioning data, the relative quantum yields of Prodan were determined under conditions in which more than 99% of the probe was bound to the particles. The fluorescence quantum yield of Prodan in POPC SBV, POPC-ether SBV, and R-HDL was two to three times higher than in the native lipoproteins, LME, and SME (Table 1).

The fluorescence spectra of Prodan in native lipoproteins and lipoprotein models were also different (Fig. 4). For POPC SBV, POPC-ether SBV, and R-HDL, the fluorescence emission maxima appeared between 495 and 500 nm, a spectral range that is characteristic of the probe in a fluid-phase phospholipid (Massey et al., 1985c; Jonas et al., 1987). The emission maxima for these particles did not change substantially with temperature (Fig. 5, *A* and *B*), and the spectral halfwidth ($\nu_{1/2}$), which varies according to the heterogeneity of the probe environment, decreased with increasing temperature (Fig. 5, *C* and *D*). For VLDL and LDL, the emission maxima, which appeared at 420 nm, were invariant with respect to temperature (Fig. 5 *A*) and were slightly blue-shifted relative to the maximum observed for HDL_3; the fluorescence maximum of Prodan in HDL_3 was red-shifted at higher temperatures. The maxima in these spectra are typical of Prodan in a hydrophobic environment, and similar to those previously reported for this probe in gel-phase phospholipids or phospholipids containing a high concentration of cholesterol (Jonas et al., 1987). The spectral width was much broader for HDL_3 than for VLDL or LDL, suggesting that the environment of Prodan in HDL_3 is more heterogeneous than for VLDL or LDL.

LME and SME exhibited contrasting fluorescence behavior in the temperature dependence of Prodan fluorescence (Fig. 4 *B*; Fig. 5, *B* and *D*). At 22°C, the fluorescence

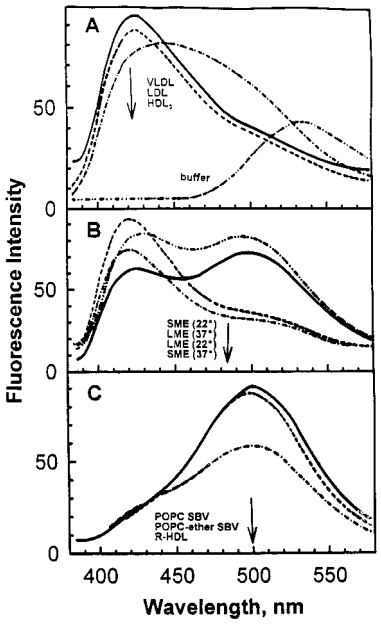

FIGURE 4 Fluorescence spectra of Prodan bound to native lipoproteins and lipoprotein models. Except where noted, all spectra were recorded at 37°C. (*A*) VLDL (——), LDL (– – –), and HDL_3 (– · –) and in buffer (– ·· –). (*B*) LME at 37°C (——) and 22°C (– – –); SME at 37°C (– · –) and 22°C (– ·· –). (*C*) POPC SBV (——), POPC-ether SBV (– – –), and R-HDL (– · –). The Prodan concentration was 1 μM, and the phospholipid concentration was 1.3 mg/ml for VLDL, LDL, HDL_3, POPC SBV, POPC-ether SBV, and R-HDL. The phospholipid concentration was 0.5 mg/ml for LME and 0.4 mg/ml for SME. The relative quantum yields and the partition coefficients are listed in Table 1.

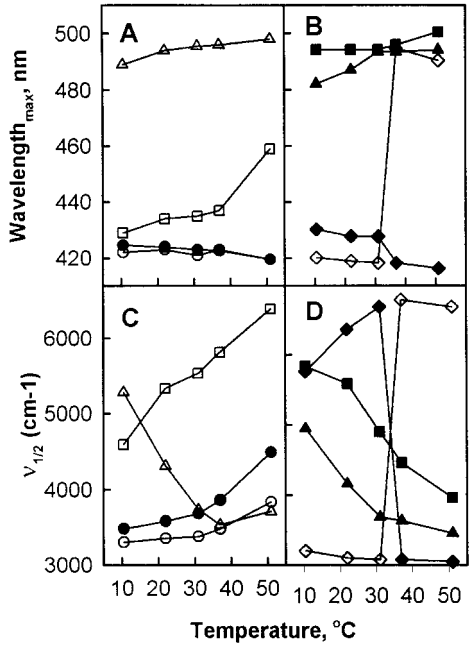

FIGURE 5 Fluorescence wavelength maxima (*A* and *B*) and spectral halfwidth (bandwidth at half peak height in cm^{-1}; *C* and *D*) for Prodan at different temperatures and in different lipid environments. (*A* and *C*) POPC SBV (△), VLDL (○), LDL (●), and HDL_3 (□). (*B* and *D*) POPC-ether SBV (▲), LME (◇), SME (◆), and R-HDL (■).

spectrum of Prodan in LME contained a single maximum at 420 nm; at 37°C, the spectrum was bimodal. On the other hand, the spectrum of Prodan in SME was bimodal at 22°C, but contained only one peak at 37°C. The maxima in the bimodal spectra, which were observed at 420 nm and 500 nm, correspond to the fluorescence of Prodan in two different environments. The spectral position of one at 500 nm was similar to that of the phospholipid-water interface in SBV and R-HDL (Fig. 4 *C*). The other environment, which on the basis of its blue shift was more hydrophobic, could correspond to the triolein core. However, the fluorescence of Prodan in two native lipoproteins with hydrophobic cores, VLDL and LDL, did not contain a peak in this spectral region. In VLDL and LDL, therefore, either all of the probe partitions into the neutral lipid core, or the surface monolayers of the two lipoproteins are more hydrophobic than those of microemulsions. The latter assignment is made more likely by the observation that the fluorescence maximum of Prodan in model HDL composed of 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC), apoA-I, and large concentrations of cholesterol is characteristic of an environment that is more hydrophobic than POPC SBV and similar to that of the microemulsion core (Massey et al., 1985c). As expected, the spectral bandwidths of the bimodal spectra were broader than those containing a single peak and exhibited a temperature dependence similar to that of the shift in spectral maxima (Fig. 5 *D*). For both LME and SME, the change in spectral shape occurred near 35°C.

To circumvent the ambiguities associated with the partitioning of Prodan between the surface and core, fluorescence spectra of Patman, a probe that localizes exclusively to the lipid surfaces, were recorded as a function of temperature in native lipoproteins and lipoprotein models (Fig. 6). The fluorescence spectra of Patman in POPC SBV, LME, and SME were essentially independent of tempera-

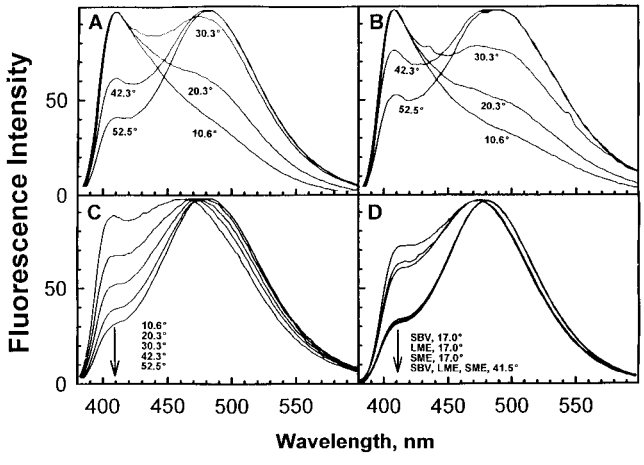

FIGURE 6 Fluorescence of Patman in native lipoproteins and lipoprotein models as a function of temperature. (*A*) VLDL. (*B*) LDL. (*C*) HDL_3. (*D*) POPC SBV, LME, and SME. The temperatures at which the spectra were recorded are noted in the panels.

ture (Fig. 6 *D*). The emission maxima recorded at 37°C were at 480 nm and shifted slightly to shorter wavelengths at lower temperatures. The fluorescence spectra of Patman in VLDL and LDL (Fig. 6, *A* and *B*) were bimodal; the emission maxima were between 480 nm and 490 nm at higher temperatures and around 410 nm at lower temperatures. In HDL_3 (Fig. 6 *C*), the changes in the emission maximum with temperature were similar to those in lipoprotein models containing POPC; on the basis of spectral width, the environment of Patman in HDL_3 was more heterogeneous than for lipoprotein models.

Phospholipase A_2 kinetics

The initial rates of phospholipase A_2-mediated hydrolysis of PC in the native lipoproteins, POPC SBV, and SME were determined by pH-stat titration. In the concentration range studied (1–6 mM phospholipid), the enzymatic activity changed little with respect to the amount of phospholipid present. Therefore, any observed differences in reaction velocity can be attributed to differences in maximum reaction velocity (V_{max}). POPC SBVs, with a relative activity of 100%, were the best substrate; the addition of 10 or 20 mol% cholesterol to POPC SBV did not appreciably alter V_{max} (Fig. 7). The relative activities of other substrate particles studied were SME 85%, VLDL 65%, HDL_3 15%, and LDL 8%. It is worth noting that the enzymatic activity varied inversely with the fluorescence polarization of TMA-DPH (Fig. 7, *inset*), indicating that catalysis was a direct function of surface fluidity.

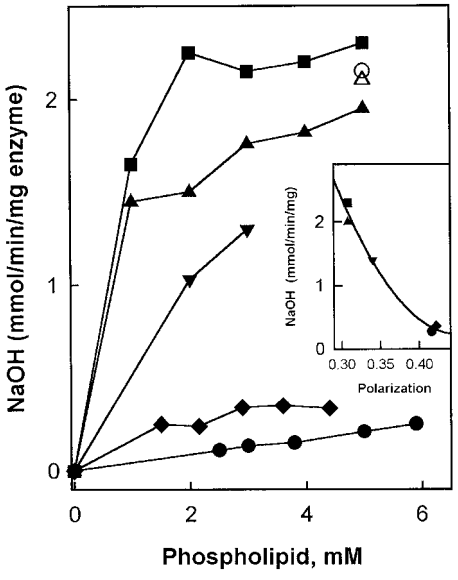

FIGURE 7 Kinetics of phospholipase A_2–mediated hydrolysis of PC in native and model lipoproteins determined by pH-stat titration. ▼, VLDL; ●, LDL; ♦, HDL_3; ■, POPC SBV; △, POPC SBV containing 10 mol % cholesterol; ○, POPC SBV containing 20 mol% cholesterol; ▲, SME. (*Inset*) Enzymatic rate at 3 mM phospholipid versus fluorescence polarization of TMA-DPH at 20°C.

The effects of apoC-III on the phospholipase A_2-mediated hydrolysis of MPNPC in lipoprotein models containing a PC-ether matrix and MPNPC were determined. MPNPC exhibits a change in its excimer fluorescence when cleaved by lipolytic enzymes. PC-ethers have frequently been used as stable matrices to study the interactions of lipolytic enzymes with phospholipid surfaces, because lipolysis causes little change in the macromolecular structure of these particles (Jain et al., 1982; DeBose and Roberts, 1983; Massey et al., 1985a; McLean and Jackson, 1985; Pownall et al., 1985; Bhamidipati and Hamilton, 1989). The rates of hydrolysis were directly proportional to the amount of apoC-III added, and even at a molar ratio of one apoC-III per vesicle, activity was profoundly increased (Fig. 8). In the absence of apoC-III, the rates (both measured and extrapolated to the *y* intercepts) were identical for POPC-ether SME, POPC-ether SBV, and DMPC-ether SBV. With the addition of apoC-III, the slopes differed by a factor of 2. POPC-ether LMEs were hydrolyzed at a much higher rate in both the absence and presence of apoC-III. In similar systems, the addition of apoC-III or other apolipoproteins to SBVs activates the CETP-mediated exchange of cholesteryl esters (Ohnishi and Yokoyama, 1993).

DISCUSSION

During the catabolism of plasma lipoproteins, apolipoproteins and lipids are transferred and exchanged among lipoprotein classes and subclasses. It has been shown that the size, surface properties, and chemical compositions of lipoproteins are important determinants of the distribution of some apolipoproteins among lipoprotein classes (Patsch et al., 1978). The physicochemical properties of the surface

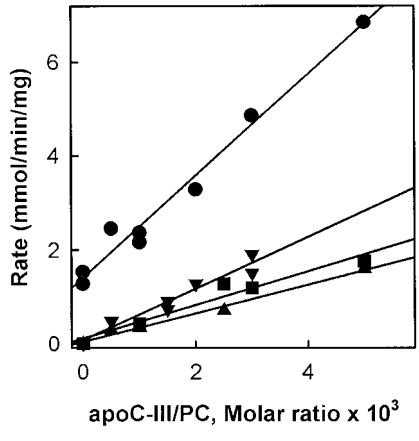

FIGURE 8 Stimulation of phospholipase A_2–mediated hydrolysis of MPNPC (3 mol%) by apoC-III. ■, DMPC-ether–MPNPC SBV (0.61 mM phospholipid); ▼, POPC-ether–MPNPC SBV (1.0 mM phospholipid); ●, POPC-ether–MPNPC–triolein LME (0.61 mM phospholipid); ▲, POPC-ether–MPNPC–triolein SME (0.61 mM phospholipid). The respective equations by sample for linear regression analysis of the data were ($y = 1060x + 1500$), ($y = 260x + 150$), ($y = 520x + 160$), and ($y = 310x + 170$), where *y* (mmol/min/mg) is the enzymatic activity and *x* (mol/mol) is the apo C-III/PC molar ratio.

monolayers of lipoproteins are also likely to determine the activities of many plasma proteins and their distribution among lipoproteins. These proteins include lipolytic enzymes, lipid transfer proteins, and apolipoproteins that are ligands for cell surface receptors or activators of lipid-catabolizing enzymes (McKeone et al., 1988; Derksen and Small, 1989; Ibdah et al., 1989). As reported here, studies of lipoprotein models identified several correlations between the compositional and structural features of the particle surface and the fluorescence behavior of several probes that localize to this region. Having established these correlations in model systems, we used them to determine the surface properties of native lipoproteins. Parallel studies on native lipoproteins and lipoprotein models can provide the groundwork for identifying the molecular and macromolecular properties that regulate lipoprotein catabolism.

Surface fluidity

Fluorescence polarization of TMA-DPH varies inversely with lipid fluidity. Unlike the commonly used probe 1,6-diphenyl-1,3,5-hexatriene (DPH), which partitions between the surface monolayer and the core in native lipoproteins and lipoprotein models (Li et al., 1990), the charged probe TMA-DPH is anchored at the monolayer surface, and the polarization values reflect only the fluidity of this region. This property is especially important for particles such as lipoproteins, which contain a hydrocarbon-like core. In the native lipoproteins studied here, the TMA-DPH fluorescence polarization measurements revealed increases in surface fluidity in the order $HDL_3 < LDL < VLDL$ (Fig. 2 *A*). HDL_3 and LDL showed much lower surface fluidity than VLDL; the rank order of fluidity is the same as that in which the ratio (protein + free cholesterol)/phospholipid decreases in these lipoproteins. These results are consistent with studies of model systems showing that the addition of cholesterol or proteins to phospholipids decreases surface fluidity (Mantulin et al., 1981; Wetterau and Jonas, 1983; Massey et al., 1985b).

Comparison of the fluorescence polarization of TMA-DPH in our POPC-containing lipoprotein models revealed increased surface fluidity in the order R-HDL < POPC SBV ≈ SME < LME (Fig. 2 *B*). As previously reported, increasing the apolipoprotein content of model HDL reduces the fluidity of the phospholipid matrix (Mantulin et al., 1981; Wetterau and Jonas, 1983; Massey et al., 1985b). POPC SBVs and SMEs, which have similar diameters, and VLDL exhibited nearly the same surface fluidities. The linearity of plots of fluorescence polarization according to temperature confirmed the absence of surface lipid-phase transitions from all native lipoproteins and lipoprotein models containing unsaturated phospholipids.

In LDL, HDL_3, POPC SBV, POPC-ether SBV, and R-HDL, the fluorescence polarization of HC was similar to that of TMA-DPH (Fig. 2, *C* and *D*). With both HC and TMA-DPH, the fluidity of R-HDL inferred from the fluorescence polarization was lower than that found in POPC SBV, again demonstrating that the proteins increase the order of the lipid phase. In VLDL, LME, and SME, the fluorescence polarization of HC was much lower than that of TMA-DPH. The differences in polarization are due to differences in the structures of the particles and the partitioning of these two probes between surface and core regions. HC contains a hydroxyl group that anchors the probe to the lipid-water interface in pure phospholipid matrices (Jonas et al., 1987). However, as with cholesterol, which also contains a single hydroxyl group, a fraction of HC may partition into the core of the particle (Lund-Katz and Phillips, 1986; Li et al., 1990), where the motion of the probe is no longer anisotropic. As a consequence, the fluorescence polarization of HC is lower than that of the cationic probe TMA-DPH, which is confined to the surface. Therefore, we assign the lower polarization and attendant higher fluidity to contributions from that fraction of the probe residing in the highly fluid core of neutral lipids.

Surface lateral mobility

The lateral diffusion of PUTA in the lipid surface was estimated from measurements of *E*/*M* versus mol% PUTA, a cationic probe that resides at the lipid-water interface and does not partition into the neutral lipid core. Among the lipid particles that were studied, the *E*/*M* ratio increased linearly with the microscopic concentration of PUTA (Fig. 3 *A*). There were only small differences in the calculated slope of *E*/*M* versus mol% PUTA among VLDL, LDL, and HDL_3. However, the values were two to three times less than those of model systems, indicating a much lower lateral mobility of PUTA in the surface lipids of these native lipoproteins than in models that are cholesterol free.

The similarity of surface monolayers of VLDL, LDL, and HDL_3 in the lateral mobility of PUTA (Fig. 3 *A*) contrasts with the differences in fluidity found with TMA-DPH (Fig. 2 *A*). This contrast may represent differences in the lateral organization of PUTA in different lipoprotein surfaces that are a function of the amount of surface-associated protein (Barenholz et al., 1996). Furthermore, the lateral diffusion and axial rotation rates of a phospholipid within a cholesterol-rich PC phase are similar to those of liquid-crystalline PC, even though the phospholipid acyl chains are highly ordered (Vist and Davis, 1990). According to both ^{31}P NMR spectroscopy and the excimer fluorescence of pyrenyl phospholipids, the phospholipids in the surface monolayer of LDL are sensitive to the phase transition of the neutral lipid core (Vauhkonen and Somerharju, 1989, 1990; Fenske et al., 1990). Although excimer fluorescence studies showed little difference in the surfaces of LDL and a variety of biological membranes (Vauhkonen and Somerharju, 1989; Vauhkonen et al., 1989; Fenske et al., 1990), ^{31}P studies have implied that the lateral diffusion of a phospholipid was much slower in LDL than in VLDL, HDL_3, and PC-triolein microemulsions.

Surface polarity measurements

The fluorescence spectra of Prodan and Patman are sensitive to the polarity of the headgroup region of phospholipids (Massey et al., 1985c; Jonas et al., 1987; Parasassi et al., 1994). The high quantum yields and spectral maxima of Prodan fluorescence in lipoprotein models consisting of only POPC or POPC-ether were consistent with location of the probe at the lipid-water interface of liquid-crystalline-phase phospholipids with similar degrees of hydration (Fig. 4 *C*, Table 1). Within the microemulsions, Prodan distributed between two distinct environments. The fluorescence maxima indicated that one was similar to that of the surface phospholipid in POPC SBV; the second was a much less polar environment. Compared with POPC SBV, the quantum yields were decreased by more than 50% (Table 1). The relative amounts of the two environments changed with temperature (Figs. 4 *B* and Fig. 5, *B* and *D*) and with the POPC/triolein ratio. The fluorescence properties of Patman were nearly the same in POPC SBVs and the microemulsions (Fig. 6 *D*). These results are consistent with the partitioning of Prodan almost exclusively into the neutral lipid core (Li et al., 1990).

For Prodan, the native lipoproteins exhibited only the less polar environment, which implies either that the probe partitioned solely into the neutral lipid core or that the environment within the interfacial region was less polar than that of POPC vesicles. The quantum yields in native lipoproteins and microemulsions were similar (Table 1). The spectroscopic properties of Patman, which is confined to the surface region by its charge, revealed the environment of this probe in the surface monolayer to be less polar in native lipoproteins than in lipoprotein models. Cholesterol is known to partially exclude water from phospholipid surfaces (Straume and Litman, 1987; Jonas, 1992; Parasassi et al., 1994); according to our Prodan fluorescence data, cholesterol reduces surface polarity, even in the liquid-crystalline phase. We conclude that the fluorescence spectra of Prodan and Patman in native lipoproteins were different from those observed in lipoprotein models that are cholesterol free, because the cholesterol excludes water from the phospholipid monolayer.

Phospholipase A_2 kinetics

Snake venom phospholipase A_2 was used as a structural probe of the surface of native plasma lipoproteins and lipoprotein models. POPC SBVs were the best substrate particles; their hydrolysis was little affected by the presence of up to 20 mol% cholesterol (Fig. 7). The reactivity of SMEs containing POPC was similar to that of POPC SBVs. By comparison, PC was poorly reactive in LDL and HDL_3. A comparison of the polarization values of TMA-DPH with the enzymatic rate indicates a good inverse correlation between lipid mobility and substrate reactivity (Fig. 7, *inset*).

The effects of apoC-III on phospholipase A_2 hydrolysis of PC in stable PC-ether matrices were studied in greater detail. In these systems, which contained only phospholipid, small amounts of apoC-III altered the surface properties in a dose-dependent manner and rendered the PC more reactive (Fig. 8). Thus, in our model systems, the addition of small amounts of the individual components—apolipoproteins or cholesterol—to the surface monolayer had little effect or an enhancing effect on the reactivity of PC to phospholipase A_2. In native lipoproteins, which contain higher (cholesterol + protein)/phospholipid ratios, the PC was much less reactive, suggesting that the presence of cholesterol blocks the stimulatory effect of apolipoproteins on phospholipolytic activity.

Several principles emerge from these studies. First, the higher the microviscosity of a phospholipid surface, the poorer it is as a phospholipase substrate. Second, the surface monolayer of a VLDL particle is much more fluid and a better substrate for lipolytic enzymes than are the surfaces of LDL and HDL_3. Third, the commonly used lipoprotein models, including SBVs, LMEs, and SMEs, have surface monolayers that are qualitatively similar to those of VLDL but are poorer models of LDL or HDL_3, which may limit their usefulness for some investigations in which surface properties of lipoprotein models must emulate those of native lipoproteins with great fidelity.

The surface compositions and structures of plasma lipoproteins are determined, in part, by fatty acids that are derived from diet. During lipolysis, fatty acids can accumulate at the lipoprotein surface because the rate of fatty acid transfer is much slower than lipolysis (Zhang et al., 1996; Massey et al., 1997). The surface monolayer differences between native lipoproteins and model systems illustrate the importance of parallel studies of both systems in the identification of compositional and structural factors that regulate lipoprotein catabolism. The many studies of the association of apolipoproteins with model lipoproteins have demonstrated that molecular packing of lipids and the structures of lipids and apolipoproteins are important determinants of the equilibrium binding and kinetics of transfer of apolipoproteins (McKeone et al., 1988; Derksen and Small, 1989; Ibdah et al., 1989; Small et al., 1991). This study suggests that similar correlations of surface structure with the distribution of other proteins that are involved in lipolysis, with lipid transfer proteins, and with other aspects of lipoprotein metabolism will help better define the relation between diet and lipoprotein metabolism.

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