Physical States of Surface and Core Lipids in Lipid Emulsions and Apolipoprotein Binding to the Emulsion Surface

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Plasma triglyceride-rich lipoproteins vary in lipid composition during their metabolism. We investigated the effects of the lipid composition of emulsion particles, specifically those of cholesterol enrichment and core replacement (replacing core triglyceride with cholesterol oleate), on the physical states of surface and core lipids. Steady-state and time-resolved fluorescence anisotropies were measured in lipid emulsions using 1,6-diphenylhexatriene to probe the core and 1,6-diphenylhexatriene analogues for the outer and inner hydrophobic portions of surface phospholipids. In the absence of cholesterol, core replacement had little effect on the surface rigidity, despite the large difference in core mobility. However, core replacement caused a marked increase in surface rigidity in the presence of cholesterol. Quenching experiments using the fluorescent cholesterol analogue, dehydroergosterol, indicated that core replacement allowed surface dehydroergosterol to redistribute from the inner to the outer regions in the emulsion surface. These results indicated that core replacement modulates the surface properties of the emulsion particles through the redistribution of cholesterol in the surface layers. Furthermore, core replacement significantly decreased the binding of apolipoprotein E to the emulsion surface, whereas the binding of apolipoprotein CII responded to the cholesterol enrichment. This binding behavior of exchangeable apolipoproteins may closely correlate with the location of surface cholesterol and the mobility of core lipids.

Human plasma chylomicrons, very low density lipoproteins, and high density lipoproteins contain apolipoprotein E (apoE), which plays a crucial role in lipoprotein metabolism through specific interactions with cell membrane receptors (1). In triglyceride (TG)-rich lipoproteins such as chylomicrons and very low density lipoproteins, the hydrolysis of core TG by lipoprotein lipase produces remnant particles that are rapidly recognized by cell receptors and taken up into the liver through receptor-mediated endocytosis (2–4). Alternatively, the apoE-mediated uptake of TG-rich particles by cell receptors is partially inhibited by other apolipoproteins that are associated with these lipoproteins, e.g. apolipoprotein Cs (5, 6). Thus, the transfer of apolipoprotein Cs and apoE to and from TG-rich lipoproteins exerts profound effects on their capacity to interact with cell receptors and, therefore, has a major regulating effect on plasma TG transport and on the remnant removal processes.

Several studies have shown that the lipid composition of lipoproteins and lipid emulsions influence their metabolic properties. The modulation of core TG content of low density lipoproteins (LDL; Ref. 7) and the lipid emulsions (8) alters the cellular uptake of these particles. The increase in the cholesterol (Chol) concentration of LDL leads to reduced uptake by macrophages (9), and the amount of free Chol also regulates the emulsion metabolism (10, 11). We have recently demonstrated the effects of Chol and cholesterol oleate (CO) on the metabolic process of the emulsions through lipolysis of TG and organ uptake (12).

These alterations in the metabolic behavior are thought to arise from changes in the conformation and binding properties of apolipoproteins. A close relationship between conformational changes in apolipoproteins and the thermal transition of surface and core lipids has been suggested (13, 14). Although the modification of surface and core lipid composition may play a role in the organization and the dynamics of lipids, little is known about the effect of the surface and core modifications on the motional state of lipids in lipoproteins and the emulsions.

In this study, we examined the effect of Chol enrichment and of replacing core TG with CO on the physical states of surface and core lipids in lipid emulsions. Emulsion particles containing only TG or CO represent extremes in the composition of lipoprotein core lipids. We assessed differences in the surface and core properties using the fluorescent probes: 1,6-diphenylethynylhexatriene (DPH) for probing the core lipids, as well as 1-[4-(trimethylamino)phenyl]phenylethynylhexatriene (TMA-DPH) and 1-palmitoyl-2-[3-(diphenylethynyl)propanoyl]-sn-3-phosphatidyldicholine (DPhPC) for different portions of the surface layers. The DPH moiety of TMA-DPH is located near the phospholipid-water interface, whereas that of DPhPC is aligned with the phospholipid acyl chains. Thus, the motion of the outer and inner hydrophobic portions of the surface phospholipids can be monitored using TMA-DPH and DPhPC, respectively. Furthermore, we estimated the relationship between the physical states of the surface and core lipids and the binding affinity of the exchangeable apolipoproteins, apolipoprotein CII (apoCII) and apoE. The results of these fluorescence and apolipoprotein binding studies demonstrated that the physical states of not only surface but also core lipids significantly affect the binding behavior of apolipoproteins.
EXPERIMENTAL PROCEDURES

Materials—Egg yolk phosphatidylcholine (PC) was kindly provided by Asahi Kasei Co. The purity (over 99.5%) was determined by thin-layer chromatography. Chol and CO were purchased from Sigma and used without further purification. Soybean TG obtained from Nacalai Tesque was purified using silica (Wakogel C-200; Wako Pure Chemicals) column to remove fatty acids, diglycerides, and monoglycerides. DPH, TMA-DPH, and DPHpPC were purchased from Molecular Probes. Dehydroergosterol was purchased from Sigma. All other chemicals were of special grade from Wako Pure Chemicals. Water was double distilled using a quartz still.

Preparation of Emulsions and Vesicles—TG emulsion particles were prepared as described (15) using a high pressure emulsifier (Nanomizer; Nanomizer Inc., Tokyo, Japan). Emulsion particles containing CO instead of TG were prepared in a similar manner: PC (or PC + Chol) and CO were dissolved in chloroform at a molar ratio of 1:2. After removing the solvent, the dried lipids were resuspended in 30 ml of 10 mM Tris-HCl, 150 mM NaCl, and 1 mM EDTA, pH 7.4, and emulsified at 1200–1300 kg/m² and 60–70 °C. The crude emulsions were placed in polycarbonate tubes and washed twice with the buffer in a Beckman SW 28 rotor at 27,000 rpm for 1 h to remove any contaminating vesicles. After removing large emulsions by centrifugation in a Beckman 50.1 rotor at 32,000 rpm for 10 min, homogeneous emulsion particles with a diameter of 100–130 nm (determined from quasi-elastic light scattering measurements; Photol LPA-3000/3100; Otsuka Electronic Co.) were obtained. PC vesicles with a diameter of about 100 nm were prepared by an extrusion method as described (15).

Steady-state Fluorescence Measurements—Diluted vesicle or emulsion suspensions were labeled with DPH (in tetrahydrofuran) or TMA-DPH (in dimethyl sulfoxide) to yield PC:probe molar ratios of 200–400:1 or 100–200:1, respectively. For the samples labeled with DPH or DPHpPC, lipids and DPH or DPHpPC stocks were mixed in chloroform at a PC:probe molar ratio of 200–400:1 before the preparation of vesicles or emulsions. Steady-state fluorescence anisotropy was measured using a SHIMADZU RF-5000 spectrofluorometer at 25 °C. Excitation was set at 360 nm through HOYA U360 and TOSHIBA UV-31 filters, and emissions were measured through a cutoff filter HOYA L42 at 434 nm. Samples were diluted to ensure that anisotropy values were not affected by light scattering.

Time-resolved Fluorescence Measurements—Fluorescence lifetimes and anisotropy decay were measured by a time-correlated single-photon counting method on a HORIBA multichannel-TAC NAES-550 system. The probe was excited through HOYA U360 and TOSHIBA UV-31 filters and detected using a cutoff filter (HOYA L42). The temperature was maintained at 25 °C. The total fluorescence decay S(t) was analyzed with an exponential decay function with the fractional amplitudes αi and the lifetimes τi.

\[
S(t) = \sum_{i=1}^{n} \alpha_i \exp(-t/\tau_i) \quad \text{(Eq. 1)}
\]

The mean lifetime (\(\tau\)) was defined as follows.

\[
\tau = \sum_{i=1}^{n} \alpha_i \tau_i = \sum_{i=1}^{n} \alpha_i \tau_i \quad \text{(Eq. 2)}
\]

The fluorescence anisotropy r(t) is expressed by the equation,

\[
r(t) = \frac{I_r(t) - G_l(t)}{I_l(t) + 2G_l(t)} \quad \text{(Eq. 3)}
\]

where I_r(t) and I_l(t) are the parallel and perpendicular polarized components of the fluorescence, respectively, and G_l represents the compensating factor for the anisotropy sensitivity of the instrument. According to the wobble-in-cone model, r(t) is expressed as (16–18),

\[
r(t) = (r_0 - r_c) \exp(-t/\phi) + r_c \quad \text{(Eq. 4)}
\]

where \(r_0\) is the rotational correlation time, \(r_c\) is the initial anisotropy at \(t = 0\), and \(r_c\) is the residual anisotropy at infinite time. The order parameter S and the cone angle \(\theta_c\) were calculated using the following equation.

\[
S = \left( \frac{r_c}{r_0} \right)^{1/2} = \frac{1}{2} \cos(\phi)(1 + \cos \theta_c) \quad \text{(Eq. 5)}
\]

The wobbling diffusion constant \(D_w\) was estimated as follows (16),

\[
D_w = \frac{(1 - S^2)^{1/2}}{2 \cos(\phi)} \quad \text{(Eq. 6)}
\]

RESULTS

Steady-state Fluorescence Anisotropy—Fig. 2 shows the steady-state anisotropy of DPH, TMA-DPH, and DPHpPC for vesicles and emulsions. Although both the surface and core phases of emulsions are accessible to DPH, we confirmed that...
over 95% of DPH was located in the core from the partition analysis of DPH between the surface and core phases. The anisotropy value of DPH in CO-PC emulsions was much higher than that for TG-PC emulsions, consistent with the fact that TG is a disordered liquid, whereas CO exists as a smectic-like ordered structure at that temperature (20). However, the difference in the core mobility between these emulsions had a small effect on the anisotropy of the surface layers. As shown in Fig. 2, the anisotropy value in CO-PC emulsions was similar for TMA-DPH and slightly higher for DPHpPC compared with those in TG-PC emulsions. For both surface probes, the anisotropy in TG-PC and CO-PC emulsions exhibited higher values than that in vesicles (15).

Fig. 3 shows the fluorescence anisotropy of TMA-DPH in TG-PC and CO-PC emulsions as a function of the surface mole percent of Chol. We have reported the characteristics of TG-PC emulsion particles in terms of the lipid composition and surface distribution of Chol (15). Although PC and Chol compositions of CO-PC emulsion particles were similar to those in TG-PC emulsions, the surface content of Chol in CO-PC emulsions tends to be smaller than those in TG-PC emulsions due to the smaller distribution coefficient of Chol between the surface and core (2). Chol showed a delayed rigidification of the TG-PC emulsion surface (15), while the anisotropy values in CO-PC emulsions increased rather linearly with the increasing surface content of Chol (Fig. 3). In contrast, the anisotropy values of DPHpPC increased with surface Chol in a similar manner in both emulsions (Fig. 3, inset). These results indicated that, in general, Chol affected the rigidity of the surface layers both in TG-PC and CO-PC emulsions, but this depended on the depth of the surface layers, i.e. the outer and inner layers in TG-PC emulsions.

Time-resolved Fluorescence Anisotropy—We further investigated the effect of Chol on the molecular orientation and dynamics of DPHpPC in surface layers of vesicles and emulsions using time-resolved fluorescence measurements. Since DPHpPC itself is a phospholipid analogue, it should reflect the motional dynamics of phospholipid molecules in the membrane. Fig. 4 shows the typical fluorescence decay of DPHpPC, and Table I summarizes the results of the time-resolved fluorescence anisotropy and lifetime measurements of DPHpPC.

Both in vesicles and emulsions, the fluorescence decay of DPHpPC was accurately represented by the biexponential fit with the minor lifetime component in the absence of Chol, whereas the monoeponential fit showed satisfactorily low $\chi^2$ values in the presence of Chol. An increasing amount of Chol led to a modest increase in the mean lifetime $\tau$, both in vesicles and in the emulsions as shown in Table I. Since Chol is known to exert a “homogenizing” effect in lipid bilayers due to a decrease in membrane water permeability (21, 22), these results indicated that Chol exerts the same effect in emulsion surface layers. In addition, the lifetime of DPHpPC was shorter in the emulsion surface than in the vesicles, indicating that the more polar environment of DPHpPC probably arose from the presence of adjacent core lipids with ester bonds.

The values of the order parameter, $S$, the rotational correlation time, $\phi$, the cone angle, $\theta_c$, and the wobbling diffusion constant, $D_W$, are also given in Table I. In the absence of Chol, DPHpPC was more ordered in the emulsion surface than in vesicles, as reflected by the increase in the order parameter and the decrease in the cone angle. However, the rate of rotational motion reflected in the values of $\phi$ and $D_W$ remained unaltered in TG-PC emulsions but decreased in those in CO-PC, as compared with the vesicles. Chol increased the order and decreased the rotational motion for all vesicles and emulsions. In the
presence of a high content of Chol (40 mol%), the difference in
the order between the vesicles and the emulsions disappeared,
but the rotational motion in CO-PC emulsions seemed to
remain slower than that in vesicles and TG-PC emulsions.

Quenching of Dehydroergosterol—Based on the finding that
the effect of Chol on the surface rigidity differed between
TG-PC and CO-PC emulsions, we considered that the location
of Chol might be different in the surface layers between the two
emulsions. To investigate this possibility, we performed
quenching experiments using the fluorescent Chol analogue,
dehydroergosterol. This compound is a close structural ana-
logue of Chol, and it has a similar surface-core distribution
coefficient values of 19.4 for TG-PC emulsions (15) and
10.4 for CO-PC emulsions (2). For all vesicles and emulsions, the
KSV values were similar, but dehydroergosterol in TG-PC emul-
sions seemed to be less exposed to the quencher than that in
those of CO-PC. In PC vesicles and CO-PC emulsions, the fa values obtained from modified Stern-Volmer plots were identical
to the predicted values, whereas the observed fa value was
significantly lower than that predicted for TG-PC emulsions,
indicating that there is a compartment of dehydroergosterol
inaccessible to the aqueous phase in surface layers of TG-PC
emulsions.

Apolipoprotein Binding to Emulsion Particles—We exam-
ined the binding of the exchangeable apolipoproteins, apoCII and
apoE, to the emulsion surface in human serum to investi-
gate the effect of the lipid composition of the emulsions on the
binding behavior of apolipoproteins. As shown in Table III,
apoCII and apoE readily associated with TG-PC emulsions
when incubated with serum, where apoCII had a markedly
higher binding capacity than apoE for all emulsions examined
(24, 25). The addition of Chol to TG-PC emulsions remark-
ably reduced the amount of bound apoCII, whereas there was a
slight decrease in apoE binding. Replacing core TG with CO in
the emulsions more effectively reduced the binding of apoE
than of apoCII. Both apoCII and apoE failed to bind to the
surface of emulsion particles consisting of a high content of
Chol (40 mol%) and core CO.

DISCUSSION

Structure and Dynamics of Emulsion Surface—Previous
study has shown that the surface monolayers of TG-PC emul-

### Table I

| PC vesicles<sup>a</sup> | 0% Chol | TG-PC emulsions<sup>b</sup> | 0% Chol | CO-PC emulsions<sup>c</sup> | 0% Chol |
|------------------------|---------|---------------------------|---------|---------------------------|---------|
| <sup>a</sup> | S (ns) | f<sub>W</sub> (deg) | D<sub>b</sub> (10<sup>7</sup> s<sup>-1</sup>)<sup>0</sup> | S (ns) | f<sub>W</sub> (deg) | D<sub>b</sub> (10<sup>7</sup> s<sup>-1</sup>)<sup>0</sup> | S (ns) | f<sub>W</sub> (deg) | D<sub>b</sub> (10<sup>7</sup> s<sup>-1</sup>)<sup>0</sup> |
|------------------------|---------|---------------------------|---------|---------------------------|---------|
| PC vesicles<sup>a</sup> | 0% Chol | 7.0 | 0.50 ± 0.02 | 3.8 ± 0.2 | 52 | 4.6 |
| 20% Chol | 7.5 | 0.73 ± 0.01 | 2.6 ± 0.1 | 36 | 3.9 |
| 40% Chol | 7.8 | 0.85 ± 0.02 | 2.3 ± 0.2 | 26 | 2.4 |
| TG-PC emulsions<sup>b</sup> | 0% Chol | 6.3 | 0.61 ± 0.03 | 3.5 ± 0.3 | 45 | 4.1 |
| 20% Chol | 6.6 | 0.75 ± 0.02 | 2.8 ± 0.2 | 35 | 3.3 |
| 40% Chol | 7.0 | 0.86 ± 0.02 | 2.6 ± 0.3 | 26 | 2.1 |
| CO-PC emulsions<sup>c</sup> | 0% Chol | 6.3 | 0.64 ± 0.04 | 4.8 ± 0.3 | 42 | 2.7 |
| 20% Chol | 6.7 | 0.78 ± 0.04 | 4.0 ± 0.3 | 33 | 2.1 |
| 40% Chol | 7.1 | 0.86 ± 0.02 | 3.0 ± 0.3 | 25 | 1.7 |

<sup>a</sup> Lifetime values were reproducible within ±0.1 ns.
<sup>b</sup> The estimated uncertainties of S and D<sub>b</sub> were 3 degrees and 10%, respectively.
<sup>c</sup> The percentage of Chol represents the initial molar ratio of PC and Chol. For example, TG-PC emulsions (20% Chol) represent the TG-PC emulsions prepared at an initial PC:Chol molar ratio of 4:1.

### Table II

| Accessibility of dehydroergosterol in vesicles and emulsions to iodide quenching at 25 °C |
|-----------------|-----------------|-----------------|-----------------|
| K<sub>SV</sub> (10<sup>-1</sup>)<sup>a</sup> | f<sub>a</sub> | f<sub>a</sub>(cal) |
|-----------------|-----------------|-----------------|
| PC vesicles (Chol = 20%) | 20.2 ± 0.8 | 0.43 ± 0.01 | 0.44<sup>b</sup> |
| TG-PC emulsions (Chol = 20%) | 17.8 ± 1.6 | 0.56 ± 0.03 | 0.82<sup>b</sup> |
| CO-PC emulsions (Chol = 20%) | 22.5 ± 1.1 | 0.55 ± 0.02 | 0.53<sup>b</sup> |

<sup>a</sup> K<sub>SV</sub> and f<sub>a</sub> are the Stern-Volmer quenching constant and the fraction of accessible dehydroergosterol, respectively, determined from modified Stern-Volmer plots. f<sub>a</sub>(cal) represents the fraction of accessible dehydroergosterol calculated from: (a) the lamellarity of vesicles determined by 31P NMR; and (b) the lipid composition of emulsions (see text for details).

### Table III

| Binding of apolipoproteins to emulsions when incubated with human serum at 37 °C |
|-----------------|-----------------|-----------------|
| Emulsion | ApoCII | ApoE |
|-----------------|-----------------|-----------------|
| g"/g PC | mol/mol PC | g"/g PC | mol/mol PC |
| TG-PC | 0.159 | 13.9 | 0.030 | 0.68 |
| TG-PC (Chol = 40%) | 0.049 | 4.3 | 0.024 | 0.54 |
| CO-PC | 0.119 | 10.4 | 0.011 | 0.25 |
| CO-PC (Chol = 40%) | 0.026 | 2.3 | 0 | 0 |

apoCII and apoE readily associated with TG-PC emulsions when incubated with serum, where apoCII had a markedly higher binding capacity than apoE for all emulsions examined (24, 25). The addition of Chol to TG-PC emulsions remarkably reduced the amount of bound apoCII, whereas there was a slight decrease in apoE binding. Replacing core TG with CO in the emulsions more effectively reduced the binding of apoE than of apoCII. Both apoCII and apoE failed to bind to the surface of emulsion particles consisting of a high content of Chol (40 mol%) and core CO.

Apofactord is more rigid than PC bilayers by means of steady-state anisotropy and lifetime measurements of TMA-

DPH (15). In this study, we measured the time-resolved anisotropy decay of DPHPC in bilayers and emulsion surface layers to obtain information about the structure and dynamics in the inner surface layers. The S and ϕ values in Table I show that the surface layers of TG-PC emulsions were more ordered than PC bilayers, whereas the rotational motion of surface lipids in TG-PC emulsions was similar to that for bilayers. These results could be explained on the basis of the interaction between surface and core lipids. The surface monolayer of vesicles abuts the fatty acyl chains of the inner phospholipid monolayer, whereas the monolayer of emulsion particles abuts the neutral lipid core. The acyl chains of core TG, therefore, could partially penetrate into the hydrophobic interior of surface monolayers in TG-PC emulsions, resulting in a restriction of motional freedom and consequently, an increase in the order of acyl chains of the surface PC in the emulsions. However, the rotational motion itself of surface layers in TG-PC emulsions was not affected by partial penetration of the core TG, probably because of the similar mobility of TG and PC acyl chains (see the data for DPH in Fig. 2). This interpretation is further supported by the data for CO-PC emulsions. That is, the partial penetration of core CO increased the order of surface PC in
CO-PC emulsions in a similar manner to TG-PC emulsions, whereas the rotational motion of surface PC in the former was more restricted than in those of the latter, because the structure of core CO is more ordered than that of TG. Another interpretation of the different effect of core TG and CO on the rotational motion of surface PC is as follows. The Chol moiety of CO penetrates into surface layers as well as the acyl chain moiety of CO, whereas TG has only acyl chains that penetrate into surface layers. The interaction of core CO with surface PC thus may cause an increase in the order and a decrease in the rotational motion of PC acyl chains, as usually seen in the effect of Chol on PC acyl chains (Table I). The interaction of the surface phospholipids with the core lipids in the emulsion particles is known to cause broadening (26) and an elevation in the temperature (20, 27) of the phase transition of dimyristoylphosphatidylcholine monolayers in the emulsions. We also found that the phase transition of the dipalmitoylphosphatidylcholine in TG-dipalmitoylphosphatidylcholine emulsions was significantly broadened as compared with that for dipalmitoylphosphatidylcholine bilayer vesicles.2

In contrast to these profound differences in the organization and dynamics of the surface lipids between the emulsion particles and the vesicles, the physical state of the core lipids seemed to have a minor influence on the surface properties. Although the core lipids were more ordered in CO-PC emulsions than in TG-PC emulsions, there was little difference in the anisotropy of the outer surface probe, TMA-DPH (Fig. 2). These results are consistent with the findings that the motional states of the surface and core lipids are relatively independent in reconstituted LDL (28). It is likely that the physical state of core lipids in LDL influences the apolipoprotein B conformation, not through an alteration in surface structure but through the direct interaction of apolipoprotein B with the core lipids, as suggested by Bañuelos et al. (14).

Chol Location in Emulsion Surface—Chol can distribute between the surface and core phases in lipid emulsions (29, 30) and lipoproteins (31). Although Chol preferentially partitions into the surface, the incorporation of CO into core TGs increases the distribution of Chol into the core phase, arising from the association of Chol with CO in the core (2). From the surface-core phase equilibrium consideration, we estimated that over 80% of the Chol was associated with the surface phase in TG-PC emulsions studied here, whereas only about one-half of the Chol was located at the surface in CO-PC emulsions.

Chol significantly affects the structure and physical properties of bilayers. Chol is considered to be located so that the 3-hydroxy11 is in the immediate vicinity of the phospholipid ester carbonyl (32, 33), probably due to hydrogen bonding between the hydroxyl of Chol and the carbonyl groups (34, 35), and it markedly enhances the orientational order and the packing of lipid acyl chains in the liquid-crystalline state (36–38). In contrast, the effects and location of Chol in the emulsion surface layers remain uncertain. Reisinger and Atkinson (39) have revealed that Chol broadens the thermal transition of dimyristoylphosphatidylcholine in CO-dimyristoylphosphatidylcholine microemulsions, suggesting that Chol is located primarily in the microemulsion surface. As shown in Table I, Chol increased the order parameter and decreased the rotational motion of DPHpPC in emulsion surface layers as well as in bilayers (40, 41). We identified increases in the fluorescence anisotropy of TMA-DPH and DPHpPC in CO-PC emulsions with increasing surface Chol content (Fig. 3). In TG-PC emulsions, however, the increase in the anisotropy of TMA-DPH caused by Chol tended to be delayed (15), whereas the fluorescence anisotropy of DPHpPC changed in a manner similar to that seen in CO-PC emulsions, indicating that the ordering effect of Chol is significant in the inner hydrocarbon region, rather than in the outer region.

One explanation for these findings is that Chol is located in a different manner on the surface layers of TG-PC and CO-PC emulsions. For TG-PC emulsions, we have proposed (15) that Chol can be accommodated in the surface membranes spanning the surface-core interface with the hydroxyl group in the opposite direction, presumably by hydrogen bond formation between the carbonyl groups of TG and the hydroxyl group of Chol. If so, the different effects of Chol on the molecular order of TMA-DPH and DPHpPC could be explained by an arrangement in which Chol is associated with surface phospholipids but located deep in the inner hydrocarbon region. Since only one carbonyl group is present in CO compared with three for TG, CO is thought to be less effective in terms of hydrogen bonding with Chol. Thus, replacing core TG with CO would cause the core-adjacent surface Chol to be squeezed toward the outer parts of the surface layers. The marked differences in the anisotropy of TMA-DPH between TG-PC and CO-PC emulsions shown in Fig. 3 are likely to result from this redistribution of Chol in the surface layers rather than the direct interaction of surface and core lipids.

The quenching studies using dehydroergosterol indicated that some surface-associated Chol is inaccessible to the aqueous phase in TG-PC emulsions, whereas all of it is accessible in CO-PC emulsions. This suggested that surface Chol in TG-PC emulsions is located in a deep hydrocarbon region of surface layers, consistent with the notion of the Chol location discussed above. However, the fraction of surface Chol with a hydroxyl group facing the aqueous phase is not known from the quenching results because the location of Chol could vary continuously with the depth in the hydrocarbon region. Direct information using nuclear magnetic resonance is required to determine the precise location of Chol in the emulsion surface monolayers.

Apolipoprotein Binding to Emulsion Surface—The data in Table III indicate that the core mobility modulated by replacing TG with CO is a major determinant for apoE binding to the emulsions. apoE is a 299-residue protein that contains the 22-kDa receptor-binding and carboxyl-terminal lipid-binding domains. The lipid-binding domain contains two types of amphipathic helices, class A and G, which are responsible for lipid binding affinity (42). The 22-kDa domain contains a four-helix bundle arrangement. This bundle is thought to undergo a conformational change when associated with lipid, such that it opens up with a broad hydrophobic face (about 3000 Å2) that can interact with lipid (4). Thus, the binding of apoE to the lipid surface will create large packing defects in the hydrophobic interior of the surface layers. If so, the core TG could fill the packing defects by deeply penetrating toward the surface to allow apoE to bind to the emulsion surface. The penetrating ability of CO for filling the packing defects would be weaker than that of TG due to the ordered structure of CO. The large difference between the mobility of TG and CO, therefore, must result in the preferential binding of apoE to TG-PC, rather than to CO-PC emulsions. This interpretation may be closely related to the finding that 3-4-fold more pig apolipoprotein AI (261 residues) binds to TG-PC emulsions than to PC vesicles.2 This may be because the lack of core penetration in PC vesicles causes it to have a poor binding capacity for apolipoproteins.

In contrast, replacing the core lipids had little effect on the binding of apoCII to the emulsions (Table III). apoCII is a 78-residue small protein with high affinity for the lipid surface.

2 H. Saito, T. Minamida, I. Arimoto, T. Handa, and K. Miyajima, unpublished data.

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through a well defined class A helical domain (42). Based on the snorkel model proposed by Segrest et al. (43), the class A helices of apoCII are thought to insert into surface layers such that the amphipathic helices are buried within the hydrophobic interior of phospholipid monolayers. The binding of apoCII to the lipid surface will not, therefore, need as much penetration of core lipids to fill the packing defects in the hydrophobic interior of surface monolayers as apoE, resulting in the high binding affinity of apoCII to CO-PC emulsions.

Previously, we have demonstrated that PC has large lateral attractive interactions with apolipoprotein AI, whereas very low interactions between Chol and apolipoprotein AI occurred at the triolein-saline interface (44). In this study, Chol reduced the binding affinity of apolipoproteins, but the binding behavior of apoCII and apoE responded differently to Chol enrichment. Chol increases the lipid molecular order and thereby reduces the conformational flexibility of lipid acyl chains. This motional restriction of PC by Chol may be unfavorable for the lipid binding of class A amphipathic helix with the "snorkeling" structure, presumably because of the inhibition of the helix insertion into the hydrophobic interior of surface layers. In this respect, the presence of Chol in the emulsion surface will significantly decrease the binding affinity of class A helices in apoCII. The weak effect of Chol on the binding affinity of apoE may be due to the higher content of the class G helix than the class A helix in apoE (42). For the complete dissociation of apoE from the emulsion surface, not only Chol enrichment but also replacing core TG with CO is needed.

Physiological Significance—During the metabolism of the plasma TG-rich lipoproteins, chylomicrons, and very low density lipoproteins, such as Chol enrichment, plays an important role in lipoprotein metabolism. The redistribution of Cholin on the surface layers of the emulsions and thereby modulates the surface properties. Furthermore, the reduced mobility of core lipids arising from replacing TG with CO appears to decrease the penetration of the core lipids for filling packing defects in the surface layers. As a result, exchangeable apolipoproteins such as apoCII and apoE will dissociate from the Chol-enriched, CO-core emulsions that represent the lipid composition of LDL.

In TG-rich lipoproteins, the hydrolysis of core TG produces Chol-enriched remnant particles. During this process, the bound apolipoprotein CS begins to leave the remnant particles, while apoE remains bound to the remnant surface, thus mediating the uptake of the particles. This binding behavior of exchangeable apolipoproteins during the metabolic process is consistent with the present findings that apoCII dissociated from the Chol-enriched emulsion particles, whereas apoE remained bound. Our results suggested that the binding sensitivity of apolipoproteins for the lipid composition of lipoproteins, such as Chol enrichment, plays an important role in lipoprotein metabolism.
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