Fluorescence Study of the Motional States of Core and Surface Lipids in Native and Reconstituted Low Density Lipoproteins†

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ABSTRACT: Low density lipoproteins (LDL) consist of an apolar core of cholesterol esters and triglycerides surrounded by a monolayer of phospholipid, cholesterol, and a single molecule of apolipoprotein B (apoB-100). To determine the influence of core and surface constituents on the surface order of LDL, we have measured core and surface order parameters for native LDL, and reconstituted LDLs (rLDL) whose apolar core lipids were extracted and replaced with either cholesterol oleate (CO) or triolein (TO). Order parameters were measured by fluorescence depolarization of diphenylhexatriene (DPH), which is located primarily in the core, and of trimethylammoniumdiphenylhexatriene (TMA-DPH), which is anchored at the water–phospholipid interface. DPH order parameters for LDL reconstituted with TO (r-[TO]LDL) were much lower than those for LDL reconstituted with CO (r-[CO]LDL), consistent with the physical properties of TO, a nonviscous liquid at all temperatures studied, and CO, which exists in a liquid crystalline or viscous liquid state at the temperatures studied. Although core cholesterol esters in r[CO]LDL and native LDL undergo distinct order–disorder transitions, these transitions were not detected by DPH. This is most likely due to the difference between the time scale for end-over-end tumbling of cholesterol esters and the fluorescence lifetime of DPH. Despite the fact that the core lipids of r-[CO]LDL were much more ordered than those of r-[TO]LDL, surface order parameters for both lipoproteins were similar. We conclude that the motional states of the core and surface lipids are relatively independent. Surface order parameters for native LDL were higher than those for reconstituted LDLs. This was attributed to the presence of unesterified cholesterol in the surface of native LDL, and its absence in reconstituted LDL. Finally, the outer surface of r-[CO]-LDL was shown to be more ordered than that of unilamellar vesicles. We suggest that this is due to the presence of apoB-100 and neutral lipid molecules in the highly curved surface of LDL which reduce the motional freedom of surface phospholipids.

Low density lipoprotein (LDL)[1] is the major carrier of cholesterol esters in plasma. It exists as a quasispherical particle with a diameter of about 210 Å and contains two domains: a central apolar core composed of cholesterol esters and triglycerides, and an external shell composed of phospholipid, cholesterol, and a single molecule of apolipoprotein B-100 (apoB-100). ApoB-100 ($M_r \sim 550\,000$) plays a structural role in LDL and also contains a binding site which is recognized by LDL receptors and thus mediates the removal of LDL from circulation by the liver. This process plays a crucial role in the maintenance of cholesterol homeostasis (Brown & Goldstein, 1986).

A number of studies have shown that the triglyceride content of the cholesterol ester-rich core LDL modulates the interaction of LDL with its receptor. Triglyceride-enriched LDL obtained from hypertriglyceridemic patients as well as LDL which has been enriched in vitro are taken up less efficiently by cultured cells (Aviram et al., 1990; Kinoshita et al., 1990); conversely, LDL which has been depleted of triglyceride in vitro is taken up more efficiently (Avriam et al., 1990). In vivo, LDL core triglyceride levels are not only altered in hypertriglyceridemic individuals but are also affected by diet (Teng et al., 1983; Pownall et al, 1980., Tall et al., 1978; Kroon & Seidenberg, 1982).

The mechanism whereby the core lipids modulate binding of LDL to the LDL receptor is not known, although the unique properties of the LDL core lipids suggest that a direct interaction between the core and surface domains may play a role. LDL cholesterol esters undergo a thermotropic order–disorder transition close to physiological temperatures (Deckelbaum et al., 1977). Below the transition temperature, the cholesterol esters exist in a radial smectic state, while they exist in a liquid state above the transition temperature (Deckelbaum et al., 1977; Kroon, 1981). The fact that the transition temperature depends on the core triglyceride content (Deckelbaum et al., 1977; Tall et al., 1978) suggests that the physical state of the core lipids may influence the surface structure of LDL and hence the interaction of apoB-100 with cell membrane LDL receptors (Deckelbaum et al., 1977). An altered LDL surface structure could also affect the interaction of LDL with tissues by receptor independent mechanisms as well as its susceptibility to in vivo oxidative modification. Recent studies have emphasised the role of LDL lipid oxidation in atherogenesis (Steinberg, 1989). For a full understanding of these phenomena a detailed knowledge of the effects of the core structure on the molecular structure of the surface and of apoB-100 is required.

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[1] Abbreviations: ApoB-100, apolipoprotein B-100; LDL, low density lipoprotein; rLDL, reconstituted LDL; r-[CO]LDL and r-[TO]LDL, heptane extracted LDL reconstituted with cholesterol oleate and triolein, respectively; TG, triglyceride; CE, cholesterol ester; C, cholesterol; PL, phospholipid; CO, cholesterol oleate; TO, triolein; DPH, 1,6,-diphenyl-hexa-1,3,5-triene; TMA-DPH, 1-[4-(trimethylamino)phenyl]-6-phenyl-hexa-1,3,5-triene.

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Table 1. Chemical Composition of Native and Reconstituted LDL

sample	mass ratio (CE + TG)/protein	mass ratio C/PL
r-[CO]LDL	1.6	0
r-[TO]LDL	1.7	0
native LDL	2.2	0.45

In the present studies we investigate the core and surface structures of LDL and specifically address the question of whether the LDL core lipids influence the organization and motional state of the surface phospholipids. To assess differences in these properties, we utilized differential phase fluorimetry to measure order parameters for the fluorescent probes 1,6-diphenylhexa-1,3,5-triene (DPH) and a charged analogue 1-[4-(trimethylamino)phenyl]phenylhexa-1,3,5-triene (TMA-DPH). The latter by virtue of its charged trimethylammonium group is anchored at the phospholipid headgroup–water interface, while DPH itself is located within the lipophilic domain bounded by the phospholipid headgroups. Thus the degree of order of the surface shell and of the hydrophobic interior of the LDL particle could be measured independently.

To compare the effects of core lipids on the surface properties of LDL, we used the procedure of Krieger et al. (1978) to prepare reconstituted LDLs (rLDL) which differed only in their core lipid contents. Specifically, we compared LDLs reconstituted with either CO or TO. These are referred to as r-[CO]LDL and r-[TO]LDL, respectively, and represent the compositional extremes of LDL core lipids (the normal triglyceride content of LDL is less than 10% by weight). These particles should therefore display the largest possible differential effects on the LDL surface properties.

EXPERIMENTAL PROCEDURES

Materials. Cholesterol oleate and triolein were purchased from Applied Science Inc. The fluorophores DPH and TMA-DPH were from Molecular Probes, Inc. (Eugene, OR). Blood was obtained from healthy volunteers. Other reagents used were obtained from the same sources as described previously (Krieger et al., 1978; Kroon & Krieger, 1981).

Lipoproteins. Human LDL (d = 1.019–1.063 g/L) was prepared by ultracentrifugation as previously described (Kroon, 1981). rLDL was prepared as described previously (Kroon & Krieger, 1981). Endogenous neutral lipids, including cholesterol esters (CE), triglycerides (TG), and unesterified cholesterol (C), were first removed by extraction with heptane and subsequently replaced with either cholesterol oleate or triolein. In each reconstitution, 1.9 mg of LDL protein was extracted and incubated with 200 μL of heptane containing 6 mg of cholesterol oleate or triolein. Heptane was evaporated with a flow of nitrogen for 30 min, and final traces were removed overnight under high vacuum. The rLDL was dissolved in 10 mM sodium phosphate, pH 7.5, and filtered through a 1-μm Unipore membrane (Bio-Rad). Samples were stored at 4 °C and were used within 1 week. The chemical compositions of the rLDLs (see Table 1) were similar to those reported previously (Kroon & Krieger, 1981).

LDL lipids were extracted by the Bligh–Dyer procedure (Bligh & Dyer, 1959). Phospholipids (PL) were isolated by silicic acid (Unisil, Williamsburg, PA) column chromatography. Chloroform was used to elute neutral lipids prior to elution of phospholipids with methanol. The elution profile was monitored by TLC using chloroform–methanol–water (65:25:4 v/v/v) as a solvent. Lipids were dissolved in chloroform and dried under nitrogen and subsequently overnight under high vacuum. Buffer (10 mM sodium phosphate, pH 8.5) was added to give a concentration of 0.2 mg of phospholipid/mL of buffer. Unilamellar vesicles were prepared by sonication under an atmosphere of nitrogen with a microtip probe (Heat System Ultrasonics Inc., Plainfield, NY), with cooling at 4 °C. Following sonication, the solution was centrifuged at 50000g for 20 min. The supernatant fraction was used for subsequent experiments on the same day.

Differential Phase Fluorimetry. Fluorescence measurements were made on an SLM 4800 phase-shift fluorimeter operated in the T format (Lakowicz et al., 1979) and controlled by an HP45 computer. Measurements were made in a thermostated sample holder. The sample temperature was measured using a digital thermocouple temperature monitor (Newport Laboratories Inc.) interfaced with the HP45. Steady-state anisotropies (r), fluorescence lifetimes (τ), and differential lifetimes ($\Delta\tau$), the difference between lifetimes measured perpendicular and parallel to the exciting light, were measured as previously described (Lakowicz et al., 1979). Fluorescence lifetimes were obtained by using incident light modulated at 30 MHz and by measuring the phase shift of light emitted by the sample relative to that of a glycogen scattering solution.

Excitation was at 360 nm for DPH and at 340 nm for TMA-DPH. Emission filters consisted of 2 mm of 1 M $NaNO_2$ and a Corning 3–73 filter. Samples were purged with nitrogen for 10 min before measurements were made by slowly bubbling nitrogen through the samples. The sample chamber was maintained under an atmosphere of nitrogen during each experiment.

Using values of r, τ, and $\Delta\tau$, the limiting anisotropy r_∞ was calculated as described (Lakowicz et al., 1979) using a computer program kindly provided by Dr. Frank Prendergast, Department of Biochemistry and Molecular Biology, Mayo Foundation. Order parameters (S) were calculated from the relationship

$$S^2 = r_\infty / r_0$$

where r_0 is the fluorescence anisotropy observed in the absence of depolarizing rotations and was taken to be equal to the theoretical value of 0.390.

Analytical Techniques. Protein concentrations were measured using a modification of the Lowry procedure (Petersen, 1977) with bovine serum albumin as a standard. Phosphate was determined by complexation of the phospholipids with ammonium ferrothiocyanate (Stewart, 1980). The esterified and unesterified cholesterol content of the lipoproteins was determined as previously described by gas–liquid chromatography after lipid extraction by the method of Folch (Kroon et al., 1982). Triglyceride concentrations were measured using a Worthington Diagnostics Kit (Freehold, NJ).

RESULTS AND DISCUSSION

In this study we examined the motional state of lipids in the core and surface domains of native and reconstituted LDLs with the fluorophores DPH and TMA-DPH. These molecules probe different regions of LDL; TMA-DPH is anchored at the surface–water interface because of its charged trimethylammonium group (Prendergast et al., 1981) and reaches into the phospholipid monolayer down to approximately carbon 10 of the fatty acyl chains (Cranney et al., 1983), while DPH is located within the interior of the lipoprotein (Jonas, 1976), at an average depth of 40–50 Å from the surface (Pownall et

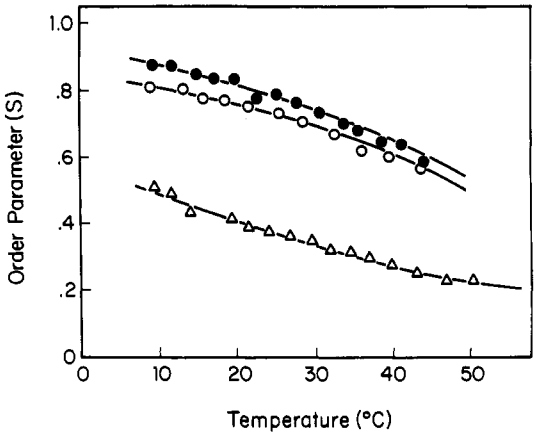

FIGURE 1: Temperature dependence of DPH order parameters in native and reconstituted LDL. Native LDL (●), r-[CO]LDL (○), and r-[TO]LDL (△). DPH was incorporated into LDL as described under Experimental Procedures. Fluorescence measurements were made by equilibrating the sample at the highest temperature indicated and then lowering the temperature in steps for subsequent measurements. Subsequently, several measurements were made with increasing temperature to ensure that measurements were reproducible. Order parameters were calculated as described under Experimental Procedures.

al., 1980). Because the hydrophobic domain of LDL also includes the shell of phospholipid fatty acyl chains which surround the core, both the core and surface layers are accessible to DPH.

To compare the surface properties of LDLs with different core contents, it was essential for the surface compositions of the LDLs studied to be the same. For this reason we used the reconstitution procedure of Krieger et al. (1978), in which neutral lipids are extracted and replaced, leaving the surface phospholipid–protein complex unaltered. Such LDLs retain their ability to bind to cellular LDL receptors (Krieger et al., 1978). Unesterified cholesterol was not incorporated because of its potential ordering effects on phospholipids which could have masked effects due to coupling between core and surface lipids. Furthermore, since cholesterol partitions between the core and surface domains (Lund-Katz & Phillips, 1986), any differences between the core/surface partition coefficients for reconstituted lipoproteins would have complicated an analysis of the results.

LDL core lipids were replaced with CO to give r-[CO]LDL or with TO to give r-[TO]LDL. Since the triglyceride content of the LDL core ranges between 0 to 10% (w/w), LDL particles which contain only cholesterol ester or triglyceride represent extremes in the composition and physical properties of LDL core lipids.

Order Parameters for DPH in Native and Reconstituted LDL. Order parameters for DPH incorporated into native LDL, r-[CO]LDL, and r-[TO]LDL are shown in Figure 1. Over the entire temperature range studied, the degree of order sensed by DPH in r-[TO]LDL was much lower than that for native LDL and r-[CO]LDL. Thus, despite the fact that the region accessible to DPH is structurally heterogeneous, the DPH order parameters for r-[TO]LDL and r-[CO]LDL are consistent with the properties of the core lipids. TO is a nonviscous liquid over the temperature range studied, while CO exists either in liquid crystalline or viscous liquid state. In consonance with the current results, we have previously shown that the motional state of pure cholesterol esters is similar to that of cholesterol esters incorporated into rLDLs (Kroon & Krieger, 1981).

What the DPH order parameters in Figure 1 do not show is the presence of clear order–disorder transitions for the core lipids in r-[CO]LDL and native LDL. At 47 °C pure CO changes from a viscous liquid to a cholesteric phase, and at 42 °C from a cholesteric to a smectic phase. However, because cholesterol ester molecules within the core of LDL are constrained by the highly curved phospholipid surface layer, they undergo only a single transition, from a liquid to a radial smectic phase (Deckelbaum et al., 1977; Kroon, 1981). Using NMR spectroscopy, we have shown that CO in r-[CO]LDL undergoes an order–disorder transition between 39 and 46 °C (Kroon & Krieger, 1981). Our inability to detect a transition in the current study is consistent with previous DPH fluorescence depolarization studies of LDL (Pownall et al., 1980; Jonas, 1976; Sklar et al., 1982). This apparent disparity can be attributed to differences between the time scales for cholesterol ester motion and the fluorescence lifetime of DPH, given the usual premise that probe molecules such as DPH provide a reliable measure of the dynamic behavior of their environment. The molecular dynamics of cholesterol ester molecules in the liquid state has been characterised by ^{13}C NMR spectroscopy (Kroon et al., 1982; Quinn, 1982). This motion is anisotropic but unrestricted; if cholesterol ester molecules are approximated as ellipsoid, the correlation time for motion about the long axis is 3×10^{-9} s, and for the short axis 2×10^{-7} s. In contrast, in the liquid crystalline state cholesterol ester molecules are aligned along their long axes, severely restricting end-over-end tumbling about the short axes, while rotation about the long axis is much less hindered (Kroon et al., 1982; Quinn, 1982). Since the fluorescence lifetime of DPH is 10^{-9} s, end-over-end tumbling of cholesterol ester molecules in the liquid state ($\tau_c \sim 10^{-7}$s) is too slow to affect DPH depolarization. Restriction of this motion in the liquid crystalline state therefore has little effect on the order parameters. In contrast, the ^1H NMR time scale is such that line widths of cholesterol ester proton resonances are very sensitive to end-over-end tumbling of cholesterol ester molecules (Kroon, 1981), resulting in relatively narrow peaks in the liquid state which broaden dramatically when end-over-end tumbling is restricted in the smectic state. The order–disorder transition is therefore readily apparent from proton NMR peak line widths.

The data in Figure 1 also show that order parameters for native LDL were higher than those for r-[CO]LDL. This finding was unexpected since the core of native LDL contains triglyceride which increases the mobility of cholesterol esters (Deckelbaum et al., 1977; Hamilton et al., 1977). Native LDL also contains a high percentage of cholesterol linoleate and more highly unsaturated cholesterol esters, which have lower transition temperatures and a greater degree of motional freedom than CO (Hamilton et al., 1977). These observations led us to conclude that since DPH is able to move between the core and the fatty acyl region of the outer phospholipid monolayer, the surface structure of native LDL might be responsible for the higher DPH order parameters. The surface layers of native and rLDL particles do indeed differ; while native LDL has a molar cholesterol to phospholipid ratio of about 0.55 (Lund-Katz & Phillips, 1986), the surface of rLDL contains no cholesterol. The ability of cholesterol to decrease the order of phospholipid bilayered membranes has been well established, and in the next section we show that unesterified cholesterol also increases the order of the surface monolayer of LDL. We conclude therefore that the differences between DPH order parameters for native and rLDL are determined by DPH molecules located in the cholesterol-rich monolayer

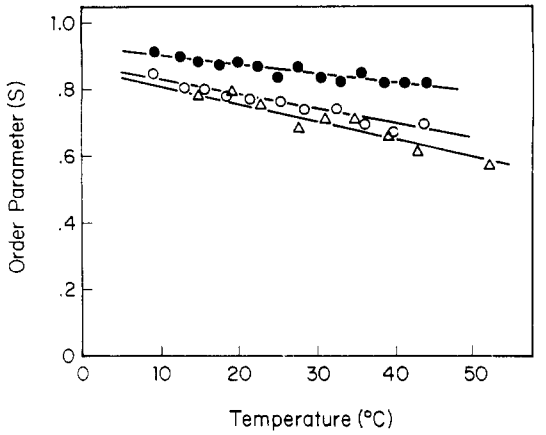

FIGURE 2: Temperature dependence of TMA-DPH order parameters in native and reconstituted LDL. Native LDL (●), r-[CO]LDL (○), and r-[TO]LDL (△). TMA-DPH was incorporated into LDL as described under Experimental Procedures. Fluorescence measurements were made as described for Figure 1.

of native LDL and in the cholesterol-depleted monolayer of rLDL.

Order Parameters for TMA-DPH in Native and Reconstituted LDL. A central issue addressed in the current research is whether the motional states of the LDL core and surface lipids are coupled. To address this, TMA-DPH order parameters were determined at temperatures between 10 and 50 °C for native LDL, r-[TO]LDL, and r-[CO]LDL. The results are shown in Figure 2. These data demonstrate (i) that the order–disorder transition of the core cholesterol esters of both r-[CO]LDL and native LDL has no discernible effect on the order of the surface domains, and (ii) that surface order parameters for r-[CO]LDL are only slightly larger than those for r-[TO]LDL. Thus despite the fact that TO is a nonviscous liquid over the entire temperature range, while CO is a viscous liquid above 46 °C, and a liquid crystal below this temperature, the motional states of the surface domains are similar. We conclude that the motional states of lipids in the core and surface domains of LDL are at best only weakly coupled.

The issue of core–surface coupling has also been addressed by Morrisett et al. (1984). These investigators demonstrated that surface order parameters of triglyceride-rich VLDL from normal rabbits were smaller than surface order parameters for cholesterol ester-rich VLDL from cholesterol-fed rabbits and attributed this difference to core–surface coupling. While coupling in these much larger lipoproteins cannot be excluded, we note that the calculated cholesterol to phospholipid molar ratios for VLDLs from normal and cholesterol-fed rabbits differ substantially; ratios calculated from the data of Morrisett et al. (1984) are 1.0 and 1.7, respectively, thus predicting a more ordered surface for VLDL from cholesterol-fed rabbits irrespective of core–surface coupling.

The only property of the LDL surface phospholipids which does appear to be affected is the the lateral diffusion coefficient. Cushley et al. (1987) have reported an order of magnitude difference in lateral diffusion rates of LDL phospholids between 25 and 45 °C and attributed this to the order–disorder transition of the core cholesterol esters. The magnitude of this change is similar to that observed during the liquid crystalline to crystalline phase transition of phospholipid bilayers (Cullis, 1976). Despite this similarity, the mechanisms responsible for the changes in diffusion rates must be fundamentally different; the decrease in diffusion rates in bilayers is accompanied by a dramatic increase in the order of phospholipid molecules, while there is no such increase in order for LDL phospholipids. It is possible that interactions between phospholipid fatty acyl chains and fatty acyl chains of the core cholesterol esters modulate the lateral diffusion rate without affecting the much faster motions measured with the fluorophore TMA-DPH.

Finally, we note that order parameters for TMA-DPH in rLDLs are significantly smaller than the corresponding order parameters in native LDL (Figure 2). In view of the insensitivity of the surface order to the core components in the rLDLs, it is unlikely that this is due to differences in their core lipid compositions. Instead, since the surface layer of native LDL contains unesterified cholesterol which is not present in the surface layer of the rLDL, we conclude that cholesterol increases the order of the surface phospholipids of native LDL. Lund-Katz and Phillips (1986) have shown that approximately 70% of the total unesterified cholesterol is present in the LDL surface. These cholesterol molecules interact particularly strongly with LDL phospholipids in part because of their high content of saturated phospholipids and sphingomyelin (Ibdah et al., 1989).

Surface Structure of LDL. Our understanding of the phospholipid monolayer which surrounds the LDL core remains incomplete. In part this reflects the fact that LDL particles are quasispherical multimolecular complexes consisting of 3000–4000 lipid molecules and a single 550-kDa protein. The highly curved surface monolayer resembles the outer monolayer of small (200 Å) bilayered phospholipid vesicles; their radii of curvature are similar, and both appose a lipid phase. The vesicle surface monolayer abuts the fatty acyl chains of the inner phospholipid monolayer, while the LDL monolayer abuts the neutral lipid core. What clearly differentiates these two particles is (i) the presence of apoB-100 in the LDL surface and (ii) the ability of the LDL core lipids to diffuse partially or completely into the surface monolayer, while phospholipids within the inner monolayer of small vesicles are limited to relatively infrequent flip-flop interchanges with phospholipids from the outer monolayer (Rothman & Dawidowicz, 1975). To determine how these differences in composition and structure affect surface order, we measured the TMA-DPH order parameters for phospholipid vesicles prepared from LDL phospholipids and compared them with corresponding order parameters for r[CO]LDL. Vesicles were prepared from LDL phospholipids as described under Experimental Procedures, and TMA-DPH was incorporated into the outer vesicle surface by incubating the fluorophore with preformed vesicles. Because the rate of exchange of charged molecules between the outer and inner vesicle monolayers is extremely slow (Rothman & Dawidowicz, 1975), the measured order parameters reflect the order of the outer monolayer only.

Order parameters for phospholipid vesicles measured between 10 and 50 °C are shown in Figure 3. Corresponding data for r-[CO]LDL are replotted from Figure 2. These data show that the order parameters for r-[CO]LDL are much higher than those for corresponding phospholipid vesicles over the temperature range studied. We conclude that the presence of apoB-100 and core lipids have a major influence on the surface order.

To discuss these results, we consider the structural implications of the highly curved surfaces of LDL and vesicles. A phospholipid monolayer with an outer radius of curvature of 105 Å, and a monolayer width of 23 Å (half the width of a phospholipid bilayer) has an outer surface area which is 1.64 times that of the inner surface area. Thus the area occupied

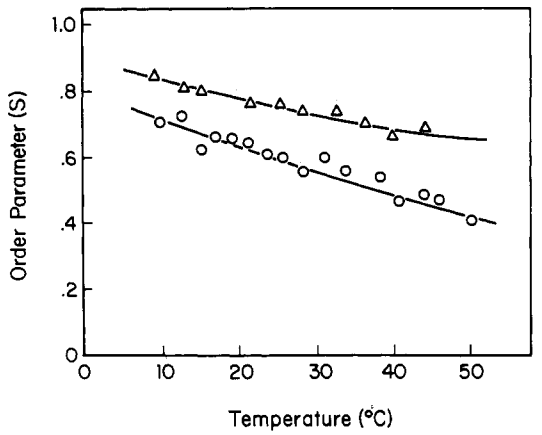

FIGURE 3: Temperature dependence of TMA-DPH order parameters in sonicated vesicles prepared from LDL phospholipids. Sonicated vesicles (○), r-[CO]LDL (△). TMA-DPH was incorporated into vesicles as described under Experimental Procedures. Fluoresecence measurements were made as described for Figure 1. r-[CO]LDL data are replotted from Figure 2.

by a phospholipid headgroup is 1.64 times that occupied by the methyl-terminal region of the fatty acyl chains. For phospholipid vesicles this results in an increased headgroup mobility when compared to a planar bilayer (Kroon et al., 1976). In contrast, apoB-100 and neutral lipids within the rLDL monolayer are able to occupy excess space surrounding the phospholipid headgroups, preventing a similar increase in mobility and predicting a more ordered rLDL surface. The larger order parameter observed for rLDL supports this model. The existence of extensive phospholipid–apoB interactions is consistent with the finding that, unlike other apolipoproteins, apoB cannot exchange between lipoproteins (Van't Hooft & Havel, 1981) and that detergents and denaturants are required to obtain apoB in a lipid free state. An analysis of the amino acid sequence of apoB shows that there are numerous domains throughout its sequence which can interact with the lipid domain. Among these are sequences capable of forming amphipathic β-pleated strands and α-helices, and hydrophobic domains (Knott et al., 1986). An analysis of the lipid-binding properties of proteolytic fragments of apoB-100 has shown that lipid-binding regions are widely distributed within the protein (Chen et al., 1989). Regions of apoB which interact with the phospholipid headgroups may also exist; ^{13}C and ^{31}P NMR studies indicate that 20% of the phospholipid polar headgroups are immobilized by interactions with apoB (Yeagle et al., 1977; Lund-Katz & Phillips, 1986), although more recent studies dispute this (Fensky et al., 1990). Finally, the notion that neutral core lipids may increase the surface order of LDL is supported by studies of Li et al. (1990). These investigators showed that the surface of microemulsions prepared from dimyristoylphosphatidylcholine (DMPC) and CO is more ordered than that of DMPC vesicles, above the DMPC phase transition temperature.

IMPLICATIONS FOR THE STRUCTURE OF NATIVE LDL

Taken together, the current studies support a model for LDL in which cholesterol, the core lipids, and apoB increase the degree of order of the surface phospholipids. The motional state of lipids in the core domain depends on the physical properties of the constituent lipids, although the relatively limited variation in core triglyceride content in native LDL (up to 10% w/w) means that the range of order parameters will only be a fraction of the difference observed between

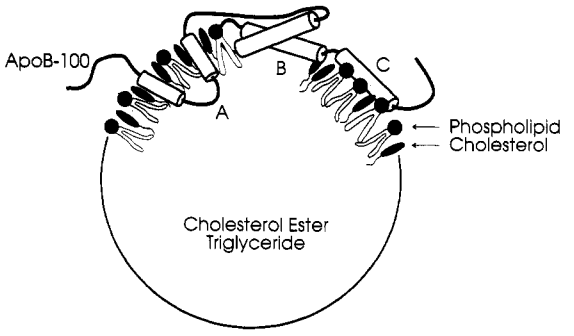

FIGURE 4: Model for interaction of apoB-100 with LDL. (A) Domains which traverse the pjopholipid monolayer into the interior core of LDL; (B) domains which interact with neutral lipid domains in the surface layer; (C) domains which interact with phospholipid headgroups.

r-[CO] LDL and r-[TO] LDL. Despite the large differences between the properties of the core lipid domains of r-[CO] LDL and r-[TO] LDL, their effects on surface order parameters are similar. These data are consistent with weak core–surface coupling and indicate that the motional states of lipids in the core and surface domains are relatively independent. We conclude that neither the conformation of apoB-100 nor its ability to bind to the LDL receptor are likely to be affected by changes in the surface order caused by alterations in the core lipids.

The question as to how alterations in the core triglyceride composition can modulate the conformation of apoB-100, and its affinity for the LDL receptor, (Aviram et al., 1988) remains. In the absence of core–surface coupling, we propose two possible mechanisms. The first involves a redistribution of cholesterol between the core and surface layers with a concomitant change in the surface order and conformation of apoB-100. This mechanism requires a core/surface phase equilibrium for cholesterol which depends on the triglyceride content of the LDL core. Although the solubility of cholesterol in cholesterol esters does not change in the presence of small amounts of triglyceride (Small, 1970), a change in the core/surface phase equilibrium is not easily ruled out, given the unique structural properties of the LDL particle. The second mechanism involves direct interactions of apoB-100 with the core lipids. This can occur in two distinct ways as illustrated in Figure 4: (i) the first involves segments of apoB-100 which traverse the monolayer into the core region and subsequently loop back into the aqueous phase, and (ii) the second depends on the existence of domains of neutral lipid in the surface layer, which are stabilized by interactions with amphipathic domains of apoB-100. Such domains are likely to exist because LDL does not contain enough phospholipids and cholesterol molecules to cover the entire surface. LDL particles with a radius of 105 Å contain approximately 665 phospholipid and 561 cholesterol molecules (Baumstark et al., 1990). Using molecular areas of 65 and 40 Å^2 for phospholipids and cholesterol, respectively (Ibdah et al., 1989), a phospholipid monolayer thickness of 23 Å, and the fact that about 70% of the cholesterol molecules are located in the surface layer (Lund-Katz, 1986), the total core surface area covered by these lipids is calculated to be about 70%. Thus there is ample space for small domains of neutral lipid in the surface layer. There is no evidence for such domains in phospholipid bilayers which contain cholesterol esters and triglycerides. However, the unique structural properties of lipoproteins, in which a core of neutral lipids is apposed to a surface monolayer which contains a large hydrophobic protein, may allow such domains to exist. Both (i) and (ii) would allow the core lipids to

influence the conformation of apoB-100 and potentially alter its affinity for the LDL receptor. Regions of apoB-100 unlikely to be affected by the core lipids are those which interact directly with phospholipid headgroups on the surface of LDL (Figure 4).

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