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Properties of the Products Formed by the Activity of Serum Opacity Factor against Human Plasma High Density Lipoproteins

Henry J. Pownall^a, Harry S. Courtney^b, Baiba K. Gillard^a, and John B. Massey^a

^aSection of Atherosclerosis and Vascular Medicine, Department of Medicine, Baylor College of Medicine, Houston, Texas, 77030.

^b Veterans Affairs Medical Center and Department of Medicine, University of Tennessee Health Science Center, Memphis, Tennessee 38104.

Abstract

Serum opacity factor from *Streptococcus pyogenes* transfers the cholesteryl esters (CE) of ~100,000 plasma high density lipoprotein particles (HDL) to a CE-rich microemulsion (CERM) while forming neo HDL, a cholesterol-poor HDL-like particle. HDL, neo HDL, and CERM are distinct. Neo HDL is lower in free cholesterol and has lower surface and total microviscosities than HDL; the surface polarity of neo HDL and HDL are similar. CERM is much larger than HDL and richer in cholesterol and CE. Although the surface microviscosity of HDL is higher than that of CERM, they have similar total microviscosities because cholesterol partitions into the neutral lipid core. Because of its unique surface properties apo E preferentially associates with the CERM. In contrast, the composition and properties of neo HDL make it a potential acceptor of cellular cholesterol and its esterification. Thus, neo HDL and CERM are possible vehicles for improving cholesterol transport to the liver.

Keywords

Serum opacity factor; HDL remodeling; fusion; cholesterol transport

Plasma high density lipoproteins (HDL) are the vehicles for reverse cholesterol transport (RCT), the mechanism by which peripheral tissue-cholesterol is transferred to the liver for recycling or disposal (Cuchel and Rader, 2006). Human HDL comprise free cholesterol (FC), cholesteryl esters (CE), phospholipids (PL), small amounts of triglyceride (TG), and apolipoproteins (apos)—mainly apos A-I, A-II, C, and E (Havel et al., 1980, Gotto and Pownall, 2003). Unlike other plasma lipoproteins, all HDL components are exchangeable by spontaneous (Massey et al., 1984) or protein-mediated mechanisms (Tall, 1995). HDL are further distinguished from other lipoproteins by their instability, which has been identified by chaotropic (Mehta et al., 2003, Pownall et al., 2007), detergent (Pownall, 2005) and thermal perturbations (Mehta et al., 2003, Sparks et al., 1992, Reijngoud and Phillips, 1984), which induces HDL fusion with the concomitant release of lipid-free (LF)-apo A-I.

Serum opacity factor (SOF), a substance produced by *Streptococcus pyogenes*, spontaneously clouds serum (Ward and Rudd, 1938). Studies with a recombinant (r) SOF showed that HDL is a specific plasma target of opacification, which is associated with the liberation of apos (Courtney et al., 2006). rSOF is a heterodivalent fusogen that catalyzes the disproportionation of HDL into a very large cholesteryl ester (CE)-rich microemulsion (CERM) and an HDL-like

particle, neo HDL, with the concomitant release of LF apo A-I (Gillard et al., 2007). During opacification the CE of >100,000 HDL particles transfer to a single CERM particle that contains apo E but no other detectable apo. rSOF is potent; at 37 EC; opacification of HDL (1-mg/mL) by rSOF (1 g/mL ~ 10 nM) is complete in ~1 hour. rSOF achieves its opacification by a physical process and without breaking covalent bonds; rSOF is neither a protease nor a lipase (Courtney et al., 2006). The products of rSOF opacification could have some therapeutic value in the management of impaired RCT (Gillard et al., 2007). However, the rational designs of cell- and animal-based studies of neo HDL and CERM in RCT require a knowledge of their compositions, structures and properties. Herein, we provide the first physicochemical characterization of neo HDL and CERM, the products of rSOF-mediated HDL opacification.

Materials and Methods

Materials

DPH, TMA-DPH, Laurdan, and Patman were purchased from Molecular Probes (Grand Junction, OR). HDL was isolated according to its density by sequential flotation of human plasma obtained from The Methodist Hospital Blood Donor Center (Schumaker and Puppione, 1986); fractions from multiple injections (0.5 mL) were pooled as needed. TBS (100 mM NaCl, 0.01% NaN₃, 0.01% EDTA, and 10 mM Tris) was used throughout. A polyhistidine-tagged, truncated form of sof2 encoding amino acids 38–843 was cloned, expressed in *Escherichia coli*, and purified by metal affinity chromatography as described (Courtney et al., 1999, 2006).

Methods

Apolipoprotein compositions were determined by SDS PAGE using 15% Tris-Glycine Ready Gels (BioRad). Particle charge was measured as previously described (Gaubatz et al., 2007) by electrophoresis in 0.79% agarose (90 mM Tris, 80 mM borate [pH 8.2]). HDL and neo HDL (5 g protein in <20 L) were loaded onto the gels and electrophoresis was performed at 4 C at 90 volts for 90 minutes. Electrophoretic bands were visualized with Pierce GelCode Blue stain reagent, destained, and recorded by photography. The compositions of HDL and the products of opacification were determined using commercial kits for protein (BioRad DC Protein Assay) and for cholesterol, cholesteryl ester, triglyceride, and PC (Wako Chemicals USA, Inc. Richmond, VA).

HDL Opacification and Isolation of Neo HDL and CERM

Both HDL and the partially purified neo HDL were purified by SEC as previously described (Pownall, 2005, Gillard et al., 2007). HDL (100 mg) was incubated with rSOF (16 µg) in 5.7 mL TBS for 24 h at 37 EC after which the sample was adjusted to $d = 1.063$ g/mL by the addition of KBr, overlaid with 3 mL TBS ($d = 1.006$ g/mL), and centrifuged at 40,000 rpm in a Beckman Ti 50.2 rotor for 18 hours. The CERM (~ 2 mL) was removed from the top by pipetting. The infranatant was siphoned from the bottom of the tube into 2 mL fractions, which were analyzed by SEC. Those richest in neo HDL were pooled and fractionated in a gradient of KBr in TBS ($d = 1.21$ to 1.12 g/mL; 48 h @ 40,000 rpm, Beckman SW 50.2). The supernatant containing neo HDL was removed from the top of the tube by pipette.

Lipid Analysis

Lipid compositions were determined by high performance thin layer chromatography (HPTLC) as previously described (Gaubatz et al., 2007). HDL was split into two equal fractions (45 mg/2 mL). One was untreated and the other was incubated with rSOF (5 g) for 24 h; SEC showed quantitative conversion of HDL to CERM and neo HDL. The CERM was separated from the neo HDL by floatation in TBS containing KBr at $d = 1.063$ g/mL for 18 hours at

32,000 rpm (Beckman SW 40.1). The CERM, which appeared as a compact pad at the top of the tube, was removed by pipette. Another ~8 mL was removed by aspiration and the bottom fraction containing neo HDL was collected. The CERM, neo HDL, and HDL were dialyzed vs. ammonium bicarbonate, lyophilized, and extracted twice with two parts chloroform plus one part methanol. The solvent was reduced to dryness under a stream of nitrogen and the residue dissolved in ~0.5 mL chloroform. Aliquots (100 μ L) of CERM-, neo HDL-, and HDL-lipids were applied to plates containing a thin layer of silica and the lipids eluted using a two solvent system that separates polar and non polar lipids (Gaubatz et al., 2007). The lipids were quantified by staining with primulin and measuring the lipid-associated fluorescence by phosphorimaging (GE Healthcare Storm 840). The identity of the lipids was confirmed by comparing their elution positions with authentic lipid standards. The lipid compositions are expressed as a percent of total composition. Compositions were compared by Student's t-test with a $p < 0.05$ being considered significant.

Fluorescence Spectroscopy

Several well characterized fluorescent probes of the properties of lipids that were previously used to characterize native and model human plasma lipoproteins (Massey and Pownall, 1998) were used to compare HDL, neo HDL, and CERM. The polarization of a fluorescent probe increases with increasing environmental microviscosity. The fluorescence polarization of DPH, which partitions equally between surface and core lipids of plasma lipoproteins, reflects average microviscosity of the surface and core; the fluorescence polarization of TMA-DPH senses the microviscosity of the acyl chain and the headgroup regions of surface phospholipids (Prendergast et al., 1981, Massey et al., 1985a); Patman and Laurdan are fluorescent probes of interfacial polarity (Parasassi et al., 1994, Massey et al., 1985b). The probes in ethanol were added to purified HDL, neo HDL, and CERM with vortexing at the rate of ~1 probe molecule/500 phospholipid molecules.

Fluorescence measurements were performed on a Jobin Yvon Spex Fluorolog-3 FL3-22 spectrofluorometer (Edison, NJ), equipped with Glan-Thompson polarizing prisms as previously described (Massey and Pownall, 2005, 2006); polarization (P) was corrected for monochromator effects on polarized light. Using a Peltier controller, the sample temperature was increased in 1 C increments and equilibrated for 1 min after which the polarization was recorded. Slopes of P vs. T were determined by linear regression analysis of the data (Sigma Plot 8.0). The excitation and emission settings are given in the Figure legends. The general polarization (G. P.) of Laurdan was calculated from the intensities of the short and long wavelength peaks in its fluorescence spectra according to $G. P. = (I_{430} - I_{480}) / (I_{430} + I_{480})$ where I_{430} and I_{480} respectively are the fluorescence intensities at 430 and 480 nm. The G. P. of Patman was calculated similarly and based on the intensities for short and long wavelength peaks, which were different for HDL, neo HDL, and CERM; these were respectively 441 and 464 nm, 451 and 489 nm, and 419 and 462 nm.

Results

Compositions of HDL, Neo HDL, and CERM

According to SEC analysis, the HDL and neo HDL were homogeneous (Figure 1). Neo HDL is more apo A-II-rich than HDL (Figure 1 **insert a**); their respective Stokes' radii were 9.7 and 10.8 nm. According to agarose gel electrophoresis (Figure 1 **insert b**), neo HDL has pre β mobility. The compositions of neo HDL and CERM were distinct from those of HDL (Table 1). Neo HDL contained less CE and its calculated mol% FC compared to PL was less than half that of HDL. In contrast, the CERM were very CE-rich and contained very little PL or protein. Moreover, the mol% FC in CERM was ~3 times that of HDL; the ratios of the core to surface lipid masses, exclusive of FC, increase in the order neo HDL < HDL << CERM. rSOF produced

a subtler but still significant segregation of phospholipid species with sphingomyelin (SM) preferentially transferring to CERM (Figure 2). The PC contents of HDL, neo HDL and CERM were similar whereas there was a non significant enrichment of neo HDL with phosphatidylethanolamine.

Intrinsic Fluorescence Spectra

Apos A-I and A-II contain two near ultraviolet chromophores tyrosine and tryptophan, which have respective molar extinction coefficients 1280 and 5690 cm^2M^{-1} at 280 nm (Edelhdoh, 1967). Unlike apo A-I, which contains four tryptophan and six tyrosine residues, apo A-II contains no tryptophan but has eight tyrosine residues (Brewer et al., 1986). Thus, neo HDL, which is apo A-II rich (Figure 1, **Insert a**), might have a fluorescence spectrum that is distinct from that of HDL. As shown in Figure 3 both HDL and neo HDL exhibit fluorescence maxima at 341 nm. However, the fluorescence spectrum of neo HDL is distinguished by a shoulder on the short wavelength side of its spectrum, and the difference between the normalized fluorescence spectra of HDL and neo HDL reveals an underlying spectrum with a peak at 303 nm.

Lipid Microviscosities of HDL, CERM and Neo HDL are Distinct

The microviscosities of the products of HDL opacification were determined as a function of temperature according to the polarization of the fluorescence of the lipophilic probe, DPH, which diffuses equally between surface and core compartments and provides an average microviscosity of the surface and core lipids, and TMA-DPH, which senses changes in the microviscosity of the surface monolayer that surrounds lipoproteins. According to DPH fluorescence polarization, the total microviscosities of HDL and CERM and their temperature dependence are similar and much higher than that of neo HDL (Figure 3; Table 2). In contrast, the polarization of TMA-DPH shows that the microviscosities of the lipoprotein surfaces decrease in the order HDL > neo HDL > CERM. The temperature dependence of the surface microviscosity as assessed from fluorescence TMA-DPH polarization is similar for all three particles (Figure 4; Table 2).

Surface Polarity

Increasing G.P. of Laurdan and Patman corresponds to a decreasing polarity of the probe environment. G. P. also senses changes in the physical state of phospholipid in the glycerol region of the phospholipid molecule (Parasassi et al., 1991); during thermal transition of phospholipids from the gel to the liquid crystalline phase, the polarity of the interfacial region is increased by increased hydration, an effect that is reversed in part by the addition of cholesterol (Parasassi et al., 1990, 1991, Massey, 2001). Patman contains a positively charged quaternary amino group that confines it closer than Laurdan to the more hydrated region closer to the lipid-water interface

At 37 EC, the G. P. (Laurdan) for HDL and neo HDL were similar indicating similar polarities in the glycerol backbone region (Figure 5 A, B). Similarly, the G. P. (Patman) were similar for HDL and neo HDL. Within probe comparison showed that the slopes of the curves for the temperature dependence of G. P. were also similar for HDL and neo HDL (Figure 5 D; Table 3). The G. P. (Laurdan) for the CERM was much higher G. P. (Figure 5 C; Table 3) In contrast, Patman does not discriminate between particles as well as Laurdan with the G. P. (Patman) being similar for HDL, neo HDL and CERM and nearly identical at 37 EC (Figure 6; Table 3).

Discussion

Plasma lipoproteins comprise a central neutral lipid core of CE and TG surrounded by a surface monolayer of phospholipids and apos. rSOF catalyzes the formation of two new particles from HDL, neo HDL and CERM, which are profoundly different from HDL with respect to both size and composition (Courtney et al., 2006, Gillard et al., 2007, Table 1). Neo HDL are smaller and contain less free cholesterol and neutral lipid than do HDL (Figure 1; Table 1). The apo A-II is preferentially associated with the small particles because it is nonexchangeable on the time scale of our studies. Thus, when the apo A-I and CE are removed from HDL by SOF, the remaining particle is smaller with the apo A-II irreversibly associated with it.

According to our previous data, rSOF produces a profound segregation of lipid species with the fraction of a given HDL-lipid transferring to CERM increasing in the order PL < FC < TG < CE. HP-TLC analysis confirmed this finding (Table 1) that the more hydrophobic lipids preferentially associate with the CERM. rSOF also catalyzed a small, but significant segregation of SM, the most hydrophobic HDL-phospholipid. Thus, the mechanism for opacification must involve a sorting step that is based on lipid hydrophobicity.

Relative to HDL, neo HDL is rich in apo A-II (Figure 1, **insert a**), which has no tryptophan but contains eight tyrosine residues. Consequently, a shoulder due to tyrosine fluorescence can be discerned by difference spectroscopy. In contrast, the CERM are very large and heterogeneous with dimensions approaching 500 nm (Courtney et al., 2006, unpublished results).

DPH Polarization

Among model lipoproteins, cholesterol exerts a viscogenic effect that increases the polarization of fluorescence of embedded probes (Mantulin et al., 1981, Massey et al., 1985c). In contrast, the cholesterol content of neo HDL, HDL, and CERM was a poor predictor of total microviscosity as assessed by DPH fluorescence polarization. The mol% FC increased in the order, neo HDL < HDL << CERM (Table 1), but the total microviscosity increased as neo HDL < HDL ~ CERM. According to the calculated core-to-surface ratios (Table 1), neo HDL have little or no core, whereas HDL have nearly equal amounts of core and surface lipids. The discrepancy is particularly profound for CERM and HDL, which have similar total microviscosities, whereas the mol% FC for CERM is much higher (45%) than that of HDL (14.5%). This is likely due to the distribution of DPH into both the core and surface of the particles so that the DPH preferentially senses the core lipids which are in great excess of the surface lipids and are apparently highly fluid. In contrast, the viscogenic effects of cholesterol are seen when HDL ($P = 0.24$; mol% FC = 14.5%) and neo HDL ($P = 0.15$; mol% FC = 6.7%), which have very little core material, are compared. Although the major component of the CERM is CE, which exhibit thermal transitions between 20 and 40 °C, no discontinuities indicative of a transition were seen in the temperature dependence of the DPH fluorescence polarization in CERM or HDL and neo HDL.

TMA DPH Polarization

The major determinants of the surface microviscosities of lipid bilayers and lipoproteins are the phospholipid composition and the cholesterol content (Massey, 2001, Massey and Pownall, 2006). Although rSOF segregates phospholipids (Figure 2) the magnitude of the segregation too small to substantively alter surface microviscosity. On the other hand, differences in the FC content of neo HDL, HDL, and CERM are expected to be reflected in the surface microviscosities. For example, according to TMA DPH polarization, the surface microviscosities of human lipoproteins increase in the order of their increasing mol% FC—VLDL ~ LDL < HDL₃ (Massey and Pownall, 1998). TMA-DPH fluorescence polarization

reveals that the surface microviscosity of neo HDL is lower than that of HDL. This difference is no doubt due to the lower mol% FC in neo HDL. The surface microviscosity of CERM is even lower ($P = 0.31$) than those of HDL ($P = 0.39$) and neo HDL ($P = 0.36$) despite having the highest mol% FC, 14.5, 6.7, and 45% respectively. It appears that the high mass of the CERM core also modulates the surface viscosity but in an indirect way. Whereas the surface microviscosities increase as $\text{CERM} < \text{neo HDL} < \text{HDL}$, mol% FC increases in the order $\text{neo HDL} < \text{HDL} \ll \text{CERM}$, with CERM having by far the highest FC content but the lowest surface microviscosity according to TMA DPH fluorescence polarization. This discrepancy can be rationalized by observing that free cholesterol is soluble in neutral lipids such as CE and TG. Nuclear magnetic resonance studies show that about one half of FC is in the particle core of human HDL (Hamilton and Cordes, 1978), which based on composition (Havel et al., 1980) has a core-lipid to surface-lipid ratio of ~ 1.5 . This gives a Surface/Core partition coefficient $K = [1/1]/[1/\sim 0.66] \sim 1.5$. According to this partition coefficient and the Core/Surface ratio for CERM ($4.3/85 \sim 0.05$; Table 1), only $\sim 5\%$ of the FC resides in the PL surface and 95% is in the neutral lipid core. This gives a calculated surface mol% FC ~ 3 , a value that is consistent with the low microviscosity that is observed according to TMA DPH fluorescence polarization.

Surface Polarity

The much higher G. P. (Laurdan) of CERM (Figure 5 C) is consistent with an environment in the glycerol backbone region that is much more polar than those of HDL and neo HDL. There are two major determinants of this. The first is the radius of curvature; surfaces like those of the very large CERM are flat and would tend to be less hydrated, the opposite of what is observed. However, this effect is apparently overcome by the very low free cholesterol content of the CERM, which may be lower than our calculated value of 5% (see above). The very similar values observed for the G. P. (Patman) are likely due to its location closer to the interface where there is equally high water penetration into all three types of particles. Thus, the only major difference between the interfacial polarity of the particles is that revealed by G. P. Laurdan; the glycerol backbone of the CERM is much less hydrated than that of HDL and neo HDL.

The Physical Properties of Neo HDL Support Cellular Cholesterol Efflux

Their distinct composition, size, and pre β mobility (Figure 1, insert b) suggest that neo HDL is similar to discoidal HDL formed via the interaction of apo A-I with ABCA1 (Duong et al., 2006). Comparison of the properties of HDL and neo HDL suggest that the latter would better support the first step in RCT—cholesterol efflux. First, PL are the essential FC-binding component of lipoproteins (Pownall, 2006; Fournier et al., 1996, 1997); our data show that relative to protein, neo HDL contain nearly twice as much PL as HDL. Second, as the FC content of rHDL increases, it becomes a poorer acceptor of net cellular cholesterol efflux from human skin fibroblasts, and at ~ 15 mol% rHDL converts from acceptor to donor. Concurrently, fibroblast 3-hydroxy-3-methylglutaryl coenzyme A reductase declines and cellular CE formation via acyl CoA:cholesterol:acyltransferase increases (Picardo et al., 1986). HDL-FC is close to the 15 mol% “switch” (Table 1). In contrast, neo HDL-FC is lower (Table 1) so that its capacity for additional FC is expected to be greater. Third, small particles, e.g., rHDL, are better acceptors of cholesterol than large particles such as single bilayer vesicles (Davidson et al., 1997), and according to our SEC data (Figure 1), neo HDL is smaller than HDL. Although neo HDL, which has pre β mobility, is more electronegative than HDL, the effects of charge difference on FC efflux and other components of RCT are difficult to predict. HDL with pre β mobility is a preferred FC acceptor (Castro and Fielding, 1988), and increasing rHDL electronegativity by addition of phosphatidyl inositol potentiates efflux. However, no mechanistic link between particle charge and efflux has been established. Neo HDL is also expected to better support the second step in RCT, remodeling by LCAT. The lower

microviscosity and cholesterol content of neo HDL is expected to make it a better substrate for LCAT, which preferentially esterifies FC in an environment of low microviscosity (Pownall, et al., 1985) and low cholesterol content (Simard et al., 1989).

Apo E is a minor component of HDL and following HDL opacification CERM contains apo E as its only apo (Gillard et al., 2007). This occurs in spite of the presence of other proteins, especially apo A-I, which is LF and in great excess. Given that apos associate with lipoprotein surfaces, preferential association with CERM must reflect distinctive surface qualities not present in neo HDL. Lipoprotein size is an important macromolecular determinant of apo E binding; as reflected in the CERM composition, apo E preferentially associates with large particles (Asztalos et al., 2007). However, the molecular basis for this and the specific lipid-protein interactions involved have not yet been identified. Surface microviscosity is not likely important; the microviscosity of neo HDL lies between those of HDL and CERM, both of which bind apo E (Table 2). Clues may be provided by our G.P. measurements. According to the G. P. of Patman and Laurdan, the surface polarization of CERM is much greater than those of HDL or neo HDL. What molecular determinant that is reflected in G. P. could support preferential apo E binding to CERM? Laurdan and Patman are sensitivity to the polarity and the molecular dynamics of the dipoles in their environment so that dipolar relaxation is reflected in large spectral shifts that are expressed in terms of G. P. Water molecules are the main solvent dipoles around Laurdan and Patman in lipid surfaces. In the absence of relaxation, GP values are high, indicating low water content at the interfacial region. Thus, CERM are distinguished from neo HDL by a lower interfacial polarity that would be expected to enhance associations with apos mediated by the hydrophobic effect. Neo HDL has a G. P. and hence an interfacial hydrophobicity that is lower than those of HDL and especially CERM. The absence of apo A-I in the CERM may be due to the interplay of two factors. First, apo E is more lipophilic than apo A-I, particularly with respect to association with large particles (Oran and Vaughan, 2006). Second, as a consequence the CERM surface is saturated with apo E. Thus, apo A-I is sterically excluded from the CERM by higher affinity binding of apo E. Apo E would be expected to target CERM to LDL-receptors, which, following rSOF treatment, could hepatically clear large amounts of plasma cholesterol.

Pharmacologic Potential

Accumulation of cholesterol in arterial macrophages produces an atherogenic state unless there is a mechanism for its disposal. Although there may be others, one mechanism for arterial cholesterol disposal is RCT, which comprises cellular cholesterol efflux to early forms of HDL in plasma where it is esterified by LCAT, and disposal of mature forms of HDL by the liver. Identifying new therapeutic strategies that enhance RCT is an important public health priority; the rSOF reaction produces three potentially anti atherogenic products. Neo HDL has a lower free cholesterol to phospholipid content than the HDL from which it was derived and is, as a consequence, a better acceptor for cholesterol efflux than HDL (data not shown). The CERM could clear large quantities of cholesteryl esters via the hepatic LDL-receptor. Moreover, the LF apo A-I released by rSOF could enhance RCT via interactions with the ABCA1 lipid transporter (Oram and Vaughan, 2006). It is unlikely that rSOF-mediated opacification will be used therapeutically because of a likely immune response to a foreign protein. However, given that the mechanism for opacification is known (Gillard et al., 2007) there is some potential for identification of small molecules with a high affinity docking site and a lower affinity delipidation site that catalyze the opacification reaction that leads to LF apo A-I, neo HDL, and CERM. Future tests in cellular and animal models of lipoprotein metabolism and atherogenesis will determine the value of opacification therapy.

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Abbreviations

apo	apolipoprotein
rSOF	recombinant serum opacity factor
HDL, LDL, and VLDL	high, low, and very low density lipoproteins
LF	lipid-free
TG	triglyceride
PL	phospholipid
CE	cholesteryl ester
PC	phosphatidylcholine
SM	sphingomyelin
SEC	size exclusion chromatography
TBS	Tris-buffered saline
DPH	1, 6-diphenyl-1,3,5-hexatriene
TMA-DPH	1-(4-trimethylammoniumphenyl)-1,3,5-hexatriene
Laurdan	6-dodecanoyl-2-dimethylamino-naphthalene
Patman	6-palmitoyl-2-((2-trimethylammonium)ethyl) methylaminonaphthalene chloride

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Figure 1.

Schematic Representation of the Opacification Reaction. rSOF (10 nM) catalyzes the conversion of human HDL (~20 μ M) to a CERM that contains the CE of ~100,000 HDL particles, a new apo A-II-rich particle called neo HDL, and LF apo A-I. Apo E is the major protein associated with the CERM. The reaction is complete in 90 min. Apos A-I and A-II are shown as black and gray helices respectively.

Figure 2.

SEC of HDL and Neo HDL. Purified HDL and neo HDL (0.2 mL) were analyzed by SEC using an Amersham-Pharmacia ÄKTA chromatography system with two Superose HR6 columns in tandem and eluted with TBS at a flow rate of 0.45 mL/min; the column effluent was monitored by absorbance (280 nm). The left vertical arrow denotes the void volume. Insert a: SDS PAGE of HDL and neo HDL as labeled (5 µg protein/lane); presence and absence of β-mercaptoethanol (βME) is indicated as labeled below the gel. From left to right the samples were protein standards, HDL, neo HDL and apos A-I and A-II as monomer (AII) and dimer (AII₂). Insert b: Agarose (0.79%) gel electrophoresis as labeled, with the direction of migration indicated by right vertical arrow; the horizontal arrow locates the pre β position of neo HDL.

Figure 3. Phospholipid Compositions of HDL, Neo HDL, and CERM. PE, phosphatidylethanolamine; PC, phosphatidylcholine, SM, sphingomyelin.

Figure 4. Intrinsic Fluorescence Spectra of HDL and Neo HDL at 22 EC. Excitation was at 280 nm using excitation and emission slits of 4 and 2 nm respectively. The difference between the spectra (Δ Intensity) was calculated after normalizing the peaks to the same intensity.

Figure 5. Fluorescence Polarization as a Function of Temperature. HDL, neo HDL, and CERM were labeled with DPH and TMA-DPH and the polarization of fluorescence at their respective spectral maxima was measured.

Figure 6. Fluorescence and G. P. of Laurdan as a Function of Temperature. (A-C) Fluorescence of Laurdan in HDL, neo HDL, and CERM respectively. Grey Arrows show the spectral changes with increasing temperature (16, 22, 27, 32, 37, 42, and 47 EC). (D) Temperature dependence of the G. P. of Laurdan in HDL (M), neo HDL (F), and CERM (M).

Figure 7. Fluorescence and G. P. of Patman as a Function of Temperature. Labels are the same as in the legend to Figure 5.

Table 1

Chemical Compositions (%) of HDL, Neo HDL, and CERM

Analyte	HDL	Neo HDL	CERM
PL	25.5 ± 1.3	43.9 ± 0.8	4.3 ± 0.01
FC	2.2 ± 0.1	1.6 ± 0.06	1.8 ± 0.13
CE	14.5 ± 1.6	2.2 ± 0.16	78.1 ± 2.7
TG	4.8 ± 0.5	4.2 ± 0.39	6.9 ± 0.8
Pro	53.0 ± 6.8	48.0 ± 4.2	8.8 ± 0.8
Mol% (FC) ^a	14.5	6.7	45
Core/Surface ^b	0.76	0.15	19.8

^a Mol% (FC) = $100 \times [\%FC/386]/[\%FC/386 + \%PL/760]$; 387 and 760 are the molecular masses of FC and PL.

^b Core/Surface = (CE + TG)mass/PL mass.

Table 2

Microviscosity Parameters for HDL, Neo HDL, and CERM

	DPH		TMA DPH	
	P (37 EC)	(P/EC) $\times 10^3$	P (37 EC)	(P/EC) $\times 10^3$
HDL	0.24	-4.3 [*]	0.39	-0.97 [*]
Neo HDL	0.15	-3.5 [*]	0.36	-1.4 [*]
CERM	0.24	-3.7 [*]	0.31	-1.5 [*]

^{*} Calculated by a linear regression fit of the data; $r^2 > 0.97$.

Table 3

G. P. Parameters for HDL, Neo HDL, and CERM

	Laurdan		Patman	
	G. P. (37 EC)	(G.P./EC) $\times 10^3$	G. P. (37 EC) $\times 10$	(G.P./E C) $\times 10^3$
HDL	0.2133	-6.32 *	-0.515	-4.68 *
Neo HDL	0.1794	-7.33 *	-0.965	-9.39 *
CERM	0.6712	-2.88 **	-0.123	-14.3 *

* $r^2 > 0.98$ ** $r^2 > 0.87$