Remodeling of Human Plasma Lipoproteins by Detergent Perturbation†

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ABSTRACT: Detergent perturbation, the treatment of total human plasma lipoproteins (TLP) with sodium cholate and its subsequent removal, has been used to study lipoprotein dynamics and stability. At physiological TLP concentrations, detergent perturbation converts low-density lipoproteins (LDL) and high-density lipoproteins (HDL) to higher-particle weight species with the concomitant release of apo A-I but not apo A-II as a lipid-poor species. Detergent perturbation of isolated HDL also releases lipid-poor apo A-I and forms larger HDL species, whereas detergent perturbation of an isolated LDL has no effect on its size. A model is presented in which detergent perturbation induces transfer of PC from metastable HDL and LDL to mixed micelles with sodium cholate. The remaining LDL and HDL are unstable because of the loss of their surface components, phospholipid and/or apo A-I, and fuse to give larger LDL and HDL particles. These effects on HDL, i.e., PC transfer, apo A-I dissociation, and particle fusion, emulate the activity of human plasma phospholipid transfer protein. Thus, detergent perturbation is a new and potentially powerful method for determining lipoprotein stability, studying the mechanisms for remodeling of plasma lipoproteins, and preparing new forms of HDL and LDL with unique interactions with lipoprotein transporters and receptors.

In human plasma, early forms of lipoproteins are remodeled by multiple enzymes and transfer proteins that convert them to mature particles that are recognized by cell surface receptors that mediate their uptake and catabolism (*1*−*3*). Lipoprotein lipase converts very low-density lipoproteins (VLDL)[1] to intermediate-density lipoproteins, which are further lipolyzed by hepatic lipase giving low-density lipoproteins (LDL), which are removed by hepatic LDL receptors. Early forms of high-density lipoproteins (HDL), which receive cholesterol via interactions with ABC transporters in peripheral tissue (*4*−*6*), are substrates for lecithin: cholesterol acyltransferase, which esterifies HDL-cholesterol while converting the HDL to a mature form that is recognized by hepatic HDL receptors (*7*). This process, reverse cholesterol transport, is an important component of normal lipid metabolism and a potential therapeutic target. Thus, characterization of the structure, dynamics, and stability of plasma lipoproteins is an important key in understanding how interacting biomolecules determine function.

Although physical techniques that include calorimetry, mass spectrometry, nuclear magnetic resonance, X-ray crystallography, and fluorescence spectroscopy have been used to study biomolecular structures, these methods are sometimes inadequate, and less direct methods of chemical perturbation such as protein denaturation or proteolytic and lipolytic probes of structure and stability must be used; this is particularly true for complex assemblies of lipids and proteins that are found in membranes and plasma lipoproteins. Some of these methods are still overly harsh and can be misleading because they break covalent bonds. Thus, we sought a method for studying lipoprotein structure and stability that uses a detergent, which perturbs lipid−protein structures without altering covalent structure.

Detergents are amphiphilic substances that are monomeric at low concentrations but at higher concentrations self-associate to form noncovalently associated oligomeric structures known as micelles (*8*). Detergents have been used to reconstitute the proteins and activities of cell membranes (*9*) and human plasma lipoproteins, particularly HDL; e.g., sodium cholate "catalyzes" the association of apolipoprotein (apo) A-I with lipids giving rHDL (*10*, *11*) as well as cholesterol-containing rHDL (*10*, *12*). Herein, we report on a new method, detergent perturbation, that we believe will be useful for studies of complex lipid−protein assemblies. Applied to normolipidemic human plasma lipoproteins, detergent perturbation reveals differences in the structures and stabilities of lipoproteins and emulates the activity of human plasma phospholipid transfer protein.

EXPERIMENTAL PROCEDURES

Materials. High-purity sodium cholate was from Anatrace. [^{3}H]Cholate was from Amersham. Buffers and salts were from Fisher Scientific. Tubular dialysis membranes (Spectra/Por) with nominal retention limits of 6−8 kDa (D = 14.6 mm) were from Spectrum Laboratories (Rancho Dominguez, CA).

Plasma for lipoprotein isolation was from The Methodist Hospital Blood Donor Center. Except where noted, all

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[1] Abbreviations: apo, apolipoprotein; HDL, LDL, and VLDL, high-density, low-density, and very low-density lipoproteins, respectively; PC, phosphatidylcholine; LCAT, lecithin:cholesterol acyltransferase; TBS, Tris-buffered saline; CMC, critical micelle concentration; rHDL, reassembled HDL; SDS−PAGE, sodium dodecyl sulfate−polyacrylamide gel electrophoresis; SEC, size exclusion chromatography; TLP, total lipoproteins; PLTP, phospholipid transfer protein.

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experiments were conducted with the total plasma lipoprotein (TLP) from one normolipidemic volunteer. The plasma lipids of the single donor at the time of collection were total cholesterol (184 mg/dL), triglycerides (48 mg/dL), HDL-C (62 mg/dL), and calculated (*13*) LDL-C (112 mg/dL). TLP was isolated and concentrated by flotation at a *d* of 1.21 g/mL; the concentrated stock TLP−protein and −choline lipid concentrations were 12.2 and 8.2 mg/mL, respectively. LDL and HDL were isolated by sequential flotation at *d* values of 1.019, 1.063, and 1.21 g/mL or by preparative size exclusion chromatography (SEC) over Superose HR6. Experiments were conducted over a time interval during which the SEC profile of the TLP on Superose HR6 was unchanged. Tris-buffered saline (TBS) [100 mM NaCl, 10 mM Tris-HCl, 0.01% azide, and 0.01% EDTA (pH 7.4)] was used throughout. Key findings were confirmed using TLP from the plasma of 10 other volunteers. The clinical protocol was reviewed and approved by the institutional review board; all volunteers provided informed consent.

Kinetics. The kinetics of cholate dialysis with and without TLP were determined by dialyzing 66 mM cholate containing [^{3}H]cholate at 4 °C, and collecting aliquots at various time intervals. Radioactivity associated with cholate was determined by liquid scintillation counting. TLP and sodium cholate were mixed in 2.23 mL of TBS to final concentrations of 1.95 mg/mL and 90 mM, respectively, by the addition of 20% sodium cholate containing [^{3}H]cholate as a tracer. After brief vortexing, a 50 μL sample was removed and the remainder was transferred to a dialysis sack. At various time intervals, 50 or 100 μL aliquots of the retentate were removed and counted. The kinetics of detergent perturbation of lipoprotein structure were also followed by SEC. Cholate and TLP were combined to final concentrations of 90 mM and 2.0 mg/mL, respectively, and an aliquot was removed and analyzed by SEC; the remainder was dialyzed for various times, during which aliquots were collected and analyzed by SEC.

Lipoprotein Analysis after Detergent Perturbation. The effects of detergent perturbation on lipoprotein compositions and SEC profiles were determined by mixing sodium cholate with TLP on ice and transferring the samples to dialysis sacks. Unless otherwise indicated, all samples were twice dialyzed for 24 h against a >1000-fold excess of TBS at 4 °C and then analyzed by SEC using an Amersham-Pharmacia ÄKTA chromatography system equipped with two Superose HR6 columns in tandem. The elution volumes for HDL, LDL, and VLDL were determined by chromatography of lipoprotein standards isolated by sequential flotation at *d* values of 1.006, 1.063, and 1.21 g/mL (*14*); other standards are indicated in the figure legends. Typically, a sample was filtered (0.2 μm), injected into the chromatograph using a 0.2 mL sample loop, and eluted with TBS. The column effluent was monitored by absorbance at 280 nm. For preparative chromatography in which the eluant was collected for further analysis, a 0.5 mL sample loop was used; individual fractions or pooled fractions from multiple runs were analyzed for protein according to the method of Lowry as modified by Markwell (*15*) and for cholesterol, triglyceride, and PC using commercial kits (Wako Chemicals USA, Inc., Richmond, VA). Protein composition was determined by SDS−PAGE using a 4 to 15% gradient or 18% Tris-Glycine Ready gels (Bio-Rad). Bands were visualized with Pierce GelCode Blue stain reagent, destained, and recorded with the Kodak Electrophoresis Documentation and Analysis System (EDAS) 290.

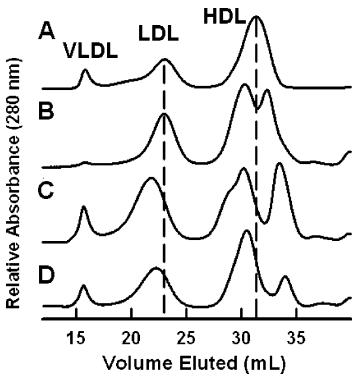

FIGURE 1: SEC of TLP after detergent perturbation. TLP and sodium cholate were combined to give final concentrations of 1.95 mg/mL and 90 mM, respectively, and split into three samples. Cholate was removed from one sample by SEC over a column of BioGel P6 DG; cholate was removed from another sample by exhaustive dialysis at 4 °C, and the third sample was diluted below the CMC of cholate by the addition of a 9-fold excess of TBS. A control sample in which TBS was substituted for the same volume of cholate was also prepared. SEC profiles of each sample over Superose HR6 are as follows: (A) control TLP, (B) TLP and cholate after removal of the cholate by SEC, (C) TLP and cholate after removal of the cholate by dialysis, and (D) TLP and cholate after dilution to 9 mM cholate. Peaks for VLDL, LDL, and HDL are labeled.

RESULTS

Effects of Detergent Perturbation on TLP. The SEC elution profile of untreated TLP contained prominent peaks that were identified as VLDL, LDL, and HDL by comparison with authentic lipoproteins isolated by sequential flotation (Figure 1). Two other samples of TLP were treated with cholate, which was removed by SEC over BioGel P6 DG or dialysis, the most common methods used for detergent-mediated formation of rHDL (*10*, *11*). Detergent perturbation of TLP by P6 SEC had little effect on the elution positions of VLDL and LDL, whereas the peak for HDL was apparently split into two poorly resolved peaks (Figure 1B). The effects of detergent perturbation by dialysis were more profound (Figure 1C). The peak for LDL was shifted to a smaller elution volume, which corresponds to a shift to a higher-molecular weight species, and HDL eluted as two nearly resolved peaks. The effect of dialysis and SEC could be due to the total removal of detergent or to the reduction of the detergent concentration below its critical micelle concentration (CMC = 15 mM) where it loses its solubilizing power. This was tested by mixing TLP and detergent to the same initial concentrations that were used for SEC and dialysis and rapidly diluting the mixture to 9 mM cholate. Dilution of the detergent/TLP mixture altered the elution profile of both LDL and HDL (Figure 1D), an effect that was similar though not identical to that of dialysis (Figure 1C). Given its experimental simplicity and its more profound effects on the lipoprotein profiles of TLP, the remaining studies of detergent perturbation were conducted using dialysis to remove the detergent.

Dialysis Kinetics. We studied the kinetics of two processes: the rate of cholate dialysis and the rate of lipoprotein

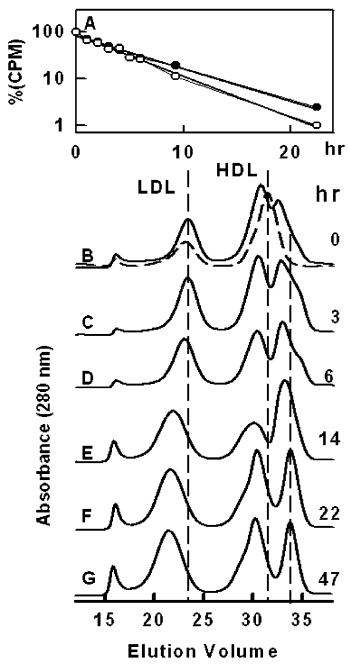

FIGURE 2: Kinetics of detergent perturbation. (A) [^{3}H]Cholate and TLP were mixed to final concentrations of 66 mM and 1.95 mg/mL, respectively, and dialyzed for 48 h, during which samples were removed for liquid scintillation counting. A control in which TLP was omitted was also tested. According to a first-order regression analysis of the data, the half-times for the disappearance of [^{3}H]-cholate were 3.5 and 4.5 h for cholate only (○) and cholate with TLP (●), respectively. (B−G) Time dependence of the SEC of TLP (2.0 mg/mL) after mixing with sodium cholate to a final concentration of 90 mM. The dialysis times are indicated to the right of each plot. Solid lines are the profiles as a function of time. The dashed line in panel B is TLP before addition of cholate.

remodeling. The rate of escape of [^{3}H]cholate from a dialysis sack was determined (Figure 2A). According to a first-order regression analysis of the data, the half-times for the disappearance of [^{3}H]cholate were 3.5 and 4.5 h for cholate only and cholate and TLP, respectively. According to the measured rate constant for dialysis, ≪0.01% (<9 μM) of the original cholate remained inside the dialysis sack after 48 h. The calculated molar cholate-to-particle ratio, given molar concentrations of HDL (25 μM) and LDL (1.6 μM), is ∼0.5. Given the solubility of cholate, most of this is likely to be unbound.

We studied the kinetics of lipoprotein remodeling by detergent perturbation using SEC to follow changes in the lipoprotein profile of TLP as a function of dialysis time. SEC of TLP after addition of 90 mM cholate but before dialysis shows the HDL peak split into two poorly resolved components and an unchanged LDL peak. With an increase in the dialysis time, the split in the HDL peak grows and by 22 h is well resolved and little changed by additional dialysis until the 47 h time point. Between 6 and 14 h, the peak for LDL shifts to an earlier elution time with little additional change up to the 47 h time point. On the basis of these results, subsequent detergent perturbation studies were conducted by dialysis for 2 days with a change of dialysis buffer after 24 h at a minimum sample-to-buffer ratio of 1000.

SDS−PAGE of Lipoproteins after Detergent Perturbation. Following detergent perturbation, three fractions were collected for SDS−PAGE analysis under reducing and nonreducing conditions (Figure 3). Analysis of pooled fraction 1 (F1) by SDS−PAGE revealed the presence of one protein, apo B-100, which remained intact following detergent perturbation (data not shown). Proteins in pooled fractions 2 and 3 (F2 and F3, respectively) were identified by comparison with authentic samples of apo A-I and A-II (Figure 3, lanes 1 and 2, respectively). SDS−PAGE of fraction 2 under nonreducing conditions revealed two bands with mobilities that coincided with those of apo A-I and A-II. Under reducing conditions, the band coinciding with that of apo A-II disappeared and a new lower-molecular weight band appeared; this further confirmed the identity of apo A-II, which splits from a disulfide-linked dimeric protein into monomeric species under reducing conditions. In contrast, fraction 3, which on Superose HR6 SEC eluted later than native HDL, was composed almost exclusively of one protein, apo A-I, with a barely visible trace of apo A-II (Figure 3, lanes 4 and 6).

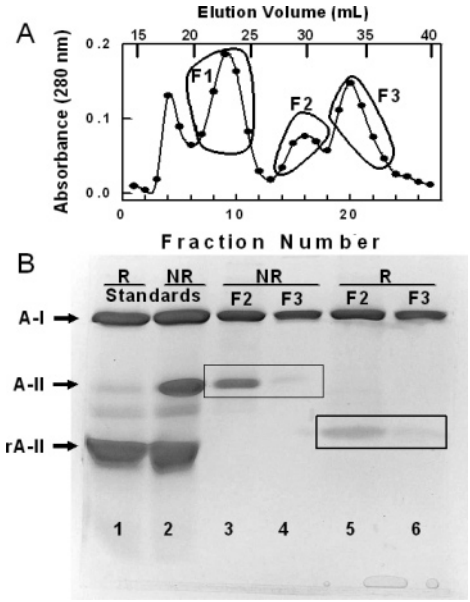

FIGURE 3: SDS−PAGE of pooled TLP fractions after detergent perturbation under nonreducing (NR) and reducing (R) conditions. The encircled curves, labeled F1−F3 (A), indicate the pooled fractions that were used for compositional analysis and SDS−PAGE (B). In lanes 1 and 2, the standards were apo A-I, apo A-II, and reduced (r) A-II. Lanes 3 and 5 contained fraction 2. Lanes 4 and 6 contained fraction 3. Lanes 3−6 were loaded with equal amounts of protein. These data reveal the preferential release of apo A-I with little or no loss of apo A-II (compare bands in boxes).

Lipoprotein Composition after Detergent Perturbation. The effluent from SEC before and after detergent perturbation was analyzed for protein, total cholesterol, and phospholipid. These data (Figure 4) revealed that detergent perturbation also shifts the distribution of TLP lipids. As expected, before detergent perturbation LDL and HDL proteins coelute with total cholesterol and phospholipids (Figure 4A). After detergent perturbation, the shifted peaks for LDL and HDL proteins coelute with those for total cholesterol and phospholipid (Figure 4B). However, the late-eluting peak, corresponding to fraction 3 in Figure 3A, appears to contain little or no cholesterol or phospholipid; its peak elution volume coincides with that of isolated apo A-I (0.36 mg/mL, data not shown).

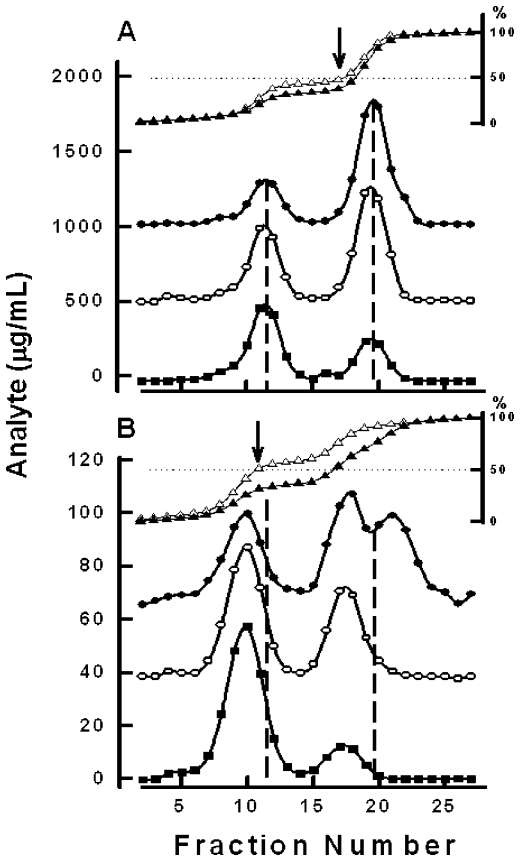

FIGURE 4: Effect of detergent perturbation on the association of protein (●), phospholipid (○), and total cholesterol (■) with lipoprotein fractions isolated by SEC: (A) TLP and (B) TLP after detergent perturbation. Cumulative protein (▲) and phospholipid (△) as a percentage of the total. The curves for protein and phospholipid (as choline) are shifted upward for easier comparison; the vertical scales are the same for all curves in each panel. Initial TLP and cholate concentrations were 2.0 mg/mL and 90 mM, respectively.

Table 1: Compositions of Isolated TLP Fractions before and after Detergent Perturbation[a]

	control TLP		TLP and detergent perturbation	
analyte	LDL	HDL	LDL	HDL
PL	18.5 ± 0.4	27.6 ± 1.5	21.1 ± 0.1	32.4 ± 0.35
FC	6.0 ± 0.7	1.8 ± 0.4	4.8 ± 1.3	3.1 ± 0.7
CE	50.7 ± 4.2	16.7 ± 3	41.1 ± 2.5	15.2 ± 0.2
TG	5.2 ± 1.2	1.7 ± 0.9	6.6 ± 0.6	5.7 ± 1.1
protein	19.6 ± 0.8	52.2 ± 0.9	26.4 ± 2.9	43.6 ± 0.8

[a] The fractions pooled for control LDL and HDL and for detergent-perturbed LDL and HDL were 10−12, 19 and 20, 9 and 10, and 19 and 20, respectively (Figure 4).

Analysis of the peak tubes revealed major compositional changes (Table 1). As expected, with the release of apo A-I from HDL, the relative amounts of the lipid components increase; this effect is greatest for phospholipid, which increases significantly but not as much as would be predicted on the basis of the release of 50% of the apo A-I, suggesting that some of the phospholipid transfers to other lipoprotein fractions. This can be seen by comparing the cumulative phospholipid (PL) and protein (Pro) content of each fraction across the entire SEC profile before and after detergent perturbation. As shown in the top curve of Figure 4A, 50% of the phospholipid in TLP appears in fractions 1−18; there is a similar distribution of protein. In contrast, after detergent perturbation, 50% of the phospholipid appears in fractions 1−11, whereas the protein is split nearly evenly between fractions 1−16 and 17−28 (Figure 4B, top curve). Thus, detergent perturbation shifts phospholipid from HDL to the apo B-100-containing lipoproteins.

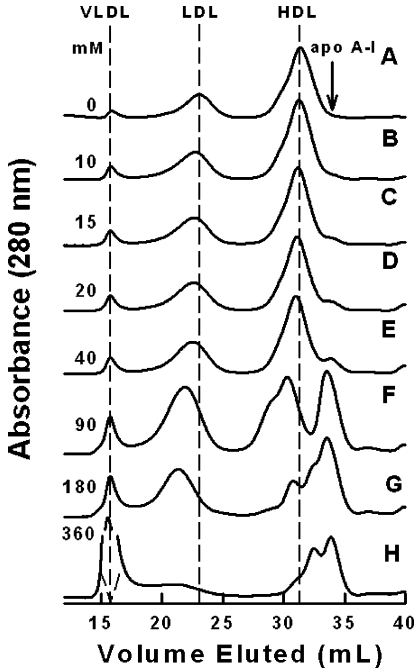

FIGURE 5: Cholate dose dependence of TLP SEC profiles. TLP (final concentration of 1.95 mg/mL) and various aliquots of 465 mM cholate were mixed at ice temperature and exhaustively dialyzed in a cold room for 48 h, after which each sample was analyzed by SEC over Superose HR6. The initial cholate concentrations are indicated to the left of each plot. The elution positions for control VLDL, LDL, and HDL are indicated in the top panel.

Cholate Dose Dependence of Detergent Perturbation. TLP at a final concentration of 1.95 mg/mL was mixed with sufficient cholate to give final concentrations of 0, 5, 10, 15, 25, 40, 60, 90, 120, and 360 mM. The cholate was removed by dialysis, and aliquots of each sample were analyzed by Superose HR6 chromatography in which the relative protein concentrations were estimated from the absorbance at 280 nm (Figure 5). Between 0 and 25 mM cholate, the SEC profiles of TLP were practically indistinguishable. At these low cholate concentrations, there were modest changes in the size of LDL, whose elution volume decreased as a function of increasing initial cholate concentration. Higher cholate concentrations produced larger shifts in the LDL peak so that at 360 mM, the LDL peak was barely discernible. Detergent perturbation also produced profound changes in the elution profile of HDL. As the initial cholate concentration increased, a new peak appeared in the chromatogram at fraction 22 and the major peak, corresponding to HDL, shifted to earlier elution volumes that correspond to higher particle weights (Figure 5). Gradient gel electrophoresis (4 to 20% acrylamide) under nondenaturing conditions corroborated the SEC data and showed that detergent perturbation increased the size of the HDL (data not shown). The shift in the HDL peak and the magnitude of the peak with an elution volume of 34 mL were greatest at 90 mM cholate; at 180 mM cholate, the two well-resolved peaks were replaced by a broad band of at least three components, which declined in magnitude when the initial cholate concentration

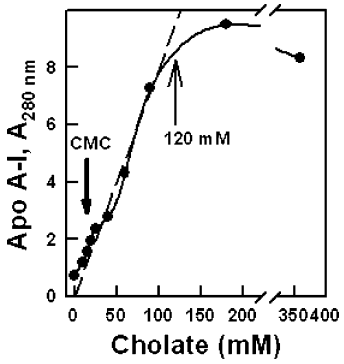

FIGURE 6: Correlation of apo A-I peak absorbance (280 nm) (Figure 4) with initial cholate concentration. The dashed line is a first-order line of regression for the change in absorbance between 0 and 90 mM. The concentrations for the CMC and the plateau of absorbance are denoted with arrows.

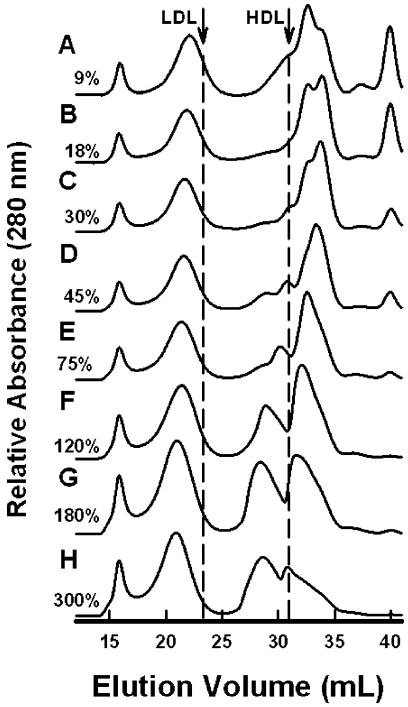

FIGURE 7: Dose dependence of the SEC profile of TLP as a function of its concentration at a constant initial cholate concentration (90 mM). The concentrations of TLP vary from 9 to 300% of the plasma concentration of the donor (1.95 mg/mL TLP protein). The arrows and adjacent vertical lines locate the elution positions of untreated LDL and HDL.

was increased to 360 mM. Comparison of the magnitude of the peak having an elution volume of 34 mL with the initial cholate concentration reveals a small but distinct change just above the CMC (Figure 6), suggesting that micelle formation plays a role, albeit minor, in the remodeling of HDL and release of apo A-I.

TLP Dose Dependence of Detergent Perturbation. The solubilization power of a given cholate concentration with respect to the distribution of lipoproteins was tested by using a constant dose of cholate while varying the TLP concentration from 9 to 300% of the plasma concentration (100% = 1.95 mg/mL TLP protein). As shown in Figure 7, the distribution of lipoprotein absorbance showed a slight shift in the peak for LDL at all TLP to cholate ratios. In contrast, the distribution of absorbance in the vicinity of the elution volume of HDL was different at all ratios that were tested,

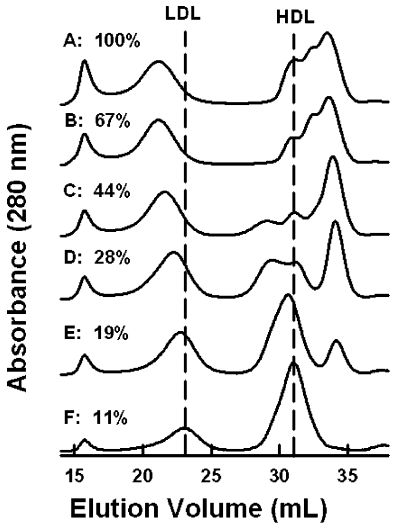

FIGURE 8: Effect of dilution on detergent perturbation at a constant TLP:cholate ratio. TLP and cholate were combined to final concentrations of 1.95 mg/mL and 180 mM, respectively, diluted to the amount shown to the left of each plot, dialyzed for 48 h as described in Experimental Procedures, and analyzed by SEC. The dashed vertical lines mark the elution positions of LDL and HDL.

with the smallest dose (9%) being associated with a shift in the absorbance toward a larger elution volume, which corresponds to a lower particle weight. As the TLP concentration was increased to 300%, the peak absorbance split into two peaks that were shifted toward a higher molecular weight. SEC after detergent perturbation of TLP at very high concentrations, ~40 mg/mL protein, revealed a profile similar to that in Figure 2F, except that the late-eluting peak containing apo A-I is absent. Centrifugation of the detergent-treated, dialyzed sample prior to SEC sedimented material that was not readily soluble in TBS but dissolved in 3 M guanidine hydrochloride. SDS−PAGE analysis showed that this precipitate contained apo A-I as the sole protein (data not shown). Thus, detergent perturbation at high TLP concentrations releases apo A-I that precipitates from solution upon removal of the detergent.

Effect of Detergent Perturbation at Various Dilutions at a Constant TLP:Cholate Ratio. Various concentrations of TLP and cholate were combined while maintaining the same TLP:cholate ratio. The samples were then dialyzed and analyzed by SEC on Superose HR6 as described above. With a decrease in the concentration of the sample from 100 to 11% of the plasma TLP protein concentration (1.95 mg/mL), the peak corresponding to LDL shifted to larger elution volumes, i.e., lower particle weights (Figure 8). At 100% plasma TLP, the late-eluting peak was composed of three poorly resolved peaks that collapsed to a single peak at 11% plasma TLP. At 11% TLP (0.2 mg/mL protein, 20 mM cholate), the Superose HR6 profile was indistinguishable from that of TLP without detergent perturbation (Figure 1A).

Effect of the HDL:LDL Ratio on Detergent Perturbation. The changes in the SEC profile of LDL and HDL after detergent perturbation suggested that there may be a transfer of mass between these two particles. Figure 9A compares the elution profiles of HDL and LDL after detergent perturbation with that of LDL and HDL without detergent perturbation. As with TLP, detergent perturbation of isolated HDL splits the HDL peak into two species, one eluting earlier and the other later than HDL. In contrast, detergent perturba-

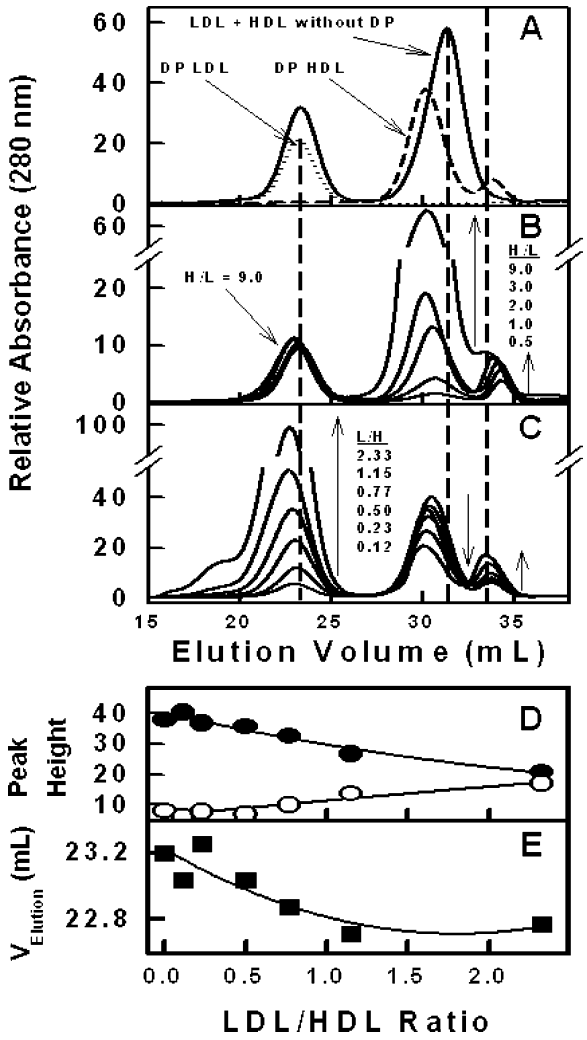

FIGURE 9: Effect of LDL and HDL concentrations on the detergent perturbation of lipoproteins as assessed by SEC: (A) control LDL with HDL (—), isolated LDL (•••), and isolated HDL (– – –) after detergent perturbation (DP). (B) Detergent perturbation at constant LDL and various HDL concentrations; arrows indicate the direction of the change in the peaks to their left with an increase in the HDL:LDL ratio. (C) Detergent perturbation at constant HDL and various LDL concentrations. H is HDL, and L is LDL; arrows indicate the direction of the change in the peaks to their left with an increase in the LDL:HDL ratio. (D) Relative peak heights of HDL (●) and lipid-poor apo A-I (○). (E) Peak elution volume (*V*) of LDL as a function of the LDL:HDL ratio (■).

tion of isolated LDL had no effect on its elution profile. In both instances, detergent perturbation was conducted at approximately the plasma concentrations of LDL and HDL (0.3 and 1.3 mg/mL protein, respectively). The effects of HDL:LDL ratio on detergent perturbation were tested by varying the HDL concentration at a constant LDL concentration (Figure 9B) and vice versa (Figure 9C). Qualitatively, the effects were similar. In both instances, the HDL peak split into two components, and the LDL peak shifted to an earlier elution volume. However, according to Figure 9B, the largest fraction of protein appearing in the late HDL peak (lipid-free apo A-I) was at the lowest HDL:LDL ratio (0.5) with the fraction of total absorbance in the late peak decreasing as the HDL:LDL ratio was increased. The LDL peak was shifted only slightly even at the highest HDL:LDL ratio (9.0). At a constant HDL concentration, the effects of detergent perturbation in the presence of increasing concentrations of LDL revealed systematic changes in the elution profiles. As the LDL:HDL ratio increased, the magnitude of the peak for HDL, which was shifted to earlier elution volumes, decreased while the peak for lipid-free apo A-I increased (Figure 9C,D). Concurrently, LDL eluted earlier (Figure 9C,E). Thus, the increase in the amount of lipid-free apo A-I that occurred with an increase in the LDL:HDL ratios correlated with an increase in the size of both LDL and HDL.

Table 2: Lipid Values for TLP Donors

volunteer no. (gender)	plasma analyte concentration (mg/dL)			
	total cholesterol	TG	HDL-C	LDL-C
1 (M)	165	52	49	106
2 (M)	163	167	36	94
3 (M)	127	44	39	79
4 (M)	185	61	46	127
5 (M)	127	185	26	64
6 (M)	151	52	34	107
7 (M)	116	101	23	73
8 (M)	192	151	45	117
9 (F)	137	58	38	87
10 (M)	133	100	23	90
11 (F)	122	44	35	78

Generalizing Detergent Perturbation. The effects of detergent perturbation on the plasma TLP from 11 volunteers was tested to determine whether the observed effects of this process could be generalized. On the basis of the lipid data in Table 2, the mean ± standard deviation plasma lipid levels for the donors were as follows: 147.1 ± 26.0 mg/dL total cholesterol, 92.3 ± 52.7 mg/dL triglyceride, 35.8 ± 9.0 mg/dL HDL-C, and 92.9 ± 19.5 mg/dL LDL-C. The TLP concentration used for detergent perturbation was adjusted to the original plasma lipoprotein level of each donor. The SEC of all 11 TLPs before detergent perturbation revealed three peaks corresponding to VLDL, LDL, and HDL with elution volumes of ∼16, ∼23, and ∼32 mL, respectively; detergent perturbation shifted both LDL and HDL peaks to earlier elution volumes in all TLP samples with the concurrent appearance of a peak corresponding to apo A-I (Figure 10).

DISCUSSION

Our data, collected on a single normolipidemic donor, show that detergent perturbation of TLP converts LDL into larger particles, while HDL is converted to a larger apo A-I-depleted species with the concomitant release of lipid-poor apo A-I. The composition of the lipid-poor apo A-I peak was as follows: 4.1% PC, 0.80% free cholesterol, 0.56% cholesteryl ester, and 94.5% protein; triglyceride was not detectable. However, analysis of individual fractions (Figure 4) showed that there was no detectable PC beyond fraction 21, i.e., elution volumes of >32.5 mL. Therefore, it is likely that the pooled fractions contained a portion of the trailing tail of the main HDL peak centered around an elution volume of 30 mL, and that lipid-poor apo A-I is, in fact, lipid-free, a conclusion that is supported by the identical elution volumes for the released apo A-I and for an authentic sample of apo A-I. The transforming properties of detergent perturbation were not unique to the single donor that was used for the majority of the studies; similar SEC profiles were

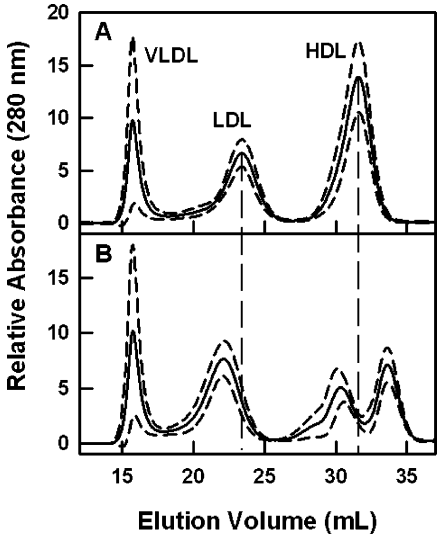

FIGURE 10: Mean SEC profiles of TLP from 11 volunteers before (A) and after (B) detergent perturbation. For detergent perturbation, the TLP concentration was adjusted to the original plasma TLP concentration by dilution with TBS. The plots show the mean (—) ± the standard deviation (— — —). The vertical lines mark the peaks for native LDL and HDL.

observed with the detergent perturbation of TLP from 11 normolipidemic subjects (Figure 10). Although the effects of detergent perturbation on the lipoprotein profiles of normolipidemic subjects are expected to be similar, similar tests of lipoproteins from patients with dysregulated lipoprotein metabolism may reveal distinct effects.

SEC, dialysis, and dilution (Figure 1) gave analytical SEC profiles that were qualitatively the same, suggesting that solubilization and detergent removal drive the perturbation process. There were prominent quantitative differences. Dialysis and dilution below the CMC of cholate produced the highest and lowest yields, respectively, of the fraction corresponding to lipid-poor apo A-I. Chromatography, which removes cholate at a rate between those of dilution and dialysis, gave an intermediate yield of released apo A-I. According to the kinetics of cholate removal (Figure 2A), between 14 and 22 h, the cholate remaining in the dialyzate is reduced below the CMC (from 23 to 5 mM) and, by the 22 h time point, the SEC profile has plateaued. Thus, formation of lipid-poor apo A-I is optimized by slower removal of the detergent and reaches equilibrium once the detergent concentration is below its CMC.

Detergent perturbation occurs in three distinct phases (Figure 5). The first, between 0 and 15 mM, does not affect the elution profile of TLP. Between 15 and 90 mM detergent, there is a gradual change in the SEC profile that corresponds to increased particle weights for LDL and HDL and the appearance of lipid-poor apo A-I. At and above 180 mM, the peaks for HDL and lipid-poor apo A-I collapse into at least three poorly resolved species while the size of LDL changes little. The effect of the detergent concentration with respect to the CMC on the appearance of lipid-poor apo A-I was small (Figure 6). This can be understood in terms of the theory of mixed micelles, which under the conditions of ideal mixing yields CMCs between those of the components according to their mole fraction in the micelle (*8*). Even though cholate and natural phospholipids, which have CMCs of $\leq 10^{-9}$ M, form mixed micelles, ideal mixing is not expected. Rather, cholate partitions between the aqueous and lipoprotein phospholipid phases and at higher cholate concentrations separates as a mixed cholate/phospholipid micelle. We suggest that the key step in detergent perturbation is the formation of mixed micelles, which can begin to appear at detergent concentrations below the CMC but increase in number nearly linearly with detergent concentration. According to this model, the amount of lipid-poor apo A-I should increase linearly with detergent with little or no effect of the CMC, which is observed (Figure 6).

Differences in the TLP concentration used for detergent perturbation did not greatly affect the SEC profiles. Over an ~30-fold range of TLP concentrations at a constant cholate concentration, the peak for LDL was always shifted to a larger particle size (Figure 7A−H). At high TLP concentrations, two separate peaks for HDL were observed, but at low TLP concentrations, the two peaks collapsed into one with an elution volume that corresponded to a particle that is smaller than HDL (Figure 7A). This effect is similar to that observed at high detergent concentrations (Figure 5G,H), suggesting that high detergent:TLP ratios, whether by variation of the TLP or detergent concentration, collapse HDL into a mixed population of particles.

Studies of the effects of HDL:LDL ratios on the SEC profile after detergent perturbation provided clues about the process and a possible mechanism. The first is that detergent perturbation of LDL alone had no effect on its elution profile (Figure 9A). The second was that detergent perturbation of HDL alone produced a peak corresponding to lipid-poor apo A-I. However, the height of the apo A-I peak was lower than those observed in the detergent perturbation of TLP, suggesting that LDL and HDL must in some way interact. This was tested by measuring the effects of detergent perturbation as a function of HDL concentration at a constant LDL concentration. Increasing concentrations of HDL had almost no effect on the SEC profile of LDL and only modestly increased the magnitude of the peak corresponding to lipid-poor apo A-I (Figure 9B); an 18-fold increase in HDL produced an only 2-fold increase in the extent of apo A-I release. The greatest proportion of apo A-I release occurred at the highest LDL:HDL ratio (Figure 9C). The importance of this was confirmed by studies of the effects of LDL concentration at a constant HDL concentration. As the LDL concentration increases, the relative heights of the peaks corresponding to lipid-poor apo A-I and to apo A-I-depleted HDL increase and decrease, respectively (Figure 9C−E), while the particle weight of LDL increased. Thus, LDL potentiates detergent perturbation release of apo A-I from HDL.

We formulated a model for the effects of detergent perturbation on TLP (Figure 11). According to SDS−PAGE (Figure 3) and chemical analysis (Table 1 and Figure 4), HDL loses a portion of its apo A-I and phospholipid. The loss of surface components, phospholipid and apo A-I, leaves a particle in which the neutral lipid core is not completely shielded from the surrounding aqueous phase. The exposed cores of two apo A-I/phospholipid-depleted HDL could coalesce and fuse giving a larger particle. Although this seems to be a likely mechanism, more definitive kinetic studies that follow the mixing of surface and core components (*16*) would confirm this model.

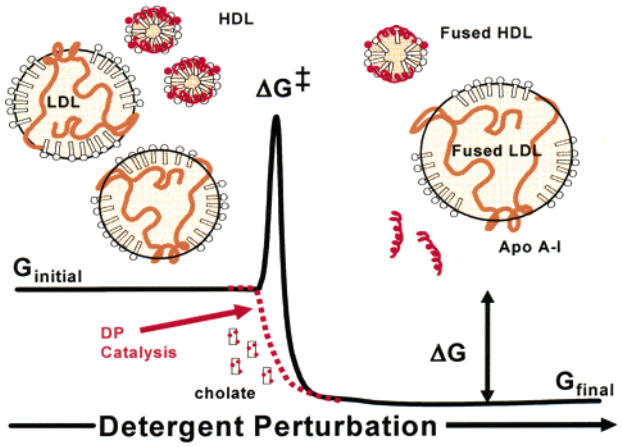

FIGURE 11: Net effect of detergent perturbation on TLP remodeling. Transfer of HDL phospholipid to LDL reduces the affinity of apo A-I for HDL and leaves portions of the neutral lipid core exposed to water. Contact between the exposed neutral lipid cores of two HDL particles initiates fusion. Although the LDL formed by detergent perturbation are shown as spherical, conversion of LDL to phospholipid-rich ellipsoids cannot be ruled out.

Detergent perturbation also converts LDL to a larger species in which apo B-100 is the sole protein. Because of its very high lipophilicity, apo B-100 is nontransferable and as a consequence cannot be displaced by lipid-poor apo A-I. On the other hand, according to their rates of desorption, LDL has a higher affinity for phospholipids than HDL (*17*). Thus, if detergent perturbation is catalytic, transfer of phospholipids from HDL to LDL is predicted, and detergent perturbation could provide a mechanism by which phospholipids associate with the particles having the highest lipophilicity. The effects of detergent perturbation on TLP might be expected to be greater at a higher HDL:LDL ratio because HDL provides the phospholipid that transfers to LDL. However, the effect is small compared to the effect of increasing the LDL:HDL ratio. We assign the greater effect of increasing the LDL:HDL ratio to the much higher affinity of LDL for phospholipid. At high LDL:HDL ratios, there is a high concentration of high-affinity phospholipid acceptors (LDL) in the presence of donors (HDL) with low affinity. Unlike HDL, LDL, which gains surface components (phospholipids from HDL), would not be as likely to fuse as surface-depleted HDL because it has adequate coverage of its neutral lipid core. Thus, the accretion of phospholipids may induce a change in the shape of LDL from a particle that is roughly spherical to one that is slightly larger but more extended, a hypothesis that can be addressed by a direct method such as electron microscopy.

In vitro, HDL fusion can be induced by simple chemicals and by physiologically relevant reagents, and comparison of products formed by these disparate reagents reveals clues about the fusion mechanism. HDL denaturation by rapid concentration jumps of guanidinium hydrochloride occurs in two irreversible kinetic steps: a fast phase of particle rupture and release of the apolar lipid core and a slow step, particle fusion (*18*). Thermal denaturation experiments indicate high enthalpic barriers for the particle rupture that may arise from the transient disruption of lipid and/or protein packing interactions that lead to the irreversible release of surface and core components as separate phases. Thermal denaturation of LDL also induces particle fusion with the unfolding of apo B-100 and, with additional heating, its dissociation (*19*). These and other studies suggest that HDL and perhaps other plasma lipoproteins exist in kinetic traps between the native state and the most stable state, which is formed by fusion (*18*, *19*).

Although each of these paths to lipoprotein fusion and the release of free or lipid-poor apo A-I involves different mediators (heat, lipid transfer, lipolysis, and denaturation), there are similarities that suggest that they are all driven by the same thermodynamic factors. Studies of the formation of rHDL from dimyristoylphosphatidylcholine (DMPC) and apo A-I suggest that detergent perturbation is a catalyst (*10*). DMPC and apo A-I rapidly associate at the transition temperature of the lipid, giving a fairly monodisperse rHDL. Although the reaction is extremely slow above and below the transition temperature, the rHDL formed by detergent treatment above and below the transition temperature is identical to that formed spontaneously at the transition temperature. Hence, the proposal of Gursky and co-workers that normal lipoprotein metabolism leads to particles that reside in kinetic traps is likely valid (*18*, *19*), and the term catalyst for detergent perturbation, which allows lipoproteins to escape from kinetic traps, is appropriate.

Fusion is an important step in the normal processing of human plasma HDL, and physiologically, several plasma activities reduce the kinetic barrier to fusion. In the absence of an adequate supply of apo A-I, lecithin:cholesterol acyltransferase mediates the conversion of small HDL to large HDL by fusion (*20*); cholesteryl ester transfer protein modification of rHDL involves particle fusion (*21*). The phospholipid transfer reactions catalyzed by PLTP and by the insect lipid transfer particle (*22*, *23*) are a potent inducer of HDL fusion. Like detergent perturbation, PLTP induces HDL fusion with the concomitant release of lipid-poor apo A-I (*16*), an effect that is more profound with TG-rich HDL (*24*), which appear to have a lower affinity for apo A-I (*25*). When apo A-II is substituted for apo A-I, the rate of fusion is reduced (*26*), a result that is consistent with our finding that apo A-I but not apo A-II is released from HDL during detergent perturbation. This effect is likely due to the greater lipophilicity of apo A-II compared to apo A-I. There are two essential parts in the PLTP-mediated conversion mechanism: the early release of phospholipids and apo A-I but not apo A-II followed by the slow fusion of apo A-I-depleted particles with pre-β mobility. It is not known whether the loss of apo A-I and phospholipids is concurrent or whether one precedes the other. The high solubility of apo A-I in guanidine hydrochloride suggests that transfer of apo A-I to the aqueous phase is an initiating factor that is followed by the transfer of phospholipids to lipid-free apo A-I to give lipid-poor apo A-I. On the other hand, activities that deplete HDL of phospholipids, lecithin:cholesterol acyltransferase and PLTP, could also be initiating factors that form a pool of phospholipids that associate with a fraction of apo A-I that is less tightly bound to HDL.

As a probe of macromolecular structure and stability, detergent perturbation of TLP offers some advantages over other methods. It is a simple method of studying HDL fusion and the concomitant formation of lipid-poor apo A-I in a way that does not require other plasma mediators, lecithin:cholesterol acyltransferase, CETP, or PLTP, which can be isolated in only small quantities by laborious multistep

procedures. Detergent perturbation does not break covalent bonds or use harsh conditions such as chemical denaturation or heating to high temperatures. Detergent perturbation by dilution is rapid (Figure 1D) and provides a means of studying HDL fusion and the release of lipid-poor apo A-I in real time. Finally, detergent perturbation can be used on a large scale to form new particles for the detailed study of their structure and properties and their interactions with cellular lipid transporters and lipoprotein receptors. The new species derived from lipoprotein fusion might be cardioprotective, and their interactions with cholesterol transporters and hepatic lipoprotein receptors might enhance reverse cholesterol transport by known or alternative pathways that would be determined by their unique structures.

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