Published in final edited form as:

Biochemistry. 2013 June 25; 52(25): 4324–4330. doi:10.1021/bi4006732.

Free Cholesterol Determines rHDL Phospholipid Phase Structure and Stability

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Abstract

Reassembled high density lipoproteins (rHDL) of various sizes and compositions containing apo A-I or apo A-II as their sole protein, dimyristoyl phosphatidylcholine (DMPC), and various amounts of free cholesterol (FC) have been isolated and analyzed by differential scanning calorimetry (DSC) and by circular dichroism to determine their stability and the temperature dependence of their helical content. Our data show that the multiple rHDL species obtained at each mol% FC usually do not have the same mole% FC as the starting mixture and that the size of the multiple species increases in a quantized way with their respective mol% FC. DSC studies reveal multiple phases or domains that can be classified as virtual DMPC, which contains a small amount of DMPC that slightly reduces the melting temperature T_m , a boundary phase that is adjacent to the apo A-I or apo A-II that circumscribes the discoidal rHDL, and a mixed FC + DMPC phase that has a T_m that increases with mol% FC. Only the large rHDL contain virtual DMPC whereas all contain boundary phase and various amounts of mixed FC + DMPC according to increasing size and mol% FC. As reported by others, FC stabilizes the rHDL. For rHDL (apo A-II) compared to rHDL (apo A-I), this occurs in spite of the reduced number of helical regions that mediate binding to the DMPC surface. This effect is attributed to the very high lipophilicity of apo A-II and the reduction in the polarity of the interface between DMPC and the aqueous phase with increasing mol% FC, an effect that is expected to increase the hydrophobic associations with the non polar face of the amphipathic helices of apo A-II. These data are relevant to the differential effects of FC and apolipoprotein species on intracellular and plasma membrane nascent HDL assembly and subsequent remodeling by plasma proteins.

Keywords

Cholesterol; Apolipoprotein A-I; Apolipoprotein A-II; Size Exclusion Chromatography; Circular Dichroism; Fluorescence; Differential Scanning Calorimetry

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Supplemental Information.

Supporting supplemental information is accessible free of charge online at <http://pubs.acs.org>.

Introduction

Although cardiovascular disease (CVD)(1) is negatively correlated with plasma high density lipoprotein-cholesterol (HDL-C), the correlation is not axiomatic because HDL functionality, which is likely related to the properties of HDL, is also important.(2) Thus, the structure and properties of various HDL are relevant to identifying their functional determinants. Apolipoproteins (apos) A-I and A-II, the most abundant HDL-apos (~50 and ~25 μ M respectively in human plasma), microsolubilize macrophage phospholipids and free cholesterol (FC) via ABCA1 giving nascent HDL.(3, 4) Nascent HDL formation in hepatocytes begins by intrahepatic ABCA1-independent apo lipidation in the endoplasmic reticulum followed by ABCA1-dependent lipidation in the Golgi and at the plasma membrane.(5) Half of apo A-I is secreted lipid-free and later transferred to spherical HDL in a lecithin:cholesterol acyltransferase (LCAT)-dependent manner.(6, 7) In contrast, apo A-II is fully lipidated and dimeric early during its intrahepatic processing and occurs on particles without apos A-I and E and only after secretion fuses with apos A-I- and E-containing particles.(7, 8) In human plasma, about two thirds of HDL particles contain apo A-I plus apo A-II with the remainder containing apo A-I without apo A-II.(9) In vitro microsolubilization of dimyristoylphosphatidylcholine (DMPC) multilamellar vesicles (MLV) by apos A-I and A-II produces rHDL, the in vitro analog of nascent HDL.(10) FC has a profound effect on the kinetics of rHDL formation, which is fastest at ~12.5 mol% cholesterol,(11) the concentration that produces the maximum number of defects for apos to insert between lipid clusters.(11) As with ABCA1-mediated apo A-I lipidation,(3) FC increases the size and number of rHDL species formed by the association of apos A-I and A-II with DMPC.(12, 13) rHDL formation is rapid up to 20mol% FC above which the rate decreases to nil.(12, 13)

Ample data shows that apo A-II is more lipophilic than apo A-I. Prolonged centrifugation of HDL sheds apo A-I but not apo A-II;(14) apo A-II displaces apo A-I from HDL;(15) denaturants or heat disrupt HDL structure with the release of lipid-free apo A-I but not apo A-II.(16) Many proteins that target HDL, such as LCAT, hepatic lipase, lipid transfer proteins,(17–20) and streptococcal serum opacity factor,(21) disrupt HDL with the concomitant release of lipid-free apo A-I but not apo A-II. Given its greater lipophilicity versus apo A-I, we hypothesized that rHDL formed from apo A-II would be more stable than those formed from apo A-I. Using high sensitivity differential scanning calorimetry (DSC) and the temperature dependence of their circular dichroic spectra, we compared the effects of FC on the stability of rHDL prepared from apos A-I and A-II.

Materials & Methods

Chemicals

Glycine and sodium chloride were obtained from Sigma Aldrich; sodium acetate and EDTA were from ICN Biomedicals and dibasic sodium phosphate was from Fisher Scientific. All chemicals were of analytical grade or higher purity. All measurements, if not stated differently, were performed using a temperature stable buffer mixture containing 10mM sodium acetate, 10mM Na₂PO₄, 10 mM glycine, 150 mM NaCl, 2 mM EDTA, pH = 8.

rHDL Preparation and Labeling

MLV were prepared from ethanolic solutions of DMPC and FC that were reduced to dryness by a stream of nitrogen and over a vacuum for > 15min. The dried lipids were dispersed into Tris-buffered saline (TBS, 10mM Tris, 100mM NaCl, 1mM Azide, 1mM EDTA) by vortexing after which the lipids were subjected to 3 cycles of warming to 50 °C with vortexing and freezing to ~20 °C.

Preparation and Analysis of rHDL formed from DMPC-Apo-A-I and -Apo-II

Apos A-I and A-II were isolated as described.(22, 23) Apo concentrations were determined spectrophotometrically.(24) rHDL were prepared by incubating DMPC containing 0 to 30 mol% FC at the transition temperature of the DMPC, 24 °C, with a DMPC to apo ratio of 10 (w/w). Unbound DMPC was sedimented at 16,000xg for 30min in an Eppendorf 5415C centrifuge at 4 °C. The rHDL in the supernatant were separated into various rHDL by SEC over tandem columns of Superose HR6 (GE Healthcare) and fractions collected.(12, 13) Protein and lipid compositions of the various rHDL species were determined using the BioRad DC protein assay and WAKO Phospholipids C and Free Cholesterol E kits. Molar compositions were calculated using molecular weight values of apo A-I 28,016, apo A-II 17,414, PC 760, DMPC 678 and FC 387.

Spectroscopy

Protein concentration was quantified spectrophotometrically according to Edelhoch(25) and modified by Pace(26) as $A_{280\text{nm}} - 2 \times A_{333\text{nm}}$ (to correct for light scattering) on a Shimadzu UV2101PC spectrophotometer. Thermal denaturation of rHDL was followed by circular dichroism at 222 nm (Aviv Biomedical Model 420C) using a 1 mm quartz cell at a scan rate of 1 °C/min; data was collected every 3 s with an integration time of 0.1 s. Ellipticity θ (millideg) was converted to mean residue molar ellipticity (MRME; $[\text{deg-cm}^2]/[\text{dmol-res}] = [100 \times \theta(\text{millideg}) \times \text{MW}(\text{g/mol})]/[\text{c}(\text{mg/mL}) \times 1(\text{cm}) \times \text{number of residues}]$). We used a Savitsky-Golay numerical derivative to smooth and differentiate the data by a simplified least squares procedure.(27) The numbers of amino acid residues in apo A-I (28,016 Da) and apo AII (17,414 Da) are 243 and 168 respectively.

Calorimetry

The stability of the rHDL lipid component was determined by differential scanning calorimetry (DSC) on a TA Instruments NanoDSC equipped with a fixed capillary cell design at a pressure of 300 kPa using a scan rate of 1 °C/min. Prior to DSC measurements, buffers and samples were degassed with moderate stirring for 10min in a vacuum dessicator. The calorimeter was calibrated by performing a cell balance scan and residual scans as described in the manual. The calorimeter was equilibrated overnight with buffer in both the reference and sample cells with repeated thermal scans until a stable baseline was obtained after which the buffer in the sample cell was replaced with protein solution during the equilibration step between scans. One measurement per second was recorded between 1 – 95 °C. DSC traces were corrected by subtracting the baseline background buffer scan. Calorimetric power compensation (microJ/s) was converted to molar heat capacity C_p (kJ/mol/°K) = $\text{Power}(\text{microJ/s})/[\text{scan rate}(\text{°C/min})]/60 \times V(\text{milliL}) \times c(\text{microM}) \times 1000$. Excess molar heat capacity was obtained by subtracting an 11th order polynomial fit to the pre- and post-transition regions of the heat capacity traces using a Mathematica (Wolfram, Inc.) script developed in our laboratory. The transition enthalpies were determined from the total integral of the excess heat capacity.

Results

rHDL Preparation and Isolation

Apos A-I and A-II were incubated with DMPC containing 0, 5, 10, and 15 mol% FC at 24° C. Excess DMPC was sedimented and the rHDL separated by SEC a method in which the elution volume (EV) decreases with increasing particle size (Figure 1). Like rHDL (apo A-I), the EV for rHDL (apo A-II) shifts to earlier elution volumes indicative of increased rHDL size as the mol% FC in the initial incubation mixture increases. The effect of FC on rHDL size appears to be quantized; at some compositions, a peak in the chromatogram is

observed whereas at a higher or lower mol% FC, only a shoulder may appear. Fractions indicated by the horizontal bars were collected and analyzed by SEC for homogeneity and composition. The collected fractions though broadly distributed are homogeneous and almost normally distributed (Figure 2). According to compositional analyses (Figure 3), the DMPC-apo ratio increased with particle size, with ratios for the largest and smallest rHDL (apo A-I) being 140 and 340 to 1. The corresponding values for rHDL (apo A-II) were 48 and 100 to 1. These data also show that at a given initial mol% free cholesterol (FC), multiple semi discrete rHDL species form that increase in size as their mol% FC increases. For example, at an initial composition of 5 mol% FC, three species form (Figure 1B). After isolation (Figure 2) the FC content in the three species (smallest to largest) were 4.5, 5.2, and 5.8 mol%.

Differential Scanning Calorimetry (DSC)

DSC of DMPC revealed two endotherms, one at 12.5 °C, a pre transition and a larger main transition temperature (T_m) at 23.9 °C (Supplementary Figure 1A, 0 Mol% FC scan). The pre transition was absent from the DSC of DMPC containing 5 mol% FC whereas the main transition was broadened, its enthalpy reduced, and its T_m shifted to a slightly lower temperature. This trend continued up to 20 mol% FC where a transition was barely discernible; at 25 mol% FC, there was no detectable transition (Supplementary Figure 1B–F).

The endotherms for all rHDL were broader than those of DMPC $\pm$ FC. Small and/or FC-rich rHDL have symmetrical endotherms. More specifically, the endotherms for the small rHDL (apo A-I) containing 0 and 4.5 mol% FC and for the medium and large rHDL (apo A-I) containing 10 and 14.5 mol% respectively were symmetrical with T_m = 28.1, 27.3, 27.8, and 28.6 °C (Figure 4A). In contrast, endotherms for medium and large rHDL (apo A-I) with 0, 5.2 and 5.8 mol% FC were comprised of two components (Figure 4), better discerned in the first derivatives of the endotherm (Supplementary Figure 2). Large rHDL (apo A-I) with 0 mol% FC exhibited T_m = 23.0 °C, the same as that of pure DMPC, and at 27.0 °C. The endotherms for large and medium size rHDL (apo A-I) containing 5.8 and 5.2 mol% FC exhibited respective T_m at 25.0 and 28.0 °C and at 26.4 and 28.0 °C. In a second thermal scan of each rHDL broad endotherms of the first scan were replaced by sharp transitions, similar to that of pure DMPC, superimposed on a broader higher thermal transition whose T_m increased with mol% FC (Figure 4B).

Similar calorimetric measurements were performed on rHDL (apo A-II). At 0 mol% FC, the T_m appeared as a single broad symmetrical peak, and unlike rHDL (apo A-I) there was no hint of a transition below that of pure DMPC. Like rHDL (apo A-I), the T_m increased with increasing mol% FC (0, 5.2, 13.4, and 18.5 mol % had T_m = 28.9, 29.3, 29.5, and 31.1 °C respectively) and the attendant enthalpies of melting decreased to nil with increasing FC content up to 22.2 mol% (Figure 5A; Supplementary Figure 3). According to the scans and confirmed by the first derivative of the scans, two weaker thermal transitions appear at 32.4 and 35.0 °C in rHDL (apo A-II) containing 13.4 and 18.5 mol% FC respectively. The second thermal scan for rHDL (apo A-II) was also distinct from that of rHDL (apo A-I); in the absence of FC, a sharp peak coinciding with that for pure DMPC appeared followed by a higher temperature shoulder (Figure 5B). With increasing mol% FC the enthalpy of the sharp peak decreased and that of the shoulder increased until at 19 mol%, only the broad higher temperature endotherm was observed. The enthalpies of melting of DMPC and each of the rHDL as a function of mol% FC are summarized in Figure 6. These data show that the enthalpy of DMPC melting at a given mol% FC decreases in the order, DMPC > rHDL (apo A-I) > rHDL (apo A-II). According to the slopes of the curves, the decrease in the enthalpy of melting with added FC for DMPC was greater than that of either rHDL.

Circular Dichroism

Both apo A-I and apo A-II in rHDL have a high α -helical content that can be used to follow temperature-dependent unfolding (Figure 7A, B). The loss of the α -helical content with increasing temperature reflects the unfolding of the rHDL protein component. The midpoint for the unfolding curve calculated from the second derivative of MRME vs. temperature (Supplementary Figure 4) defines the unfolding transition temperature, which was little changed with respect to FC content for rHDL (apo A-I) but very FC-dependent for rHDL (apo A-II), which exhibited a sharp increase in the T_m as a function of mol% FC (Figure 7C). As discussed below, previous studies have shown that rHDL formed by the reactions of apo A-I and apo A-II with cholesterol containing DMPC MLV are unstable to heat and dissociate into lipid-free or poor apos and fusion products at $T > 75^\circ\text{C}$.

Discussion

Calorimetric Data

The DSC data are both profound and subtle. In connection with the latter, our data shows that both FC and apos produce a profound broadening of the main DMPC transition. On the subtle side, a more thorough analysis of the data, in part according to the first derivatives of the DSC data, uncovered evidence for multiple lipid melting transitions that were a function the size of the rHDL, and the mol% FC contained therein. The rHDL have been studied previously. However, the present study differs from those previously reported in a number of ways. First, our rHDL were prepared by incubation of a great molar excess of DMPC with the apos so that multiple rHDL with different sizes and mol% FC were formed at each initial mol% FC. Second, noting that the mol% FC in the rHDL does not always reflect that of the starting mixture, we determined the mol% FC in each of the isolated complexes, which were relatively homogeneous according to SEC. Lastly, we determined the enthalpies of DMPC melting within the various rHDL complexes. These experimental differences enabled us to refine the current model of FC-containing discoidal rHDL.

Whereas DMPC dispersed in buffer occurs as MLV, according to current models, rHDL has a discoidal structure even in the presence of up to ~20 mol% FC.(12) The effects of FC on the endotherms of pure DMPC are similar to those reported by others who observed that at 14 mol% FC the endotherms can be decomposed into narrow and broad peaks with the T_m of the former, a “virtual DMPC” phase, being equal to or slightly lower than that of pure DMPC and containing a trace of FC and the latter, a more FC-rich phase, having a higher T_m that increases with mol% FC.(28) Others, like us, have observed that the DMPC (0 mol % FC) T_m for rHDL (apo A-I) is lower than that of pure DMPC and that the thermal unfolding of apo A-II within rHDL occurs at a lower temperature than that of apo A-I.(29, 30) Moreover, increasing the FC content increases particle size(12, 13, 31) and the negative charge on apo A-I while reducing the free energy of apo A-I α -helix stabilization.(31)

rHDL (apo A-I) and rHDL (apo A-II) share many similarities. In the absence of FC, they both occur as quasi-discrete particles and as the mol% FC increases, additional larger particles are formed. Small, medium and large rHDL (apo A-I) are nearly the same size as the respective rHDL (apo A-II). The particle sizes do not increase continuously with increasing mol% FC but rather appear to be quantized so that they occur as discrete species. This has been attributed to an increase in domain size with the addition of FC so that additional apos are needed to fully circumscribe the domain(12, 13) and form a disc.

The calorimetric data collected on rHDL support a model with DMPC in three different phases (Figure 8). These are a] virtual DMPC, b] boundary DMPC, i.e., DMPC with $T_m \sim 28^\circ\text{C}$ that is in direct or near-direct contact with the apolipoprotein from which FC is largely excluded, and c] a mixed FC + DMPC phase that has a transition temperature that increases

from ~25 to ~35 °C with increasing mol% FC. Both boundary and mixed FC + DMPC phases have peak T_m 3 to 5 °C above that of the virtual DMPC. According to this model (Figure 8), the large rHDL (apo A-I) with 0 mol% FC contains a virtual DMPC phase with $T_m = 23.0$ °C and a boundary phase with $T_m = 27.0$ °C. Large (5.8 mol% FC) and medium (5.2 mol% FC) rHDL (apo A-I) contain mixed FC + DMPC phases with $T_m = 25.0$ and 26.4 °C and a boundary phase with $T_m = 28.0$ °C. The small (4.5 mol% FC), medium (10.0 mol% FC), and large (14.5 mol% FC) rHDL (apo A-I) have both boundary and mixed FC + DMPC phases melting at 27.3, 27.8 and 28.6 °C. As the size and FC content of rHDL (apo A-I) increases, one would expect the boundary lipid to increase linearly with size and the mixed FC + DMPC phase to increase as the square of the size so that the T_m of the largest, most FC-rich rHDL (apo A-I) would be dominated by the melting of the mixed FC + DMPC phase. This was the large rHDL (apo A-I) containing 14.5 mol% FC, which has $T_m = 28.6$ °C. Thus, it is likely that the T_m of the mixed FC + DMPC phase is higher than that of boundary DMPC, particularly at a high mol% FC. In the context of our three phase model, the calorimetric data for rHDL (apo A-II) was distinct from that apo A-I. Unlike rHDL (apo A-I), rHDL (apo A-II) exhibits no T_m for virtual DMPC. However, like the endotherms for rHDL (apo A-I), the T_m for rHDL (apo A-II) also increase with increasing mol% FC thereby corroborating the hypothesis that the T_m for the FC + DMPC phase increases with increasing mol% FC.

The DSC of both rHDL (apo A-I) and rHDL (apo A-II) after the first heating and cooling cycle were different (Figures 4B and 5B) from the first scans indicating that the rHDL structure has been disrupted in a way that was not reversed over the time scale of the cooling cycle.

Circular Dichroic Data

Like others,(29) we observed that the protein unfolding transition is lower for rHDL (apo A-II) than for rHDL (apo A-I) and that the size and unfolding transition temperature increases with increased mol% FC in rHDL (apo A-II). In addition, we found a linear increase in the protein T_m with mol% FC for both rHDL with the rate being greater for rHDL (apo A-II) even though its T_m in the absence of FC is lower (Figure 7).

According to their respective MRME at 0 mol% FC, the α -helicity of rHDL (apo A-I) at 10 °C is greater than that of rHDL (apo A-II). Moreover, the α -helicity of rHDL (apo A-I) is independent of FC content at all temperatures studied whereas the α -helicity of rHDL (apo A-II) decreases with increasing mol% FC. The T_m for rHDL (apo A-I) and rHDL (apo A-II) increase with mol% FC with respective slopes of 1.4 ± 0.13 and 5.1 ± 0.14 °C/mol% (Figure 7C). Gursky et al(16) showed that spherical HDL resides in a kinetic trap from which it escapes by thermal and chaotropic perturbation during which some but not all apo A-I are transferred to the aqueous phase. Moreover, thermal perturbation of rHDL (apo A-I) and rHDL (apo A-II) also liberates lipid free apos.(32) Thus, the changes observed in the temperature-dependence of the CD melting curves are a superposition of apo A-I and apo A-II desorption and likely changes in apo A-I and apo A-II that remain lipid-bound. During heating, the total α -helical content of the system is reduced because the desorbed apos have less α -helicity than lipidated apos.

A major effect of FC is increased rHDL stability, especially for rHDL (apo A-II). This may be due to the well-documented effect of FC on the quality of the water-DMPC interface. FC modulates the depth of water penetration into lipid bilayers(33) and decreases their interfacial polarity(34) and hydration.(35) Association of the apos with phospholipids is mediated by the hydrophobic effect whereby the nonpolar face of the amphipathic helix inserts into and associates with the phospholipid interface. Reducing the polarity and increasing the hydrophobicity of the interfacial region would be expected to amplify the

hydrophobic effect and stabilize rHDL (apo A-II). The greater stabilizing effect of FC on rHDL (apo A-II) may be due to the greater enhancement of the already stronger hydrophobic association of apo A-II, the more lipophilic of the two apos.

Our studies are relevant to the mechanisms by which HDL are assembled. Early intracellular forms of HDL include those that contain apo A-II but no apo A-I or apo E.(7) Thus, the rHDL (apo A-II) is a rational biophysical model of a nascent HDL. However, to date the lipid composition of hepatocyte nascent HDL (apo A-II) is not known so that firm conclusions cannot be formulated. The surface of mature forms of human HDL is ~25 mol% FC, and according to our data, nearly saturated in terms of the amount of FC that can be spontaneously incorporated into a phospholipid. Although based on a non physiological PC, our data suggests that FC affects the conformation of apo A-II, which would have decreasing helical content as the FC content is increased. In addition, the presence of FC in HDL could increase the lipophilicity of apo A-II for HDL thereby stabilizing LpA-II structure. Thus, it would be interesting to determine whether increasing or decreasing the HDL FC content effects HDL stability and apo A-II conformation and to determine whether macrophage-derived HDL is more or less stable than mature forms of HDL. Future studies will reveal the effects of adding or removing FC from HDL on apo A-II vs. apo A-I conformation and stability.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Funding: Supported by grants-in-aid from the National Institutes of Health (HL-30914 and HL-56865 to H.J.P. and HL109109 to M.A.).

Abbreviations Used

ABCA1	ATP-binding cassette transporter A1
apo	apolipoprotein
C	cholesterol
CD	circular dichroism
CVD	cardiovascular disease
DMPC	dimyristoyl phosphatidylcholine
DSC	differential scanning calorimetry
EV	elution volume
FC	free cholesterol
HDL	high density lipoprotein
LCAT	lecithin: cholesterol acyltransferase
MLV	multilamellar vesicle
PC	phosphatidylcholine
SEC	size exclusion chromatography
T_m	melting temperature

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Figure 1.

Isolation of rHDL Prepared from DMPC and Various Initial Starting Mol% FC as Indicated: A—D, SEC of rHDL (apo A-I); E—H, SEC of rHDL (apo A-II). Bars indicate the fractions that were pooled for analysis by DSC and CD. According to SEC principles, the elution volume of the rHDL increases as its particle size decreases.

Figure 2.

Analytical SEC of the Various rHDL. Left panels contain the SEC profiles of rHDL (apo A-I); each panel indicates the initial mol% FC of each rHDL and its respective elution volume. Right panel are the same data as for the left panel but for rHDL (apo A-II). The compositions of each peak are given in Figure 3.

Figure 3.

Stoichiometry of rHDL. The analyzed rHDL are those isolated according to Figure 1 and tested for purity in Figure 2. Note that a given initial mol% FC can yield multiple, differently sized rHDL with FC compositions that are different from those of the starting mixture. E.g., at 5 mol% FC three species of rHDL (apo A-I) are obtained from a starting FC content of 5 mol%. Note also that typically the elution volume decreases (larger size) as mol % FC of each rHDL increases.

Figure 4.

A, DSC of rHDL (apo A-I). The vertical arrows indicate the melting points, T_m , of each species identified according to the first derivative of the excess heat capacity vs. temperature. The long-dash gray line is the T_m of DMPC. Note that the larger particles exhibit more than one T_m . The legend associated with each scan denotes the mol% FC in the rHDL tested and the elution volume (EV) at which it was isolated. B, Repeat DSC scans of the same rHDL as in A. The most prominent features are the replacement of the broad, multicomponent endotherm of A with a composite endotherm that includes a T_m for a virtual DMPC phase appearing slightly below that of pure DMPC (vertical dashed gray bar). Note the appearance of a second transition defined as the FC + DMPC phase at temperatures higher than that of pure DMPC for the medium (5.2 mol% FC) and large (0 and 5.8 mol% FC).

Figure 5. Same as Figure 4A, B but for rHDL (apo A-II). The long-dash gray line is the T_m of DMPC. The dotted gray line is the T_m for 0 mol% FC rHDL. Again, like the rHDL (apo A-I) second scan, the broad first scan endotherm is replaced by two peaks, a sharp one similar to that of DMPC and a broader peak that represents an increasing fraction of the total endotherm as the mol% FC increases.

Figure 6.

Enthalpy of DMPC Melting within rHDL as a Function of Mol% FC. Notable features are a lower melting enthalpy for rHDL (apo A-II) than for rHDL (apo A-I). The slopes for the two rHDL are similar but distinct from the much greater slope for DMPC.

Figure 7.

Thermal Unfolding of rHDL as Assessed by the magnitude of the CD spectrum at 222 nm, which senses α -helical content. Notable features are the similarity of the MRME vs. temperature plots for all rHDL (apo A-I) (Panel A) irrespective of FC content. The data for rHDL(apo A-II) are more complicated (Panel B) and show loss of α -helical content with increasing mol% FC and a profound increase in T_m with mol% FC (See also Supplementary Figure 4). C, Differences in stability assessed from the temperature dependence of the T_m from Panels A and B vs. mol% FC. Both rHDL are stabilized by FC, i.e., have higher T_m . rHDL stability increases with added cholesterol faster for rHDL (apo A-II) than for rHDL (apo A-I).

Figure 8.

Model of rHDL Phases Based on DSC data. This model shows that the number of phases within rHDL increases with mol% FC and particle size. In B, increasing shading intensity represents increased FC content within the FC + DMPC phase. The smallest rHDL contain only a boundary DMPC phase.