Pressure Perturbation Calorimetry of Lipoproteins Reveals an Endothermic Transition without Detectable Volume Changes: Implications for Apolipoprotein Adsorption to Phospholipid Surface

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SUPPLEMENTAL DATA

Protein:lipid complex		Partial specific volume, $cm^3\ g^{-1}$
Lipid-free apoA-I (1)		0.735
apoA-I:DMPC	1:5	0.767
	1:10	0.792
	1:20	0.830
	1:40	0.877
	1:50	0.891
apoA-I:DPPC:Ch		0.949
HDL		0.880
LDL		0.963
VLDL		0.990

Table S1. Partial specific volumes of apolipoprotein:lipid complexes used for quantitative analysis of the PPC data. The values for the apoA-I:DMPC and apoA-I:DPPC:Ch complexes were calculated based on the reported partial specific volumes of 0.735 $cm^3\ g^{-1}$ for 100% protein (1) and 1.02 $cm^3\ g^{-1}$ for 100% lipid (2). The published values for HDL (3, 4), LDL (4) and VLDL (1, 4) were used.

Lipoprotein or protein	d, nm	Volume expansion coefficient α_V, 10^3/deg 5 °C	40 °C	80 °C
Plasma lipoproteins				
VLDL	30-100	6.36	3.94	2.61
IDL	25-40	4.14	2.18	1.28
LDL	22-24	2.65	2.2	1.89
HDL	8-12	1.5	1.14	0.89
Reconstituted HDL				
A-I : DMPC:Ch	9-12	1.91	3.14	2.1
A-I : DMPC	9-12	1.56	2.34	2.0
Lipid-free protein				
apoA-I		1.15	0.71	0.53

Table S2. Lipoproteins used in this study and their volume expansion coefficients determined from the PPC studies.

Figure S1. Heat-induced remodeling of human plasma VLDL analyzed by negative stain electron microscopy (A), differential scanning calorimetry (B), and pressure perturbation calorimetry (C). VLDL (1.2 mg/mL protein in 10 mM Na phosphate, pH 7.5) were either intact or heated at a rate of 90 °C/h from 5-100 °C and cooled to 22 °C prior to EM analysis. EM data of the heat-denatured VLDL from this and earlier study (5) show large lipid droplets that are products of lipoprotein rupture. (B) DSC data show heat capacity peaks corresponding to reversible smectic-to-disorder phase transition in the core cholesterol esters and triacylglycerides ("core") and to irreversible VLDL remodeling, fusion and rupture ("remodeling"). PPC data (C) were recorded under similar conditions as the DSC data but at a rate of about 80 °C/h. Volume change involved in the core transition in VLDL, which was determined by integrating the $\alpha_v(T)$ peak and using partial specific volume of VLDL listed in Table S1, is $\Delta V/V$ = 1.62 ± 0.5%. The accuracy in $\Delta V/V$ determination reflects batch-to-batch variations in the lipoprotein composition which affect the absolute value of $\alpha_v(T)$ and $\Delta V/V$.

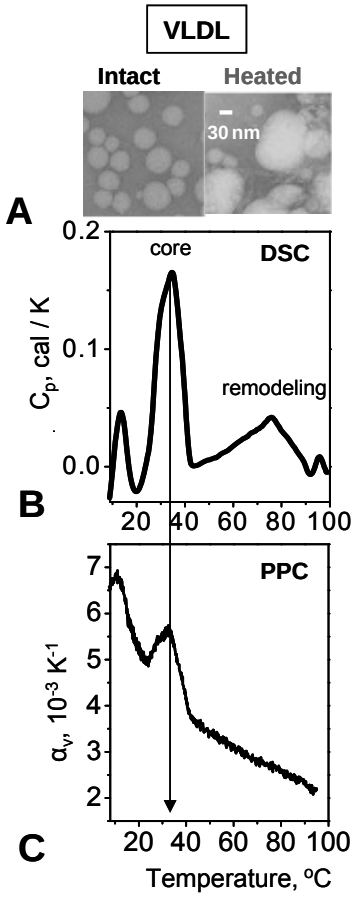


Figure S2. Biochemical analysis of model discoidal rHDL reconstituted from apoA-I, DPPC and cholesterol, and of the products of their thermal denaturation. ApoA-I:DPPC:Ch molar ratio was 1:80:4; protein concentration was 0.5 mg/mL. (A) SEC profile of lipoproteins that were intact (black) or were irreversibly denatured upon heating to 115 °C (grey). The fractions corresponding to peak positions 1 (vesicles), 2 (intact rHDL) and 3 (dissociated apoA-I) were collected in 0.5 mL aliquots and analyzed by thin-layer chromatography (TLC) (B). Known amounts of DPPC and unesterified cholesterol (marked PC and Ch) were used as standards (St). Lipids were extracted by Folch method (6), dried and used for spotting in TLC plates. The numbers 1-3 correspond to peaks in the SEC profile. TLC analysis shows the presence of DPPC and cholesterol in fractions 1-3.

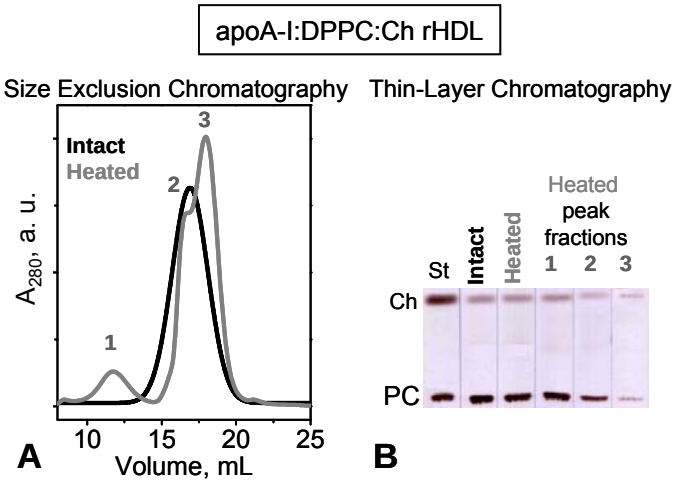


Figure S3. Size characterization of apoA-I: DMPC complexes by negative stain EM and non-denaturing gel electrophoresis. Protein to lipid molar ratios ranged from 1:5 to 1:50 as indicated; 1:0 stands for lipid-free protein. (A) Electron micrographs show discoidal apoA-I:DMPC complexes stacked on edge, with occasional face-up views of round particles. The quantity of discoidal complexes increased with increasing the relative amount of lipid. (B) NDGE shows that such an increase leads to particle size re-distribution, from the predominantly small rHDL (d<8 nm) to larger ~10 nm rHDL. Lipid-free apoA-I is self-associated at 0.5 mg/mL concentrations that were used in NDGE experiments.

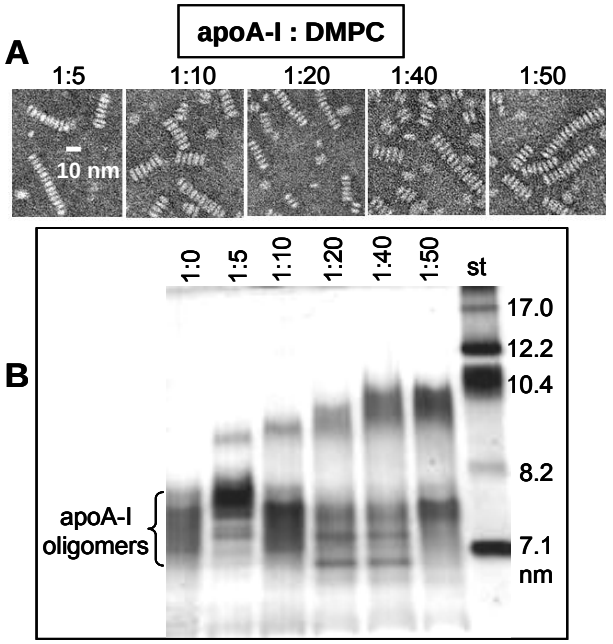


Figure S4. Effects of DPPC MLV on the self-association and thermal stability of apoA-I. The protein in standard buffer is either lipid-free (black) or mixed with DPPC MLV at 1:100 molar ratio of protein to lipid (grey). (A) NDGE, which was run at 0.5 mg/mL apoA-I concentration in standard buffer, indicates that the presence of DPPC MLV helps disperse protein oligomers. (B) Far-UV circular dichroism heating and cooling data, which were recorded at 222 nm for α-helical unfolding in apoA-I, indicate that the protein stability does not change in the presence of lipid vesicles. The arrows indicate the direction of temperature changes. The temperature of apoA-I unfolding, T_m=60 ^oC, is indicated.

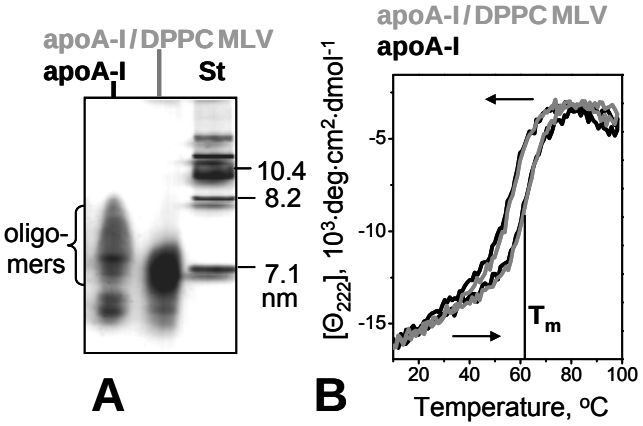
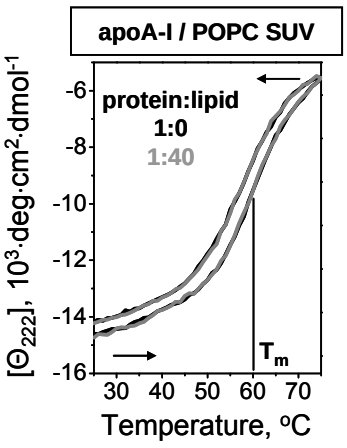


Figure S5. Thermal stability of apoA-I is not affected by adsorption to POPC SUV. ApoA-I was lipid-free (black) or in the presence of POPC SUV at 1:40 molar ratio of protein to lipid (grey). Protein unfolding during heating and cooling was monitored by CD at 222 nm. The arrows indicate the directions of temperature changes. The unfolding temperature of apoA-I, T_m=60 ^oC, is indicated.



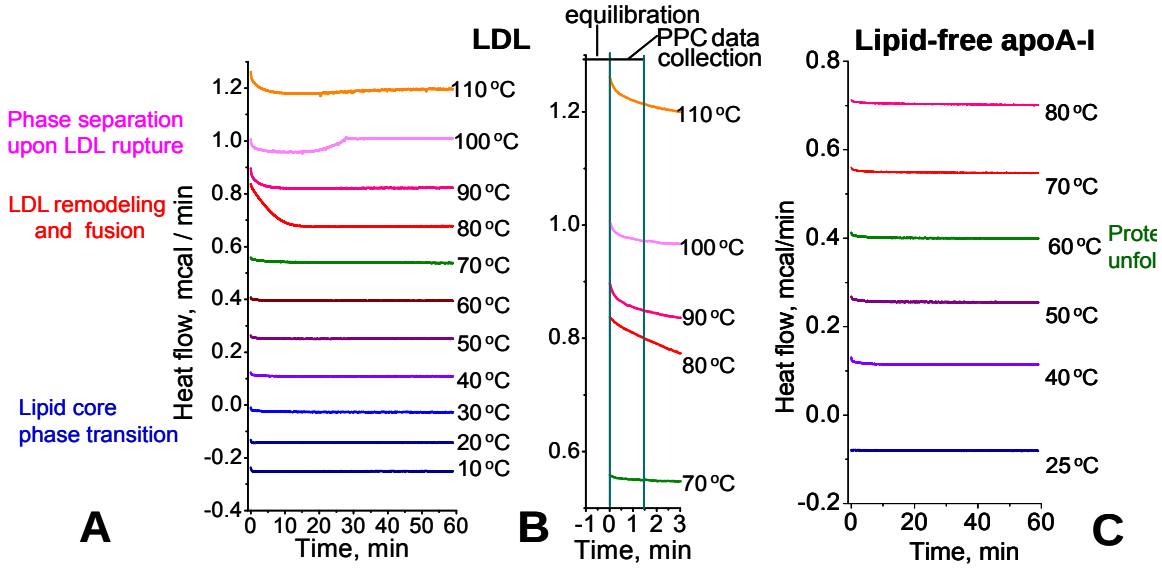


Figure S6. Isothermal scans recorded by using VP-DSC at constant temperatures. Complete 60 min scans of LDL (3 mg/ml protein in standard buffer) (**A**) and the first 3 minutes of these scans (**B**). Lipid-free apoA-I (1 mg/ml protein in standard buffer) (**C**). To assess the time course of the heat flow during PPC experiments (in which the measurements are performed at a constant temperature over an approximately 90 sec cycle of compression and decompression), we used isothermal scan mode of the VP-DSC instrument. The heat flow as a function of time was recorded from the same sample at several constant temperatures from 10 to 110 ^{o}C. At each temperature, the instrument was equilibrated for 1 min and the data were recorded for 60 min prior to increasing the temperature by 10 ^{o}C to the next constant value. Similar data recorded from the buffer showed no detectable variations after the 1 min equilibration, indicating that the instrument was adequately equilibrated by the beginning of the data collection (buffer data not shown). Selected data are shown in Fig. S6. As expected, fast thermodynamically reversible transitions such as the unfolding of lipid-free apoA-I at T_m=60 ^{o}C (**C**) or smectic-to-disorder phase transition in LDL core lipids near room temperature (**A**), display no slow time-dependent changes in the transition temperature range. In contrast, slow irreversible transitions whose relaxation time is comparable or longer than 1 min, such as LDL heat denaturation, manifest themselves as slow time-dependent changes in the transition temperature range (red line in **A**). Thus, the isoscan data at 80 ^{o}C (which approaches $T_{m,app}$ =85 ^{o}C observed for this LDL batch by DSC upon heating at a rate of 90 ^{o}C/h) show that the relaxation time of the heat-induced LDL remodeling and fusion is close to 10 min; the progress of the reaction during the 1st minute is illustrated in zoomed-in portion of the data (**B**, red line). These data suggest that at $T \leq T_{m,app}$, PPC is not well-suited for the analysis of lipoprotein heat denaturation since the relaxation rate of this denaturation exceeds the data collection time in PPC.

In contrast, our earlier spectroscopic data recorded in temperature-jumps of various lipoproteins show that at $T > T_{m,app}$, the reaction rate rapidly increases and the relaxation time becomes shorter

than 1 min (5, 7, 8), i. e. shorter than the data collection time in PPC. Therefore, at $T>T_{m,app}$, lipoprotein denaturation is fast enough to be detectable by PPC. This suggests that the lack of observable changes in volume expansion coefficient of lipoproteins at high temperatures cannot be accounted for solely by the slow progress of the reaction during the data collection time in PPC.

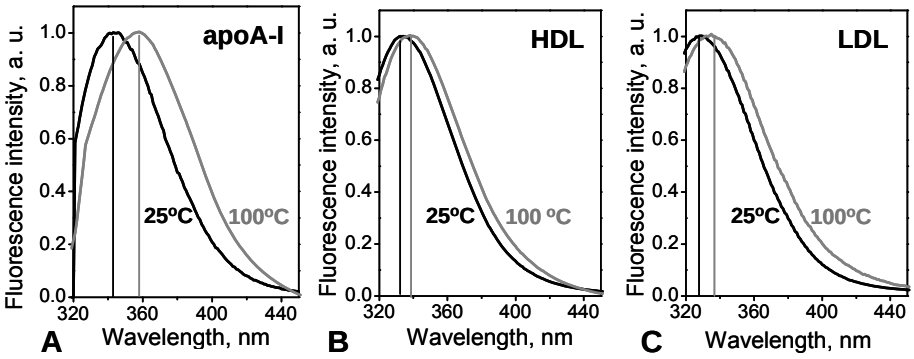


Figure S7: Solvent exposure of apolipoprotein aromatics assessed by intrinsic Trp fluorescence in the native and in thermally denatured states. Fluorescence emission spectra were recorded using a Fluoromax-2 spectrofluorimeter from 320-500 nm, with 280 nm excitation wavelength and 5 nm bandwidth for excitation and emission. The protein or lipoprotein solutions (0.05 mg/mL protein in standard buffer) were equilibrated at 25 ^{o}C (black) or 100 ^{o}C (grey) for 1 h before the data collection. (A) Lipid-free apoA-I showed a large red shift in the wavelength of maximal fluorescence, λ_{max}, upon thermal unfolding, from 343 nm at 25 ^{o}C to 358 nm at 100 ^{o}C, indicating complete solvent exposure of Trp in the heat-denatured state at 100 ^{o}C. In HDL (B) and LDL (C) proteins, smaller red shifts in λ_{max} were observed, from 333 to 339 nm in HDL and from 328 to 335 nm in LDL. Furthermore, λ_{max} at 100 ^{o}C was shorter than that corresponding to full exposure of Trp (~360 nm). Hence, upon thermal denaturation of HDL and LDL, the Trp groups remain partially buried. This results, at least in part, from the protein-lipid association retained in the heat-denatured lipoprotein state.

References

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