Folded Functional Lipid-Poor Apolipoprotein A-I Obtained from Heated High-Density Lipoproteins: Relevance to HDL Biogenesis

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Supplemental Data

Figure S1. Lipid analysis by thin-layer chromatography (TLC) of human HDL before (intact) and after heating to 115 ^{o}C in DSC experiments under N_2 pressure (heated). Lipid standards are marked as follows: CE – cholesterol ester; TG – triacylglyceride; FFA – free fatty acids; Ch – free (unesterified) cholesterol; PE – phospatidyl-ethanolamine; PC - phosphatidylcholine; SM – sphingomyelin; lysoPC – lyso-phosphatidylcholine. TLC is a sensitive tool for detecting lipid peroxidation products, such as additional FFA and lysoPC, in lipoproteins [1]. The absence of such additional products in heated HDL, together with the absence of detectable changes in absorbance at 234 nm, indicates that HDL heating to 115 ^{o}C in our DSC experiments does not lead to lipid peroxidation.

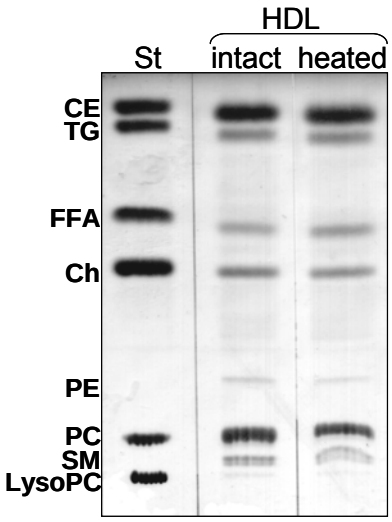
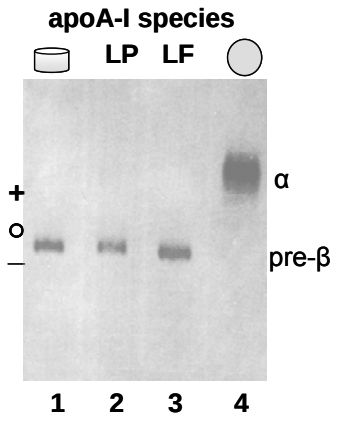
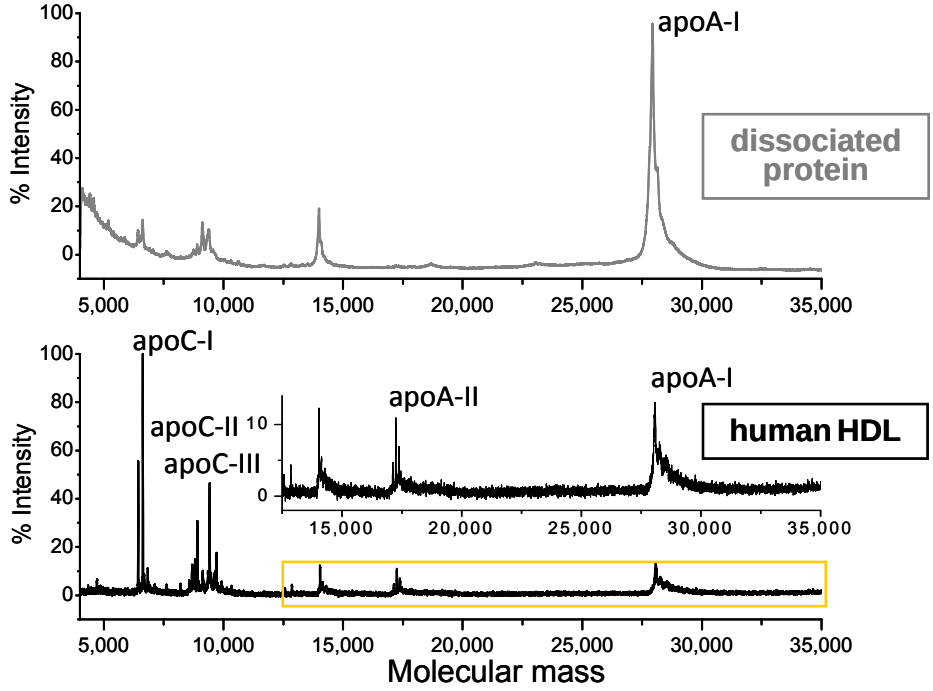


Figure S2. Net charge on various apoA-I species assessed by agarose gel electrophoresis. In contrast to plasma spherical HDL that show α electrophoretic mobility (**lane 4**), lipid-poor apoA-I isolated by SEC from heated HDL (**LP, lane 2**) shows pre-β mobility, which is characteristic of the lipid-poor apoA-I found in plasma [2]. Lipid-free apoA-I (**LF, lane 3**) and model discoidal HDL (**lane 1**) reconstituted from apoA-I, Ch, and POPC (1:4:80 molar ratio, diameter d=9.6 nm [3]) are shown for comparison.



Agarose gel electrophoresis was done using a TITAN lipoprotein gel electrophoresis system. Samples containing 4 μg of protein were loaded on the pre-cast 1% gel, and the gel was run in barbital - sodium barbital buffer at 60 V for 40 min and at 125 V for 7 min. The gel was dried at 70°C for 20 min and stained with Coomassie R-250 protein stain.

Figure S3. Protein analysis of intact human HDL (**bottom panel**) and of the protein-rich fraction isolated from the heat-denatured human HDL (**top panel**) by matrix-assisted laser desorption / ionization (MALDI) time-of-flight (TOF) mass spectrometry. The peaks observed in the spectrum of intact HDL correspond to apoA-I (28 kDa), apoA-II (17 kDa) (boxed and zoomed-in portion in the insert), and apoC-I (6.6 kDa), apoC-II (8.9 kDa) and apoC-III (8.8 kDa). Only apoA-I is observed in the protein-rich fraction isolated from the heat-denatured HDL.



MALDI TOF was performed using Reflex-IV instrument (Bruker Daltonics). Protein:lipid samples (0.51 µL solution of 1 mg/mL protein concentration) were mixed with the matrix (0.51 µL of saturated aqueous solution of cyano-4-hydroxycinnamic acid (M_r=189) in 70% acetonitrile and 0.1% trifluoroacetic acid). The instrument was optimized and calibrated in linear mode using a standard calibration mixture containing oxidized B-chain of bovine insulin, equine cyctochrome C, equine apomyoglobin, and bovine serum albumin. The spectra were collected as an average of 100 laser shots at a laser power varying from 50-80 %. Multiple spectra in the range of 3-200 kDa were collected from the same sample covering the entire sample area.

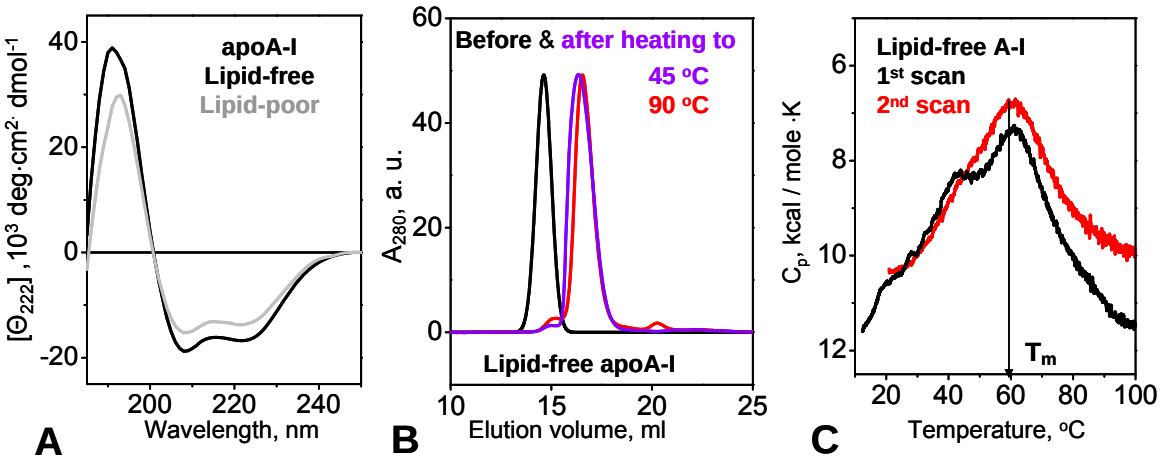


Figure S4. Secondary structure of apoA-I obtained from human plasma HDL and the effects of heating on the protein self-association. (**A**) Far-UV CD spectra of lipid-poor (grey) and free apoA-I (black) recorded from 0.02 mg/mL protein in standard buffer at 25 ^{o}C. The spectral difference suggests a reduction in the α-helical content from about 57±4 % in lipid-free to 50±4% in lipid-poor apoA-I. (**B**) SEC profile of lipid-free apoA-I recorded at 22 ^{o}C before (black) and immediately after heating to 45 ^{o}C (violet) or 90 ^{o}C (red). Lipid-free apoA-I was isolated by HDL delipidation using high Gdn HCl concentration followed by KBr density centrifugation, and was refolded as described [ref. [4] and references therein]. For SEC, protein concentration was 1 mg/mL in 10 mM Na PBS, pH 7.5. At this concentration and room temperature, lipid-free apoA-I forms oligomers (black line in panel B) [5]. Heating to 45 ^{o}C and beyond converts apoA-I oligomers into monomers (colored lines). (**C**) Consecutive heating scans of lipid-free apoA-I recorded by DSC. Protein concentration was 1 mg/mL in standard buffer. The main peak centered at T_m=60 ^{o}C represents reversible protein unfolding, and the shoulder observed in the first scan near 40 ^{o}C represents oligomer dissociation.

Figure S5. NDGE analysis of discoidal rHDL formed from DMPC and lipid-poor or free apoA-I. The complexes were obtained by spontaneous reconstitution upon co-incubation of apoA-I and DMPC (protein : lipid weight ratio 1:4) at 24 ^{o}C, as shown in Fig. 7. Samples containing 5 μg of protein were loaded in each lane. **Lane 1**: lipid-free apoA-I; **2** – lipid-poor apoA-I; **3** – rHDL formed from free apoA-I; **4** – rHDL formed from lipid-poor apoA-I; **st** – molecular size standards.

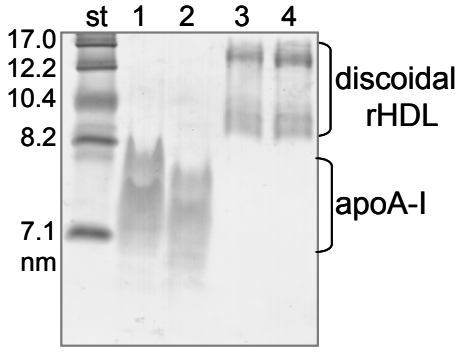
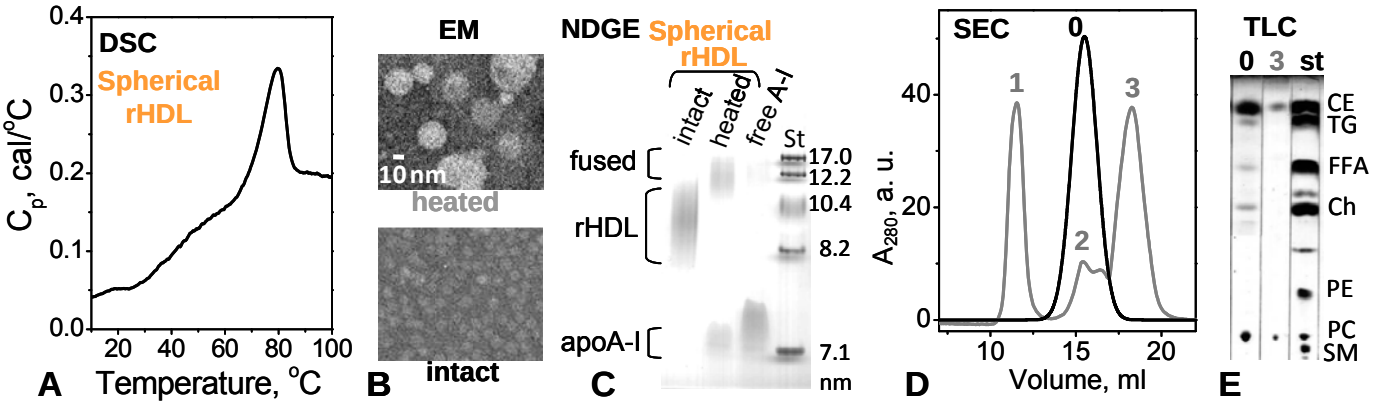


Figure S6. Thermal remodeling of spherical rHDL comprised of apoA-I, POPC, Ch, and CE. Core-containing spherical particles were obtained from discoidal apoA-I:POPC:Ch rHDL via the LCAT reaction as described [3]. Briefly, discoidal rHDL were incubated with low-density lipoprotein (LDL) and lecithin-cholestol acyltransferase (LCAT). LDL were used as a source of cholesterol that was converted by LCAT into CE and was sequestered in the particle core, converting discoidal into spherical rHDL. The reaction mixture was fractionated by sequential ultracentrifugation to isolate rHDL. NDGE and EM analysis showed that the resulting particles had size in the HDL range. SDS PAGE and mass spectrometry confirmed complete removal of LDL, LCAT and other proteins from rHDL. These rHDL were purified by SEC to obtain homogeneous population (d=9.3-9.6 nm), and were dialyzed against standard buffer for further studies. Spherical morphology of the resulting rHDL was confirmed by negative-stain EM (**B**) and the presence of apolar core lipids (mainly CE) was confirmed by thin-layer chromatography (TLC, lane 0 in panel **E**).



(**A**) DSC data of spherical rHDL (1 mg/mL protein) recorded during heating from 10 to 100 °C. The irreversible transition centered near 80 °C reflects rHDL fusion, rupture and apoA-I dissociation. (**B**) Electron micrographs of negatively stained rHDL that were intact (bottom) or heated to 100 °C and cooled to 22 °C. Intact rHDL are seen face-up, which contrasts with discoidal particles that form stacks in negative stain (Fig. 7B, C). (**C**) NDGE analysis of intact and heated rHDL. Free apoA-I is shown for comparison. (**D**) SEC profile of spherical rHDL that were intact (black) or heated 100 °C and cooled to 22 °C (grey). Fraction 3 (16-19 mL elution volumes) corresponds to apoA-I monomer. (**E**) TLC analysis of lipid composition in spherical rHDL and in the products of their thermal denaturation. **Lane 0** - intact rHDL; **lane 3** - peak fraction 3 from SEC profile of the heat-denatured rHDL (panel D); **st** - lipid standards. The results in panels C-E show that fraction 3 contains monomeic lipid-poor apoA-I with some PC and CE.

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