Effects of Acyl Chain Length, Unsaturation and pH on Thermal Stability of Model Discoidal High-Density Lipoproteins

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SUPPLEMENTAL DATA

Lipoprotein preparation

Lipid suspensions were mixed with sodium cholate and incubated overnight, leading to complete lipid clearance; the incubation temperatures (4 ^{o}C for DLPC, 24 ^{o}C for DMPC, 38 ^{o}C for DPPC, and 52 ^{o}C for DSPC) were close to the respective gel-to-liquid crystal phase transition temperatures (Table 1). Protein stock solution (0.5 mg/mL apoC-I) was added to the lipid–detergent mixture; the protein:lipid ratios were about 1:4 mg/mg for DLPC, DMPC and DPPC and 1:3 mg/mg for DSPC. The mixtures were incubated overnight and the detergent was removed by dialysis. Discoidal particles containing shorter-chain PCs were also formed by spontaneous reconstitution upon overnight incubation of protein with lipid at 4 ^{o}C for DLPC and at 24 ^{o}C for DMPC; rHDLs obtained by cholate dialysis and by spontaneous reconstitution had indistinguishable structure and stability properties. Fig. 1S shows EM images of typical apoC-I disk preparations with various PCs used in this study. Fig. 2S shows non-denaturing gel electrophoresis of apoC-I:DMPC complexes before and after heat denaturation (courtesy of Dr. Sangeeta Benjwal[30]).

Characterization of disks with different diameters

ApoA-I complexes with PCs that have identical protein and lipid composition but different diameters were obtained by varying protein:lipid weight ratio from about 1:2.5 to 1:4 mg/mg in the disk preparations to form smaller and larger particles, respectively. Similar variations in protein:lipid weight ratio in apoC-I preparations usually resulted in excess protein or lipid rather than formation of smaller or larger disks. However, occasional preparations yielded apoC-I complexes with DMPC, DPPC or DSPC that had identical composition but distinctly different diameters. These preparations

were used to test the effect of disk diameter on disk stability (Figs. 3S, 4S). Thermal denaturation data of such large and small particles, which were recorded by CD in the melting and kinetic experiments, largely superimposed, indicating similar stability. This result, taken together with our earlier studies of DMPC disks varying in protein composition (apoA-I, apoA-II, wild-type and mutant apoC-I) and/or diameters,[16,24,25] clearly shows that the binary discoidal complexes with similar protein and lipid composition but different diameters that were used in our studies had similar thermal stability.

This does not exclude the possibility that protein and lipid interactions in other complexes may differ on disks of different diameters, leading to differential stability. Thus, disks of certain sizes may be only transiently stable (for example, if they have apolipoprotein trapped in a relatively unfavorable conformation) and convert over time to smaller or larger particles and/or lipid-poor protein. Such spontaneous remodeling was recently reported by Oda and colleagues for 8.4 nm complexes of apoA-I, POPC and free cholesterol; similar disks of larger and, especially, smaller size appeared more stabile over time.[31] This result is consistent with the notion that discoidal particle as a whole may not comprise a cooperative unit during denaturation; otherwise, disks with larger diameters that contain larger number of protein and lipid molecules would have had larger activation energy and kinetic stability, which was clearly not the case in our[16,24,25] (Figs. 3S, 4S) and in other studies (31 and references therein). Taken together, these studies suggest that the particle stability is determined by specific protein and lipid interactions (which may or may not be significantly different in disks of different diameters) rather than the disk diameter per se.

REFERENCES

30. Benjwal, S. 2007. Structure and kinetic stability of model discoidal high-density lipoproteins containing human apolipoprotein C-I. Ph.D. Thesis, Boston University School of Medicine.

31. Cavigiolio, G., B. Shao, E. G. Geier, G. Ren, J. W. Heinecke, and M. N. Oda. 2008. The interplay between size, morphology, stability, and functionality of high-density lipoprotein subclasses. *Biochemistry* **47** (E-publication ahead of print Mar 27).

Figure 1S Negative staining electron micrographs of apoC-I complexes used in this study and the corresponding histograms showing disk diameter distributions.

Figure 2S Non-denaturing polyacrylamide gel electrophoresis of apoC-I:DMPC complexes (adopted with permission from ref. 30). Lane 1 – lipid-free apoC-I; lane 2 – intact apoC-I:DMPC disks; lane 3 – thermally denatured disks (same as in lane 2 after 15 min incubation at 90 ^{o}C). Protein concentration is 0.5 mg/ml in 10 mM Na Phosphate buffer, pH 7.6; human apoC-I at this concentration is self-associated. ApoC-I:DMPC disk sizes assessed from comparison with molecular size standards (St) are consistent with those observed by EM (Fig. 1S).

Figure 3S Diameter of apoC-I:DPPC complexes has no significant effect on the disk stability. EM images illustrating discoidal complexes of apoC-I:DPPC that have distinctly different diameters and the corresponding size histograms. Representative T-jump CD data of these complexes indicate similar thermal stability.

Figure 4S Diameter of apoC-I:DSPC complexes has no significant effect on the disk stability. EM images illustrating discoidal complexes of apoC-I:DPPC that have distinctly different diameters and the corresponding size histograms. Representative T-jump CD data of these complexes indicate similar thermal stability.

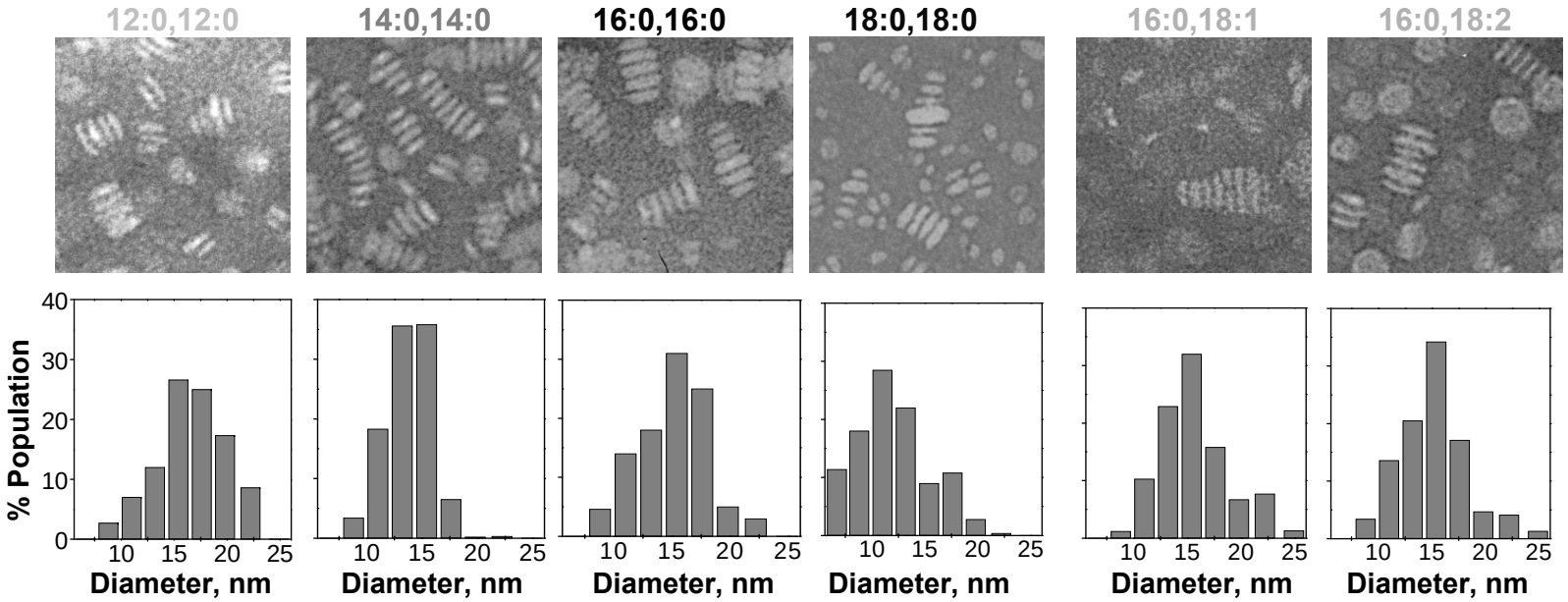
12:0,12:0
14:0,14:0
16:0,16:0
18:0,18:0
16:0,18:1
16:0,18:2
% Population
0
10
20
30
40
10 15 20 25
Diameter, nm

Fig 1S

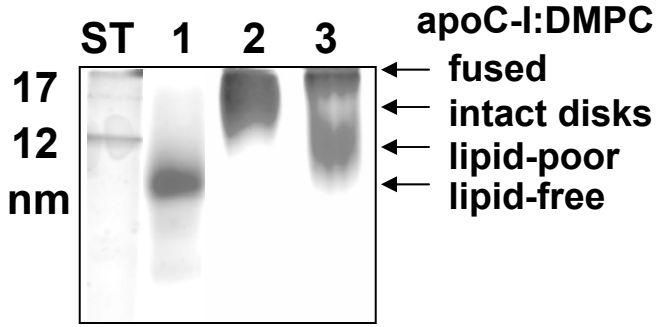
ST
1
2
3
apoC-I:DMPC
17
12
nm
fused
intact disks
lipid-poor
lipid-free

Fig. 2S

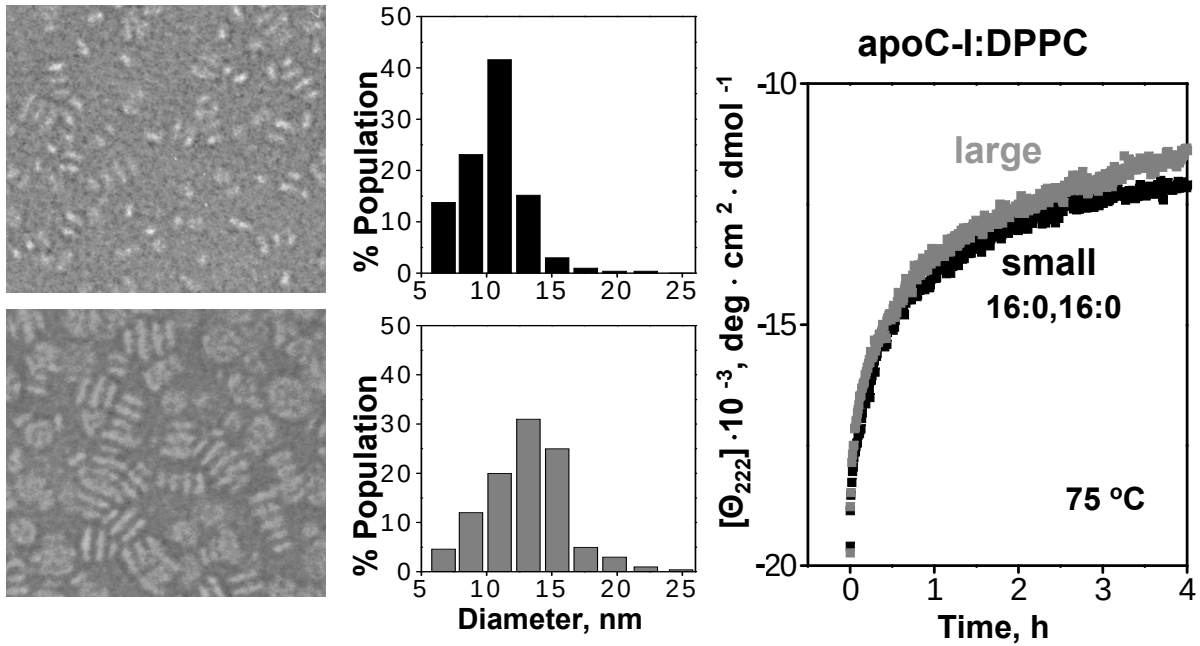
% Population
Diameter, nm
apoC-I:DPPC
large
small
16:0,16:0
75 °C
[Θ222] ·10 -3, deg · cm 2 · dmol -1
Time, h

Fig. 3S

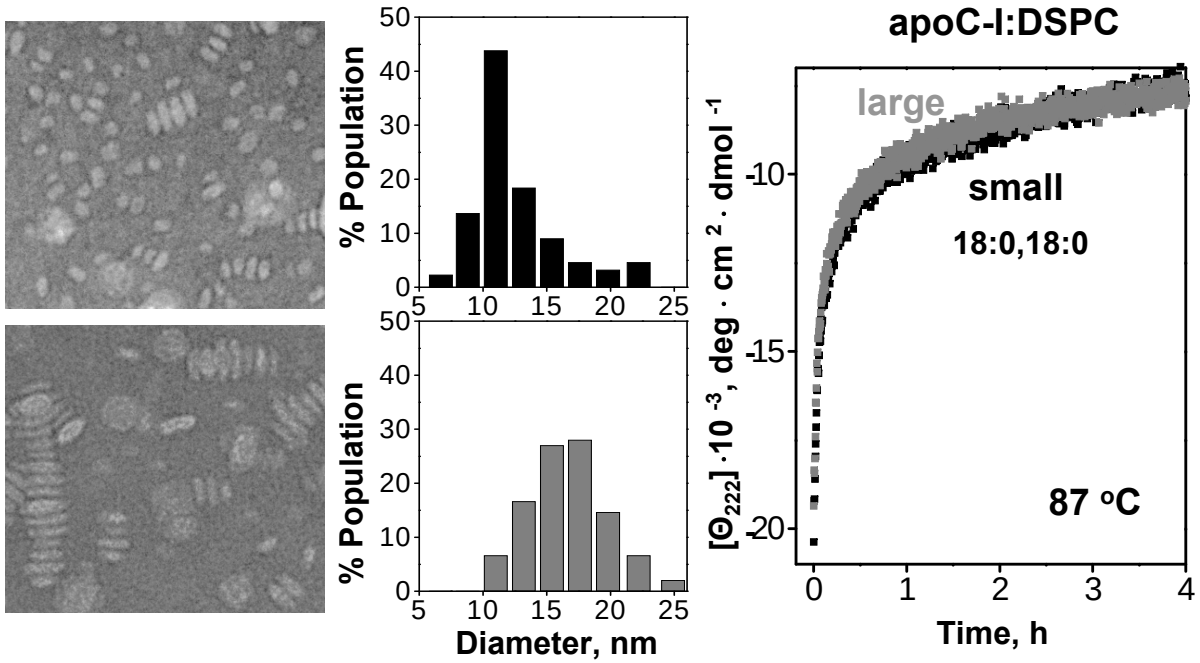
% Population
Diameter, nm
apoC-I:DSPC
large
small
18:0,18:0
87 °C
[Θ222] ·10 -3, deg · cm 2 · dmol -1
Time, h

Fig. 4S