SUPPLEMENTAL MATERIALS AND METHODS

Chemical cross-linking of apoA-I on discrete-sized particles—DMPC:apoA-I complexes were prepared by the cholate dialysis method. Chemical cross-linking of apoA-I on the particles in solution was carried out with a 100 molar excess of Bis(sulfosuccinimidyl)suberate (BS^3) for 1 hr at room temperature **(1)**. The reaction mixture was subjected to NDGGE. Gel slices containing different-sized particles were sliced out and minced in the SDS-PAGE sample buffer. After an overnight incubation, extracted apoA-I was recovered from the supernatant following a brief centrifugation and subjected to SDS-PAGE analysis.

Circular dichroism spectroscopy—The circular dichroism (CD) spectroscopy was conducted as described previously **(2,3)**. Briefly, the CD spectra were recorded using an AVIV 62DS spectropolarimeter (Lakewood, NJ) equipped with a thermoelectric temperature controller and interfaced to a personal computer. The instrument was calibrated with (1S)-(+)-10-camphorsulfonic acid. The CD spectra were measured with apoA-I or (Δ43)apoA-I in solution (PBS) or in POPC complexes from 260 to 190 nm every 0.5 nm with 4 s averaging per point and a 2 nm bandwidth. A 0.01 cm path length cell was used for obtaining the spectra. The CD spectra were signal averaged by adding four scans, baseline corrected. All the CD spectra were recorded at 25°C with protein concentration of 7.7 μM. We have confirmed that the spectra were concentration-independent in the concentration range of 2.5 to 25 μM for both apoA-I and (Δ43)apoA-I **(2)**.

The mean residue ellipticity, $[\theta]_{MRE}$ (deg cm^2 $dmol^{-1}$), was calculated using the following equation: $[\theta]_{MRE} = (MRW \times \theta) / (10cl)$,
where MRW is the mean residue weight (molecular weight of the protein divided by the number of amino acids in the protein), θ is the observed ellipticity in degrees, *c* is the concentration of the protein in grams per milliliter, and *l* is the path length of the cell in centimeters. The percent helicity of the protein was estimated from the following equation:
% α-helix= $([\theta]_{222}+3000)/ (36000 + 3000) \times 100$, where $[\theta]_{222}$ is the mean residue ellipticity at 222 nm **(4)**.

Electron microscopy—The transmission electron microscopic analysis of the particles was carried out as described previously **(5)**. Briefly, the complexes were adsorbed to hydrophilic, carbon and Formvar-coated grids. Samples were negatively stained for 20 s with 2% phosphotungstic acid, pH 7.0. Digital images were taken using a Philips CM-30 electron microscope operated at 80 keV accelerating voltage. For quantification, at least 10 arbitrarily selected fields were chosen and more than 200 particles were measured.

REFERENCES

1. Li, L., Chen, J., Mishra, V. K., Kurtz, J. A., Cao, D., Klon, A. E., Harvey, S. C., Anantharamaiah, G. M., and Segrest, J. P. (2004) *J Mol Biol* **343**, 1293-1311
2. Rogers, D. P., Brouillette, C. G., Engler, J. A., Tendian, S. W., Roberts, L., Mishra, V. K., Anantharamaiah, G. M., Lund-Katz, S., Phillips, M. C., and Ray, M. J. (1997) *Biochemistry* **36**, 288-300
3. Mishra, V. K., Palgunachari, M. N., Datta, G., Phillips, M. C., Lund-Katz, S., Adeyeye, S. O., Segrest, J. P., and Anantharamaiah, G. M. (1998) *Biochemistry* **37**, 10313-10324

4. Morrisett, J. D., Sparrow, J. T., Hoff, H. F., and Gotto, A. M., Jr. (1973) *Cardiovasc Res Cent Bull* **12**, 39-51
5. Catte, A., Patterson, J. C., Jones, M. K., Jerome, W. G., Bashtovyy, D., Su, Z., Gu, F., Chen, J., Aliste, M. P., Harvey, S. C., Li, L., Weinstein, G., and Segrest, J. P. (2006) *Biophys J* **90**, 4345-4360

SUPPLEMENTAL FIGURES

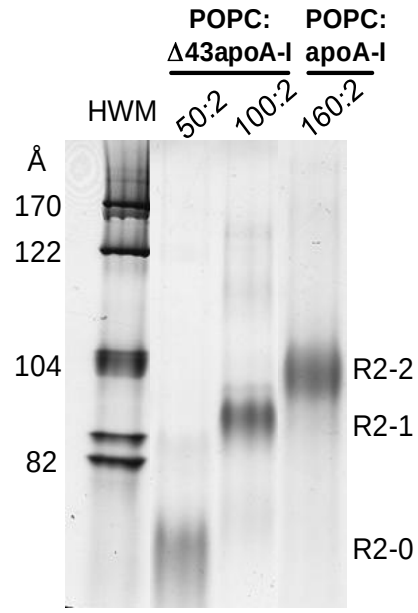

FIG. S1. Formation of relatively homogeneous R2-2, R2-1, and R2-0 assemblies with POPC. FL-apoA-I or Δ43apoA-I were used to prepare relatively pure samples of discrete-sized particle with POPC at different molar ratios by the cholate dialysis method. The figure shows a representative NDGGE analysis of such assemblies.

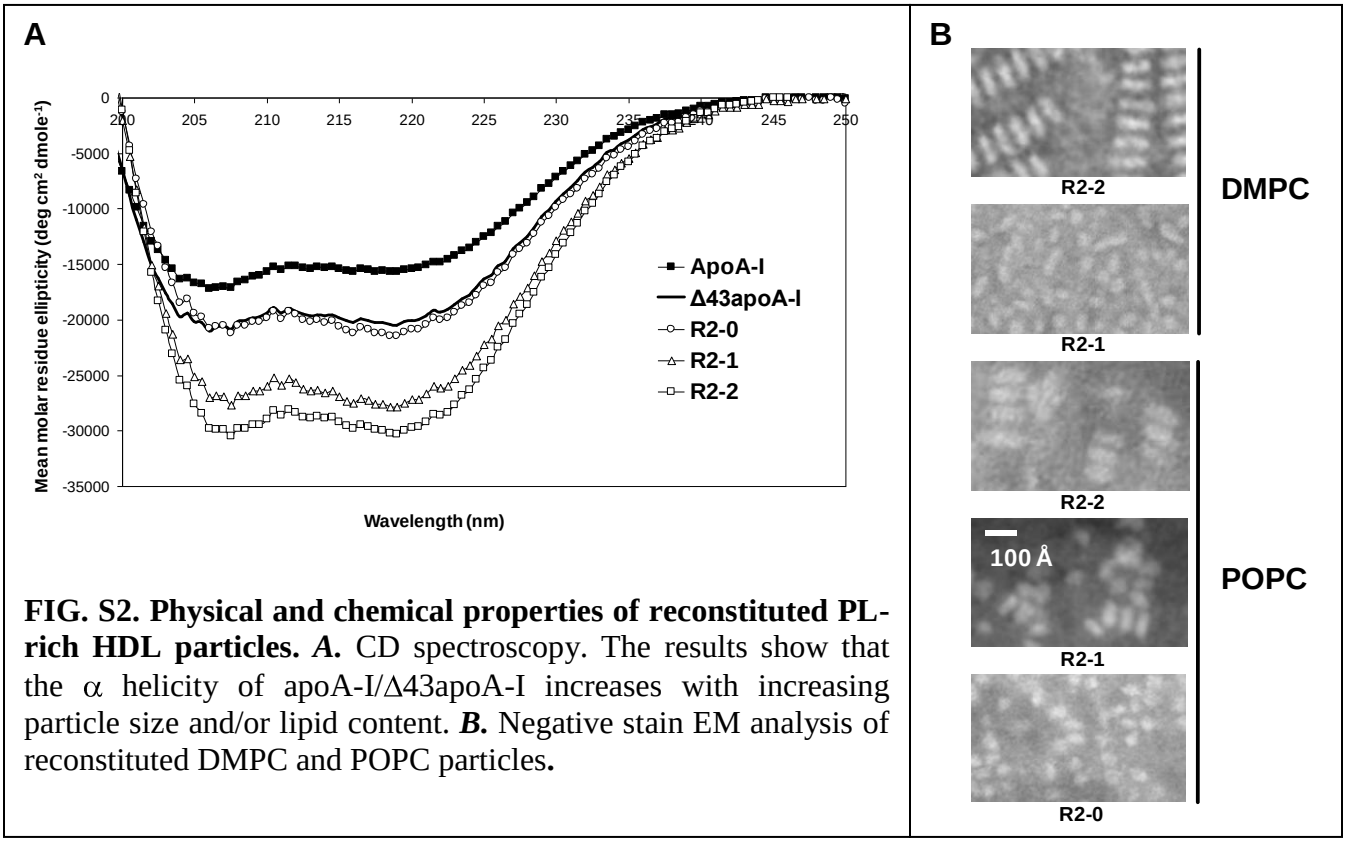

FIG. S2. Physical and chemical properties of reconstituted PL-rich HDL particles. ***A.*** CD spectroscopy. The results show that the α helicity of apoA-I/Δ43apoA-I increases with increasing particle size and/or lipid content. ***B.*** Negative stain EM analysis of reconstituted DMPC and POPC particles**.**

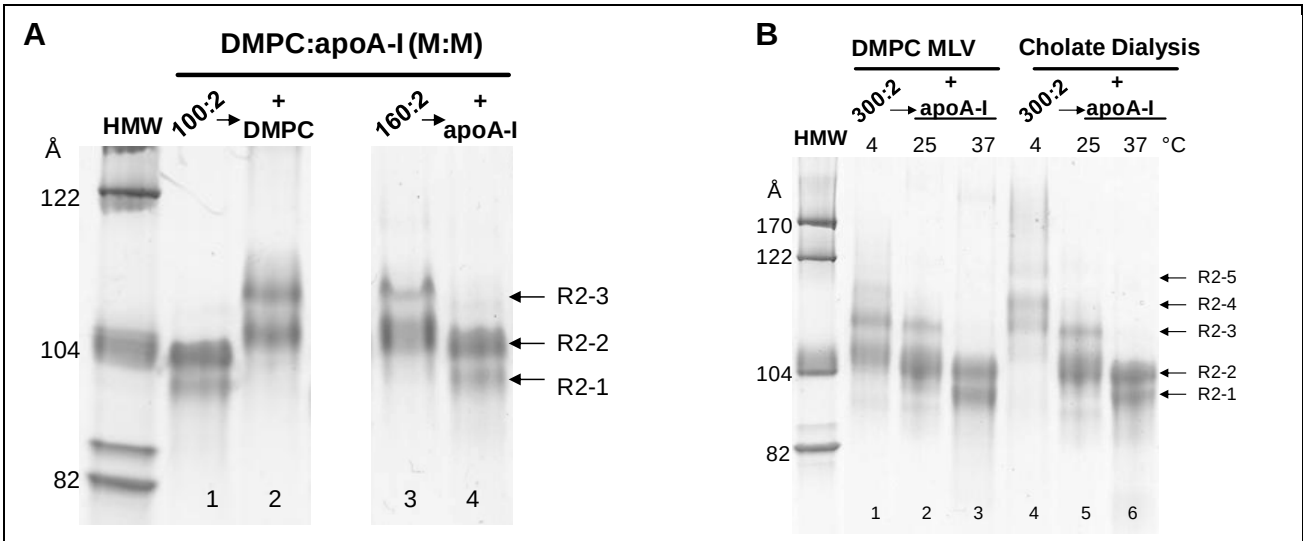

FIG. S3. Interconversion between pre-formed DMPC:apoA-I R2 assemblies. The NDGGE (4-20% polyacrylamide gels) were run for 48 hrs and stained with colloidal blue. ***A***, DMPC:apoA-I complexes at 100:2 (lane 1) and 160:2 (lane 3) molar ratios were formed by the cholate dialysis method. Additional DMPC in the form of MLV (lane 2) or apoA-I (lane 4) were added to these pre-formed assemblies to reach a final DMPC:apoA-I ratio of 200:2 (lane 2) and 80:2 (lane 4), respectively. The mixture was incubated for 16 hrs at 25°C before analyzed by NDGGE. The results showed that addition of DMPC or apoA-I to pre-formed assemblies led to the formation of larger and smaller particles, respectively. ***B***, DMPC:apoA-I complexes (300:2) were formed either by the spontaneous interaction of apoA-I with DMPC MLV (lane 1) or by the cholate dialysis method (lane 4). Additional apoA-I was added to the pre-formed assemblies to reach a final DMPC:apoA-I ratio of 150:2 and incubated for 16 hrs at 25°C (lane 2 & 5) or 37°C (lane 3 & 6). The results showed that the addition of apoA-I drove the formation of smaller particles regardless of the preparation methods and that increase of incubation temperature further reduced the size of the particles formed with additional apoA-I.

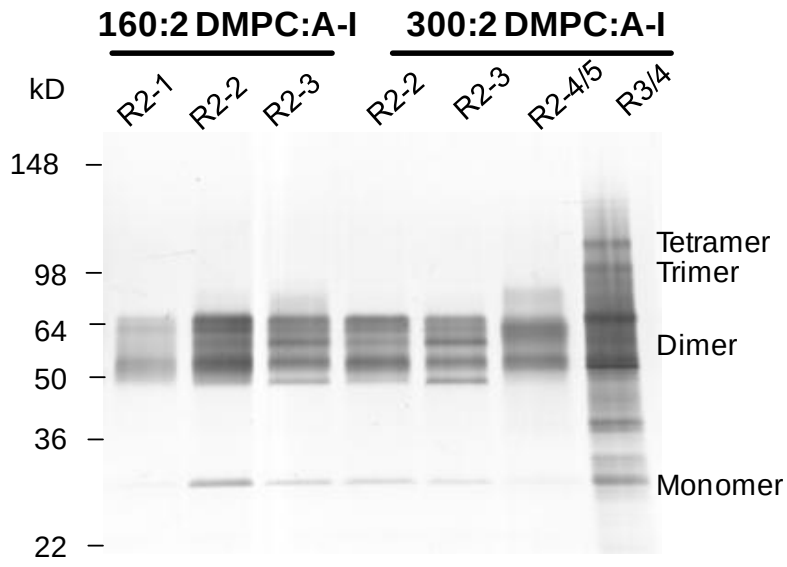

FIG. S4. SDS-PAGE analysis of chemically cross-linked apoA-I on different-sized DMPC:apoA-I particles. The gel was stained with silver. The results showed that all R2 particles have two apoA-I, whereas that R3/4 particles have three/four apoA-I.