Kinetic Analysis of Thermal Stability of Human Low-Density Lipoproteins:

A Model for LDL Fusion in Atherogenesis

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SUPPLEMENTAL MATERIALS

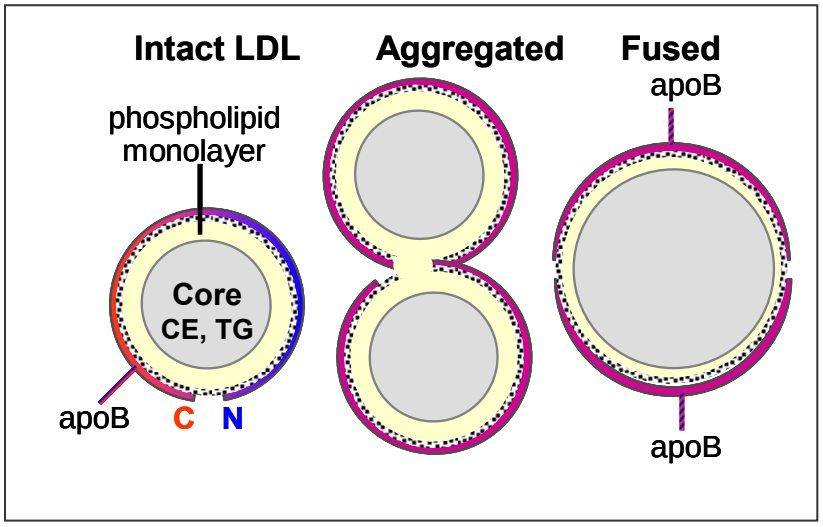


Figure S1. Cartoon illustrating intact, aggregated and fused LDL in cross-section. **Intact LDL** (diameter d=20-24 nm) has an apolar core containing mainly cholesteryl esters and triacylglycerols (gray) surrounded by an amphipathic surface containing one copy of apoB envisioned as a belt wrapped around the particle (shown by a blue and red arc from N- to C-terminus). ApoB is embedded into a cholesterol-containing phospholipid monolayer on LDL surface; phospholipid head groups are in black dots, acyl chains are in yellow. **Aggregated LDL** are similar to intact LDL in the particle size and morphology. We speculate that the contacts between the aggregated LDL are initially mediated by apoB with the possible involvement of phospholipids. **Fused LDL** retain lipoprotein-like morphology (i. e. have an apolar core and an amphipathic protein-containing surface) but have increased diameter due to fusion of two or more particles and hence, are expected to have several copies of apoB per particle. **LDL rupture** (not shown) involves collapse of the lipoprotein morphology and release of core lipids that coalesce into large (>50 nm) droplets (1).

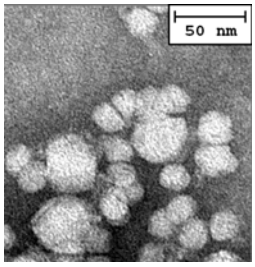


Figure S2. Negative stain electron micrograph of Peak III (void volume) isolated by SEC from the heated human plasma LDL.

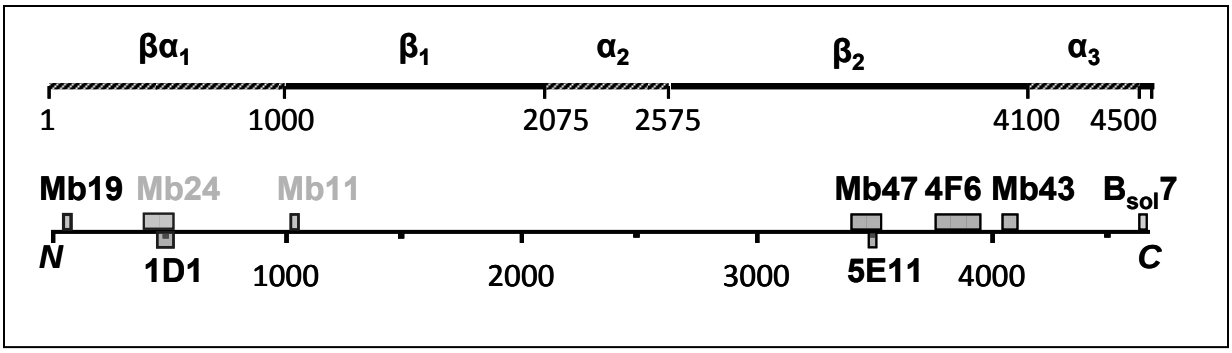
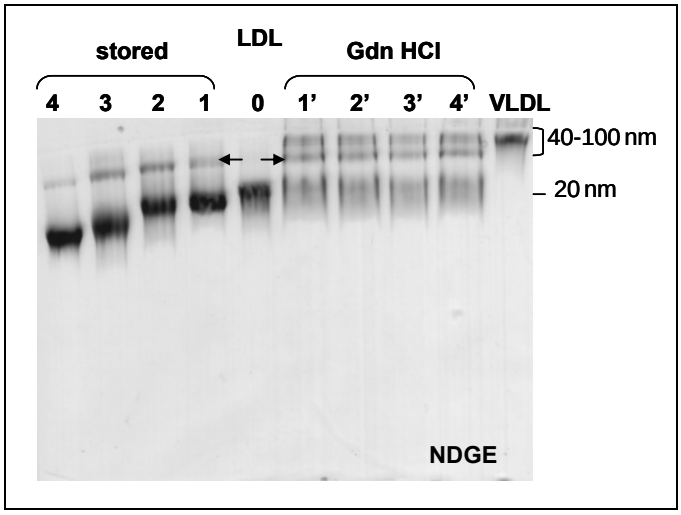


Figure S3. Schematic representation of the pentapartide structure of apoB. Top: On the basis of the amino acid sequence analysis and low-resolution structural studies, apoB has been proposed to contain five domains with alternating α-helical and β-sheet regions designated as $\beta\alpha_1$, β_1, α_2, β_2, and α_3 (2); residue numbers delineating individual domains are indicated. Bottom: The epitope map for the monoclonal antibodies used in this work.

mAb	Epitope
Mb19	71
Mb24	405-539
1D1	474-539
Mb11	1022-1031
Mb47	3429-3453, 3507-3523
5E11	3441-3569
4F6	3569-3925
Mb43	4027-4081
$B_{sol}7$	4517-4536

Table S1. Monoclonal antibodies and their apoB epitopes used in this work; see (3-5) for details.

Figure S4. Non-denaturing polyacrylamide gel electrophoresis (4%, Coomassie Blue stain) showing LDL disintegration and formation of a distinct band with hydrodynamic diameter circa 40 nm (shown by arrows) upon storage at 4 ^{o}C for 5-10 months (lanes 1-4) or upon incubation with chemical denaturant at 37 ^{o}C for 10-60 min (lanes 1'–4'). Intact human LDL (lane 0) and VLDL are shown for comparison. These samples, as well as LDL stored for months at 4 ^{o}C (lanes 1-4), are in standard buffer (20 mM Na phosphate, pH 7.5) containing 0.25 mM Na EDTA. Other samples are in standard buffer. Sample conditions are as follows:



Lane 0: 4.2 mg/ml apoB, intact LDL;

Lane 1: 2.6 mg/ml apoB after incubation at 4 ^{o}C for 5 months;

Lane 2: 3.4 mg/ml apoB, after incubation at 4 ^{o}C for 7 months;

Lane 3: 7.0 mg/ml apoB, after incubation at 4 ^{o}C for 9 months;

Lane 4: 4.2 mg/ml apoB, after incubation at 4 ^{o}C for 10 months.

Lanes 1'-4': LDL samples (3.5 mg/ml apoB) after incubation in 5.5M guanidinum hydrochloride (Gdn HCl) for 10 min (1'), 20 min (2'), 30 min (3'), or 60 min (4') at 37 ^{o}C.

References

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