Molecular Models Need to be Tested: The Case of a Solar Flares Discoidal HDL Model

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ABSTRACT In the absence of atomic structures of high-density lipoproteins in their lipid-bound states, many molecular models have been produced based on experimental data. Using molecular dynamics, we show that a recently proposed "solar-flares" model of discoidal high-density lipoprotein is implausible. Our simulations show a collapse of the protruding solar-flare loops and a notable protein rearrangement due to an energetically unfavorable orientation of the hydrophobic protein surface toward the aqueous solvent.

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High-density lipoproteins (HDL) have been the subject of intense research for many decades because low-levels of HDL are a risk factor for coronary heart disease. However, lipoprotein research, in general, has been impeded by a lack of high-resolution structures of these particles in their lipid-bound states. Hence, molecular modeling, based on experimental data and physical properties of the proteins, provides the only atomic-level structural images. For discoidal HDL, many structural models have been proposed over the years, including the generally accepted double-belt model (1,2). In the double-belt model, two strands of apolipoprotein A-I (the major protein component of HDL) wrap around the circumference of a lipid bilayer in an anti-parallel, beltlike fashion. Apolipoprotein A-I is a 243-residue protein consisting of an N-terminal globular domain and a C-terminal lipid-binding domain. The C-terminal domain is primarily α-helical in nature and has a distinct hydrophobic and hydrophilic face, which shields the hydrophobic lipid tails from the aqueous environment.

A refined structure of the double-belt discoidal HDL model was recently proposed by Wu et al. (3) (note that the originally published ''solar-flares'' model has since been corrected in a corrigendum (4); all results presented below are in reference to the original model, while results on the corrected model can be found in the Supplementary Material, Data S1). The model is developed based upon hydrogen-deuterium exchange mass spectrometry measurements. The coordinates of this refined structure were made available online at the Protein Model DataBase with code No. PM0074956 (http://mi.capsur.it/PMDB/main.php).

The proposed structure, the so-called solar-flare HDL model (i.e., ''solar flares''), proposes an interesting refinement of the generally accepted double-belt model wherein residues 159–180 protrude out, forming solar flares, which are predicted to interact with lecithin cholesterol acyl transferase, an enzyme vital to the maturation of HDL particles. The protruding disordered solar-flare loops, proposed to be stabilized by intramolecular salt bridging, allow for these residues to interact with and activate the enzyme lecithin cholesterol acyl transferase.

Upon examining the originally proposed solar-flare HDL structure (3), a major problem is immediately evident. The authors of that article chose to model the distinct hydrophobic face of the amphipathic apolipoprotein A-I lipid binding domain oriented out toward the solvent as opposed to facing in toward the hydrophobic lipid tails (Fig. 1). Previous models, including the double-belt (1,2), on which the solar-flares model is based, orient the hydrophobic face of the apolipoprotein A-I strictly toward the hydrophobic lipid tails.

The solar-flares HDL model was tested by means of equilibrium molecular dynamics using the program NAMD (5) (see Data S1 for a detailed description of the methods). A 10-ns all-atom molecular dynamics simulation revealed that the proteins immediately start to pull away from the lipids at several locations. This is most pronounced in the region near residue G129 (the residue on which the two amphipathic apolipoprotein A-Is are aligned) and water is seen to infiltrate at the protein-lipid interface due to the mismatch between the polar face of the apolipoprotein A-I strands and the hydrophobic lipid tails. In our simulations, the protruding solar-flare loops quickly collapse into the main body of the HDL particle (Fig. 2 *a*). The stabilizing intramolecular salt-bridges proposed to stabilize the solar flare loops (Fig. 2, *b* and *c*) break within 1 ns of simulation, being replaced by intermolecular salt-bridges (Fig. 2, *d* and *e*). The authors also predicted the structure of the N-terminal domain using the homology modeling program MODELLER (6). MODELLER is an excellent program for generating homology models and can even be used to generate de novo structures of short loops. However, this computational package cannot be used to

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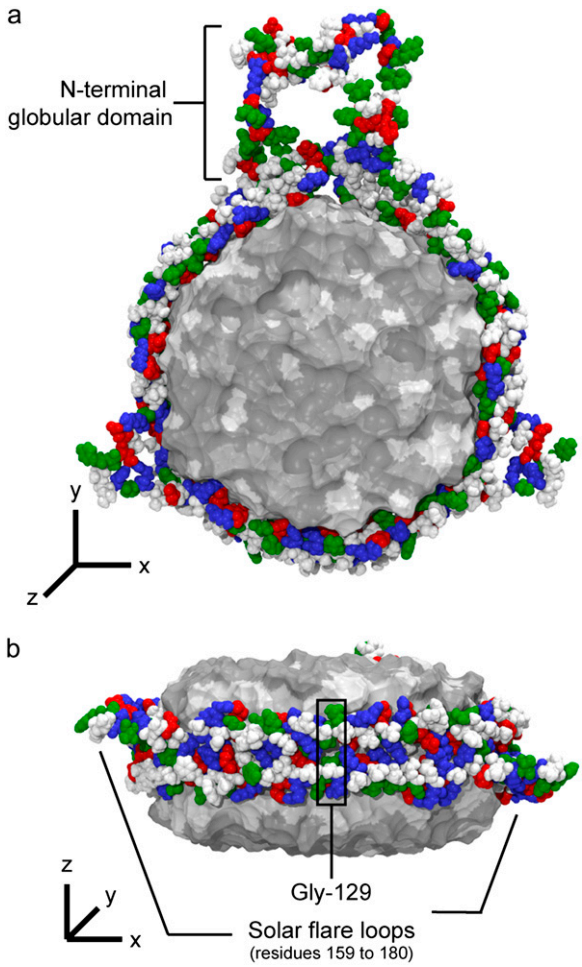

FIGURE 1 A solar-flares discoidal HDL model with proteins colored by residue type. A top-view (*a*) and side-view (*b*) of a solar-flares HDL model (3). Protein residues are colored by residue type as follows: hydrophobic residues in white, polar residues in green, basic residues in blue, and acidic residues in red. The lipid bilayer containing 200 POPC lipids and 20 cholesterol molecules is shown as a solid surface colored in gray. Solar flares orient the hydrophobic face of the amphipathic apolipoprotein A-I proteins facing out toward the aqueous environment.

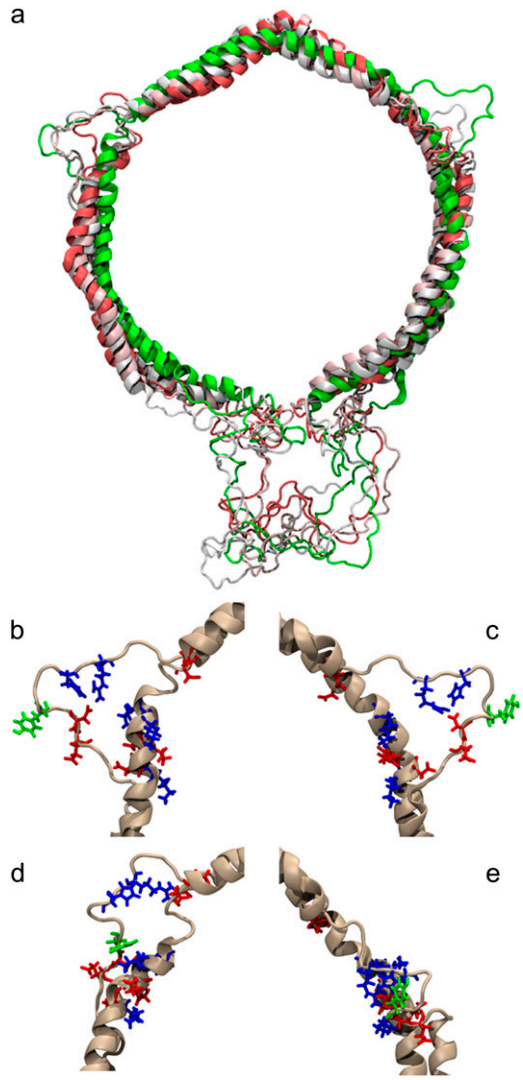

FIGURE 2 All-atom molecular dynamics simulations of a proposed solar-flares discoidal HDL model. Superimposed snapshots of the apolipoprotein A-I strands during the course of the simulation are shown in panel *a*, with the initial structure shown in green and the final structure, at 10 ns, shown in red (lipids are not shown). The simulations revealed the collapse of the solar-flare loops and the random reorientation of the N-terminal domain. In panels *b* and *c*, a closeup view of the initial structure of the proposed solar-flare loops (shown in *tan*) with intramolecular salt-bridging (with basic residues shown in *blue* and acidic residues in *red*) and with Tyr-166 (shown in *green*) protruding. By the end of the 10 ns simulation, the solar flares (*d* and *e*) have collapsed, and are now forming intermolecular salt-bridges.

reliably or accurately predict structures of entire domains, such as the ~43-residue N-terminal globular region of apolipoprotein A-I. This is evident from the all-atom molecular dynamics simulations carried out here (Fig. 2 *a*) in which the N-terminal domains are found to be structurally unstable as evidenced by excessive mobility.

After our 10 ns of all-atom molecular dynamics, the resulting solar-flares HDL model was coarse-grained (7–13) and simulated for an additional 750 ns. The N-terminal domains (first 43 residues), which are disordered loops in the solar-flares model, were removed before the coarse-grained simulations. Due to the unfavorable orientation of the apolipoprotein strands with the hydrophobic face of the proteins oriented toward the aqueous media, significant protein rearrangement was observed during the course of the 750-ns simulation (Fig. 3). A large, 45 Å, gap opened up between the N- and C-termini of the protein strands. In response, the lipids in this area formed a micellelike structure to shield the hydrophobic lipid tails and minimize the solvent-exposed hydrophobic surface area. A similar lipid response was seen in the area surrounding protein residues 120–150 in which the proteins strands pushed away from the lipid bilayer, no longer making contact. Water is seen to permeate through this opening. In numerous other locations, again due to the unfavorable protein

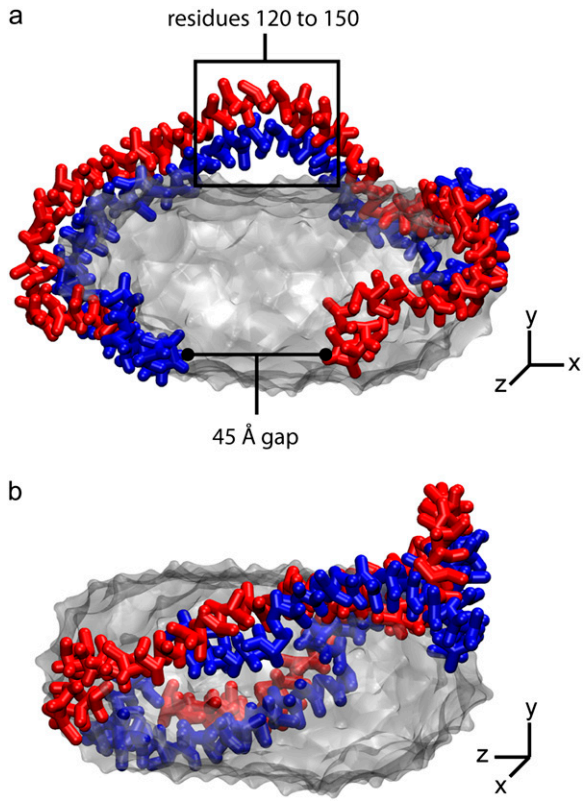

FIGURE 3 Solar-flares discoidal HDL model after 750 ns of coarse-grained molecular dynamics simulation. Two views of the HDL particle are shown, one looking directly at the protein N- and C-termini (*a*), and the other rotated by 90° (*b*). The two apolipoprotein strands are shown in red and blue. The POPC lipids and cholesterol molecules are shown as a solid transparent gray surface. Due to the unfavorable orientation of the apolipoprotein strands to the lipid bilayer, a gap (*a*) has opened up between the N- and C-terminus of the proteins and the lipids in this area form a micellelike structure. A gap has also opened up between the apolipoprotein strands and the lipid bilayer in the region between residues 120 and 150, allowing water to infiltrate and the lipids are also micellelike (*a*). Additionally, in numerous locations one of the protein strands has rotated so that it no longer makes contact with the lipid bilayer but rather interacts only with the other protein strand (*b*).

orientation, the protein strands rearranged themselves so that only one protein strand is making contact with the lipid bilayer, while the other protein strand rotated so that it is only in contact with the other strand but not with the lipid bilayer (Fig. 3 *b*).

From the all-atom molecular dynamics simulations in which the solar-flare loops collapse and the coarse-grained molecular dynamics simulations in which the proteins significantly reorient themselves, one can conclude that the proposed solar-flares discoidal HDL model is unlikely to be an accurate depiction of nascent discoidal HDL particles. In fact, the authors of the solar-flares model (3) have since published a corrigendum (4) replacing the original model with a corrected model. In all-atom simulations this corrected model undergoes a similar collapse of the solar-flare loops, breakage of the salt-bridges proposed to stabilize the loops, and a disordered fluctuation of the N-terminal domain (see Data S1) as seen in the original model. The case of the solar-flares model for discoidal HDL particles emphasizes the need for researchers suggesting molecular models based on low-resolution experimental measurements to rigorously test their models using established techniques such as molecular dynamics.

SUPPLEMENTARY MATERIAL

To view all of the supplemental files associated with this article, visit www.biophysj.org.

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