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Structural stability and local dynamics in disease-causing mutants of human apolipoprotein A-I: what makes the protein amyloidogenic?

Madhurima Das¹, Christopher J. Wilson², Xiaohu Mei¹, Thomas Wales², John R. Engen², and Olga Gursky¹

¹Department of Physiology & Biophysics, Boston University School of Medicine, Boston USA

²Department of Chemistry, Northeastern University, Boston, USA

Background

Apolipoprotein A-I (apoA-I, a single chain of 243 amino acids) is the major structural and functional protein on plasma high-density lipoprotein (HDL, a.k.a. “good cholesterol”). ApoA-I and HDL remove excess cholesterol from peripheral tissues to the liver and protect against atherosclerosis. Although nearly 95% of circulating apoA-I is bound to the HDL surface, a small fraction of the protein transiently dissociates from HDL in a labile lipid-poor/free state that is the precursor of amyloid [1]. ApoA-I misfolding *in vivo* can lead to two forms of human amyloidosis. In the most common acquired form, full-length apoA-I deposits as fibrils in tissues and arteries, which is thought to contribute to atherosclerosis. Further, apoA-I gene mutations can cause either aberrant lipid homeostasis or hereditary amyloidosis (AApoAI). AApoAI is a life-threatening disease wherein N-terminal 9–11 kDa protein fragments, typically residues 1–83 to 1–93, form fibrils in vital organs (kidneys, liver, nerves, spleen, heart, testes etc.) and damage them. The only current treatment is end-stage organ transplant. Our goal is to unravel the apoA-I misfolding pathway in hereditary and in acquired amyloidosis and to identify factors that promote or prevent this pathogenic process, which will ultimately help identify targets for therapeutic interventions.

Methods

We analyzed the structure, stability and lipid binding properties of several naturally occurring mutants of full-length human apoA-I causing either AApoAI (G26R, W50R, F71Y, L170P) or aberrant lipid metabolism (L159R, or apoA-I Fin mutation, which provided a non-amyloidogenic control). The overall protein conformation in solution and in reconstituted HDL (rHDL) was assessed by far- and near-UV circular dichroism (CD) spectroscopy for the secondary and tertiary structure. Intrinsic Tyr fluorescence and ANS binding was used to determine mutation-induced changes in the aromatic packing and in the apolar surface exposure. Turbidity was used to monitor lipid binding and formation of

Address for correspondence: Olga Gursky, Department of Physiology & Biophysics, Boston University School of Medicine, Boston USA 02118. Gursky@bu.edu.

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rHDL. rHDL were analyzed by native PAGE, electron microscopy and CD to visualize the particles and compare their structure and stability. Hydrogen-deuterium exchange mass spectrometry (HDX MS) was used to determine mutational effects on the local protein conformation and dynamics in solution [2]. We also used the 2.2Å resolution crystal structure of the C-terminally truncated human lipid-free apoA-I, (185-243)apoA-I [3], which contains all sites of known human AApoAI mutations [4] and provides an excellent model for elucidating the effects of these mutations on the protein conformation in solution.

Results

Our previous studies suggested the first molecular mechanism of apoA-I misfolding in hereditary and in acquired amyloidosis [3]. The basic premise was that perturbed native structure in amyloidogenic segments (or amyloid hot spots), particularly in residues 14–22, triggers the misfolding [1,3]. To test this premise, lipid-free and lipid-bound wild-type (WT) and mutant proteins were examined by using an array of biophysical techniques. The results showed that global protein destabilization in solution or on rHDL is neither necessary nor sufficient for amyloid formation *in vivo*. Interestingly, two “outside” mutants, L170P and L159R, whose mutation sites were located outside the N-terminal segments found in AApoAI deposits, caused the greatest structural perturbation in solution and on rHDL.

All proteins were examined by HDX MS for the local conformation and dynamics in solution. All mutations caused an increase in the amide deuteration (loss in structural protection) of the major amyloidogenic residues 14–22, supporting our idea that reduced protection of such hot spots can trigger amyloidosis. Surprisingly, the greatest loss in protection was observed in the two “outside” mutants, L170P and L159R. This raised a question: why the L159R mutant does not form amyloid *in vivo*?

To find an answer, we carefully examined the H-D exchange data of all peptic peptides produced in HDX MS experiments and found only one region where L159R was distinct from all other proteins. L159R showed local helical unfolding extending from the mutation site to the central linker near residue 125. Such an unfolding makes the protein labile to proteolysis and explains why apoA-I(Fin) is cleaved in plasma to generate the transient 1–159 fragment. This fragment is expected to be unstable since it lacks a large part of helix-IV from the native 4-helix bundle observed in the crystal structure. We generated recombinant 1–159 fragment and showed that, indeed, it loses most of its native helical structure in solution and has a melting temperature $T_m=40^{\circ}\text{C}$ (as compared to 60°C for full-length apoA-I or its 1–184 fragment). This result explains the observation that the 1–159 fragment is rapidly cleared from circulation and does not aggregate in amyloid *in vivo* despite decreased structural protection of the NT amyloid ‘hot spots’ in apoA-I(Fin) mutant.

Discussion

Collectively, these results enabled us to postulate that two factors can trigger apoA-I misfolding *in vivo*: i) decreased structural protection of the amyloid ‘hot spots’ upon mutations or post-translational modifications, and ii) the presentation of these ‘hot spots’ in the context of the native protein fold. Together, these factors shift the balance from normal

proteostasis and protein clearance to aberrant aggregation. This idea probably extends to other globular proteins. In fact, it explains why the structural destabilization of Ig light chain, β -microglobulin and other globular proteins upon mutations or other modifications is neither necessary nor sufficient for amyloidosis.

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Figure 1.

ApoA-I binding to HDL (top left corner) stabilizes the protein structure against the misfolding or proteolysis. In contrast, the apoA-I monomer released from HDL is labile. Free monomer is shown as a 4-helix bundle with a flexible C-terminal tail; this model is based on the crystal structure of the C-terminally truncated apoA-I [3]. If a mutation promotes apoA-I cleavage and formation of unstable fragments, as is the case for the non-amyloidogenic Fin mutant, L159R apoA-I, the protein is rapidly cleared from circulation (top right). If a mutation increases solvent exposure of amyloidogenic segments without producing unstable fragments, the protein has sufficient time to accumulate and deposit as amyloid (bottom).