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Lipid-free Apolipoprotein A-I Structure: Insights into HDL Formation and Atherosclerosis Development

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Apolipoprotein A-I is the major protein in high-density lipoprotein (HDL) and plays an important role during the process of reverse cholesterol transport (RCT). Knowledge of the high-resolution structure of full-length apoA-I is vital for a molecular understanding of the function of HDL at the various steps of the RCT pathway. Due to the flexible nature of apoA-I and aggregation properties, the structure of full-length lipid-free apoA-I has evaded description for over three decades. Sequence analysis of apoA-I suggested that the amphipathic α-helix is the structural motif of exchangeable apolipoprotein, and NMR, X-ray and MD simulation studies have confirmed this. Different laboratories have used different methods to probe the secondary structure distribution and organization of both the lipid-free and lipid-bound apoA-I structure. Mutation analysis, synthetic peptide models, surface chemistry and crystal structures have converged on the lipid-free apoA-I domain structure and function: the N-terminal domain [1−184] forms a helix bundle while the C-terminal domain [185−243] mostly lacks defined structure and is responsible for initiating lipid-binding, aggregation and is also involved in cholesterol efflux. The first 43 residues of apoA-I are essential to stabilize the lipid-free structure. In addition, the crystal structure of C-terminally truncated apoA-I suggests a monomer-dimer conversation mechanism mediated through helix 5 reorganization and dimerization during the formation of HDL. Based on previous research, we have proposed a structural model for full-length monomeric apoA-I in solution and updated the HDL formation mechanism through three intermediate states. Mapping the known natural mutations on the full-length monomeric apoA-I model provides insight into atherosclerosis development through disruption of the N-terminal helix bundle or deletion of the C-terminal lipid-binding domain.

Key Words: Apolipoproteins, Lipoproteins, Lipids, Structure, Atherosclerosis.

Introduction

Diseases of lipid metabolism, in particular cardiovascular disease, remain the number one cause of health problems and death, especially CHD caused by atherosclerosis (1). One of the main risk factors for atherosclerosis is high blood cholesterol. Plasma levels of high-density lipoprotein (HDL) are negatively correlated with the incidence of atherosclerosis and the mechanism of the anti-atherogenic effects of HDL are mainly related to its involvement in the pathways of reverse cholesterol transport (RCT). It has been recently demonstrated that it does not simply lower HDL cholesterol levels in plasma that correlate with the anti-atherogenic role of HDL but the cholesterol efflux ability of the HDL that determines the role in RCT (2) underscore the need for molecular understanding of the function of HDL at the various steps of the RCT pathway.

Apolipoprotein A-I (apoA-I), the major protein component of HDL, plays vital roles throughout the RCT process as follows: formation and stabilization of the HDL particle structure, interacting with the ABCA-I transporter (3), activating lecithin cholesterol acyl transferase (LCAT) (4) and acting as a ligand for the hepatic scavenger receptor (SRB1) (5).

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Plasma apoA-I (243 amino acids, 28kd) exists in lipid-free, lipid-poor and lipid-bound states and, as a consequence, has a flexible and adaptable structure similar to the molten globular state (6). This flexible nature has hindered high-resolution structural studies. Until now, the high-resolution structure of full-length lipid-free apoA-I remains enigmatic. A lipid-free apoA-I structure in full-length is crucial to understanding HDL formation and atherosclerosis development.

Structural Motif of Exchangeable Apolipoproteins

Amphipathic α-helices that exist in the exchangeable apolipoproteins (apoA-I, apoA-IV and apoE) were first proposed as a unique structural and functional motif involved in lipid interaction by Segrest (7,8). The class A amphipathic helix is a major lipid-binding motif and is characterized by basic residues near the hydrophobic/hydrophilic interface with acidic residues at the center of the polar face. The hydrophobic interface provides the lipid-binding surface. Based on the sequence analysis of apoA-I, apoA-IV and apoE-3, consensus sequence units A (PLAEELRARLR) and B (AQLEELRERLG) were classified in early studies (9). A helix-wheel representation of the AB repeat shows the typical class A amphipathic helix (Figure 1). In addition, salt bridges between acidic and basic residues at positions (i, i + 3/i, i + 4) may provide additional stabilization of the helical structure. X-ray, NMR and MD simulations have confirmed the formation of helix-loop-helix structure by the ABAB repeat sequence peptide (10−12).

Secondary Structure

Figure 2 summarizes the helical distribution of apoA-I probed by different methods and the details are as the follows. Mature apoA-I contains 243 residues that are encoded by two exons. The first 43 residues are endcoded by exon-3 and the 44−243 region is encoded by exon-4 (13). Segrest first proposed a G* helix in the exon-3 encoded region followed by ten continuous tandem repeat helices (22 or 11 residues) in the exon 4 encoded region separated by prolines: H1 [44−65], H2 [66−87], H3 [88−98], H4 [99−120], H5 [21−142], H6 [143−164], H7 [165−187], H8 [188−208], H9 [209−219], and H10 [220−243] (Figure 2) (7). The 22 amino acid repeat was further classified into the two 11-mer repeats sequence units A and B with different homology to consensus sequence units (9). The exon-4 encoded region is thus divided into a series of putative helical segments with different homologies: H1 (BB), H2 (AA), H3 (B), H4 (AB), H5 (AB), H 6(AB), H7 (AB), H8 (BB), H9 (A), H10 (BB) (Figure 2). This classification results in five AB (22 residues) repeating motifs in the center of the apoA-I sequence: AB1 (H2/H3), AB2 (H4), AB3 (H5), AB4 (H6) and AB5 (H7).

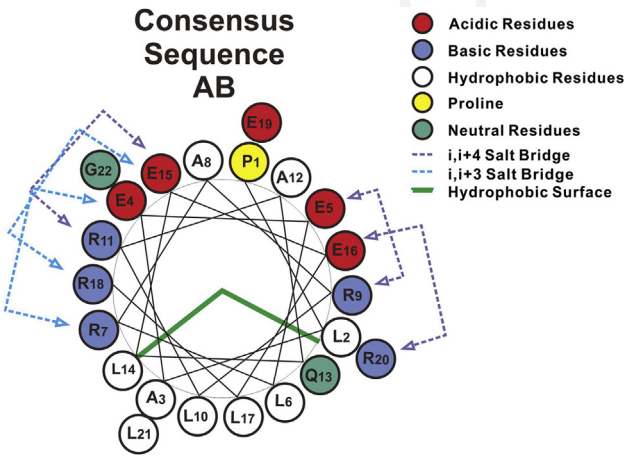

Figure 1. Helix wheel diagrams of the consensus sequence AB model. Consensus sequence AB shows typical class A amphipathic helix.

Nolte and Atkinson proposed a secondary structure model of lipid-free apoA-I based on circular dichroism data and computer modeling (Figure 2) (9). N-terminal residues 1−59 contained the most ambiguously defined structure composed mostly of random coil or β-structure with a short region of amphipathic alpha-helix in residues 9−13. The central region of apoA-I [60−184] was suggested to be composed solely of amphipathic α-helix. C-terminal residues 185−243 were predicted as helices from residues 187−223 and 231−243. Residues 224−230 are very hydrophobic and assigned to a random coil conformation. Mutation and deletion studies in our laboratory further refined the secondary structure model. Double substitution (G185P, G186P) increased the protein stability without altering the secondary structure, suggesting G185 and G186 are located in a loop/disordered region (14). Deletion of residues 136−143 led to stabilization without altering the number of residues in helical conformation, suggesting that this region is unstructured in lipid-free apoA-I (15). The quadruple substitution E125K/E128K/K133E/E139K led to ~17 additional residues in helical conformation consistent with a disordered structure in the segment of residues 123−142 that becomes helical as a result of the quadruple mutation or upon lipid binding (15). Deletion of residues 121−142 led to a loss of ~8 residues in helical conformation compared to WT apoA-I (16). Considering that the region [136−143] is unstructured in lipid-free apoA-I suggests that these eight helical residues are in the region [123−130], whereas P121 and L122 may form a kink between H4 and H5. Thus, the segment of residues 131−143 is disordered and may function as a "hinge domain." Furthermore, single and double terminal truncations (Δ[1−41], Δ[1−59], Δ[198−243], Δ[209−243], Δ [1−41 and 185−243], and Δ[1−59 and 185−243]) suggested the close proximity of the N- and C-termini in the lipid-free apoA-I tertiary conformation (17). With these mutation studies, our laboratory suggested an updated secondary structure model of lipid-free apoA-I (Figure 2).