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The central type Y amphipathic α-helices of apolipoprotein AI are involved in the mobilization of intracellular cholesterol depots

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Abstract

We studied the role of a central domain of human apolipoprotein AI (apoAI) in cholesterol mobilization and removal from cells. In order to check different protein conformations, we tested different sized and cholesterol-content reconstituted apoAI particles (rHDL). Meanwhile cholesterol-free discs were active to induce mobilization, only small cholesterol-containing rHDL were active. To test the influence of a central domain in such events, we used two apoAI variants: one, with its central Y helix pair replaced by the C-terminal domain, and the other having a lysine deleted in central region. The helix-swapping variant decrease the cholesterol pool available to acyl-CoA cholesterol acyl transferase and increase mobilization of newly synthesized cholesterol. Instead, the deletion mutant had no effect on both events. We conclude that the central domain of apoAI is involved in cholesterol cell traffic and solubilization, and that a Y-type charge distribution in polar face may be required, as well as a correct helices-polar face orientation.

Keywords: Apolipoprotein AI; Cholesterol efflux; Chinese hamster ovary cells; Intracellular cholesterol mobilization; Mutants; Reverse cholesterol transport; Reconstituted high density lipoprotein

Apolipoprotein A-I (apoAI)[1] is the major protein component of high density lipoproteins (HDL) which play a key role in reverse cholesterol transport (RCT), a process of great antiatherogenic importance that results in the removal of cholesterol (Chol) excess from peripheral cells, and its transport toward Chol metabolizing organs as liver or steroidogenic tissues [1,2]. Chol efflux mediated by lipid-free apoAI or other apolipoproteins can be considered as the consequence of at least three partial events: (1) Initial apolipoprotein lipidation or loading with phospholipids at the cell membrane, resulting in the formation of discoidal HDL particles, (2) Stimulation of mobilization of intracellular Chol depots toward the cell membrane, and (3) Chol uptake from the cell membrane by discoidal HDL or other lipoprotein particles. There is a large body of evidence that the first process is catalyzed by ATP Binding Cassette A1, (ABCA1) [3]. It was proposed that ABCA1 catalyzes the concurrent loading of apolipoproteins with phospholipids and Chol [4], but there are also different pieces of evidence indicating that these events occur separately in time and in different specialized domains of the cell membrane [5]. Further Chol efflux toward the apoAI acceptors is proposed to occur mediated by the ABCG1 transporter, which should act sequentially to the ABCA1 [6]. Chol transfer from the cell membrane toward HDL can be produced by simple aqueous diffusion, in a process facilitated by Scavenger Receptor Type B1 (SR-B1) [7] and,

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[1] *Abbreviations used:* ApoAI, apolipoprotein AI; rHDL, reconstituted high density lipoprotein; RCT, reverse cholesterol transport; CHO, chinese hamster ovary; MEM, minimal essential medium; FBS, fetal bovine serum; Chol, cholesterol; cholne, 4-cholesten-3-one; BSA, bovine serum albumin; POPC, 1-palmitoyl-2-oleyl sn-glycero-3-phosphocholine; PBS, phosphate-buffered saline; ACAT, acyl-CoA cholesterol acyl transferase; IPTG, isopropyl-beta-D-thiogalactopyranoside; ABCAI/GI, ATP binding cassette transporter A-I/G-I; FPLC, fast performance liquid chromatography.

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as we proposed for cholesterol-poor discoidal HDL [8–11], by the insertion in Chol-rich membrane domains of the central Y-helix of apoAI which facilitates its desorption.

The molecular mechanisms of intracellular Chol pools mobilization toward the cell membrane induced by apoAI are poorly understood. It was proposed that apoAI induces the transport of intracellular Chol to cell-surface caveolae, possibly in part through the stimulation of caveolin expression [12]. Mobilization of intracellular pools also seems to be dependent on the expression of ABCA1, but can be separated from ABCA1/apoAI interaction or ABCA1-mediated phospholipid efflux under certain circumstances [13]. Activation of protein kinase C (PKC) by apoAI was reported to be involved in the mobilization of intracellular cholesterol pools, as the one available to be esterified by ACAT [13–15].

ApoAI contains several repeats which are predicted to form amphipathic α -helices [16,17] (see Fig. 3). Most of them (helices 1, 2, and 5–8) are 22mer repeats forming type A helices, a very common motif in all exchangeable apolipoproteins, characterized by two clusters of positively charged residues at the hydrophobic/hydrophilic interface and a cluster of negative charges at the center of the hydrophilic face. Besides, apoAI contains a G^* helix at its N-terminus which is similar but not identical to the helices present in globular proteins, and two Y type helix pairs: one in the central region (residues 88–120, helices 3 and 4) and the other located at the C-terminus (residues 209–241, helices 9 and 10). Type Y helices are similarly organized to type A helices, but they have positive residues disrupting the cluster of negative charges at the center of the hydrophilic face.

The relative exposition of the different regions of apoAI to the aqueous/and or lipid environments is probable to play key roles in mediating cellular signals prone to redistribute the Chol among domains with different accessibility to be exported. Previously we have shown, using monoclonal antibodies, that the conformation of the central region, is strongly dependent on lipid environment in discoidal, reconstituted particles. This domain is highly exposed in small-cholesterol-free particles, and becomes masked as particles get larger and-or enriched in Chol [11]. Later, our group showed that the central region spanned residues 87–112 deeply inserted into the lipid bilayer of phospholipid membranes [10]; moreover, a synthetic peptide having the sequence of apoAI comprised from residues 77 to 120 (AI 77–120) promoted Chol efflux from Chinese hamster ovary (CHO) cells with high efficiency [8]. Thus, it is conceivable that a sequence spanning this peptide, could actively participate also in cell events destined to promote cholesterol efflux.

Based on these previous results, we tested here the ability of different reconstituted particles to induce mobilization of the internal Chol pool from cells. We hypothesize that the exposition and/ or conformation of the central domain could play a role for inducing cellular events involved in cholesterol exportation. We also explored the structural requirements of this central region by using two mutants. One of them (ΔK107) presents a deletion in helix 4, which changes by about 100° the helix registry and the orientation of the hydrophilic and hydrophobic faces of this amphipathic helix at both sides of the deletion point. In the second mutant (H9–10@H3–4) the central Y-helix pair 3–4 was replaced by the C end Y-helix pair. Altogether, our results suggest that the central type Y helix pair, which may be highly exposed in lipid-poor particles, contributes to the mobilization of intracellular Chol pools toward the cell membrane. Its functionality would not require a specific sequence, rather a type Y charge distribution in the polar helix face and a correct polar/hydrophobic face orientation throughout helix 4.

Materials and methods

Preparation of apoAI

ApoAI was obtained from human serum (donated by Banco de Sangre, Instituto de Hemoterapia de la Provincia de Buenos Aires, La Plata, Argentina) as previously described [11]. It shows more than 95% purity as estimated by SDS polycrylamide gel electrophoresis (SDS–PAGE) using Coomasie blue staining.

Reconstitution of high density lipoprotein particles (rHDL)

The cholate dialysis method was used to obtain rHDL containing apoAI. Discoidal rHDL of 96 and 78 Å Stokes diameter, with or without cholesterol, were obtained starting from 95/1/150 and 40/1/65 POPC/apoAI/sodium cholate, or 95/24/1/150 and 40/10/1/65 POPC/chol/apoAI/sodium cholate molar ratios, respectively. rHDL (120 Å) were purified from the 96 Å reconstitution mixture. Particles were purified by molecular size exclusion chromatography by FPLC as described before [10] and the molecular weight determined by polyacrylamide gel gradient electrophoresis in non-denaturing conditions (PAGGE). The composition was determined by the usual analytical methods.

Construction and expression of proapoAI variants

Wild type proapoAI and ΔK107 DNA sequences were a generous gift from Dr. A. Jonas, University of Illinois at Urbana-Champaign, IL. The respective inserts, ligated to pET30a vectors were transformed into BL21 (DE) *Escherichia coli* cells (Novagen, Madison, WI), then expressed by induction with IPTG and purified as described elsewhere before [18]. Proteins were reconstituted into lipid particles and dialyzed against 10 mM Tris buffer, pH 8.0 (TNB). The fusion tag was removed by digestion with enterokinase followed by further elution through Ni-chelate columns (Novagen). Finally, proteins were delipidated as described previously [18].

The helix-swapping H9–10@H3–4 mutant inserted on a pET30 vector was a gift from Dr. W.S. Davidson (University of Cincinnati, OH). As explained above, in this construction amino acids spanning residues 88–120 were replaced by residues 209–241, in such a way that the original central region of the protein was absent, but the variant kept the molecular weight preserved. A region with the sequence for an Igase protease cleavage site was designed directly upstream of the pro segment of the apoAI construction in order to specifically eliminate the histidine tag. BL21 (DE) *E. coli* cells were transformed with the vector containing the insert and plated in the presence of Kanamicyn. A colony was grown in Luria Bertani (LB) media at 37° C to an absorbance of 0.2 at 600 nm. Temperature was lowered to 25° C, and induced for 3 h with 0.4 mM IPTG. Proteins were purified through Ni-chelate columns in the presence