Characterization of Oligomeric Human ATP Binding Cassette Transporter A1

POTENTIAL IMPLICATIONS FOR DETERMINING THE STRUCTURE OF NASCENT HIGH DENSITY LIPOPROTEIN PARTICLES

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Maxime Denis‡§, Bassam Haidar‡, Michel Marcill‡, Michel Bouvier§, Larbi Krimbou‡, and Jacques Genest‡‡

From the 3Cardiovascular Genetics Laboratory, Cardiology Division, McGill University Health Centre/Royal Victoria Hospital, Montréal, Québec H3A 1A1, Canada and the §Department of Biochemistry, Université de Montréal, Montréal, Québec H3C 3J7, Canada

The oligomeric structure of ABCA1 transporter and its function related to the biogenesis of nascent apoA-I-containing particles (LpA-I) were investigated. Using n-dodecylmaltoside and perfluoro-octanoic acid combined with non-denaturing gel, the majority of ABCA1 was found as a tetramer in ABCA1-induced human fibroblasts. Furthermore, using chemical cross-linking and SDS-PAGE, ABCA1 dimers but not the tetramers were found covalently linked. Oligomeric ABCA1 was present in isolated plasma membranes as well as in intracellular compartments. Interestingly, apoA-I was found to be associated with both dimeric and tetrameric, but not monomeric, forms of ABCA1. Neither apoA-I nor lipid molecules did affect ABCA1 oligomerization. Immunoprecipitation analysis showed that oligomeric ABCA1 did not contain other associated proteins. We next investigated the relationship between the oligomeric ABCA1 complex and the structure of LpA-I. Lipid-free apoA-I incubated with normal cells generated LpA-I with diameters between 9.5 and 20 nm. Subsequent isolation of LpA-I followed by cross-linking revealed the presence of four and eight apoA-I molecules per particle, whereas apoA-I incubated with ABCA1 mutant (Q597R) cells was unable to form such particles and remained in the monomeric form. These results demonstrate that: 1) ABCA1 exists as an oligomeric complex; and 2) ABCA1 oligomerization was independent of apoA-I binding and lipid molecules. The findings that the majority of ABCA1 exists as a tetramer that binds apoA-I, together with the observation that LpA-I contains at least four molecules of apoA-I per particle, support the concept that the homotetrameric ABCA1 complex constitutes the minimum functional unit required for the biogenesis of high density lipoprotein particles.

ABCA1 is a 240-kDa protein belonging to a large family of conserved transmembrane proteins that transport a wide variety of substrates, including lipids, ions, amino acids, peptides, sugars, vitamins, steroid hormones, and drugs across cell membranes. ABC transporters have been associated with many diseases such as drug-resistant cancer (2), diabetes (3), and cystic fibrosis (4).

Apolipoprotein (apo) A-I binding to the extracellular domain of ABCA1 results in the activation of apoA-I lipidation, a key step in the reverse cholesterol transport process, one of the major mechanisms by which high density lipoprotein (HDL) may protect against atherosclerotic vascular disease (5–7). The molecular interaction of apoA-I with ABCA1 promotes cholesterol and phospholipid efflux from peripheral cells and macrophages. However, Brewer and colleagues (8) recently reported that hepatic ABCA1 is a key protein for the formation and maintenance of plasma HDL levels. Moreover, the importance of ABCA1 in the lipidation of apoA-I is highlighted by the finding that over 50 mutations in the ABCA1 gene have been associated with a variety of clinically distinct HDL deficiency diseases including Tangier disease and familial HDL deficiency (9–11). These patients are characterized by excess cholesterol accumulation in macrophages, low plasma HDL levels, and increased risk of coronary artery atherosclerosis (12).

ABCA transporters typically consist of two multispanning membrane domains that serve as a pathway for the translocation of substrates across membranes and two ATP binding cassettes or nucleotide binding domains that provide the energy for substrate transport (13, 14). These domains are found either on a single long polypeptide chain, as in the case of cystic fibrosis transmembrane conductance regulator and the multidrug resistance proteins, P-glycoprotein and MRP1, or as a complex of two identical or similar “half-molecule” subunits each having a multispanning membrane domain and an nucleotide binding domain, as found in the TAP1/TAP2 ABC transporters associated with peptide antigen processing. ABCA1 belongs to the first category because it consists of a single polypeptide composed of two arranged halves. Each half contains a multispanning membrane domain followed by a cytoplasmic nucleotide binding domain. A distinguishing feature of ABCA1 is the presence of a large exocytoplasmic domain that connects the first transmembrane segment to the multispanning membrane domain in each half of the protein (15).

Although the ABCA1 molecule is well characterized, very little is known concerning its quaternary structure and its apo, apolipoprotein; DSP, dithiobis(succinimidylpropionate); DTT, di-thiothreitol; HDL, high density lipoprotein; LpA-I, nascent apoA-I-containing particle; DMEM, Dulbecco's modified Eagle's medium; PBS, phosphate-buffered saline; MWCO, molecular weight cut-off.
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EXPERIMENTAL PROCEDURES

Patient Selection—For the present study, we selected fibroblasts from three normal control subjects and one patient with Tangier disease (homozygous for Q597R at the ABCA1 gene). The protocol for the study was reviewed and accepted by the Research Ethics Board of the McGill University Health Centre. Separate consent forms for blood sampling, DNA isolation, and skin biopsy were provided.

Cell Culture—Human skin fibroblasts were obtained from 3.0-mm punch biopsies of the forearm of patients and healthy control subjects and were cultured in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 0.1% non-essential amino acids, penicillin (100 units/ml), streptomycin (100 μg/ml), and 10% fetal bovine serum. Human green fluorescent protein (GFP)-ABCA1 expressing Chinese hamster ovary cells were generously provided by Dr. Karl H. Weisgraber (Gladstone Institutes of Cardiovascular Disease, San Francisco, CA).

Solubilization of Cell Proteins by n-Dodecylmaltoside and Perfluoro-octanoic Acid—Normal fibroblasts in 100-mm diameter dishes were stimulated or not with 2.5 μM R-9-cis-retinoic acid for 20 h. Cells were then lysed at 4°C with PBS containing 0.5% n-dodecylmaltoside in the presence of a protease inhibitor mixture followed by low speed centrifugation to remove insoluble materials. In separate experiments, cells were lysed with 0.8% perfluor-octanoic acid as described by Ranjeesingh et al. (17). After solubilization of cell proteins and centrifugation at 11,000 × g, 4 °C, for 10 min, the supernatants were treated or not with 50 mM dithiorthioletritol (DTT) for 30 min at 37 °C, and then the samples were separated by non-denaturing gradient gel electrophoresis (3–15%) as described previously (18).

Chemical Cross-linking and Immunoprecipitation Analysis—Chemical cross-linking and immunoprecipitation analysis was performed as described by Tall and colleagues (19) with a minor modification. Fibroblasts were grown to confluence in 100-mm diameter dishes and then stimulated or not with 2.5 μg/ml 22-R-hydroxycholesterol and 10 μM 9-cis-retinoic acid for 20 h in DMEM/bovine serum albumin. Cells were incubated in the presence or absence of 10 μg/ml 125I-labeled apoA-I in DMEM/bovine serum albumin for 2 h at 37 °C. After 2 h, cells were then placed on ice for 15 min and washed three times with PBS. Dithiobis(succinimidylpropionate) (DSP, cross-linker) was dissolved immediately before use in Me2SO and diluted to 500 μM in PBS. Six ml of DSP solution was added in each well. Cells were then incubated at room temperature for 30 min; the medium was removed, and the cells were washed twice with PBS. Cells were then lysed at 4°C with 20 mM Tris, 5 mM EDTA, 5 mM EGTA; pH 7.5) containing 0.5% n-dodecylmaltoside, and the suspension was allowed to stand for 10 min at 4°C in the presence of a protease inhibitor mixture followed by low speed centrifugation to remove insoluble materials. The supernatants were immunoprecipitated with an anti-ABCA1 antibody. Immunoprecipitated samples were separated on 4–22.5% SDS-PAGE, and 125I-labeled ABCA1 was directly detected by autoradiography.

Separation of Lipoproteins by Two-dimensional Non-denaturing Gradient Gel Electrophoresis (PAGGE)—ApoA-I-containing particles were separated by two-dimensional-PAGGE, as described previously (18). Briefly, either stimulated or unstimulated normal cells were labeled with 150 μCi/ml [35S]methionine for 4 h. Cells were then lysed at 4°C with lysis buffer containing 0.5% n-dodecylmaltoside in the presence of a protease inhibitor mixture followed by low speed centrifugation to remove insoluble materials. The supernatants were immunoprecipitated with an anti-ABCA1 antibody. Immunoprecipitated samples were separated on 4–22.5% SDS-PAGE, and 125I-labeled ABCA1 was directly detected by autoradiography using XAR-2 Kodak film.

RESULTS

In the present study, we have examined the multimeric status of human ABCA1 transporter in normal intact fibroblasts stimulated with 22-R-hydroxycholesterol and 9-cis-retinoic acid (22OH/9CRA) by using both n-dodecylmaltoside and perfluoro-octanoic acid combined with non-denaturing gel electrophoresis. These detergents at appropriate concentrations do not break the covalent interactions between protein subunits of an oligomer allowing determination of the oligomeric structure of ABCA1 complex. As shown in Fig. 1A, detection of ABCA1 by anti-ABCA1 antibody, after separation of total cell lysate solubilized by a non-ionic detergent n-dodecylmaltoside (0.5%) on non-denaturing gel (3–15%), revealed both a major and minor bands. The major band migrated as an ~950-kDa complex, consistent with the molecular mass of tetramers. The minor band migrated as a larger complex, possibly an oligomer higher than tetramer, whereas the band with an apparent molecular mass of ~550 kDa is likely a dimer. On the other hand, using DTT as a reducing agent, we observed that all the oligomeric forms were reduced to the monomeric form with a functional properties related to the formation of nascent apoA-I-containing particles. To date, no studies have directly assessed the multimeric status of human ABCA1. It was therefore the aim of the present study to provide evidence for the existence of oligomeric ABCA1 complex, to demonstrate how ABCA1 forms could be affected by apoA-I or lipid molecules, and to examine the impact of the oligomeric ABCA1 complex on the structure of nascent apoA-I-containing particles in a cell culture model.
The supernatants were treated or not with 50 mM DTT for 30 min at 37 °C. Affinity-purified polyclonal anti-ABCA1 antibody. Stimulation of fibroblasts was performed by incubation with 22-OH/9CRA for 20 h. Cells were then lysed at 4 °C with PBS containing 0.5% n-dodecylmaltoside in the presence of a protease inhibitor mixture followed by low speed centrifugation to remove insoluble material. The supernatants were treated or not with 50 mM DTT for 30 min at 37 °C and then separated by non-denaturing PAGE. After electrophoresis, ABCA1 was detected with an affinity-purified polyclonal anti-ABCA1 antibody. B, stimulated fibroblasts were lysed at 4 °C with PBS containing 0.8% perfluoro-octanoic acid. The supernatants were treated or not with 50 mM DTT for 30 min at 37 °C and then separated by PAGE. ABCA1 complex was detected as described in A. Thyroglobulin (669 kDa), ferritin (440 kDa), catalase (232 kDa), lactate dehydrogenase (140 kDa), and bovine serum albumin (67 kDa) were used as markers.

To determine further whether the oligomeric ABCA1 complex exists in living cells, chemical cross-linking was performed as described by Tall and colleagues (19). As shown in Fig. 2A, using SDS-PAGE under non-reducing conditions, ABCA1 migrated at either the monomeric or dimeric molecular masses (−250 and −500 kDa, respectively), whereas the monomer was predominant in the presence of DTT, indicative of disulfide bond contribution in dimer formation. On the other hand, a chemical cross-linker, DSP, was applied to the surface of intact normal fibroblasts to assess the quaternary structure of ABCA1. We found that immunoreactive ABCA1 in cells treated with DSP migrated primarily as a monomer (−250 kDa), as dimers (−500 kDa), or as a larger complex with a mass greater than that predicted for either a monomer or a dimer (Fig. 2B). This larger band is likely a tetramer. DTT reduced all the oligomeric forms to the monomeric ABCA1. To assess further the subcellular distribution of oligomeric ABCA1, we employed surface biotinylation to isolate ABCA1 associated with plasma membrane. Cross-linking of intact normal stimulated fibroblasts followed by biotinylation and detection of ABCA1 on SDS-PAGE showed three bands associated with plasma membrane, corresponding to monomeric, dimeric, and tetrameric ABCA1 (Fig. 2C, left panel). The three ABCA1 forms were also detected in the intracellular compartments (Fig. 2C, right panel).

Having determined that oligomeric ABCA1 complex was present in normal human fibroblasts treated with 22OH/9CRA, the question was posed whether ABCA1 induction in fibroblasts may cause self-association events that are non-physiological. We next examined the presence of oligomeric ABCA1 complex in Chinese hamster ovary cells overexpressing human ABCA1. We found that the monomeric and dimeric ABCA1 forms were present on SDS-PAGE under non-reducing conditions, whereas tetrameric ABCA1 was detected in the presence of the cross-linker reagent (DSP) (data not shown). To verify that the oligomerization of ABCA1 is not due to oxidation during cell lysis and membrane preparation, 5,5-dithiobis-2-nitrobenzoic acid was used as an agent that inhibited disulfide bond formation and the dimerization (25). We found that the absence or presence of 5,5-dithiobis-2-nitrobenzoic acid did not prevent the dimerization of ABCA1.

Because the physical interactions between apoA-I and ABCA1 have been proposed to be important in the lipidation of apoA-I (26), the question was raised whether lipid-free apoA-I could bind to different ABCA1 forms. Stimulated cells were incubated or not with 10 μg/ml 125I-apoA-I for 2 h at 37 °C, and then cross-linking with DSP was performed. As shown in Fig. 3B, 125I-apoA-I associated with ABCA1 co-localized with both dimeric and tetrameric ABCA1 complex (Fig. 3A), whereas 125I-apoA-I was not found associated with monomeric ABCA1 (Fig. 3). Moreover, the absence or presence of apoA-I did not affect ABCA1 oligomerization (Figs. 2B and 3A, respectively). To verify that ABCA1 oligomerization was not dependent on the presence of lipids, cells lysates were delipidated or not (three times) with ethanol-ether 3:1, and then cross-linking was performed. Removal of total cellular lipids did not prevent ABCA1 oligomerization (data not shown). To determine whether the oligomeric ABCA1 is a homo- or hetero-oligomer, either stimulated or unstimulated normal fibroblasts were labeled with [35S]methionine, and then 35S-labeled ABCA1 was immunoprecipitated with an anti-ABCA1 antibody. As shown in Fig. 4, the human anti-ABCA1 antibody specifically precipitated no other proteins except human ABCA1. Although it cannot be ruled out that other proteins co-migrate with ABCA1 on SDS-PAGE, the low amount of detectable 35S-labeled material in unstimulated cells did not support this possibility.

To determine the relationship between oligomeric ABCA1 complex and the structural properties of nascent apoA-I-containing particles in our cell culture model, stimulated cells either from normal or from Tangier disease (Q597R) subjects in 100-mm diameter dishes were incubated with 10 μg/ml 125I-apoA-I in 6 ml of DMEM for 24 h at 37 °C. The medium was concentrated by ultrafiltration (spiral ultrafiltration cartridge,
MWCO 10,000, Amicon), and $^{125}$I-apoA-I-containing particles were electrophoretically separated by two-dimensional-PAGE. As shown in Fig. 5, upper panels, apoA-I-containing particles generated by stimulated normal cells exhibited $\alpha$-electrophoretic mobility with a particle diameter ranging from 9.5 to 20 nm (second gel). In contrast, lipid-free apoA-I incubated with stimulated ABCA1 mutant (Q597R) cells was unable to form such particles (third gel), which had a molecular diameter and charge similar to the lipid-free apoA-I incubated in the same conditions without cells (first gel). We next isolated LpA-I particles by using ultrafiltration (spiral ultrafiltration cartridge, MWCO 100,000, Amicon) to discard any lipid-free apoA-I. LpA-I particles were further dialyzed by using a dialysis membrane with a MWCO of 50,000 to remove any remaining lipid-free apoA-I. As shown in the fourth gel, isolated LpA-I particles did not contain any significant amount of lipid-free apoA-I.

FIG. 2. Chemical cross-linking of ABCA1 in intact fibroblasts and the cellular localization of the oligomeric ABCA1 complex. A, normal fibroblasts in 100-mm diameter dishes were stimulated with 2.5 µg/ml 22-(R)-hydroxycholesterol and 10 µM 9-cis-retinoic acid for 20 h. Cells were then lysed at 4 °C with lysis buffer containing 0.5% n-dodecylmaltoside in the presence of a protease inhibitor mixture followed by low speed centrifugation to remove insoluble material. The supernatants were treated or not with 50 mM DTT for 30 min at 37 °C and then separated by SDS-PAGE (4–22.5%) in triplicate. ABCA1 was detected as in Fig. 1. B, stimulated cells were cross-linked or not with 500 mM DSP, and the cells were lysed and reduced or not with DTT as described above for A. After electrophoresis on SDS-PAGE, ABCA1 was detected by an anti-ABCA1 antibody. C, stimulated cells were cross-linked with DSP, and surface biotinylation was employed to isolate ABCA1 associated with the plasma membrane (PM) as described under “Experimental Procedures.” ABCA1 associated with intracellular compartments (ICC) was immunoprecipitated by an anti-ABCA1 antibody. Samples containing either plasma membrane or intracellular compartments were reduced or not with DTT and then separated by SDS-PAGE. ABCA1 was detected by an anti-ABCA1 antibody.

FIG. 3. Association of apoA-I with the oligomeric ABCA1 complex. Stimulated normal cells were incubated with 10 µg/ml $^{125}$I-apoA-I for 2 h at 37 °C. Cross-linking with DSP was performed as described above. Samples containing $^{125}$I-apoA-I cross-linked to ABCA1 (200 µg of total protein) were incubated with 10 µl of affinity-purified human anti-ABCA1 antibody for 20 h at 4 °C followed by the addition of protein A bound to Sepharose (30 µl). Immunoprecipitated samples were reduced or not with 50 mM DTT for 30 min at 37 °C and then separated on 4–22.5% SDS-PAGE. A, the ABCA1 protein was detected by an anti-ABCA1 antibody. B, $^{125}$I-apoA-I/ABCA1 complexes were directly detected by autoradiography.

FIG. 4. Immunoprecipitation of $^{35}$S-labeled ABCA1. Either stimulated or unstimulated normal cells were labeled with 150 µCi/ml [35S]methionine for 6 h as described under “Experimental Procedures.” Cells were then lysed at 4 °C with lysis buffer in the presence of a protease inhibitor mixture followed by low speed centrifugation to remove insoluble material. The supernatants were immunoprecipitated with an anti-ABCA1 antibody. Immunoprecipitated samples were separated on 4–22.5% SDS-PAGE, and $^{35}$S-labeled ABCA1 was directly detected by autoradiography.
DSP, a homobifunctional cross-linker that interacts with the ε-amine group on the side chain of lysine residues. The cross-linking can occur both intra- and intermolecularly but only between Lys residues within the reagent spacer arm length of 12 Å (27). This homobifunctional amine-specific cross-linker also possesses a cleavable disulfide bond. Either lipid-free apoA-I incubated without cells or lipid-free apoA-I incubated with ABCA1 mutant (Q597R) cells remained in the monomeric form following cross-linking. To rule out the

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possibility that cross-linking conditions or iodination of apoA-I might affect the number of apoA-I molecules per particle, the molar ratio of DSP to apoA-I was varied from 5/1 to 20/1, and incubations were conducted at different temperatures (4 °C, 37 °C, and room temperature). On the other hand, unlabeled apoA-I was used in some experiments. We found that neither the amount of the cross-linker nor the iodination of apoA-I might affect the number of apoA-I molecules per particle, the possibility that cross-linking conditions or iodination of apoA-I affected significantly the number of apoA-I molecules per particle (data not shown).

**DISCUSSION**

The lipid translocase activity of ABCA1 transporter has been implicated in important functions, including the regulation of intracellular lipid trafficking and the lipidation of lipid-poor apolipoproteins to form nascent HDL-particles (20, 28, 29). It is key that we understand the functional properties of this protein and structural basis for its activity. For the first time, we present evidence that a majority of human ABCA1 exists in intact fibroblasts as a homo-tetramer with a possible higher order of oligomerization (Fig. 1). Similar results were also observed with Chinese hamster ovary cells overexpressing human ABCA1, suggesting that the oligomeric ABCA1 complex observed was not due to the use of specific cell types. Interestingly, the absence of ABCA1 monomer as assessed by non-denaturing gel electrophoresis (Fig. 1) suggests that the oligomeric ABCA1 complex state could even be an essential prerequisite for its sorting, in the trans-Golgi-network and to secretory vesicles. This is consistent with previous studies demonstrating that other ABC transporters such as cystic fibrosis transmembrane conductance regulator, MRPI, or ABCG2 function as either dimers or tetramers (17, 30, 31).

Although the molecular mechanism of apoA-I binding to oligomeric ABCA1 has not been elucidated, the present study shows that lipid-free apoA-I binds to both dimeric and tetrameric ABCA1 complex (Fig. 3). We believe that these structures are physiologically relevant; it is likely that the tetrameric ABCA1 complex constitutes the minimum functional structure required for the apoA-I lipidation process. However, it is possible that the dimeric ABCA1 is a functional lipid transporter and that other oligomeric ABCA1 complexes function only as a regulator for the level of a dimeric form. Our observation that only a minor proportion of oligomeric ABCA1 exists as a dimer in living cells (Fig. 1A) did not support such a mechanism.

The proposed mechanism of tetrameric ABCA1 complex as the minimum functional unit required for the lipidation of apoA-I was further strengthened by our results demonstrating that nascent apoA-I-containing particles generated by the lipid translocase activity of ABCA1 contain either four or eight molecules of apoA-I per particle. Thus, we provide further evidence for a functional link between oligomeric ABCA1 transporter and the multimeric structure of nascent apoA-I-containing particles. We postulate that functional oligomeric ABCA1 complex is required for the lipid transfer and the assembly of multiple molecules of apoA-I on the same particle. Our current results support this hypothesis. We demonstrate that lipid-free apoA-I incubated with ABCA1 mutant (Q597R) cells remained in the monomeric form (Fig. 5, lower panel). Furthermore, we have reported that lipid-free apoE3 incubated with stimulated normal fibroblasts generated pre-β-LpE3 with a particle size ranging from 9 to 15 nm (28). Interestingly, we found that pre-β-LpE3 contains four and eight molecules of apoE3 per particle, whereas lipid-free apoE3 incubated with ABCA1 mutant (C1477R) remained in the monomeric form (data not shown). It is likely that the minimum functional unit of ABCA1 is a tetramer that lipitates four molecules of apoA-I at the same time, whereas the presence of eight molecules of apoA-I per
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Although the spatial organization of apoA-I molecules within stacked on top of each other, forming a continuous amphipathic apoA-I. In this model, two ring-shaped molecules of apoA-I are giving rise to apparent higher order oligomers of apoA-I that were not normally produced by ABCA1. Although this possibility cannot be totally excluded, experiments with different molar ratios of DSP to apoA-I and experiments involving a cross-linking at 37 °C or room temperature rather than at 4 °C did not result in significant alteration of the number of apoA-I molecules per particle. Although ABCA1 mutants Q597R and C1477R were found to oligomerize normally (data not shown) and localized to the plasma membrane, they showed the total absence of binding to apoA-I (21, 23). These results indicate that the apoA-I lipidation defect observed in either Q597R or C1477R ABCA1 mutants is not caused by impaired oligomerization of ABCA1. Furthermore, C1477R, a naturally occurring mutant of ABCA1 in which cysteine 1477 within the second large extracellular loop is replaced with arginine (10), was found to dimerize normally. This suggests that cysteine 1477 is not essential for ABCA1 homodimerization. We are currently investigating the structural requirements for the ABCA1 transporter to form an oligomeric complex.

It is well documented that phosphorylation of a number of cellular receptors triggers their oligomerization, which regulates their function. Recent studies from our laboratory and others have shown that ABCA1 phosphorylation by the cAMP/cAMP-dependent protein kinase-dependent pathway plays an important role in the apoA-I lipidation process (21, 23, 32, 33). It is possible that apoA-I induces ABCA1 phosphorylation, allowing ABCA1 oligomerization. Although apoA-I binds to both the dimeric and the tetrameric ABCA1, the presence or absence of apoA-I molecules did not affect the oligomerization of ABCA1 in our cell culture model (Figs. 2B and 3A, respectively). More thorough investigations are required to establish definitively a possible role of apoA-I in the ABCA1 oligomerization process.

The molecular organization of apoA-I molecules within nascent LpA-I particles formed by the lipid translocase activity of the oligomeric ABCA1 complex has not yet been determined. However, because of the absence of cholesterol acyltransferase activity in the extracellular medium to convert unesterified cholesterol to cholesteryl ester, it is most likely that nascent LpA-I particles are discoidal. We have previously suggested that the α-electrophoretic mobility of LpA-I particles may be attributable to their high content in phosphatidylinositol (20, 34). However, it is possible that the high number of apoA-I molecules per particle as documented in the present study may contribute to increase the net negative charge of LpA-I particles and consequently cause their α-electrophoretic mobility. Although the spatial organization of apoA-I molecules within nascent α-LpA-I particles is unknown, Segrest et al. (35) published a computer model referred to as the “double belt” model for reconstituted LpA-I particles containing two molecules of apoA-I. In this model, two ring-shaped molecules of apoA-I are stacked on top of each other, forming a continuous amphipathic α helix that wraps around the perimeter of the phospholipid disc in an antiparallel orientation, resulting in the greatest potential for salt bridge connections between the two molecules. It is likely that the conformation(s) of two apoA-I molecules assumed on 96-Å discs might not be the same as that found on nascent LpA-I containing four or eight molecules of apoA-I. Of interest, the presence of heterogeneous subpopulations of nascent LpA-I having both a different apoA-I and number of apoA-I molecules supports the idea that apoA-I conformational differences on discoidal particles are highly flexible (36). However, we wish to make clear that no attempt was made to use these models to give a definitive interpretation concerning the organization of apoA-I molecules within nascent α-LpA-I. Our model presented in Fig. 6 is a simple illustration of apoA-I lipidation by the oligomeric ABCA1 complex. The detailed structural organization of apoA-I within these nascent particles requires more thorough investigations, which are currently ongoing.

The results presented in this study provide a biochemical basis for a cellular apoA-I lipidation pathway that involves oligomeric ABCA1 complex in peripheral cells. This process plays in vivo a key functional role in the biogenesis of nascent HDL particles.

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Maxime Denis, Bassam Haidar, Michel Marcil, Michel Bouvier, Larbi Krimbou and Jacques Genest

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