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Breakthroughs and Views

In search of new structural states of exchangeable apolipoproteins

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Abstract

Based upon state of the art biophysical experimentation, this article focuses on the different structural arrangements exchangeable apolipoproteins achieve when placed on Langmuir monolayers and subjected to changes in lateral pressure. We have studied the monolayers of apolipoproteins CI, CIII, AI, AII, and E that show as secondary structure a high percentage of amphipathic α-helix. This has been achieved employing techniques such as Brewster angle microscopy, synchrotron X-ray diffraction, and surface pressure measurements. In addition, the lateral order of protein arrays has been also studied by atomic force microscopy. These monolayers show that a phase transition from a two-dimensional disorder fluid to an ordered state is detected at relatively high lateral pressure, where unusual one-dimensional solid phases are discovered. While several helices that conform the apolipoprotein are confined to the interface, others are uniformly tilted toward the hydrophobic air or the phospholipid fatty acid chains. Our results suggest that a similar ordering might also occur when these apolipoproteins are attached to a lipoprotein particle such as a high density lipoprotein (HDL) particle. Therefore, changes from a nascent or discoidal HDL to a mature spherical HDL might in parallel involve structural changes as those described in our Langmuir interfaces. Current experimentation is being carried out in order to elucidate if the structural states already found are related to the efficiency of lipid transfer between lipoprotein particles or lipoproteins and the plasma membrane of cells, as well as receptor ligand recognition.

Keywords: Apolipoprotein; Monolayer; α-Helix; Lipoprotein; Interface

Lipoproteins circulating in the plasma of vertebrates and several species of invertebrates constitute non-covalent complexes of lipid and protein. Water insoluble molecules such as cholesterol, triacylglycerols, and phospholipids are transported in plasma and mobilized to and from cells in the form of lipoproteins. One of the most intriguing classes of lipoproteins is the high-density lipoprotein fraction (HDL), involved in reverse cholesterol transport (RCT) [1], a process that removes excess cholesterol from cell membranes, and is therefore proposed as a mechanism of protection against atherosclerosis [2–6]. The HDL fraction constitutes the smallest and densest of the lipoproteins. This fraction is composed of phospholipids, cholesterol, cholesterol esters, and importantly by a series of proteins called apolipoproteins (apos). ApoAI forms the major protein component of HDLs with around 70% of the total protein mass [7]. Another important component is apoAII together with varying amounts of apoCI, apoCII, apoCIII, and apoE [8,9]. These apolipoproteins called exchangeable due to their property to move between the different classes of lipoproteins apparently give lipoproteins directionality and the ability to interact with receptors at the surface of cells. The scavenger receptor class B type 1 (SR-B1) [10] and the ATP binding cassette protein (ABCA-1) [11] have been proposed as important receptor molecules for the HDLs. Together with these two molecules, interactions of apolipoproteins with enzymes like the lecithin cholesterol acyl transferase (LCAT) [12],

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and lipid transfer proteins such as the cholesterol ester transfer protein (CETP) [13] together with the phospholipid transfer protein (PLTP) [14], contribute to HDL remodeling. While the interaction of apoAI with SR-B1 and ABCA-1 has been thoroughly discussed in the last few years, the role apoAII, apoCI, and apoE play in this remodeling process still remains an open question.

Although functional studies performed on HDL propose that apoAI might adopt different conformational states, one of the problems found to explain this phenomenon has been the lack of detailed structural information on apoAI associated to lipid, situation even more critical with apoCI, apoAII, and apoCIII. The study of lipid-free and lipid-bound apolipoproteins has been important to explain both cholesterol efflux from membranes and cholesterol transfer from low- and very low-density lipoproteins (LDL and VLDL). Lipid-bound apolipoproteins and more specifically lipid-bound apoAI is the main activator of LCAT [12]. During its activation, cholesterol uptake seems to be associated with the concomitant uptake of membrane phospholipids in order to reach the formation of nascent and discoidal HDLs. These particles are considered to be a transient species in equilibrium with the mature or spherical HDLs. During this reshaping process, while the phospholipid is arranged as a discoidal particle surrounded in its acyl-chain region by the amphipathic apoAI, LCAT is responsible to transform discoidal HDLs into spherical HDLs, as cholesterol starts to move as cholesterol ester from the surface to the core of the particle [15,16]. Although throughout the years two models for the structure of discoidal HDLs have been proposed; the ''picket fence'' and the ''belt'' models including its hairpin variant [17–19], both with their own points against and in favor, have not been able to answer yet, the key structural questions that would explain the fate of HDL circulating in plasma. In an attempt to define these structural key features that would give us the possibility to explain basic issues such as receptor recognition and lipid transfer activity carried out by exchangeable apolipoproteins, our group has attempted to address these points directly measuring molecular conformational changes of apolipoproteins at the air/water and lipid/water interfaces in order to approach the possible switching mechanisms that might explain these phenomena. This has been achieved employing Langmuir monolayers studied by Brewster angle microscopy (BAM), atomic force microscopy (AFM), grazing incidence X-ray diffraction [20–23], and surface force analysis (SFA) [24].

According to our results, apolipoproteins AI, AII, CI, and CIII independently of their differences in length and number of amphipathic α turn regions, when placed as monolayers in an air/water interface, show upon lateral compression an important conformational change detected as a wide kink at pressures (π) between 3 and 35 mN/m, and areas (a) between ~1000 and 2500 Å^2/molecule [20,21]. This change can be followed by Brewster angle microscopy and grazing incidence synchrotron X-ray diffraction measurements [22]. However, if we take into account the structural differences between the apolipoproteins such as the length and number of amphipathic α turn regions in relationship with their monomeric or dimeric states, it becomes interesting to analyze the outcome of these experiments, that in general point out to the fact that all exchangeable apolipoproteins studied confine their main body to the interface (Fig. 1A). Nevertheless, there is evidence that an α-helix segment gets tilted towards the hydrophobic air secondary to changes in protein lateral pressure (Fig. 1B). Our recent data have unearthed a further interesting property of binary Langmuir monolayers composed of DPPC/apos (rac-l,2-dipalmitoyl-*sn*-glycero-3-phosphocholine/apolipoproteins) at the air/water interface, showing that apoCI and AII when injected into the subphase move and penetrate to the lipid/air interface (Fig. 1C) [23]. Upon lateral compression, both systems present wide kinks in an independent way, first as a transition exclusively associated to the phospholipid monolayer at $\pi \sim 8-11$ mN/m and $a \sim 110$–125 Å^2/molecule, and second as a transition at $\pi \sim 33$ mN/m and $a \sim 350$–600 Å^2/molecule for apoCI, and $\pi \sim 30-35$ mN/m and $a \sim 1000$–2500 Å^2/molecule for apoAII [23]. These experiments carried out in conjunction with BAM analysis put forward the fact that specific α-helix segments in both apolipoproteins changed the structure and penetrated the DPPC monolayer (Fig. 1D).

Our working hypothesis becomes relevant when we extrapolate this type of conformational change to the surface of a lipoprotein [25]. In such a case, due to the small size and rich protein composition of nascent lipid-poor apoAI particles and discoidal HDLs (pre β-2HDL), it could be proposed that lateral pressure in the phospholipid monolayer of these particles embedded with the different apolipoproteins (apoAI, AII, CI, and E) and accessory proteins (LCAT, CETP, and PLTP) could be relatively high, and only gets decreased in parallel to changes in the size and form of these lipoproteins when they start to accumulate cholesterol esters and become spheroidal HDLs (α-HDL).

Theoretical calculations employed to investigate changes in size and form of nascent, discoidal, and spheroidal or mature HDLs showing the area values obtained from the projected shadow of the different α-helix components of apolipoproteins at the surface of these HDL particles have permitted us to postulate, as experimentally presented in air/water and lipid/water interfaces, that the lateral pressure of phospholipid monolayers embedded with proteins at the surface of the different HDL particles might be very different depending on their size and form [25]. We believe this

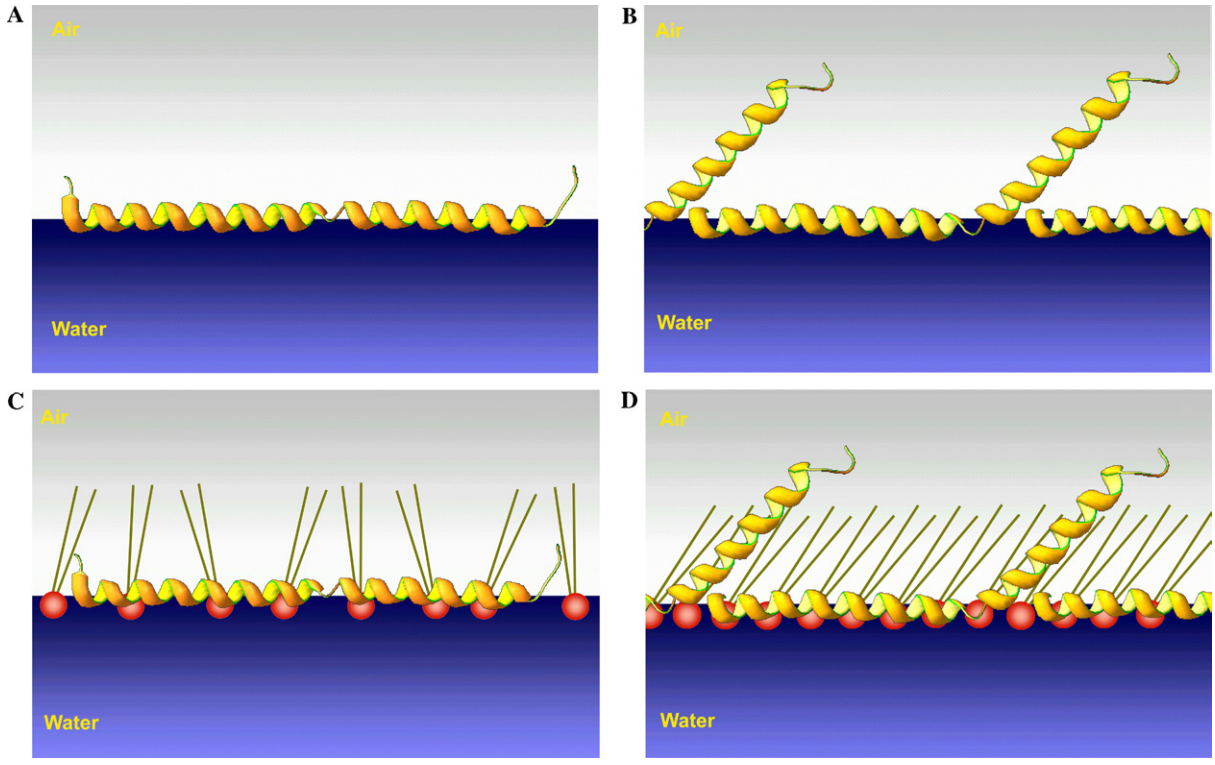

Fig. 1. Langmuir monolayers of apolipoprotein CI at the air/water interface submitted to different lateral pressures illustrating our working hypothesis. Langmuir monolayers of apoCI at the air/water interface under low (A) and high (B) lateral pressures. Binary Langmuir monolayers of DPPC/apoCI at the air/water interface at low (C) and high (D) lateral pressures [20,22,23,25].

phenomenon might define the conformation of apolipoproteins and therefore their specific function, in order to direct the fate of HDL particles in plasma.

Coming from the fact that nascent HDL particles become organized around apoAI, nowadays very little is known about the actual structural characteristics that define the behavior of apoAI under these conditions and therefore the way discoidal HDL particles start to get organized. A structural change probably related to an unfolding process of apoAI that might be important during the formation of a discoidal HDL particle remains to be investigated. Here, the possibility of a molten globule state has to be also considered, since there are examples of proteins that partially unfold near a membrane surface and are therefore proposed as a prerequisite to lipid binding [26,27]. Thermal unfolding studies of lipid-free apoAI support this proposal [28].

At this stage, let us consider an already formed discoidal HDL particle as a cylinder composed of a bilayer of phospholipids with an average diameter of 75 Å by 30 Å height, where the available lateral area calculated corresponds to 7068.5 $Å^2$. Taking into account the base and lid surfaces of this cylinder, the average total area increases to 15904.3 $Å^2$. Nevertheless, since apoAI has been postulated in discoidal particles to correspond to a double belt-like orientation, and if we consider apoAI as a molecule 319.5 $Å^2$ long by 5 Å wide, the projected area of two apoAI molecules upon the lateral mostly hydrophobic area of discoidal HDLs would only cover 3195 $Å^2$. Therefore, this calculation supports the fact that the cylinder must be distorted closer to a pill-like shape rather than a perfect cylinder in order to avoid the exposure of acyl chain of phospholipids to the hydrophilic environment. In this model, the pseudo-base and lid surfaces would give us a free area that might be occupied by other exchangeable apolipoproteins together with CETP, PLTP, and LCAT in a rather confined way.

When this HDL particle continues to grow from a discoidal shape to a spheroidal one reaching an average diameter of 12 nm, the available surface area increases to 45239 $Å^2$, roughly an area three times larger than that shown as a discoidal particle. Therefore, depending on the number of apolipoprotein copies associated to these mature particles, but considering that the protein to lipid ratio increases at the expense of lipid incorporation to these particles, we could assume first; that lateral pressure between apolipoproteins at their surface might have considerably changed, allowing a more efficient exposure of specific apolipoprotein α-regions at the surface of the lipoprotein, and second; that apolipoproteins such as apoAI now placed at the surface of spheroidal particles might have acquired a preferred rotational orientation in relationship with the phospholipid molecules [29]. This last possibility is supported by the proposal that apoAI adjusts its intermolecular interactions when

the particle changes from a discoidal to a spherical shape [19]. Further experimentation involving surface tension measurements of artificial lipoproteins showing different sizes and different contents of apolipoproteins will certainly put our hypothesis to test.

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