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Biochemical and Biophysical Research Communications 365 (2008) 8–15
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Lipid dependant disorder-to-order conformational transitions in apolipoprotein CI derived peptides

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Received 6 October 2007
Available online 29 October 2007

Abstract

In contrast to the notion established for many years that protein function depends on rigid 3D structures, nowadays there is important evidence suggesting that non-structured segments of proteins play important roles in protein function. Therefore, disorder-to-order dynamic conformational transitions have been proposed as an attractive mechanism involved in protein–protein recognition. Our laboratory using Langmuir monolayers of apolipoproteins has previously shown that upon lateral compression at the air/water and phospholipid/water interfaces, there is an important movement of the C-terminal segment of apolipoprotein CI towards the air, considered the hydrophobic region of the monolayer and the acyl-chain region of the interface when phospholipids are used. Here, in an attempt to define secondary structure changes that might occur within this C-terminal segment of apoCI while moving from the monolayer interface back and forth its hydrophobic region, employing three peptides derived from apoCI we studied by circular dichroism and dynamic light scattering their conformational properties when associated to a series of amphipathic lipids and lipid-like molecules. Our results show that a series of lysophospholipids present the ability to modulate the formation of an α helix at the C-terminal peptide of apoCI through a disorder-to-order transition while forming small lipid/peptide aggregates below 10 nm in diameter.
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Keywords: Apolipoprotein CI; Disorder-to-order transitions; Molecular switch

As an alternative to the two state view for protein folding and denaturation, partially unfolded intermediates between the ordered state and the random coil have been reported. These folding intermediates have been shown to represent compact structures, showing only slight expansions compared with their ordered state as initially determined by intrinsic viscosity and later confirmed by dynamic light scattering and small angle X-ray scattering [1]. An interpretation for the partially unfolded state originated the term "molten globule", where the protein converts from and ordered or native state into a form having some liquid-like characteristics [2]. In contrast to this view where function depends strictly on three dimensional structures or regulatory shifts between alternate structures, nowadays we have important examples in which non-structured segments of proteins play important roles in protein function [3,4]. In this respect, disorder-to-order transitions that can potentially lead to high specificity coupled with low affinity are becoming recognized as a common occurrence [5]. This phenomenon is mainly based on the fact that upon binding, disorder-to-order transitions can overcome steric restrictions and thereby enable larger interaction surfaces in protein–protein complexes than could be obtained for rigid partners [6]. Despite the extraordinary importance of these transitions, we still lack detailed biophysical studies that relate this type of disorder-to-order organization and protein function.

Therefore, in an attempt to define the possibility that these structural key features could give us the way to

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doi:10.1016/j.bbrc.2007.10.112

explain basic issues such as receptor recognition, lipid transfer activity and self-exchangeability carried out by several 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 mechanisms that might explain these phenomena. This has been achieved employing Langmuir monolayers in conjunction with Brewster angle microscopy (BAM), atomic force microscopy (AFM) of LB films of protein [7–10], grazing incidence X-ray diffraction on protein monolayers [11], and surface force measurements (SFA) [12].

Since at that time we were unable to define if secondary structure of specific segments of apolipoproteins CI and AII (apoCI and apoAII) remained stable independently of their position at the air/water and lipid/water interfaces, during the development of the present study we addressed the possibility that these segments responding to specific environment changes and following disorder-to-order transitions might function as molecular switches.

Due to its composition, plasma apoCI exhibits the highest isoelectric point (pI) among the HDL-associated apolipoproteins. ApoCI inhibits both phospholipase A2 and hepatic lipase [13,14] and activates the lecithin-cholesterol acyltransferase (LCAT) [15]. Also, it has been reported that the N-terminal fragment (residues 1–38) of human apoCI acts as an inhibitor *in vitro* of the cholesterol ester transfer protein (CETP) [16]. On the other hand, the discovery that apoE-enriched β-migrating very low density lipoprotein (β-VLDL) binds to the lipoprotein receptor related protein (LRP) [17], the effect of apoCI content upon this binding has been studied [18]. When individual members of the C apolipoprotein family were examined, it was found that apoCI is the most potent inhibitor of apoE-mediated β-VLDL binding to the lipoprotein related protein (LRP) [19].

Although, function that depends specifically on 100% disordered proteins represents the extreme case of our statement, we believe the concept of having disordered segments in proteins that only respond and acquire a well defined secondary structure secondary to the binding of specific ligands, might be more common than we thought. The acquisition of lipid-specific conformations in apoCI may provide new insights into how this protein modulates function, namely protein/enzyme modulation and self-exchangeability between lipoprotein particles. Since according to our hypothesis, the C-terminal peptide-segment of apoCI might be displaced depending on compactness and size of high density lipoproteins (HDL) [20], the present study puts forward the possibility that the non-structured C-terminal peptide of apoCI while embedded in a hydrophobic environment shows a disorder-to-order transition only promoted by its displacement to a hydrophobic/hydrophobic interface. This mechanism could start to explain the great conformational plasticity and modulator properties exerted by this apolipoprotein upon several proteins at the surface of HDL.

Materials and methods

Peptide synthesis and preparation. Based on the reported sequence for apoCI, three peptides were synthesized and purified (Peptido Genic Research) encompassing 95% of its entire sequence. Peptides were designated in function of the first letters of the amino acids that compose their primary structure and named ALDO (N-terminal segment), ARELI (hinge segment) and SAK (C-terminal segment) containing amino acids 7–24, 22–38 and 35–53, respectively. Lyophilised peptides were dissolved in water at 1 mg/ml and diluted with a solution containing the molecules to be tested, such sodium dodecylsulphate (SDS) and trifluorethanol (TFE), in order to give a final peptide concentration of 200 μg/ml.

Lipid preparation. L-α-Phosphatidylcholine (PC), cholesterol (Sigma), 1,2-dihexanoyl-sn-glycero-3-phosphocholine (DHPC), 1-hexanoyl-2-hydroxy-sn-glycero-3-phosphocholine (lyso-C_6PC) and 1-lauroyl-2-hydroxy-sn-glycero-3-phosphocholine (lyso-C_{12}PC) (Avanti Lipids) are obtained dissolved in chloroform at 100 mg/ml (PC) and 20 mg/ml (DHPC and lysophospholipids). In order to obtain the desired final concentration for each lipid, chloroform is separated from the samples using a SpeedVac equipment (Savant) and immediately reconstituted with degasified MilliQ water, before each series of experiments. Independently of the lipid concentration used in order to be working above or below their critical micellar concentration (cmc), samples were briefly sonicated using a Sonifier 250 ultrasonicator (Branson) equipped with a titanium microtip, and centrifuged at 13,000 rpm for 10 min at room temperature. All peptide samples were incubated with the different lipid, SDS or TFE solutions for 12 h at room temperature before their characterization employing circular dichroism (CD) and/or dynamic light scattering (DLS). SDS was employed at 20 mM and TFE at 40%.

Circular dichroism spectroscopy. CD spectra were registered with an AVIV62DS spectropolarimeter (AVIV Instruments) at 25 °C employing far UV wavelengths (190–250 nm). Experiments were performed at a protein concentration of 200 μg/ml in a 1.0 mm path length cuvette running AVIV software. Spectra are recorded with a 1 mm bandwidth, using 0.5–1 mm increments and 15 s accumulation time averaged over 2–5 scans. Following base line corrections the ellepticities were converted to mean ellepticities [Θ], in units of deg cm^2 $dmol^{-1}$.

Dynamic light scattering. DLS analysis [21] of every sample was immediately performed after CD measurements employing a Malvern Zetasizer Nano System at 25 °C. The intensity of dispersed light was measured at an angle of 173 °.

Results and discussion

Supported by our published results employing Langmuir monolayers of apoCI proposing the fact that the C-terminal segment of this protein desorbs from air/water as well as from water/lipid interfaces in response to lateral pressure, during this study we have investigated the structural properties of three peptides that compose most of the full-length of apoCI. For this purpose, employing synthetic peptides derived from the original sequence of apoCI and by means of CD and DLS analysis, we tested their properties in solution. Based on their amino acid sequences, together with their hydrophobic moment values (μH) and basic desorption properties shown in Langmuir monolayers, peptides (Materials and methods section) were designated ALDO (N-terminal segment), ARELI (hinge segment) and SAK (C-terminal segment) (Fig. 1A and B).

As previously shown by us, apoCI in solution shows a clear CD signal associated to a high degree of α-helix structure (Fig. 1C) [7]. Nevertheless, peptides ALDO, ARELI, and SAK when tested using the same experimental condi-

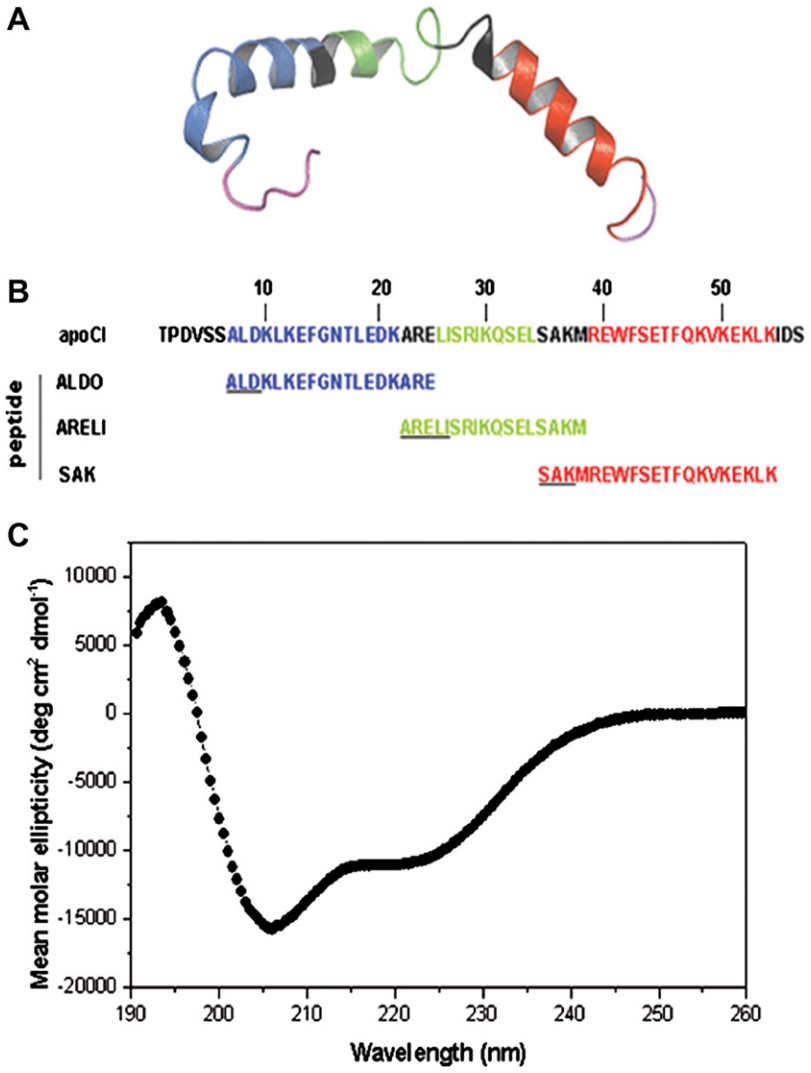

Fig. 1. ApoCI structure. ApoCI structure defined from prediction analysis [22] and lately from NMR experimentation [23]. (A) Color-coded segments show the N-terminal domain (blue), hinge domain (green), and C-terminal domain (red). (B) Sequence overview using the same color code as in (A). Underlined segments show N-terminal domains of apoCI peptides ALDO (7–23), ARELI (22–38), and SAK (35–54); aminoacids from which they received their names. (C) Far-UV CD of apoCI in solution (milli-Q water). Spectra recorded with a 1 nm bandwidth, and 0.5–1 nm increments using 200 μg/ml at 25 °C, 15 s accumulation time is employed and averaged over 2–5 scans.

tions, do not show any degree of secondary structure and remain in a non-structured form independently of pH, temperature, and ionic strength (data not shown). Nevertheless it is interesting to note that independently of their amphipathic character and high μH values (above 0.315 kcal/mol) that make them strong candidates to form α-helical structures, they remain unfolded in solution (Fig. 2A). Only after the addition of increasing amounts of SDS or TFE, their CD spectra showed a clear double minimal signal at 208 and 222 nm and a maximum at 195 nm, indicative of the formation of an α-helix with each one of the peptides tested (Fig. 2B and C). The spectra obtained in the presence of SDS show the presence of an isodichroic point at 203 nm indicative of the existence of only two molecular species that correspond to the unfolded form of the protein and the form associated to an α-helix conformation (Fig. 2C). Interestingly, DLS revealed that all peptides in solution, while keeping their unfolded characteristics, tend to form large aggregates (inset, Fig. 2A). When the solution is added with 40% TFE, peptides recover their α-helix structure and DLS reveals they remain in small aggregates (inset, Fig. 2B; Table 1). SDS solutions at concentrations below or above its micellar critical concentration (cmc), promote a disorder-to-order transition and also the formation of small aggregates (inset, Fig. 2C) (Table 1 as supplementary material). These experiments indicate that all apoCI-derived peptides tend form large aggregates in their unfolded form and only acquire a non-aggregated state and predicted α-helix conformation secondary to the formation of a hydrophobic/hydrophilic interface. Therefore, in order to test the possibility that specific lipids present at the surface of both lipoproteins and plasma membranes might support these disorder-to-order transitions, a series of phospholipids and phospholipid-like

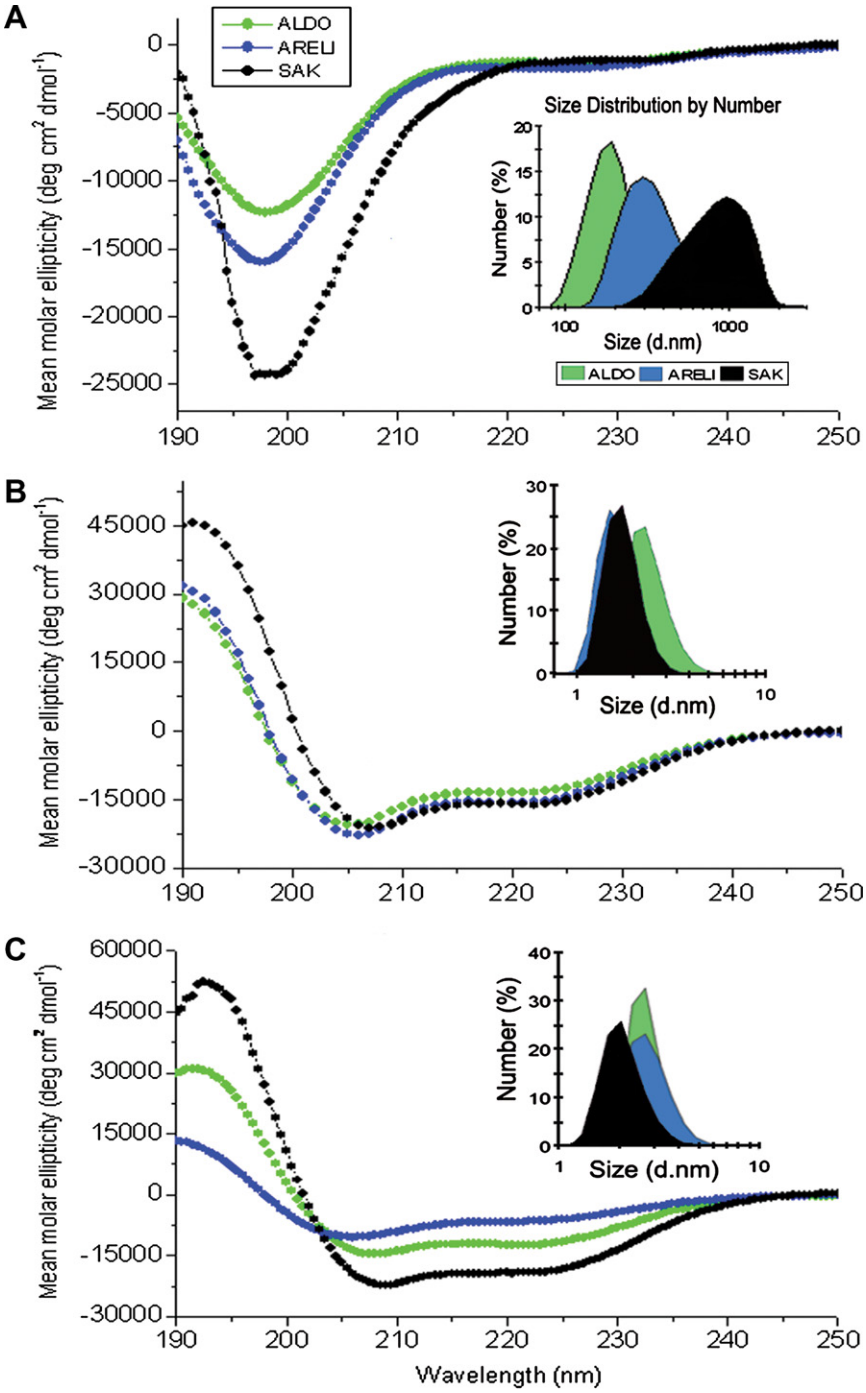

Fig. 2. Far-UV CD data of apoCI-derived peptides. (A) Spectra recorded in water as in Fig. 1 for peptides ALDO, ARELI, and SAK. (B) In the presence of 40% TFE. (C) In the presence of SDS (20 mM). Insets: DLS analysis of the same corresponding peptide solutions employed for CD experiments.

molecules below and above their cmc were incorporated to peptide solutions, and their secondary structure studied by CD (Table 1). As previously shown, the non-structured state of ALDO, ARELI, and SAK while kept in water was periodically changed towards and α-helix using increasing amounts of either TFE or SDS, acquiring an α-helix content of approximately 86% when 80% TFE is reached in the medium. Interestingly, SDS presenting a long fatty acid chain and a large charged head group, independently of the concentration range used (1.5–20 mM), allowed the recovery of the secondary structure in all peptides in a well differentiated fashion, where the lowest recovery of the α-helix corresponded to ARELI and the highest to SAK, the C-terminal peptide. According to the amount of lipid needed to work with clear solutions during CD determinations, phospholipids such as PC and DHPC were employed above and below cmc, respectively. Independently of this condition, both phospholipids were not

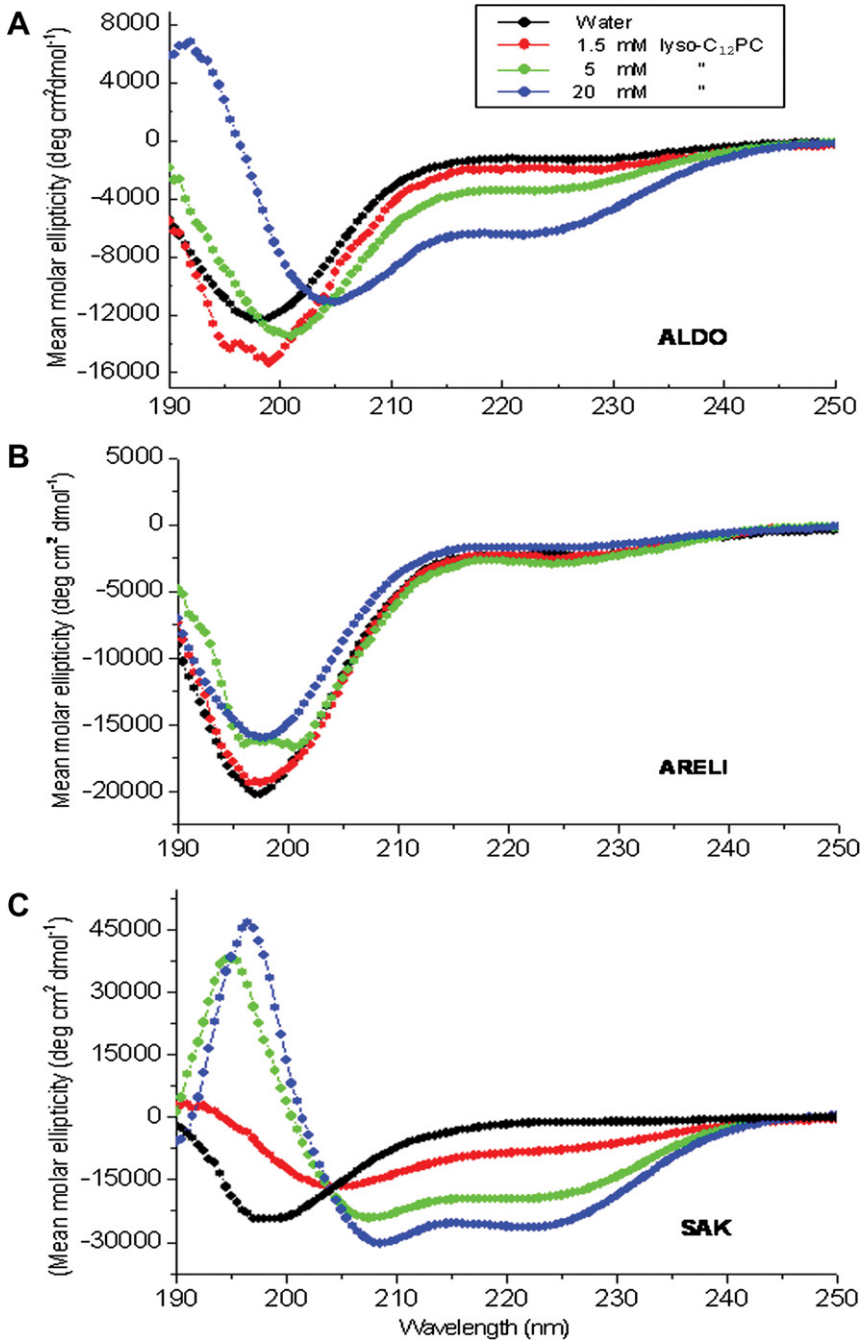

Fig. 3. Far-UV CD data of apoCI-derived peptides in the presence of lyso-C_{12}PC. Spectra of peptides recorded as in Fig. 1; (A) ALDO; (B) ARELI; (C) SAK. Inset: line coding shows peptides in water and lyso-C_{12}PC solutions (1.5–20 mM).

capable to support a well defined disorder-to-order transition, nor the definition of a well established secondary structure, with the exception of SAK. This peptide while kept in the presence of DHPC at concentrations close to and below its cmc, responded relatively well to the presence of this lipid. Since medium hydrophobicity seems to be critical for the transition to be observed, we tested if these lipids mixed with small amounts of cholesterol altered these low percentages of α-helix, finding no changes (data not shown). Nevertheless, when two lysophospholipids closer to the molecular structure of SDS were tested, depending on the length of their acyl chains and the concentration employed in solution, important changes were found specially with peptide SAK. lyso-C_6PC below its cmc did not promote any changes upon secondary structure of all peptides tested. Experiments above its cmc were not performed due to the important interference found with CD measurements. However, lyso-C_{12}PC presenting a longer fatty acid chain was capable to promote an important disorder-to-order transition towards a well established α-helical structure with peptide SAK starting at concentrations below and well above cmc (Fig. 3) (Table 1 as supplemen-

tary material). While ALDO responded slightly at high lipid concentrations ARELI did not present any changes (Fig. 3). The transition observed with SAK resulted to be more efficient than the one found using SDS and close to the maximum effect observed when 80% TFE was added to the medium. Again, in comparison to the transition found in the presence of SDS, SAK showed to be the peptide most sensitive to lyso-C_{12}PC, and ARELI the least sensitive (Fig. 3). Interestingly, in accordance to the results shown with all peptides in the presence and absence of SDS, DLS experiments showed that lyso-C_6PC, a lipid not capable to support a disorder-to-order transition at concentrations below cmc while maintaining all peptides in a non-structured state, promotes their aggregation in solution (Fig. 4). In contrast, lyso-C_{12}PC at concentrations above cmc allowing an optimal disorder-to-order transition and the promotion of a well defined α-helical conformation specially with SAK, allows the association of lipid/peptide molecules in such an orderly fashion that the system avoids aggregation (Fig. 4). Although the lyso-C_6PC concentration used is well below its calculated cmc, due to its physicochemical properties it tends to form by itself as identified by DLS large amorphous aggregates, in contrast to lyso-C_{12}PC that forms well defined micelles (control bars, Fig. 4). Interestingly, while lyso-C_6PC aggregates increase in size almost an order of magnitude in the presence of peptides, lyso-C_{12}PC micelles containing the same peptides do not change their size.

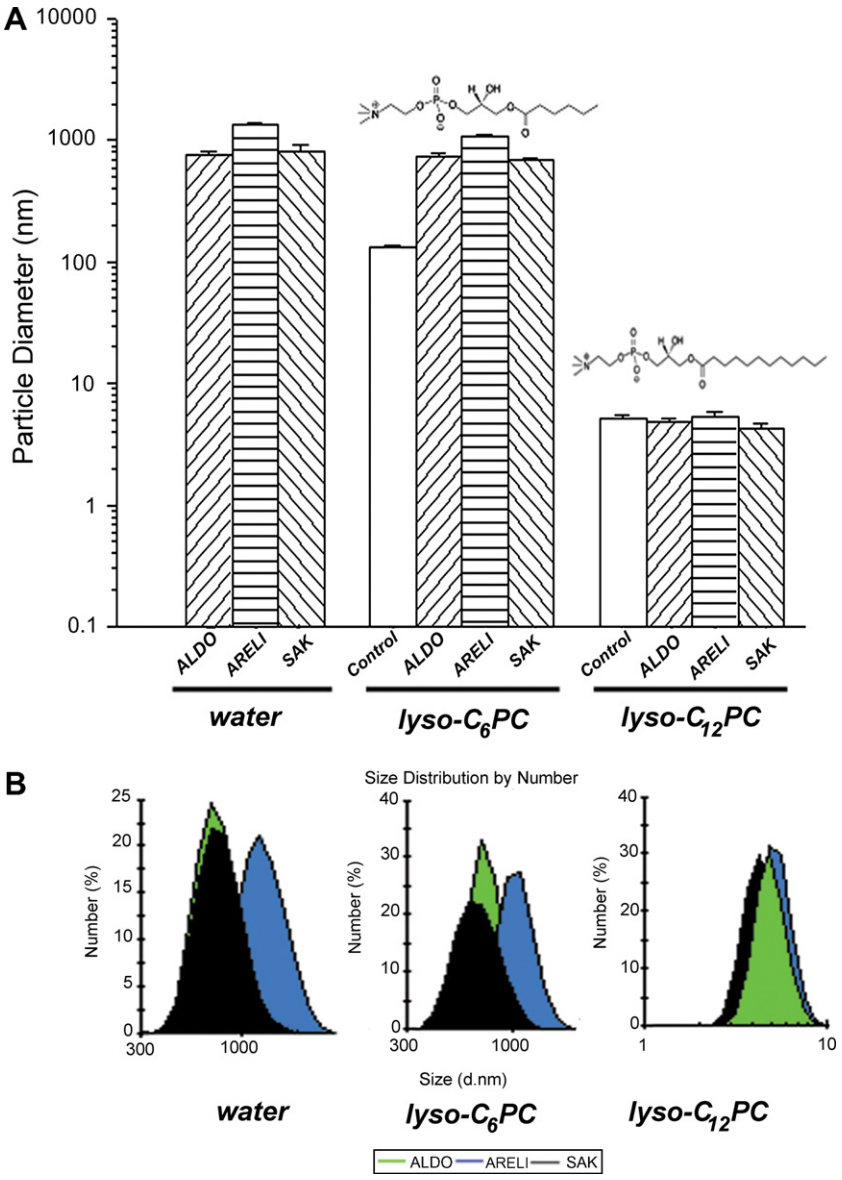

Fig. 4. Dynamic light scattering of apoCI peptides associated with lysophospholipids of different acyl-chain lengths. (A) Quantification of particle diameters given by peptide/lipid aggregates in the presence of lyso-C_6PC (20 mM) and lyso-C_{12}PC (20 mM); (B) same experiment expressed as particle size distribution by number. Error bars indicate mean of three experiments performed in duplicates. Control bars correspond to particle size in the absence of peptides.

According to our published results, most exchangeable apolipoproteins independently of their differences in length and number of amphipathic α-turn segments, when placed as monolayers at the air/water interface, show upon lateral compression an important conformational change [7–11]. Although these experiments pointed out to the fact that all exchangeable apolipoproteins we have studied confine their main body to the interface, there is evidence that α-helical segments are tilted towards the air secondary to changes in the lateral pressure of the protein monolayer [7–10]. Our data unearthed a further interesting property of binary Langmuir monolayers composed of phospholipid/apolipoprotein mixtures at the water/lipid interface, showing that apoCI when injected into the subphase, moves into the water/lipid interface in a rather fast way and upon lateral compression the C-terminal helix penetrates the monolayer [10]. When lateral pressure is released, this segment is again incorporated into the lipid/water interface.

Supported by these data, the experiments performed during the present study allow us to go even further and propose that apoCI, while responding to a decrease in lateral pressure at the surface of HDL and becoming cholesterol-ester rich, might promote the movement of its C-terminal segment, originally embedded in the phospholipid monolayer as a non-structured segment, towards the polar/non-polar interface of the lipoprotein particle, and while doing so changing to an α-helical structure. The fact that specific membrane and lipoprotein associated lipids like lyso-C_{12}PC unchain in a rather dramatic way a disorder-to-order transition in the C-terminal peptide of apoCI, might show a direct relationship to biologically active molecules such as sphingosine1-phosphate and lysosulfatide. It has been observed that when associated to HDL particles, they promote several anti-inflammatory effects and therefore proposed to have a potential atheroprotective role [24].

Since cholesterol esters are formed by the enzyme LCAT located at the surface of HDL particles, promoting the transfer of a fatty acyl group from the two position of phosphatidylcholine to cholesterol, with the consequent synthesis of lysophosphatidylcholine, it is possible that the presence of new OH groups at the polar/non-polar interface change the electrostatic properties of the interface and the way water is displaced from the interface during peptide folding. In fact, it has been proposed that in the presence of lipids, the process of peptide folding corresponds to an enthalpy driven process supported by the energy employed for water displacement [25]. Localized changes in secondary structure of a number of proteins have been found to be physiologically relevant [26,5,27], and therefore a series of conformational switches have been proposed to explain in specific cases protein activation [28] and protein folding [29].

Although further investigation is needed in order to find out the precise mechanism by which lyso-C_{12}PC induces such an important conformational change of the apoCI-derived peptides studied here, the present study proposes that while the folding of peptides is mostly given by hydrophobic forces, electrostatic interactions present mostly at the polar/non-polar interface of lipid/protein micellar aggregates could play a key role in the transition and stabilization of specific segments of apolipoprotein CI at the surface of HDL particles. These changes might be important in the understanding of the mechanisms apoCI employs to modulate protein/protein recognition directly related to enzyme activation and modulation of apoE and CETP function, while associated to the surface of HDL particles. Our proposal of a lipid dependant disorder-to-order conformational transition in apoCI might function as a conformational switch mediating enzyme activation and lipid transport, opens new ways to visualize the concert of events that take place at the surface of HDL particles during their transformation from early protein/lipid aggregates to discoidal and spherical particles, ready to be taken up by liver cells. Further investigation of this potential mechanism designed to recognize and promote localized secondary structure conformations in proteins, will undoubtedly provide new advances to better comprehend protein function at the surface of lipoproteins.

Acknowledgments

We thank Pablo Reyes-Grajeda for helpful discussions, Blanca Delgado for excellent technical assistance, and Ma. Elena Gutiérrez for invaluable help with word processing. This work was supported by grants from the National Council for Science and Technology, Mexico (CONACyT 47333/A-1) and DGAPA at the National Autonomous University of Mexico (IN228607).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bbrc.2007.10.112.

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