Biochemical and Biophysical Research Communications 441 (2013) 469–475

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Lecithin cholesterol acyltransferase (LCAT) activity in the presence of Apo-AI-derived peptides exposed to disorder–order conformational transitions

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ARTICLE INFO

Article history:
Received 9 October 2013
Available online 26 October 2013

Keywords:
Plasma LCAT activity
Apo-AI
Cholesterol metabolism
Peptide conformational change
Secondary structural transitions
Lysophospholipid

ABSTRACT

Although the association of Apo AI with HDLs has been proposed to activate LCAT activity, the detailed molecular mechanisms involved in the process are not known. Therefore, in this study we have investigated how conformational changes in several exposed regions of Apo-AI might cause LCAT activation and for this purpose, designed a strategy to investigate three Apo AI-derived peptides. Since these peptides present the ability to adopt several secondary structure conformations, they were used to determine whether LCAT activity could be modulated in the presence of a particular conformation. Circular dichroism experiments showed that Apo AI-derived peptides in PBS displayed a disordered arrangement, with a strong tendency to adopt β-sheet and random conformational structures as a function of concentration. However, in the presence of Lyso-C_{12}PC, maximal percentages of α-helical structures were observed. Performed in human plasma, time-course experiments of LCAT activity under control conditions reached the highest level of ^{3}H-cholesteryl esters after 2.5 h incubation. In the presence of Apo AI-derived peptides, a significant increase in the production of ^{3}H-cholesteryl esters was observed. The present study provides an important insight into the potential interactions between LCAT and lipoproteins and also suggests that peptides, initially present in a disordered conformation, are able to sense the lipid environment provided by lipoproteins of plasma and following a disorder-to-order transition, change their conformation to an ordered α-helix.

1. Introduction

Lecithin cholesterol acyltransferase (LCAT) is a key enzyme that catalyzes the synthesis of most of plasma cholesteryl esters. The reaction catalyzed by LCAT involves the transfer of an acyl group in the sn-2 site of phosphatidylcholine to the 3-β-hydroxyl group of cholesterol, which yields lysophosphatidylcholine and cholesterol-ester products. Cholesterol esters produced by this pathway are transported to the core of high-density lipoproteins (HDLs). Therefore, LCAT has a relevant role in the maturation of discoidal HDLs to spherical HDLs [1].

Human pre-β_1 HDL consists of a phosphatidylcholine bilayer with 47.5% apolipoprotein AI (Apo AI), 44.6% phospholipids, and 7.6% free cholesterol [2]. HDLs in plasma receive phospholipids, cholesterol, and apolipoproteins from chylomicrons and very low-density lipoproteins (VLDLs). HDLs accumulate cholesterol esters, causing them to grow and adopt spherical shapes, HDL_3 or HDL_2, which contain up to 19% esterified cholesterol. The process of cholesterol uptake carried out by HDLs from peripheral tissues, known as reverse cholesterol transport, is an important mechanism in the prevention of cholesterol accumulation in the intima of arteries [3].

Apo AI accounts for 70% of total HDL protein [4], and the anti-atherogenic effects of Apo AI have been demonstrated in animal models and humans [5,6]. In humans, mature Apo AI is a 243-residue polypeptide [7] containing a series of highly homologous 11- and 22-residue amphipathic α-helices. These repeats comprise the amino acid region 44–243, which is a lipid-binding domain [8]. The first 43 residues of the N-terminal region are relatively disordered [9], which may stabilize the lipid-free Apo AI conformation [10]. The central region of Apo AI (residues 159–180) protrudes from discoidal HDL particles [11], where the exposed loops have been considered potential sites for LCAT interaction [12].

In our group we have investigated the disorder-to-order and order-to-disorder conformational transitions that occur when apolipoproteins are exposed to air/water interfaces [13,14]. It has been

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http://dx.doi.org/10.1016/j.bbrc.2013.10.089

hypothesized that lateral pressure exerted on the phospholipid monolayer of nascent HDLs diminishes when the particles enlarge and turn into spherical lipoproteins. This pressure reduction may change the conformations of apolipoproteins and probably the functions they exert at the surface of lipoproteins [15]. We have also shown that several carboxy-end lipid-binding proteins change from non-structured conformations to well-structured α-helices in the presence of amphipatic lipid molecules following disorder-to-order transitions [16].

Although the association of Apo AI with HDLs has been proposed to activate LCAT activity [17,18], the molecular mechanisms involved are not known. Therefore, in this study we have investigated how conformational changes in several exposed regions of Apo-AI might cause LCAT activation, and designed a strategy to investigate three Apo AI-derived peptides. Since these peptides present the ability to adopt several secondary structure conformations, we used them to determine whether LCAT activity could be modulated in the presence of a particular secondary structure. We defined that peptides in an α-helix conformation present the structural ability to maintain and enhance LCAT activity.

2. Materials and methods

Salts, buffers, bovine serum albumin (BSA), and cholesterol were purchased from Sigma (St. Louis, MO). Lauryl-2-hidroxy-sn-glycero-3-phosphocholine (Lyso-C_{12}PC) in chloroform was purchased from Avanti Polar Lipids Inc. (Alabaster, AL). Digitonin was purchased from Calbiochem (Darmstadt, Germany) and [^{3}H]-cholesterol (20 Ci/mmol) in ethanol was purchased from American Radiolabeled Chemicals (St. Louis, MO). The liquid scintillation cocktail UniverSol ES was obtained from MP Biomedicals (Solon, OH, USA). Peptides used in this study were designed based on the reported sequence for Apo AI and synthesized with purities >98% by the GenScript Corporation (Piscataway, NJ). Peptides were named after the first amino acids of their primary sequence; DRV represents the N-terminal segment (amino acids 9–24), KLL the initial portion of the central segment (amino acids 45–63), and VLES the C-terminal segment (amino acids 221–239). Human plasma was obtained from healthy subjects from the Centro Médico Nacional 20 de Noviembre (ISSSTE, Mexico DF). Samples were stored at −70 °C and used before 3 weeks.

2.1. In silico analyses of Apo AI and Apo AI-derived peptides

The hydrophobicity profile of Apo AI was analyzed using the Pepinfo algorithm on the EMBOSS server. A window of 9 amino acids and the Kyte and Doolittle hydrophobicity scale were used [19]. Hydrophobic segments were predicted based on the secondary structure of the polypeptide chain of Apo AI using the hydrophobic cluster analysis server [20]. In addition, multiple alignments of human Apo AI with orthologs in other species were obtained using Clustal W 1.7 with default parameters [21]. The Pfam database was used to choose a small set of representative members from each family [22].

2.2. Circular dichroism (CD) experiments

Lyophilized peptides were reconstituted in PBS buffer (pH 7.4) for CD experiments in aqueous conditions. To carry out experiments in solutions containing Lyso-C_{12}PC, chloroform was evaporated, and 30, 60, and 90 mM solutions were prepared in water. For several experiments, a molar ratio of peptide to lipid of 1:200 was used. Peptides for CD experiments and LCAT activity measurements were prepared at final concentrations of 0.3, 0.6, and 0.9 mg/mL and incubated for 24 h at room temperature in the dark. CD spectra were recorded with a Dichroism Spectrometer model 62DS from Aviv Biomedical, Inc. (Lakewood, NJ, USA) at 25 °C employing far-UV wavelengths (260–190 nm).

Experiments were performed in a 0.01-cm path-length cell. Spectra were recorded with a 1-mm bandwidth using 1-nm increments and 2.5-s accumulation time. CD spectra were signal averaged by adding 2–5 scans, baseline corrected, and smoothed. CD measurements were reported as a mean of ellipticity in degrees centimeter squared per decimol (deg cm^2 dmol^{-1}) using the AVIV 2.94 software. The secondary structure content of peptides was calculated in a wavelength range of 260–195 nm using both the neural network based CDNN software [23] in the simple spectral analysis mode and the CDPro package, which includes the SELCON3, CDSSTR, and CONTIN software packages [24].

2.3. LCAT activity measurements

Peptides used in the LCAT activity assays were prepared from stock solutions to obtain final concentrations of peptides and lipids in plasma in a final volume of 10 μl. Before LCAT activity assays were performed, peptide–lipid mixtures were incubated at room temperature for 1 h. LCAT activity was measured according to a modified procedure described by Glomset and Wright [1]. Briefly, the procedure utilized a substrate of heat-inactivated human plasma lipoproteins equilibrated with [^{3}H]-cholesterol plus albumin inactivated plasma (1:8:1 v/v). After heating human plasma at 60 °C for 30 min, the substrate was recovered by centrifugation at 13,200 rpm for 3 min in a refrigerated 5415R Eppendorf microfuge (rotor F45-24-11). Inactivated plasma was labeled with a stabilized cholesterol emulsion prepared by adding 1 nmol of [^{3}H]-cholesterol/μl of the preparation to one volume of 5% BSA in 150 mM NaCl. Eight volumes of inactivated plasma were added to the cholesterol emulsion and incubated at 37 °C for 2–4 h.

To start the enzyme activity assays, the substrate was mixed with one volume of enzyme in a final volume of 100 μl. After incubation at 37 °C, the reaction was stopped with 800 μl of 1% digitonin (in 95% ethanol) and 40 μl of 5 mg/mL cholesterol [25]. Samples were vigorously mixed and centrifuged at 6000 rpm for 10 min in a Beckman microfuge. [^{3}H]-cholesteryl esters produced by LCAT were measured in 0.8-mL aliquots of the supernatant plus 3 mL of liquid scintillation cocktail. LCAT activity was reported as pmol of the [^{3}H]-cholesterol esterified product/h/mL of plasma.

2.4. Statistics

Statistical analyses were performed by ANOVA using GraphPad Prism 6.0c (GraphPad Software, Inc.). Tukey's test was performed to study the significance of differences between the control and the Apo AI-derived peptides treated samples. Data were expressed as mean ± SEM. $P < 0.05$ was considered statistically significant.

3. Results and discussion

3.1. Physicochemical characterization of Apo AI-derived peptides

To uncover whether structural transitions of Apo AI could activate LCAT, the physicochemical characterization of Apo AI and Apo AI-derived peptides was carried out. The *in silico* analysis of Apo AI showed a hydrophobicity profile with negative values present at the N- and C-terminal regions of the protein (amino acid segments 10–17 and 213–229). Positive values indicating hydrophobic zones were found in 3 clusters (amino acid segments 13–22, 45–49, and 216–232) where peptides designed for this study are also located (Fig. 1A). This hydrophobicity pattern is compatible with the interaction between Apo AI and discoidal HDL particles, where the N

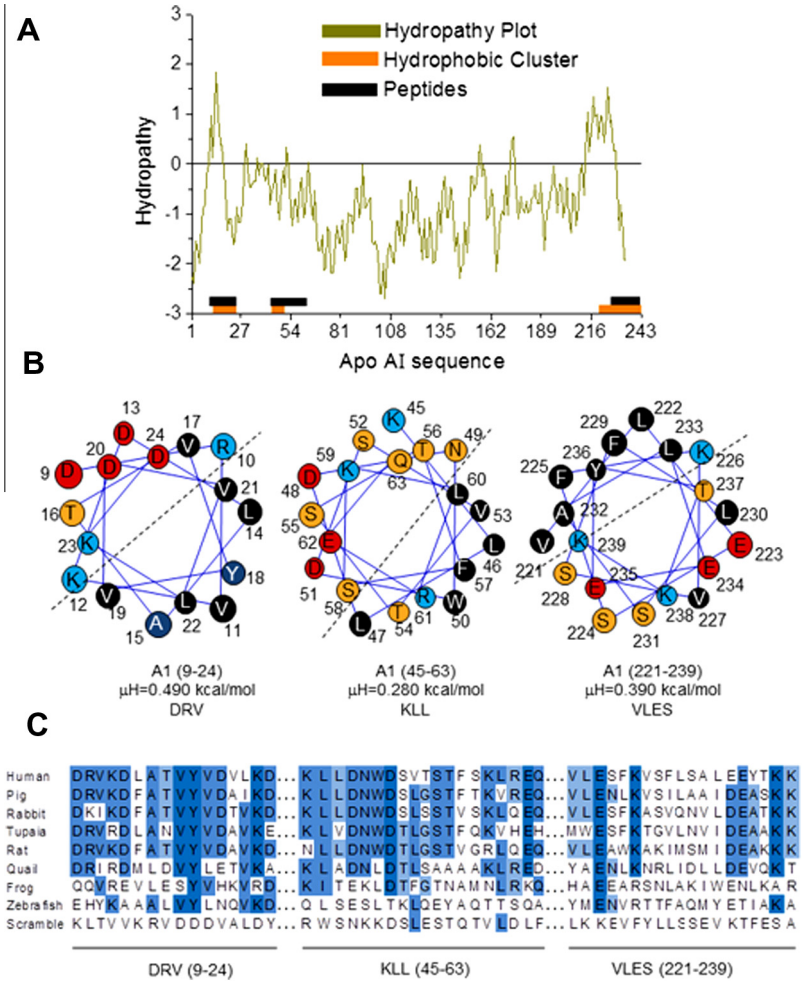

Fig. 1. Physicochemical analyses of Apo AI and Apo AI-derived peptides DRV, KLL, and VLES. (A) Hydrophobicity profile and hydrophobic clusters obtained in EMBOSS (orange boxes). (B) Helical wheel representation of Apo AI-derived peptides with their corresponding μH values. The amphipathic nature of the peptides is shown with the line indicating the polar–nonpolar interface. Black: hydrophobic residues, blue: basic residues, red: acidic residues, and yellow: polar uncharged residues. (C) Alignment of the amino acid sequence of DRV, KLL, and VLES peptides with orthologs in other species. Conserved residues are indicated.

and C-ends of Apo AI might be relevant to lipid recognition domains. Previous studies have indicated that the N-terminus of a lipid-free Apo AI is involved in the control between the opened (horseshoe-like) or closed status of the protein, and that the C-terminus facilitates its anchoring to lipid membranes [26,27].

As part of the physicochemical analysis of these peptides, their amphiphilicity was also estimated (Fig. 1B). DRV presented the highest hydrophobic moment value (μH) of 490 kcal/mol, followed by VLES and KLL (μH = 0.390 and 0.280 kcal/mol, respectively). A helical wheel representation shows that DRV and VLES presented a more asymmetrical distribution of polar and non-polar amino acids than KLL, confirming their amphiphilic properties (Fig. 1B). Since μH values >0.2 kcal/mol were found in all peptides studied, they can be considered as good candidates to form amphipathic helices [28].

In addition, the analyses performed to determine the sequence-structure–function relationship of these Apo AI-derived peptides, suggest a relevant and phylogenetically conserved function (Fig. 1C).

3.2. Circular dichroism experiments

CD experiments showed that Apo AI-derived peptides in PBS displayed a disordered arrangement, with a strong tendency to adopt β-sheet and random conformational structures as a function of concentration (Fig. 2A). This phenomenon was reflected on the solubility of peptides at high concentrations. KLL, the most hydrophilic peptide, was soluble in PBS even at high concentrations, whereas VLES, the most hydrophobic one, was difficult to dissolve even at low concentrations. According to previous work performed by our group, when amphipathic lipids are used beyond to their critical micelle concentration in a mol:mol ratio of 1:200 (peptide:Lyso-C_{12}PC), it is possible to obtain organized peptide/lipid arrangements and therefore solubility achieved [16]. Based on these data, we used peptide concentrations of 0.3, 0.6, and 0.9 mg/mL dissolved in solutions containing 30, 60, and 90 mM lysophospholipid. When compared to results obtained using PBS solutions, maximal percentages of α-helical structures were observed in the presence of 30 mM Lyso-C_{12}PC. DRV increased its α-helix content from 19.7% to 34.5%, KLL from 17% to 31.3%, and VLES from 16.4% to 30.2% (Table 1). However, there was a loss of ordered structures as a function of peptides concentration (Fig. 1B, Table 1). A similar trend was found in experiments with peptide solutions at a constant 30 mM Lyso-C_{12}PC concentration (data no shown). Therefore, the maximal content of α-helical structures was obtained at a peptide concentration of 0.3 mg/mL containing 30 mM Lyso-C_{12}PC, suggesting that the structural arrangement of the three peptides depends not only on peptide concentration, but on the presence of a specific lipid concentration at which optimal ordered structures are reached.

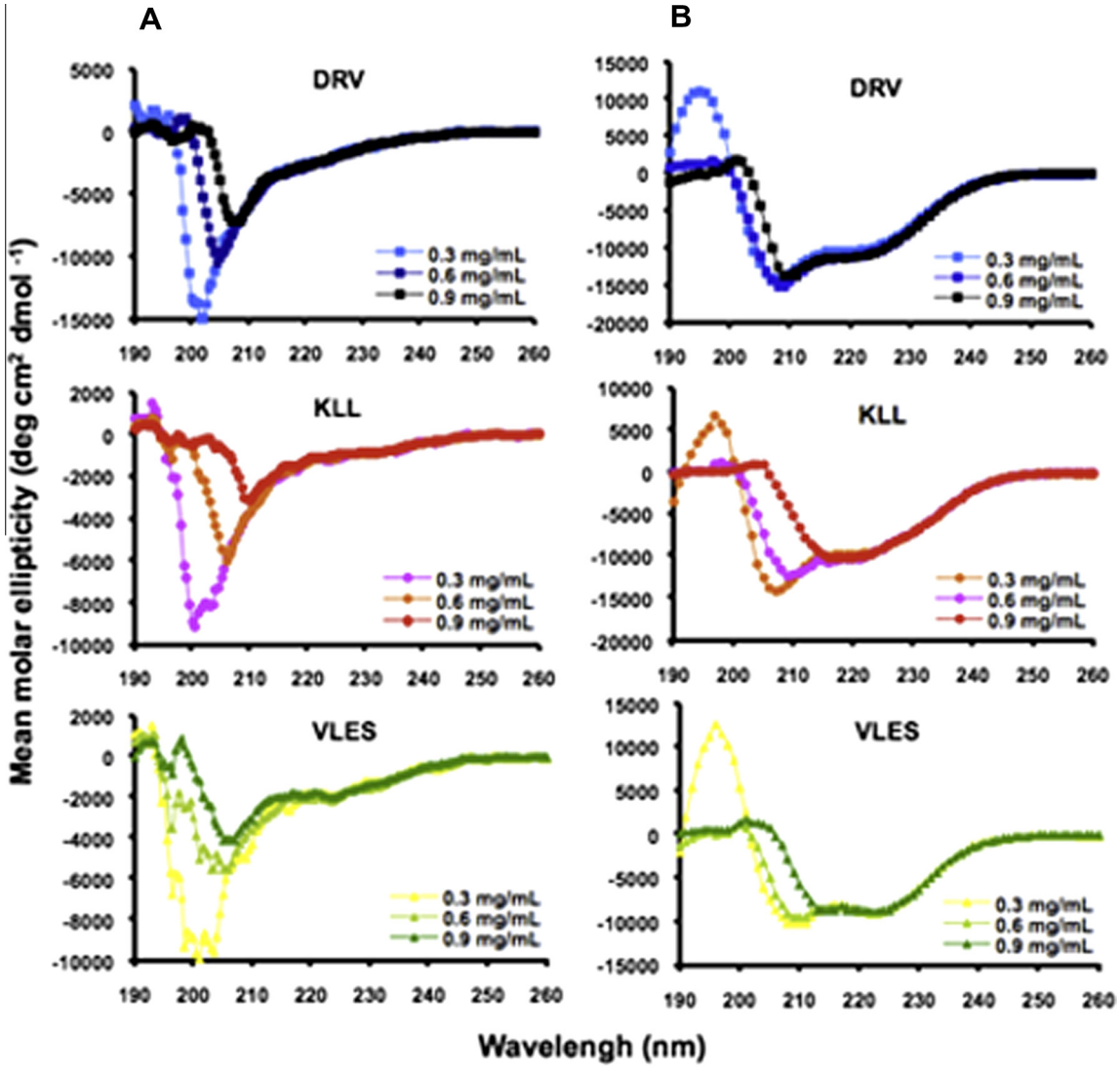

Fig. 2. CD spectra of the Apo AI-derived peptides at different concentrations. (A) In PBS, pH 7.4. (B) In peptide:Lyso-C_{12}PC ratios of 1:200 (mol:mol); 0.3, 0.6 and 0.9 mg/mL peptide in 30, 60 and 90 mM Lyso-C_{12}PC, respectively.

Table 1
Percentages of α-helical content of Apo-AI derived peptides solutions dissolved either in PBS (pH 7.4) or in Lyso-C_{12}PC in a mol/mol ratio 1:200 (peptide:lipid), determined by circular dichroism spectroscopy.

	α Helix/PBS (%)			α Helix/Lyso-C_{12}PC (%)		
Lyso-C_{12}PC (mM)	0.0	0.0	0.0	30.0	60.0	90.0
Peptide (mg/mL)	0.3	0.6	0.9	0.3	0.6	0.9
DRV	19.7	20.0	18.2	34.5	29.9	25.9
KLL	17.0	17.2	15.9	31.3	26.2	20.6
VLES	16.4	16.9	17.0	30.2	22.9	19.9

3.3. *Effect of Apo AI-derived peptides on LCAT activity*

To our knowledge, this is the first study where several interventions designed in order to modulate LCAT activity, is directly measured in plasma, considered its natural environment. Under these conditions, plasma LCAT activity was measured in the presence of all three Apo AI-derived peptides reconstituted in PBS or Lyso-C_{12}PC solutions. Time course experiments of LCAT activity under control conditions showed the highest enzyme activity increase after 2.5 h of incubation and the maximal product yield was obtained up to 5 h. Therefore, this time was used to calculate the highest production of ^{3}H-cholesteryl esters in the subsequent plasma LCAT activity assays (Fig. 3). When plasma was tested in the presence of Apo AI-derived peptides at 0.3 and 0.6 mg/mL in PBS, a significant increase in ^{3}H-cholesteryl esters production was observed (Fig. 3A and B, respectively). It is evident that LCAT activity was stimulated in a higher proportion at 0.3 mg/mL (Fig. 3A) in comparison to 0.6 mg/mL peptide solutions (Fig. 3B). According to these results and considering the hydrophobic properties of peptides tested, it is possible that stimulation of LCAT activity in their presence is related to their ability to bind lipids and to adopt and maintain an α-helical conformation.

To confirm this hypothesis we suspended the peptides in different lipid solutions and tested their abilities to activate LCAT. Assays containing a mol:mol ratio of 1:200 (peptide:Lyso-C_{12}PC) showed that plasma LCAT activity was fully abolished at peptide concentrations >0.3 mg/mL in 30 mM Lyso-C_{12}PC (Fig. 4A). Control experiments performed in the absence of peptides indicated that the lysophospholipid itself showed an inhibitory effect on the LCAT activity at concentrations higher than 30 mM (Fig. 4A). Several explanations involving product inhibition by lysophospholipids and cholesteryl esters have been proposed. It is also possible that detergent effects might be involved [29] and that the surfactant

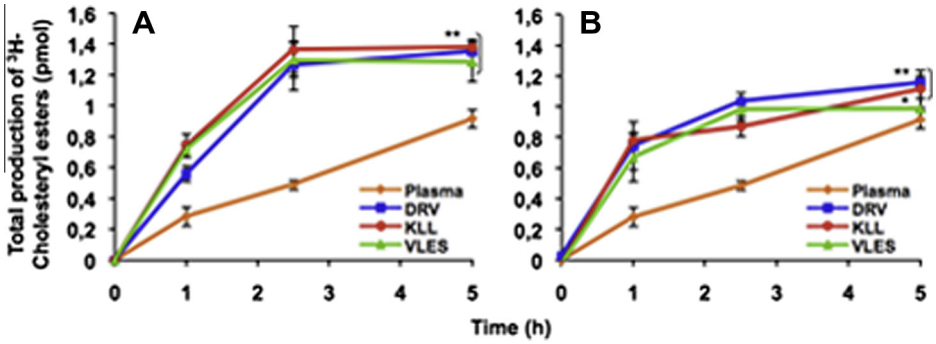

Fig. 3. Time course of LCAT activity in the presence of Apo-AI-derived peptides in PBS (pH 7.4). (A) At 0.3 mg/mL and (B) At 0.6 mg/mL. Each point represents the mean of quantifications performed in triplicate± SEM. * Significant $P \leqslant 0.01$; ** significant $P \leqslant 0.0001$, compared to plasma control.

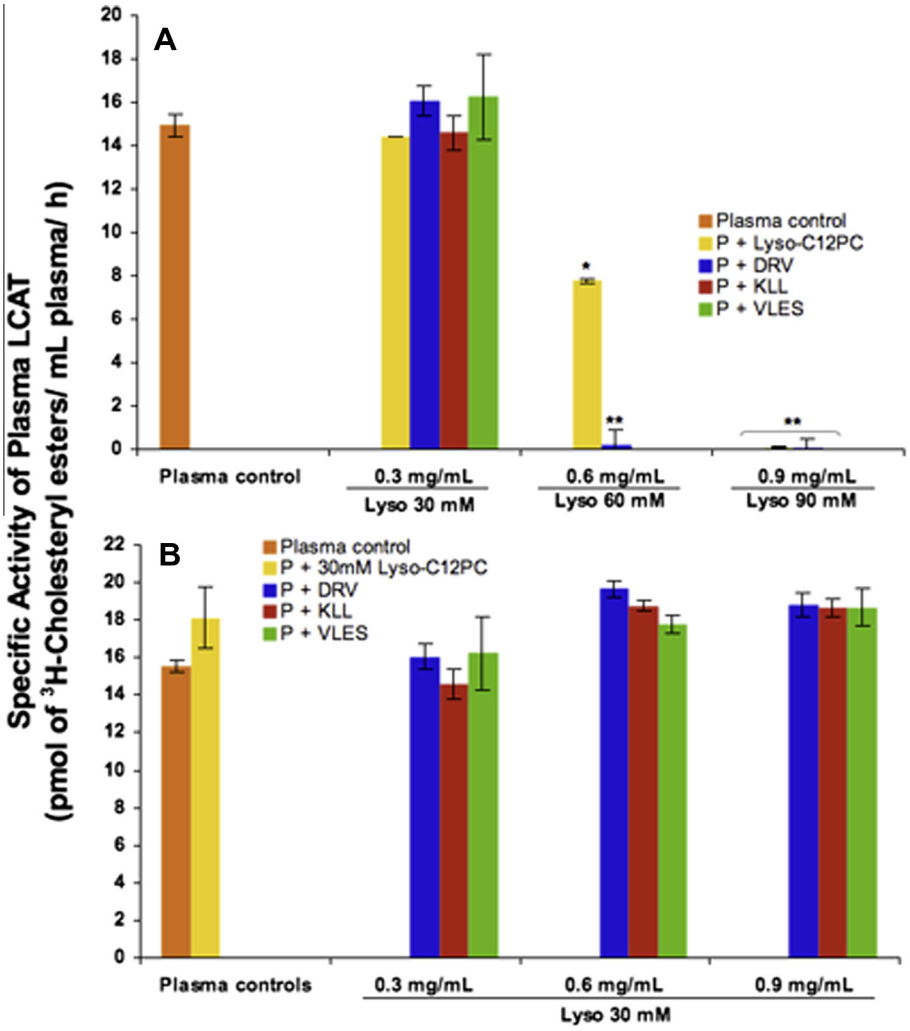

Fig. 4. Effects of Apo AI-derived peptides in the presence of Lyso-C_{12}PC on LCAT activity. (A) In a ratio peptide/Lyso-C_{12}PC of 1:200 (mol:mol). (B) Activity measured at a constant Lyso-C_{12}PC concentration (30 mM). Each point represents the mean of quantifications performed in triplicate ±SEM. * Significant $P \leqslant 0.01$; ** significant $P \leqslant 0.0001$, compared to plasma control.

properties of the lysophospholipid promote the solubilization of the substrates including HDLs and LDLs and preclude enzyme interaction [30]. Another possibility is that lysophospholipids compete with free cholesterol inhibiting the lysolecithin acyl transferase activity of LCAT [31,32].

Under our experimental conditions, inhibition was observed even in the presence of Apo AI peptides, indicating that they were unable to revert the lysophospholipid effect. This finding may be explained by the loss of α-helical conformation associated with a higher concentration of peptides in the presence of high Lyso-C_{12}PC concentrations (Fig. 2B, Table 1). Due to the inhibitory effect observed on LCAT activity followed by the presence of high lysophospholipid concentrations, we changed the peptide concentrations and maintained a Lyso-C_{12}PC concentration of 30 mM. This set of experiments showed that plasma LCAT activity was maintained in the presence of the three peptides, even at concentrations

Table 2
Quantification of [^{3}H]-cholesteryl esters produced by plasma LCAT at 5 h of incubation in the presence of Apo-AI-derived peptides in PBS (pH 7.4).

Condition		[^{3}H]-cholesteryl esters (pmol)	Increase over control (%)
Plasma		0.92 ± 0.06	–
DRV	0.3 mg/mL	1.35 ± 0.08	47.03**
	0.6 mg/mL	1.16 ± 0.04	26.46**
KLL	0.3 mg/mL	1.38 ± 0.03	50.08**
	0.6 mg/mL	1.12 ± 0.12	21.92**
VLES	0.3 mg/mL	1.29 ± 0.13	40.12**
	0.6 mg/mL	0.99 ± 0.07	7.51*

Absolute values determined ± SEM. – Not Applicable
* Significant $P \leqslant 0.01$.
** significant $P \leqslant 0.0001$.

between 0.6 and 0.9 mg/mL but with no significant differences among them (Fig. 4B). Consistent with our previous results, a high peptide concentration was associated with the loss of helical structure (Fig. 2B, Table 1) and a decrease in LCAT activity (Fig. 3B). Therefore, structural arrangements of peptides and the conservation of plasma LCAT activity were favored in the presence of specific Lyso-C_{12}PC concentration, independent of peptide sequence (Table 2).

Concerning the mechanism involved in the modulation of peptides tested upon LCAT activity, it has been suggested that α-helices modify the water/phospholipid interfaces in order to expose the substrate at the active site of LCAT [33]. Our group has shown that Apo AI and other exchangeable apolipoproteins can be maintained at the air/water interface in Langmuir pans where they are able to undergo conformational changes due to increments in lateral pressure [16]. These observations suggest that when HDLs are in a discoidal lipid-poor form, apolipoproteins are exposed to high pressures. While HDLs increase their size due to the accumulation of cholesteryl esters, apolipoproteins increase freedom of movement and respond better to microenvironmental conditions [17]. Experimentally, we observed that a segment of Apo CI reacted to lateral pressures by changing their unstructured conformation to a more ordered α-helical conformation [18]. These findings might suggest that disorder-to-order transitions are dependent on medium composition [18]. Likewise, our present results show that the conformational transitions of peptides modulated by the surrounding lipid environment are also important in the maintenance of LCAT enzyme activity.

The present study provides an important insight into the potential interactions between LCAT and lipoproteins and also suggests that peptides, initially present in a disordered conformation, when in contact with the reaction mixture are able to sense the lipid environment provided by lipoproteins of plasma and change to an ordered α-helical conformation. Then, when peptides adopt a more efficient conformation following disorder-to-order transitions, they are able to increase LCAT activity. Moreover, subtle changes in activation can be related to specific characteristics of the peptides, such as amphiphilicity and the capacity to bind lipids. Accordingly, peptides with a high μH and high affinity for lipid surfaces were more potent activators of LCAT activity. Another relevant finding was that the concentration of the activator should be precisely controlled to attain the expected disorder-to-order transition.

Acknowledgments

This work was supported by the DGAPA-UNAM (Grants IN-205711/2 and RR205711) awarded to J.M.-O. The authors thank José Gutiérrez-Salinas for the human plasma samples. SLAE was supported by a fellowship by the Consejo Nacional de Ciencia y Tecnología (CONACyT) for M. Phil. studies.

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