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

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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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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