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Influence of N-terminal helix bundle stability on the lipid-binding properties of human apolipoprotein A-I

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ABSTRACT

As the principal component of high-density lipoprotein (HDL), apolipoprotein (apo) A-I plays essential roles in lipid transport and metabolism. Because of its intrinsic conformational plasticity and flexibility, the molecular details of the tertiary structure of lipid-free apoA-I have not been fully elucidated. Previously, we demonstrated that the stability of the N-terminal helix bundle structure is modulated by proline substitution at the most hydrophobic region (residues around Y18) in the N-terminal domain. Here we examine the effect of proline substitution at S55 located in another relatively hydrophobic region compared to most of the helix bundle domain to elucidate the influences on the helix bundle structure and lipid interaction. Fluorescence measurements revealed that the S55P mutation had a modest effect on the stability of the bundle structure, indicating that residues around S55 are not pivotally involved in the helix bundle formation, in contrast to the insertion of proline at position 18. Although truncation of the C-terminal domain (Δ190–243) diminishes the lipid binding of apoA-I molecule, the mutation S55P in addition to the C-terminal truncation (S55P/Δ190–243) restored the lipid binding, suggesting that the S55P mutation causes a partial unfolding of the helix bundle to facilitate lipid binding. Furthermore, additional proline substitution at Y18 (Y18P/S55P/Δ190–243), which leads to a drastic unfolding of the helix bundle structure, yielded a greater lipid binding ability. Thus, proline substitutions in the N-terminal domain of apoA-I that destabilized the helix bundle promoted lipid solubilization. These results suggest that not only the hydrophobic C-terminal helical domain but also the stability of the N-terminal helix bundle in apoA-I are important modulators of the spontaneous solubilization of membrane lipids by apoA-I, a process that leads to the generation of nascent HDL particles.

1. Introduction

Elevated plasma concentrations of high-density lipoprotein (HDL) and apolipoprotein (apo) A-I, the major protein constituent of HDL, are inversely related to the risk of cardiovascular disease in humans [1,2]. The inherent anti-atherogenic functions of HDL and apoA-I are primarily attributed to their known involvement in the reverse cholesterol transport pathway [3]. In this pathway, HDL mediates efflux of cholesterol from peripheral cells by diffusion-mediated processes (for a review, see ref. [4]) and lipid-free or lipid-poor apoA-I molecules remove cholesterol from peripheral cells via the ATP-binding cassette transporter A1 (ABCA1) (for a review, see ref. [5]). The ability of apoA-I to solubilize membrane lipids plays a key role in cholesterol efflux from cells via ABCA1 and in nascent HDL particle assembly [6].

Human apoA-I is composed of 243 amino acids; residues 1–43 are encoded by exon 3 and residues 44–243 are encoded by exon 4 of the apoA-I gene. Analysis of the amino acid sequence reveals that residues 1–43 form a class G* amphipathic α-helix, while residues 44–243 contain a series of 22-mer and 11-mer class A and class Y amphipathic α-helices [7]. Several studies have elucidated that lipid-free apoA-I is folded into two structural domains, comprising an N-terminal part (residues around 1–189) forming a four-helix bundle and a discrete C-terminal part (residues around 190–243) [8–10]. Previously, we demonstrated that the extreme N-terminal region spanning residues 1–43 is important for maintaining the helix bundle structure [8,11]. On the other hand, the most C-terminal region spanning residues 223–243 is crucial for initiating lipid binding, which also involves the subsequent conformational opening of the N-terminal helix bundle [8,12].

Abbreviations: ABCA1, ATP-binding cassette transporter A1; ANS, 8-anilino-1-naphthalenesulfonic acid; apo, apolipoprotein; CD, circular dichroism; DMPC, dimyristoylphosphatidylcholine; GdnHCl, guanidine hydrochloride; HDL, high-density lipoprotein; HX, hydrogen–deuterium exchange; ITC, isothermal titration calorimetry; PC, phosphatidylcholine; SUV, small unilamellar vesicle; Trp, tryptophan; WT, wild-type

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A proline residue lacks an amide hydrogen atom essential for the formation of a hydrogen bond so that helix formation tends to be disrupted [13]. We reported previously that insertion of a proline residue into the most hydrophobic residue in the N-terminal domain (Y18P) disrupted the helix bundle structure [11]. Interestingly, although removal of the C-terminal domain (Δ190–243) significantly reduced the binding of apoA-I to lipids, the presence of the Y18P mutation offset the negative effects of this C-terminal truncation. Considering the two-step mechanism for apoA-I binding to lipid [8], it is conceivable that the α-helix around Y18 masks a potential lipid-binding region in the N-terminal domain by inhibiting the unfolding of the helix bundle.

Hydrogen–deuterium exchange (HX) experiments revealed that helical structures in the N-terminal domain are located in residues 7–44, 54–65, 70–78, 81–115, and 147–178 [14]. Hydropathy analysis shows that the region around residue 55 is relatively hydrophobic compared to most of the helix bundle domain [12]. In addition, a peptide corresponding to residues 44–65 binds to lipids well whereas peptides from the remainder of the N-terminal domain do not [15]. These observations led us to postulate that the segment around residues 54–65 is a potential lipid-binding region in the N-terminal domain, substituting for the lipid-binding function when the C-terminal domain is deleted. Y18 is located in the middle of the putative α-helical segment (residues 7–44), whereas S55 is located at the edge of the putative α-helical segment (residues 54–65), according to the HX results. In the present study, in comparison to the Y18P mutation, we examined the effects of proline insertion at position S55 on the stability and lipid-binding properties of apoA-I.

2. Materials and methods

2.1. Materials

Egg phosphatidylcholine (PC) and dimyristoylphosphatidylcholine (DMPC) were purchased from Sigma-Aldrich (St. Louis, MO) and NOF (Tokyo, Japan), respectively. Ultrapure guanidine hydrochloride (GdnHCl) was obtained from MP Biomedicals (Aurora, OH). 8-Anilino-1-naphthalenesulfonic acid (ANS) was purchased from Molecular Probes (Eugene, OR). [^{3}H]cholesterol and [^{14}C]formaldehyde were purchased from PerkinElmer Life Sciences (Wellesley, MA). All other reagents were special grade.

2.2. Protein expression and purification

Mutations were introduced in the N-terminal (S55P) or both of the N- and C-terminal (S55P/Δ190–243, Y18P/S55P/Δ190–243) domains in human apoA-I. Wild-type (WT) human apoA-I and engineered mutants were expressed as thioredoxin fusion proteins in the *E. coli* strain BL21-DE3 and then cleaved and purified as described previously [8]. The apoA-I preparations were at least 95% pure as assessed by SDS-PAGE. In all experiments, apoA-I was freshly dialyzed from 6 M GdnHCl solution into Tris buffer (10 mM Tris, 150 mM NaCl, 1 mM EDTA, 0.02% NaN_3, pH 7.4) or phosphate buffer (10 mM sodium phosphate, pH 7.4) before use. Protein concentrations were determined either by the Lowry procedure using bovine serum albumin (Bio-Rad, Hercules, CA) as a standard [16,17] or by absorbance measurements at 280 nm.

2.3. Preparation of lipid vesicles

A film of egg PC or DMPC on the wall of a glass tube was dried under vacuum overnight and hydrated with buffer. Egg PC small unilamellar vesicles (SUV) with a diameter of approximately 25 nm were prepared as described [18]. Multilamellar vesicles of DMPC were prepared by vortexing vigorously. The PC concentration was determined using an enzymatic assay kit for choline from Wako Pure Chemicals (Osaka, Japan).

2.4. Circular dichroism (CD) spectroscopy

Far-UV CD spectra were obtained using an Aviv 62ADS spectropolarimeter. ApoA-I samples were dissolved at 50 μg/mL in phosphate buffer. The α-helix contents were calculated from the equation using molar ellipticities $[\theta]$ at 222 nm: % α-helix $= [(-[\theta]_{222} + 3000)/(36000 + 3000)] \times 100$. For lipid-binding experiments, proteins were incubated with SUV for 1 h prior to the measurement [18].

2.5. Fluorescence measurements

All fluorescence measurements were carried out at 25 °C using a Hitachi F-7000 or F-4500 spectrophotometer. For denaturation experiments, samples were preincubated with given concentrations of GdnHCl overnight at 4 °C. The emission spectra of tryptophan (Trp) were recorded from 300 to 420 nm at the excitation wavelength of 295 nm. ANS fluorescence spectra were recorded from 400 to 600 nm at the excitation wavelength of 395 nm in the absence or presence of 50 μg/mL protein and an excess amount of ANS (250 μM) [8].

2.6. Interactions of apoA-I with lipid

The binding of apoA-I labeled with [^{14}C]formaldehyde to eggPC SUV labeled with a trace amount of [^{3}H]cholesterol was assayed by gel filtration chromatography as described [18]. The kinetics of solubilization of DMPC vesicles by the addition of apoA-I were measured by monitoring the time-dependent decrease in the turbidity at 24.0 °C [19]. Sample light scattering intensity was monitored at 325 nm on a Beckman Coulter DU-640 spectrophotometer. The 10 min time courses were fitted to a monoexponential decay equation. The 10 min decrease in absorbance was measured as a function of apoA-I concentration.

2.7. Isothermal titration calorimetry (ITC) measurements

Enthalpies of apoA-I binding to SUV were measured using a MicroCal MCS isothermal titration calorimeter at 25 °C as described [18]. Measurements were carried out by titrating 8 μL aliquots of apoA-I sample (0.8 mg/mL) into the cell (1.35 mL) containing an excess of SUV (15–20 mM) at constant time intervals of 540 s. Enthalpies of apoA-I binding to SUV were corrected for heats of apoA-I dilution and dissociation at 25 °C; these values were determined by titrating apoA-I into buffer alone.

3. Results

3.1. Effect of S55P mutation on the secondary structure and stability of apoA-I

The secondary structures of apoA-I variants were analyzed by far-UV CD spectroscopy. α-Helix contents calculated from the molar ellipticity at 222 nm are summarized in Table 1. In the lipid-free state, the α-helical content of S55P and S55P/Δ190–243 apoA-I was comparable to that of WT and Δ190–243 apoA-I, respectively (Table 1). This suggests that the influence of a single proline substitution on the secondary structure in lipid-free state is not significant, consistent with the previous study [11]. Fig. 1 shows the GdnHCl-induced denaturation curves of WT, S55P, and S55P/Δ190–243 apoA-I monitored by the change in Trp fluorescence intensity at 335 nm. The conformational stability, ΔG_D°, the midpoint of denaturation, $D_{1/2}$, and m values are listed in Table 1. All four Trp residues (positions 8, 50, 72, and 108) are located in the N-terminal half, which enabled us to estimate the stability of the N-terminal domain. It has been proposed that there are possible contributions to the stability of electrostatic interactions between the N- and C-terminal domains of apoA-I, as is seen in the apoE molecule [20,21]. However, deletion of the C-terminal domain (Δ190–243) had a negligible effect on the stability of apoA-I [8,22], because electrostatic interactions are