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Ordered opening of LDL receptor binding domain of human apolipoprotein E3 revealed by hydrogen/deuterium exchange mass spectrometry and fluorescence spectroscopy

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ABSTRACT

Apolipoprotein E3 (apoE3) is an exchangeable apolipoprotein that plays a critical role in cholesterol homeostasis. The N-terminal (NT) domain of apoE3 (residues 1–191) is folded into a helix bundle comprised of 4 amphipathic α-helices: H1, H2, H3 and H4, flanked by flexible helices N1 and N2, and Hinge Helix 1 (Hinge H1), at the N-and C-terminal sides of the helix bundle, respectively. The NT domain plays a critical role in binding to the low density lipoprotein receptor (LDLR), which eventually leads to lowering of plasma cholesterol levels. In order to be recognized by the LDLR, the helix bundle has to open and undergo a conformational change. The objective of the study was to understand the mechanism of opening of the helix bundle. Hydrogen/deuterium exchange mass spectrometry (HDX-MS) revealed that apoE3 NT domain adopts several disordered and unfolded regions, with H2 exhibiting relatively little protection against exchange-in compared to H1, H3, and H4. Site-directed fluorescence labeling indicated that H2 not only has the highest degree of solvent exposure but also the most flexibility in the helix bundle. It also indicated that the lipoprotein behavior of H1 was significnatly different from that of H2, H3 and H4. These results suggest that the opening of the helix bundle is likely initiated at the flexible end of H2 and the loop linking H2/H3, and involves movement of H2/H3 away from H1/H4. Together, these observations offer mechanistic insight suggesting a regulated helix bundle opening of apoE3 NT domain can be triggered by lipid binding.

1. Introduction

ApoE3 is an exchangeable apolipoprotein that plays a crucial role in cholesterol and triglyceride homeostasis by virtue of its ability to serve as a ligand for the low density lipoprotein receptor (LDLR) family of proteins [1,2]. These receptors mediate endocytosis of the lipoprotein particles thereby decreasing plasma cholesterol and triglycerides levels [3]. ApoE3 also bears the ability to promote efflux of cholesterol from macrophages in atherosclerotic lesions, and to mediate retrograde transport of cholesterol from macrophages to the liver [4].

ApoE3 is composed of 299 amino acids (~34 kDa) that are folded into a 22 kDa N-terminal (NT) domain (residues 1–191) and a 10-kDa C-terminal (CT) domain (residues 201–299). The two domains are linked by a protease sensitive flexible segment (192–200). The NT domain houses the LDLR binding sites, while the CT domain bears high affinity lipid binding sites, mediates protein-protein interaction that facilitates apoE3 tetramerization and efficiently promotes ABCA1-mediated cholesterol efflux [4]. X-ray crystallographic analysis of apoE3(1–191) at

Abbreviations: apoE3, apolipoprotein E3; CD, circular dichroism; CT, C-terminal; GdnHCl, guanidine hydrochloride; FI, fluorescence intensity; FP, fluorescence polarization; HDL, high density lipoprotein; HDX-MS, hydrogen/deuterium exchange mass spectrometry; IAEDANS, 5-((((2-iodoacetyl)amino)ethyl)amino)naphthalene-1-sulfonic acid; LDL, low density lipoprotein; LDLR, low density lipoprotein receptor; NT, N-terminal; PLC, phospholipase C; TCEP, tris(2-carboxyethyl) phosphine hydrochloride; WT, wild type

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2.5 Å resolution [5] and of apoE3(1–183) by NMR [6] reveal a long helix bundle (65 Å) comprised of 4 anti-parallel amphipathic α-helices (labeled H1-H4). H1 and H2 are linked by a short helix 1′ (H1′) that is roughly perpendicular to the helix bundle. H4 harbors several positively charged residues between 134 and 150, which display a large region of positive electrostatic potential; this segment, along with Arg172 [7] and additional residues between 174 and 183 [8], plays a significant role in LDLR binding.

As an exchangeable apolipoprotein, apoE3 exhibits the capability to exist in lipid-free and lipid (lipoprotein)-bound states. From a functional standpoint, it is recognized that lipid binding of the NT domain is an *a priori* requirement for apoE3 to elicit LDLR binding activity [9]. Disulfide bond engineering coupled to lipid binding studies suggest that lipid-association involves an initial interaction between protein and lipids, followed by opening of the four-helix bundle to unveil the hydrophobic interior [7]. This is analogous to that observed for insect apolipophorin III, which involves lipid-triggered opening of a five-helix bundle [10]. Fluorescence resonance energy transfer [11–13] [11–13], parallax depth quenching [14] and Attenuated Total Reflectance Fourier Transformed Infrared [15] spectroscopic analyses indicate that interaction of the NT domain helix bundle of apoE3 with phospholipids results in a dramatic conformational change. This involves re-configuration of the lipid-free helix bundle to a belt-like organization of α-helices circumscribing a discoidal bilayer of phospholipids. In this state, the helical axes are oriented perpendicular to the fatty acyl chains of the phospholipid. The transition between lipid-free and lipid-bound forms is a complex process, and likely represents a key regulatory strategy in lipoprotein metabolism.

Despite evidence that suggest lipid-triggered conformational change in apoE3, the details regarding the mode of helix bundle opening is not known. In the current study, we address the issue of helix bundle opening by applying hydrogen/deuterium exchange mass spectrometry (HDX-MS) and site-directed fluorescence spectroscopy to assess amide-backbone structural dynamics and the order of opening and lipoprotein binding of the helices.

2. Materials and methods

2.1. Design and generation of single Cys constructs

Single Cys constructs were designed by examining the X-ray crystal structure of human apoE3(1–191) (PDB ID: 1NFN) and the NMR structure of apoE3(1–183) (PDB ID: 2KC3) using the Chimera software [16]. The introduction of Cys allows covalent attachment of fluorophores containing sulfhydryl-reactive functional groups. The selected sites allow sampling of individual helices by monitoring the fluorescence behavior of the attached probe. Throughout this study apoE3(1–191) encompassing the entire LDLR binding domain and a hexa-His tag at the N-terminal was used and referred to as apoE3 NT domain. Wild type (WT) apoE3 bears a single endogenous Cys at position 112; to generate single Cys constructs for fluorescence studies, C112 was replaced by serine (with no significant change in function or protein fold) [17], and designated as apoE3C112S(1–191). Single Cys constructs were generated in apoE3C112S(1–191)/pET22b vector using the QuikChange II Site Directed Mutagenesis kit (Agilent Technology, Stratagene, La Jolla, CA) to substitute a Cys at desired locations on H1, H2, H3 and H4 (A29C, A62C, A102C and V161C, respectively). The plasmids were sequenced to confirm the presence of the desired mutations.

2.2. Expression, isolation and purification of WT and single Cys apoE3 NT domain variants

E. coli cells were transformed with the pET-22b(+) expression vector encoding WT or single Cys constructs of apoE3 NT domain and bearing ampicillin resistance. The recombinant proteins were over-expressed, isolated in the presence of 4 M guanidine HCl (GdnHCl) and purified using a Ni^{2+}-affinity matrix (Hi-Trap chelating column, GE Healthcare, Piscataway, NJ) as described previously [18]. Protein purity was verified by SDS-PAGE analysis using a 4–20% acrylamide gradient gel under reducing conditions. Protein concentration was determined in a Nano-Drop 2000/2000c spectrometer (Thermo Fisher Scientific, Wilmington, DE) using the molar extinction coefficient at 280 nm of 27,960 M^{-1} cm^{-1}. All proteins were unfolded in 6 M GdnHCl and freshly refolded by dialysis against buffer at 4 °C for 48 h with 3 changes.

2.3. AEDANS labeling

The purified single Cys apoE3 NT domain variants (5 mg protein in 10 mM ammonium bicarbonate, pH 7.4) were treated with 6 M guanidine HCl (GdnHCl) (Applied Biosystems, Eugene, OR), 2-fold molar excess of Tris(2-carboxyethyl)phosphine hydrochloride (TCEP) (Sigma-Aldrich, St. Louis, MO) and 5-fold excess of 5-((((2-Iodoacetyl)amino) ethyl)amino)napthalene-1-sulfonic acid (IAEDANS) (Invitrogen Molecular Probes, Eugene, OR) for 2 h at 37 °C. The labeled proteins were dialyzed extensively against 10 mM ammonium bicarbonate, pH 7.4. The extent of labeling was calculated using the molar extinction coefficients of apoE3(1–191) and that of IAEDANS at 340 nm (5700 M^{-1} cm^{-1}).

2.4. Circular dichroism (CD) spectroscopy

The secondary structure of labeled apoE3 NT domain variants was assessed by CD spectroscopy on a Jasco J-810-150S spectropolarimeter at 24 °C. Far-UV CD scans were recorded between 185 nm and 260 nm in 10 mM sodium phosphate buffer, pH 7.4 using protein concentrations of 0.2 mg/ml in a 0.1 cm path length cuvette. The CD profiles were the average of four independent scans recorded with a response time of 1 s and bandwidth of 1 nm. The molar ellipticity ([θ]) in deg. cm^2 $dmol^{-1}$ at 222 nm was obtained using the equation:

$$[\theta]_{222nm} = MRW\ (\theta)/10\ (l)\ (c) \quad (1)$$

where MRW is the mean residue weight (obtained by dividing molecular weight by the number of residues), θ is the measured ellipticity at 222 nm (in degrees), l is the cuvette path-length (in cm) and c is the protein concentration (in g/ml). The percent α-helix content was calculated as described previously [19].

2.5. Fluorescence measurements

Fluorescence emission intensity (FI) measurements of AEDANS-apoE3 NT domain variants were carried out in a Perkin-Elmer LS55B fluorimeter at 25 °C. Fluorescence emission spectra were recorded in 10 mM sodium phosphate buffer, pH 7.4, containing 150 mM NaCl (phosphate buffered saline, PBS) in the absence or presence of 6 M GdnHCl between 350 and 600 nm following excitation at 340 nm, at a scan speed of 50 nm/min (3 nm excitation and emission slit widths); an average of 10 scans were recorded. The samples were treated with TCEP to reduce any residual S—S bonds prior to analysis. Fluorescence polarization (FP) measurements of AEDANS labeled apoE3 NT domain variants were carried out in a Perkin-Elmer LS55B fluorimeter at 25 °C with an integration time of 0.1 s and slit width of 5 nm. The excitation and emission wavelengths were 340 nm and 480 nm, respectively.

2.6. HDX-MS

HDX was initiated by adding 10-fold deuterated buffer (10 mM sodium phosphate buffer, pD 7.4, 25 °C) to WT apoE3(1–191) (0.1 mg/ml in 10 mM sodium phosphate buffer, pH 7.4) as described previously [20]. Briefly, the exchange-in reaction was arrested with cold deuterated phosphate buffer, pD 2.5 at various time points, and samples were