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Fluorescence study of domain structure and lipid interaction of human apolipoproteins E3 and E4

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ABSTRACT

Human apolipoprotein E (apoE) isoforms exhibit different conformational stabilities and lipid-binding properties that give rise to altered cholesterol metabolism among the isoforms. Using Trp-substituted mutations and site-directed fluorescence labeling, we made a comprehensive comparison of the conformational organization of the N- and C-terminal domains and lipid interactions between the apoE3 and apoE4 isoforms. Trp fluorescence measurements for selectively Trp-substituted variants of apoE isoforms demonstrated that apoE4 adopts less stable conformations in both the N- and C-terminal domains compared to apoE3. Consistent with this, the conformational reorganization of the N-terminal helix bundle occurs at lower guanidine hydrochloride concentration in apoE4 than in apoE3 as monitored by fluorescence resonance energy transfer (FRET) from Trp residues to acrylodan attached at the N-terminal helix. Upon binding of apoE3 and apoE4 variants to egg phosphatidylcholine small unilamellar vesicles, similar changes in Trp fluorescence or FRET efficiency were observed for the isoforms, indicating that the opening of the N-terminal helix bundle occurs similarly in apoE3 and apoE4. Introduction of mutations into the C-terminal domain of the apoE isoforms to prevent self-association and maintain the monomeric state resulted in great increase in the rate of binding of the C-terminal helices to a lipid surface. Overall, our results demonstrate that the different conformational organizations of the N- and C-terminal domains have a minor effect on the steady-state lipid-binding behavior of apoE3 and apoE4: rather, self-association property is a critical determinant in the kinetics of lipid binding through the C-terminal helices of apoE isoforms.

1. Introduction

Apolipoprotein E (apoE) plays a key role in regulating lipid transport and cholesterol homeostasis in the cardiovascular and central nervous systems [1–3]. In humans, there are three major isoforms of the protein, apoE2, apoE3, and apoE4, each differing by a single amino acid substitution [4]. ApoE3, the most common isoform, contains Cys-112 and Arg-158, whereas the less common apoE2 and apoE4 contain Cys-112/Cys158 and Arg-112/Arg-158, respectively (Fig. 1). ApoE2 displays defective binding to the low-density lipoprotein (LDL) receptor superfamily and is associated with type III hyperlipoproteinemia [5]. Although apoE3 and apoE4 bind similarly to the LDL receptor [6], apoE4 reduces plasma cholesterol less in humans compared to apoE3, giving rise to a more proatherogenic lipoprotein–cholesterol distribution [7,8]. ApoE4 is also known to be a major genetic risk factor for Alzheimer's disease [9,10]. Despite the profound differences in the outcomes of these diseases, the differences in the molecular properties of the apoE isoforms are still unclear [11].

ApoE contains two independently folded functional domains: a 22-kDa N-terminal domain (residues 1–191) and a 10-kDa C-terminal domain (residues 216–299) linked by a hinge region [4,12]. The N-terminal domain is folded into a four-helix bundle of amphipathic α-helices [13,14] and contains the region (residues 136–150) that binds to the LDL receptor [15]. The C-terminal domain contains amphipathic α-helices that are involved in binding to lipoproteins with high affinity [16–18] and self-association to form predominantly tetramer in solution [16,19,20]. Studies of apoE3 and apoE4 variants containing a progressively truncated C-terminal domain demonstrated that the region spanning residues 260–299 is important for determining self-association and ability to bind to lipoprotein particles of apoE [20–23]. Recent hydrogen/deuterium exchange coupled with electron-transfer dissociation mass spectrometry indicated that residues within regions 230–270 are critical for oligomer formation of apoE isoforms [24],

Abbreviations: Ac, acrylodan; apoE, apolipoprotein E; FRET, fluorescence resonance energy transfer; GdnHCl, guanidine hydrochloride; LDL, low-density lipoprotein; PC, phosphatidylcholine; PL, phospholipid; SUV, small unilamellar vesicle; TBS, Tris-buffered saline; WMF, wavelength of maximum fluorescence

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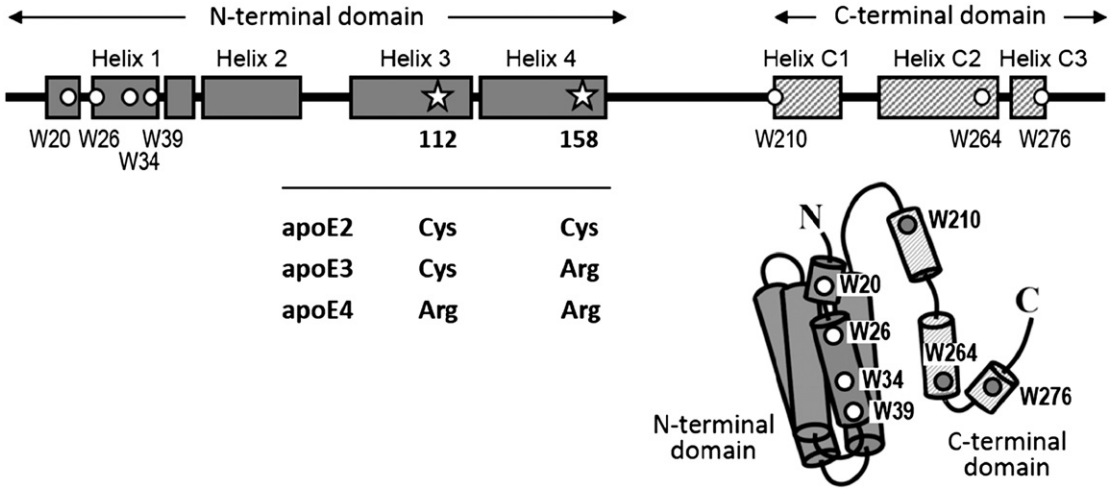

Fig. 1. Schematic representation of the location of Trp residues in the N- and C-terminal domains of human apoE. The isoform-specific differences in amino acid sequence are also shown in the linear diagram. The α-helical segments are depicted as boxes based on the NMR structure of monomeric apoE3 [27].

overlapping the major lipid binding region in the C-terminal domain. This suggests that the lipid binding and self-association in apoE isoforms are closely linked [25], consistent with the kinetic analysis of turbidity clearance of phospholipid vesicles by apoE [26].

A recent NMR structure of a monomeric variant of full-length apoE3 demonstrated that the C-terminal domain folds over and around the N-terminal domain through extensive interactions of salt-bridges and hydrogen bonds between the two domains [27]. Consistent with this, our previous deletion mutagenesis study indicated that the segment 261–272 contributes to the helix stabilization of the N-terminal domain, with this effect being much greater in apoE3 than apoE4 [28]. ApoE has a much lower thermodynamic stability compared to other globular proteins [29], and mutations in the protein have been shown to alter these inter-domain interactions, causing defective physiological functions [30–32]. The overall stability of the entire apoE molecule exerts a major influence on its lipid- and lipoprotein-binding properties [33].

In this study, we examined the conformational stability and lipid binding properties of the N- and C-terminal domains of apoE3 and apoE4 using selectively Trp-substituted variants, in which Trp residues in either the N- or C-terminal domains were mutated to Phe to allow us to evaluate each domain separately. In addition, we extended our previous site-directed fluorescence labeling approach in the N- or C-terminal helices of apoE [34] to directly compare the conformational reorganization of each domain upon binding to spherical lipid particles for apoE3 and apoE4 variants that have different tendencies to self-associate in solution.

2. Methods

2.1. Materials

Human apoE3 and apoE4 variants and their 12-kDa (residues 192–299) fragment were expressed in *E. coli* as thioredoxin fusion proteins and isolated and purified as described previously [17,35]. Cleavage of the thioredoxin fusion protein with thrombin leaves the target apoE with two extra amino acids, Gly and Ser, at the N terminus. The QuikChange site-directed mutagenesis kit (Stratagene, La Jolla, CA) was employed to introduce the S94C/C112S or C112S/S290C mutations in apoE3 and the S94C or S290C mutations in apoE4 so that a single cysteine residue was present in each molecule. To generate monomeric apoE3 and apoE4 variants, additional mutations (F257A/W264R/V269A/L279Q/V287A) in the C-terminal domain were introduced [19]. In addition, Trp-substituted apoE3 and apoE4 variants in which Trp residues were selectively substituted to Phe in the N-terminal (W20F/W26F/W34F/W39F, ΔW-NT) or C-terminal (W210F/W264F/W276F, ΔW-CT) domains were prepared. The apoE preparations were at least 95% pure as assessed by SDS–PAGE. In all experiments, the apoE sample was freshly dialyzed at 4 °C from a 6 M guanidine hydrochloride (GdnHCl) and 1% β-mercaptoethanol solution into Tris buffered saline (TBS; 10 mM Tris, 150 mM NaCl, 0.02% NaN_3, pH 7.4) before use. Egg yolk phosphatidylcholine (PC) was kindly donated from Kewpie (Tokyo, Japan). *N*-(1-pyrene)maleimide and 6-acryloyl-2-dimethylaminonaphthalene (acrylodan) were purchased from Invitrogen (Eugene, OR).

2.2. Fluorescence labeling

Cysteine-containing apoE variants were incubated with 10-fold molar excess of tris(2-carboxyethyl)phosphine hydrochloride (Thermo Scientific, Rockford, IL) for 1 h to reduce the sulfhydryl group. A 10 mM stock solution of *N*-(1-pyrene)maleimide (in dimethylsulfoxide) or acrylodan (in dimethylformamide) was added so that a final molar ratio of probe to protein was 10:1. The reaction mixtures were then incubated at room temperature for 3 h in the dark, and unreacted probe was removed by extensive dialysis at 4 °C in TBS. The degree of labeling was determined using the extinction coefficients of 38,200 M^{-1} cm^{-1} at 338 nm for pyrene and 19,200 M^{-1} cm^{-1} at 391 nm for acrylodan, respectively.

2.3. Preparation of small unilamellar vesicles (SUVs)

Egg PC SUVs were prepared as described [36,37]. Briefly, a film of egg PC on the wall of a glass tube was dried under vacuum overnight. The lipid was then hydrated in TBS and sonicated on ice under nitrogen. After removing titanium debris, the samples were centrifuged in a Beckman 70.1Ti rotor for 1.5 h at 15 °C at 40,000 rpm to separate any remaining large vesicles. The PC concentration was determined using an enzymatic assay kit from Wako Pure Chemicals (Osaka, Japan).

2.4. Fluorescence measurements

Fluorescence measurements were carried out with a Hitachi F-4500 fluorescence spectrophotometer at 25 °C in TBS (pH 7.4). Trp fluorescence emission spectra of apoE variants were recorded from 300 to 420 nm using a 290 nm excitation wavelength to avoid tyrosine fluorescence. For monitoring chemical denaturation, proteins at concentrations of 25–50 μg/ml were incubated overnight with GdnHCl or urea at various concentrations. K_D at a given denaturant concentration were calculated from the change in Trp fluorescence intensity. The free energy of denaturation, $\Delta G_D°$, the midpoint of denaturation, $D_{1/2}$,