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FOR THE RECORD

Concerning the structure of apoE

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Abstract: Apolipoprotein E (apoE), first described in 1973, is a truly fascinating protein. While studies initially focused on its role in cholesterol and lipid metabolism, one apoE isoform (apoE4) is a major risk factor for development of late onset Alzheimer's disease. Yet the difference between apoE3, the common form, and apoE4 is a single amino acid of the 299 in this 34 kDa protein. Structure determination of the two domain full length apoE3 protein was only accomplished in 2011 and supports the notion that mutations in the N-terminal domain can be propagated through the structure to the C-terminal domain. Understanding the structural differences between apoE3 and apoE4 is critical for finding ways to modulate the deleterious effect of apoE4.

Keywords: Alzheimer's disease; apoE isoforms; structural changes; salt bridges; domain interactions

Introduction

Human plasma contains a variety of proteins associated with lipoprotein particles. In the 1970s, Shore and Shore investigated the heterogeneity of proteins associated with very low density lipoproteins (VLDLs) and showed that there were mixtures of proteins associated with these particles.[1] One such protein family was classified as the apolipoprotein E (apoE). Using isoelectric focusing, Utermann *et al*. showed that the apoE proteins consisted primarily of three isoforms[2] which were later termed apoE2, apoE3, and apoE4 by Zannis *et al*.[3]

In 1981, Weisgraber *et al*.[4] demonstrated that the three major apoE isoforms, of molecular weight 34 kDa, differed only by simple arginine to cysteine changes: apoE4 does not contain cysteine while the other isoforms did. In 1982, Rall *et al*.[5] found the location of the cysteine in apoE3 (Cys112) and those in apoE2 (Cys112,158). In 1982, Weisgraber *et al*. showed that the receptor binding function was isoform-specific although apoE3 and apoE4 appeared to be essentially identical.[6] The complete sequence of apoE2 (299 amino acids) was determined by Rall *et al*. in 1982[5] and that there were two structural domains in apoE3 was suggested by the Weisgraber group in 1988.[7] Structure determination of the full-length protein, either by crystallography or NMR, was not possible due to aggregation of the protein to high molecular weight forms as investigated by Perugini *et al*.[8] Even at low nanomolar concentrations, oligomers, dimers, and tetramers form.[9] By isolation of fragments of apoE, however, it was established that aggregation occurs through the C-terminal domain since the N-terminal domain remains monomeric even at high concentrations[10] while the isolated C-terminal domain aggregates.

The first determination of the structure of the N-terminal domain of apoE3 reported in 1991 as an elongated four-helical bundle.[11] Other structures of the N-terminal domain were subsequently

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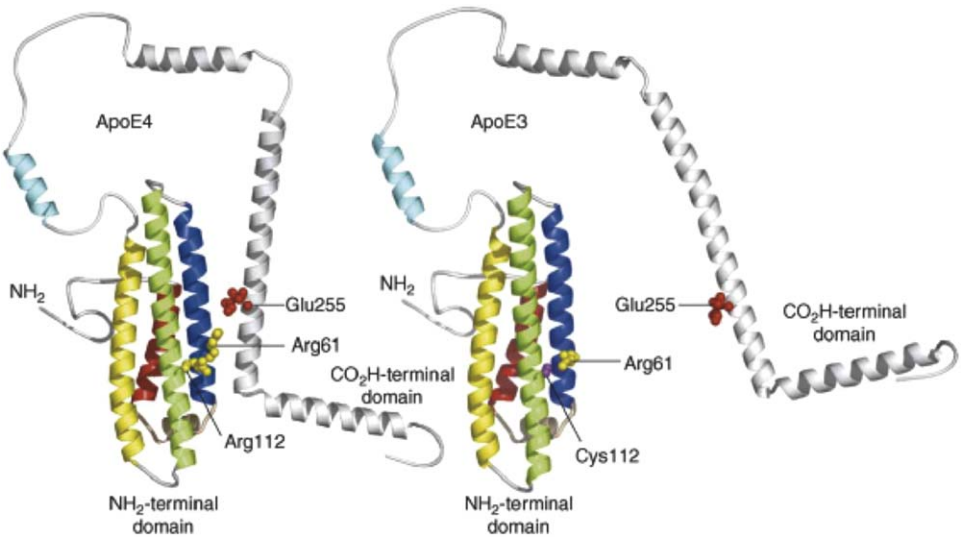

Figure 1. Proposed structures of full length apoE3 and apoE4. Reproduced with permission from Ref. 21 from Elsevier Trends Journals. The authors proposed that a salt bridge in the apoE4 structure, Glu255 of the C-terminal domain to Arg61 in helix 2 (blue), occurs in apoE4 but not in apoE3. Helices 1–4 are colored red, blue, green, and yellow, respectively.

determined by NMR for apoE3[12] and by X-ray crystallography for both apoE3[13] and apoE4.[14] Perhaps the most unusual aspect of this structure is the regions between helices were rather flexible loops rather than tight turns. Not unexpectedly, polar groups in helix 2 (residues 55–79) and regions of helix 4 (residues 140–158) were all solvent exposed. Since it was not possible to determine the structure of the full length protein there were efforts to determine the structure of the C-terminal domain using structural prediction programs. For example, Chou–Fasman predictions[15] suggested that the C-terminal domain was highly helical, a prediction supported by circular dichroism data.[10]

While lipoproteins were considered to be important for cholesterol metabolism, it is now clearly recognized that they have multiple functions related to lipid metabolism.[16] The role of apoE in lipid and cholesterol metabolism was recognized early, but it was not until 1993, in a classic paper, that Corder *et al*. found apoE4 to be the major risk factor in late-onset Alzheimer's disease.[17] This observation made the determination of the full length structure, and structural differences between apoE3 and apoE4, critically important.

The Proposed Structure of the Full Length Protein. The Arg61 Mutant

Based on mutational data, specifically an Arg61Thr mutation in apoE4, Weisgraber and coworkers[18–20] suggested that Arg61 in helix 2 of the N-terminal domain interacted with the C-terminal domain of apoE4. Hatters *et al*. discussed the interaction between the N- and C-terminal domains for apoE3 and apoE4 suggesting the importance of a salt bridge between Arg61 and Glu255 in apoE4 that was proposed not to exist in apoE3.[21]

Dong and Weisgraber showed that mutation of Glu255 alters the preference of apoE4 from VLDL to high density lipoprotein (HDL) similar to what would be expected for apoE3.[19] They concluded that the preferential association of apoE4 for VLDL is an intrinsic property of apoE4, likely related to domain interaction, and that domain interaction is probably mediated by a salt bridge between arginine 61 and glutamic acid 255. Their proposed structures of full length apoE3 and apoE4 are shown in Figure 1.

The Full Length apoE Structure

In 2004, Fan *et al*. found that changing 4–5 residues in the isolated C-terminal domain (residues 200–299) yielded material that did not aggregate and showed a well defined helical structure[22] as predicted by Chou and Fasman.[15] This mutant replaced five bulky hydrophobic residues in the region of residues 253–289 with either smaller hydrophobic or polar/charged residues (F257A, W264R, V269A, L279Q, and V287E). Zhang *et al*. using the same mutations[23] then found that apoE3 existed as a monomer at high enough concentrations for NMR studies. They also concluded that this monomeric form retained many of the biological functions as did wild-type apoE3. This observation opened the door for the determination of the structure of the full length apoE3.

In 2011, Chen *et al*., published the full length structure of the mutated apoE3.[24] This structure, determined by NMR, is shown in Figure 2. One of the surprising results was that Arg61, which had been suggested to interact with Glu255, did not, in

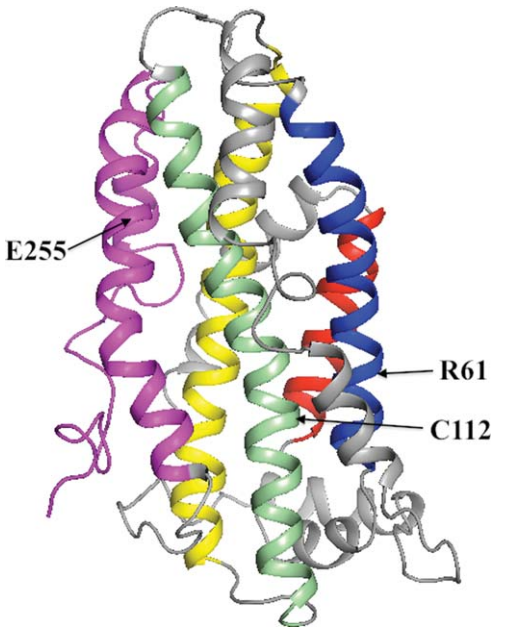

Figure 2. The average NMR structure of apoE3 as determined by Chen *et al*.[24] The helices in the N-terminal domain are shown in the same colors and similar orientation as in Figure 1. The C-terminal domain is colored magenta. To determine this structure, the authors made five mutations in the C-terminal domain to prevent apoE3 aggregation as discussed in the text.

fact, interact with that residue. Rather, Glu255 forms a salt bridge with Lys95. This result is critical to the overall structure because the C-terminal domain does not interact with helix 2 but rather with both helices 3 and 4. Surprisingly, the polar groups of helix 4 which were solvent exposed in the isolated N-terminal domain are completely buried by regions of the C-terminal domain in the full length protein. One could, and should, legitimately question whether mutations made to determine the NMR structure affect one or more of these interactions. The chances, however, that the mutant monomeric structure differs markedly from the wild-type structure are minimal since the five mutated residues are probably solvent exposed.

But if arginine 61 is located distant from glutamate 255, how does one explain the results of the mutational data described above? The explanation appears to be similar to that proposed by Frieden and Garai in their discussion of structural differences between apoE3 and apoE4.[25] These authors suggested, based on hydrogen-deuterium exchange results[26] that the charge difference between cysteine and arginine at position 112 (the difference between apoE3 and apoE4) was propagated through the highly charged helix 4 resulting in structural changes of specific residues in the C-terminal domain. Figure 2, based on the apoE3 structure determined by Chen *et al*., shows that the side chains of the solvent exposed Arg61and Cys112 are within 8 Å separated by mostly hydrophobic residues. Thus, mutating the Arg61 to a non-charged or oppositely charged amino acid may result in exactly the same change in structural differences noted for apoE3 relative to apoE4. That is, the change in behavior as a consequence of the mutation of Arg61 is not a direct effect on the interaction between the N- and C-terminal domains, but an indirect, allosteric, effect. Figure 3 shows one possible path for how the mutation of Arg61 in helix 2 can be propagated to the region 271–287 of the C-terminal domain. In this particular example, there are no contacts between helix 3 and residues 271–287. There are, however, numerous interactions between helices 3 and 4 and the C-terminal domain of apoE3[24] suggesting that there will be many structural changes in the C-terminal domain in apoE4 compared to apoE3 as a consequence of the cysteine to arginine change at position 112.

Changing the Behavior of apoE4

ApoE binds to multiple ligands. In order to understand these interactions it is essential to use the full length structure determined by Chen *et al*.[24] As noted above, Frieden and Garai suggested that the arginine/cysteine change at position 112 was propagated through the structure to regions of the C- and N-terminal domains distant from residue 112. They discussed the possibility of targeting these latter regions to alter the behavior of apoE4 relative to apoE3. Another possibility, based on the above discussion, exists: to target the region near arginines 61 and 112 in apoE4. The region around position 112 might be considered as an obvious target for compounds to change the behavior of apoE4. It is, but not for the obvious reason. The rationale for this approach is based on the same argument used by Frieden and Garai to explain how distant regions of the protein were affected by the arginine to cysteine change at position 112 via the highly charged, but buried, helix 4 as shown in Figure 3. Targeting this region would be easier than targeting regions of the C-terminal domain because there is a known structure of the N-terminal domain of apoE4 while no full length structure of apoE4 currently exists. While there may be structural differences in the C-terminal domain between apoE3 and apoE4, as suggested by the hydrogen/deuterium exchange data,[26] the expectation is that the domain interactions are similar in both apoE4 and apoE3. Structural differences in the C-terminal domain between apoE3 and apoE4 may influence uptake of Aβ via cell surface receptors such as LRP1.[27]

Since the arginine residue of apoE4 is positively charged compared to the cysteine residue of apoE3, compounds that might be useful in changing the behavior of apoE4 might be those that specifically alter the positive charge of arginine at position 112.

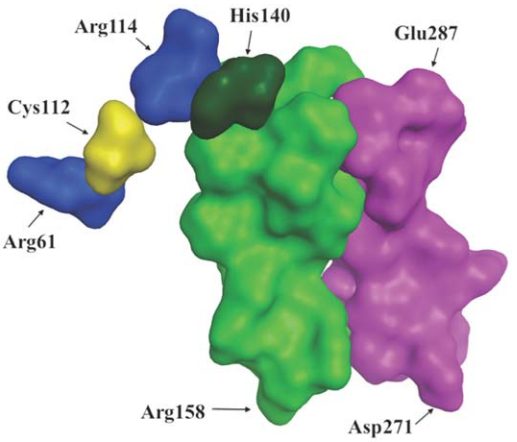

Figure 3. A proposal for those portions of the protein involved in the propagation of structural changes in apoE3 for the Arg61Thr mutant. The proposal details how the Arg61Thr change in the N-terminal domain may be propagated to a region in the C-terminal domain. The proposal is based on the structure of the full length apoE3 as determined by Chen *et al*.[24] Shown are Arg61, Cys112 (yellow), Arg114, His140, a region of helix 4 (residues 140–158) and a region of the C-terminal domain (residues 271–287). Helix 2 (not shown) does not contact this region of the C-terminal domain.

Thus, such compounds need to bind specifically to the region around 112 and carry a negative charge.

What Behavior Should be Changed?

In vitro: It was recognized as early as 1993 that apoE interacts with Aβ.[28,29] The mechanism of binding, the form of Aβ that binds, and the regions of apoE that bind remain to be definitively clarified. *In vitro* experiments using recombinant apoE show that apoE interacts with Aβ oligomers[30] but these binding experiments have been carried out under widely varying conditions by different investigators and the mechanism of the interaction is unclear. Many investigators have found isoform dependent differences in Aβ aggregation and this could be a valid test for determining whether the behavior of apoE4 resembles that of apoE3.

In vivo: Considerable evidence has accumulated suggesting apoE affects Aβ clearance[31–33] from cells and that apoE isoforms differentially affect Aβ clearance with clearance by apoE4 being slower than that by apoE3 or apoE2.[32,34] For example, LaDu and coworkers find that Aβ accumulation is apoE isoform dependent in a transgenic mouse model.[35,36] The ultimate test for whether a potential therapeutic agent alters the effect of apoE4 may be measuring the clearance of Aβ from cells. Chen *et al*.[37] have found several small molecule effectors (called correctors) that abolish detrimental effects of apoE4 in cultured neurons. Alternatively, immunotherapy targeting apoE4 specifically may be an effective therapeutic approach. Kim *et al*. have shown that intraperitoneal administration of a specific monoclonal apoE antibody HJ6.3 dramatically decreased the amyloid plaque load in mouse brain possibly by promoting clearance of Aβ associated with the plaques.[38] Mahley *et al*. suggest other possible differences between apoE isoforms in that apoE4 is less effective at redistributing lipids among cells in the central nervous system (CNS)[39,40] or in mitochondrial function.[41]

Comments on apoE2

The difference between apoE3 and apoE2 is yet again a single amino change (Arg158Cys). While not discussed here, similar arguments can be made for this change as for the change at position 112. For example, polar residues distant in the sequence but especially close to the buried residue at position 158 include Lys92, Glu96, and Arg260, the former two in helix 2 and the latter in the C-terminal domain.

Conclusion

The recent determination of the full length structure of apoE3 has not yet revealed why apoE4, which differs by a single amino acid from apoE3, is the major risk factor for late onset Alzheimer's disease. The structure does show, however, that apoE is a surprisingly complex protein in which the consequence of the single amino changes between the apoE isoforms can be propagated throughout the structure. Small molecular weight compounds that bind to specific regions of the protein may exert effects on the structure that are distant from the binding site of the compound. Such compounds could alter the behavior of the apoE4, the major risk factor for late onset Alzheimer's disease.

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