Supplementary Information

Insights into the C-terminal Domain of Apolipoprotein E from Chimera Studies with Apolipophorin III

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Comparison of DMS crosslinking and ANS fluorescence of apoLp-III/CT-apoA-I and apoLp-III/CT-apoE. A chimeric construct bearing residues 190-243 of apoA-I appended to *L. migratoria* apoLp-III (apoLp-III/CT-apoA-I) was used as described previously [1]. CT-apoA-I (residues 179-243) was isolated from a chimera that contained a methionine residue between apoLp-III and CT-apoA-I, facilitating isolation by cyanogen bromide digestion [2]. Chimera DMS cross-linking and ANS fluorescence analyses were carried out as described under Materials and Methods for apoLp-III/CT-apoE.

A direct comparison of apoLp-III/CT-apoE and apoLp-III/CT-apoA-I chimeras showed that while each CT-domain promoted self-association of the chimera, there were differences in the extent of crosslinking (Fig. S1). Based on the decrease of the density of the

monomer in the presence of DMS and the corresponding increase in intensity in high-molecular weight bands, the crosslinking analysis indicates that CT-apoA-I mediates self-association more efficiently than CT-apoE.

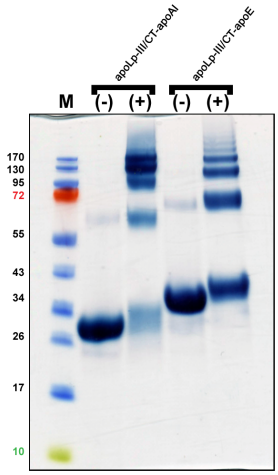

Fig. S1. Comparison of DMS crosslinking of apoLp-III/CT-apoA-I and apoLp-III/CT-apoE. The chimeric proteins were incubated with DMS in the absence (-) or presence (+) of DMS crosslinker, separated by SDS-PAGE, and visualized by staining with Amido Black.

A comparative analysis of the ANS fluorescence emission spectra of apoLp-III/CT-apoA-I with that of apoLp-III/CT-apoE was carried out, using the corresponding parent proteins (Table S1). A few interesting observations were made: i) The fluorescence intensity of isolated CT-apoA-I was very low (35.9 ± 1.2), which is likely a reflection of the lack of defined structure. In contrast, the CT-apoE ANS fluorescence intensity was much higher (198.7 ± 4.2), thus offering many ANS binding sites. ii) The ANS fluorescence intensity of apoLp-III/CT-apoE was 3-fold higher compared to apoLp-III/CT-apoAI, in agreement with the observation that CT-apoE contains more ANS binding sites compared to CT-apoAI. iii) NT-apoAI (residues 1-189), NT-apoE, and apoLp-III all displayed relatively low ANS fluorescence intensities, showing that that their helix bundle organization does not offer many ANS binding sites. Since the ANS fluorescence intensity of NT-apoAI was higher compared to the 4- and 5-helix bundles of NT-apoE3 or apoLp-III, this indicate that the helices of NT-apoA-I are organized differently, in agreement with the consensus model for apoA-I [3].

Table S1: ANS fluorescence intensity of chimeras and their parent proteins.

Sample	ANS Fluorescence Intensity
ANS	14.2 ± 1.3
apoE3	417.7 ± 18.4
NT-apoE3	56.0 ± 1.7
CT-apoE	198.7 ± 4.2
apoA-I	174.2 ± 5.3
NT-apoAI	76.6 ± 2.4
CT-apoAI	35.9 ± 1.2
apoLp-III	31.4 ± 1.3
apoLp-III/CT-apoE	353.4 ± 6.1
apoLp-III/CT-apoAI	119.2 ± 5.1

Fluorescence intensity was recorded at 468 nm. Error represents standard deviation with n = 3.

References

1. Horn JVC, Ellena RA, Tran JJ, Beck WHJ, Narayanaswami V, Weers PMM (2017) Transfer of C-terminal residues of human apolipoprotein A-I to insect apolipophorin III creates a two-domain chimeric protein with enhanced lipid binding activity. Biochim Biophys Acta Biomembr 1859:1317–1325. https://doi.org/10.1016/j.bbamem.2017.04.017

2. Sallee DE, Horn JVC, Fuentes LA, Weers PMM (2017) Expression of the C-terminal domain of human apolipoprotein A-I using a chimeric apolipoprotein. Protein Expr Purif 137:13–19. https://doi.org/10.1016/j.pep.2017.06.008

3. Melchior JT, Walker RG, Cooke AL, Morris J, Castleberry M, Thompson TB, Jones MK, Song HD, Rye K-A, Oda MN, Sorci-Thomas MG, Thomas MJ, Heinecke JW, Mei X, Atkinson D, Segrest JP, Lund-Katz S, Phillips MC, Davidson WS (2017) A consensus model of human apolipoprotein A-I in its monomeric and lipid-free state. Nat Struct Mol Biol 24:1093–1099. https://doi.org/10.1038/nsmb.3501