

Distinct Domains in Apolipoprotein E are Responsible for its Anti-Oxidation Properties and its Ability to Induce Cholesterol Efflux.

Thomas A. Pham and David Y. Hui

Department of Pathology, Lipid Research Group

Apolipoprotein E is a 34 KDa glycoprotein consisting of a defined lipid binding domain (10 KDa) and a receptor binding domain (22 KDa) separated by a 2 KDa hinge region. The motif at residues 141-155 located within the 22 kDa domain is responsible for its binding to receptors such as LDL receptor and its related receptor LRP-1. Apo E also acts as an anti-oxidant, preventing the oxidation of LDL in the vasculature. This anti-oxidant effect can be shown in vitro by monitoring the formation of conjugated dienes due to lipid oxidation. This study examined the anti-oxidative properties of different domains of Apo E testing the hypothesis that the receptor binding domain of apoE will inhibit LDL oxidation more efficiently than the lipid domain. We showed that the 22 KDa domain inhibits LDL oxidation as efficiently as native Apo E. The 10 KDa lipid binding domain did not show any significant anti-oxidation as compared to control. A synthetic peptide containing a tandem repeat of the receptor binding domain of apoE, Apo E (141-155)₂, was shown to be the most potent inhibitor of oxidation, abolishing LDL oxidation completely. The Heparin binding domain (211-243) of Apo E was studied as well and was shown to inhibit oxidation to a lesser degree than the 22 KDa region. In a separate study, we also examined the domain in apoE responsible for mediating cholesterol efflux from cells. Whereas apoE binding to LDL receptor and LRP-1 is mediated by residues 141-155 in its 22-kDa receptor binding domain, thus indicating the identical domain in apoE is responsible for mediating cholesterol transport to cells, cell signaling, and anti-oxidation, the apoE domain responsible for cholesterol efflux from cells has not been identified. This study measured apoE-induced cholesterol efflux out of human fibroblasts +/- ABCA1 upregulators. Whole Apo E was shown to induce ABCA1-dependent cholesterol efflux, confirming previous results reported by others. We found that the 22 KDa, 211-243, and (141-155)₂ peptides exhibited insignificant efflux as compared to control. The 10 KDa region exhibited slight efflux that appeared to be independent of ABCA1. These experiments demonstrated that a different domain in Apo E is responsible for its function in inducing cholesterol efflux, thereby providing evidence that its signaling action is likely due to receptor binding and not the transport of cholesterol out of the lipid rafts in cell membrane.