Limited Proteolysis of Human Apolipoprotein A-IV

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Background: Apolipoprotein A-IV (apoA-IV) has several known effects on human health including anti-atherosclerotic effects, satiety effects, and effects on the ABCA1 and LCAT cholesterol pathways. The tertiary structure of apoA-IV is still unknown, but a recent study suggests that apoA-IV has a stable core domain and loosely associated termini.

Aim: This study will use limited proteolysis to determine the differences in the structural organization of apoA-IV in its lipid-free and lipid-bound states and to elucidate information about the general domain structure of apoA-IV.

Methods: ApoA-IV was produced using a bacterial expression system and purified by His-binding columns. Discoidal human apoA-IV using POPC lipid was then generated by a sodium cholate dialysis method. ApoA-IV was added to buffered protease solutions and incubated at 37°C for specific timepoints, then a protease inhibitor was added and the solution was boiled. The timepoints were run on SDS-GEL and analyzed using SynGene software.

Results:
All lipid-bound experiments required higher protease:apoA-IV ratios and longer time intervals to achieve limited proteolysis. Under the same proteolysis conditions, lipid-bound fragments persisted at least 4x longer than lipid-free fragments. Additionally, the size of fragments found in the lipid-free vs. lipid-bound experiments are remarkably different in size.

Conclusion:
The difference in sizes of fragments found between lipid-free and lipid-bound experiments confirms a significant difference in structural organization between the two forms. The higher stability found in the lipid bound experiments suggests that the discoidal (lipid-bound) conformation imparts higher stability to the molecule. All the fragments found in this study could be consistent with the previously proposed “core domain” structure for apoA-IV. Future mass spectrometry of the fragments, when combined with other structural studies, will make a computer model of the tertiary structure of apoA-IV possible.