Structural Features of ApoA-I in HDL

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High-density lipoprotein (HDL) is known as "good cholesterol" by virtue of its involvement in reverse cholesterol transport, anti-inflammatory effects, and ability to be an effective antioxidant. Apolipoprotein A-I (apoA-I) is the most abundant protein in HDL. It accounts for more than 70% of the total HDL protein and 30% of the mass of HDL, and yet our knowledge of its structure in the context of native HDL remains limited. Through the use of a trifunctional crosslinker (Sulfo-SBED) and the employment of a streptavidin purification technique, we demonstrate the efficacy of selectively purifying crosslinked peptide fragments from apoA-I in recombinant HDL particles. This strategy has the benefit of eliminating much of the background signal that previously overpowered the signal of interest using a homobifunctional crosslinker (BS^3) during analysis by electrospray ionization mass spectrometry. The aryl azide moiety of Sulfo-SBED lacks binding specificity, which allows for much greater diversity of crosslinks to be made, but also prevents unambiguous identification of the bound peptide. The methodology still demonstrates clear promise for the study of the structure of lipid bound apoA-I. Future studies will apply this strategy to a crosslinker with two Lysine specific arms and a third biotinylated arm to yield unambiguous CID spectra from a selectively purified population of peptide crosslinks.