MACROPHAGE METALLOPROTEINASES DEGRADE HDL-ASSOCIATED APOLIPOPROTEIN A-I AT BOTH THE N- AND C- TERMINI

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Atheromatous plaque contains various cell types, including macrophages, endothelial and smooth muscle cells. To investigate the possible interactions between secreted matrix metalloproteinases and HDL components, we tested the above cell types by culturing them for 24 h. HDL_3 were then incubated in their cell-free conditioned media. Proteolytic degradation of apolipoprotein A-I was observed with macrophages, but not with endothelial or with muscle cell conditioned supernatant. Absence of calcium or addition of EDTA to incubation media prevented all proteolytic processes. The identified apolipoprotein A-I fragments had sizes of 26, 22, 14 and 9 kDa. Two-dimensional electrophoresis and mass spectrometry resolved the 26 and the 22 kDa components and identified peptides resulting both from N-terminus and from C-terminus cleavage of apolipoprotein A-I. The higher abundance of C- than N-terminus cleaved peptides agrees with literature data for a fully structured a-helix around ^{18}Y *versus* an unstructured region around ^{185}G and ^{186}G. The flexibility in the latter region of apoA-I may explain its susceptibility to proteolysis. In our experimental set-up, HDL_{3C} was more extensively degraded than the other HDL_3 subclasses (HDL_{3A} and HDL_{3B}). Proteolytic fragments produced by metalloproteinase action were shown by gel filtration and electrophoresis to be neither associated with lipids nor self-associated.