INVESTIGATING THE STRUCTURAL AND AMYLOIDOGENIC PROPERTIES OF MURINE SAA ISOFORMS TO EXPLORE THE MOLECULAR BASIS OF AMYLOID A AMYLOIDOSIS

by

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ABSTRACT

Serum amyloid A (SAA) is a major acute phase reactant protein expressed by the liver and belongs to a family of apolipoprotein that binds to high-density lipoprotein (HDL) in the plasma. During chronic inflammation, persistent high levels of SAA can occasionally give rise to amyloid fibril deposition of SAA and SAA fragments, leading to AA amyloidosis, which is one of the most common systemic amyloid diseases in the world. In mouse models of AA amyloidosis, only one SAA isoform, SAA1.1, has been found in the amyloid deposits. The CE/J mouse strain, which expresses a single isoform of SAA (SAA2.2) is resistant to the disease. However, recent studies have shown that SAA2.2 forms amyloid fibrils *in vitro*. Thus, it has been proposed that the different amyloidogenicity of SAA isoforms may arise from their structural and biophysical differences, and this may be an important factor in their *in vivo* pathogenicity. In this thesis, the differences in the refolding, oligomerization, stability, and aggregation between SAA2.2 and SAA1.1 were investigated to gain some insight about the molecular basis of their different amyloidogenicity. During refolding, SAA2.2 formed a kinetically accessible octameric species that converted over a few weeks at 4°C to a more thermodynamically stable hexamer. The SAA2.2 octamer formed amyloid fibrils with a much shorter lag phase than SAA2.2 hexamer, suggesting that it is possibly a significant intermediate in amyloid formation. Interestingly, micromolar amounts of zinc trapped the octamer and blocked its conversion to hexamer, suggesting that the octamer binds and is stabilized by zinc like was previously shown for the hexamer. In another study, an SAA2.2 mutant where leucine 11 was changed to a glutamine residue as present in SAA1.1 was investigated to determine the role of this position in determining the differences between the two isoforms. SAA2.2L11Q was found to initially refold mostly into a hexamer instead of an octamer, but had less stability than the wild type SAA2.2 hexamer and unlike the latter, the SAA2.2L11Q hexamer was unable to form fibrils after 24 hours incubation at 37°C. In a third study involving the potentially pathogenic SAA1.1 isoform, it was observed that different preps yielded protein that refolded into a mixture of hexamer, tetramer and monomer, or predominantly into a tetramer species. SAA1.1 oligomers were found to be less stable than the SAA2.2

hexamer and formed amorphous aggregates instead of amyloid fibrils *in vitro*. These results suggest that as in most amyloid diseases, the formation of toxic oligomers but not amyloid fibrils may be the pathogenic determinant in AA amyloidosis. A general conclusion of this work is that SAA proteins are marginally stable proteins that may be intrinsically disordered *in vivo*, but they appear to possess the capability to self-assemble into different oligomeric and aggregated structures. The biological and pathological relevance of this property of SAA is intriguing and remains to be determined.