STRUCTURAL AND MECHANISTIC STUDIES OF AMYLOID FIBRIL FORMATION BY SERUM AMYLOID A 2.2

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ABSTRACT

Amyloid A (AA) amyloidosis is one of the best-known systemic amyloid diseases. As of yet, the mystery of why only a small percentage of patients suffering from chronic inflammatory diseases develop AA amyloidosis remains unclear. Serum amyloid A (SAA), the precursor protein of AA amyloidosis, is a major acute phase reactant and a small apolipoprotein of high-density lipoproteins in the serum. In cases of prolonged inflammation, SAA may form amyloid fibrils, leading to AA Amyloidosis. In mouse models of AA amyloidosis, full-length or fragments of SAA1.1 is the main component of the amyloid deposits. The CE/J mouse, which produces only the SAA2.2 isoform, is resistant to AA amyloidosis. However, SAA2.2 was recently shown to form amyloid fibrils *in vitro*. Thus, we hypothesize that SAA2.2 is intrinsically amyloidogenic and that its resistance to amyloid accumulation *in vivo* may result from biochemical and biophysical differences, such as fibril stability, aggregation kinetics and ligand binding, etc, between SAA2.2 and SAA1.1.

Using the relatively more soluble protein SAA2.2 as a model system, the mechanism of SAA denaturation, misfolding, oligomerization, and fibril formation was investigated. Important intermediates, including spherical oligomers, pores, protofibrils and fibrils were identified in sequential order. The characterization of a non-native 16mer oligomer that self-assembles into various higher order species, including larger spherical bundles, pores, and fibrils were also carried out. One study focused on demonstrating the marginal stability of SAA2.2 fibrils upon exposure to mild temperatures and low urea concentrations. The finding that SAA2.2 fibrils are marginally stable may potentially explain why SAA2.2 fails to cause AA amyloidosis in the CE/J mouse. Finally, experiments supporting a potential proteolytic activity of SAA2.2 are also reported. A possible regulatory role of this proteolytic activity in the rapid serum turnover of SAA and the amyloid load in an organism is proposed. Together, the studies reported here may lead to a better understanding of the diverse functions and amyloidogenicity of different SAA isoforms and could have important implications for the design of therapeutic approaches for AA amyloidosis.