Structural Studies of Serum Amyloid A Through Chemical Cross-linking and Mass Spectrometry

by

Michael Perry

An Abstract of a Thesis Submitted to the Graduate

Faculty of Rensselaer Polytechnic Institute

in Partial Fulfillment of the

Requirements for the degree of

MASTER OF SCIENCE

Major Subject: CHEMISTRY

Approved:

_____

Dr. Wilfredo Colón, Thesis Adviser

_____

Dr. K. V. Lakshmi, Member

Rensselaer Polytechnic Institute

Troy, New York

October, 2008

(For Graduation December 2008)

ABSTRACT

Over the last 15 years it has become evident that protein misfolding and aberrant self-assembly into toxic species are pathologically linked to many diseases by a poorly understood mechanism. Serum Amyloid A (SAA) is a small apolipoprotein found predominantly circulating through the plasma bound to high-density lipoproteins (HDL). SAA is a major acute phase reactant whose functions remain poorly understood. However, SAA appears to play an important role in cholesterol metabolism and transport as well as host defense during an inflammatory response. Prolonged high levels of SAA during inflammation can lead to AA amyloidosis. AA amyloidosis results from the deposition of SAA amyloid fibrils in organs such as the liver. An isoform that is only expressed in the CE/J mouse, SAA2.2, was found to be resistant to AA amyloidosis under various stimuli.

The limited functional information for SAA2.2 results in part because its three-dimensional structure is not known. The fundamental tendency of SAA to aggregate *in vitro* has restricted high-resolution structural studies. With the help of distance constraints obtained from chemical cross-linkers and matrix assisted laser desorption/ionization mass spectrometry (MALDI-MS), acquiring a low-resolution structure of SAA2.2 might be possible. Chemical cross-linkers, such as [sulfosuccinimidyl] suberate (BS^3) and bis[sulfosuccinimidyl] glutarate (BS^2G), cross-link lysine residues at specific distances within a protein. BS^3, an 11.4 Å chemical cross-linker was found to cross-link SAA2.2 at lysine residues 33 and 56. The BS^3 cross-linker formed a second cross-link at 4378.6 Daltons. A cross-link at 4.4 kDa is indicative of a cross-link between K56 and either K29 or K33. These new distance constraints for SAA2.2 enhance the understanding of the structure and biophysical properties of SAA, and should be useful in determining the three-dimensional structure of SAA. Elucidation of the three-dimensional structure of SAA may be used to develop potential therapeutics for AA amyloidosis.