Albumin Adsorption: Inferences of Protein Interactions Measured by Sedimentation both Between Species and Induced by Denaturing

Kristin Dianne McKeon

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Brian J. Love, Committee Chair
David R. Bevan, Committee Member
John Y. Walz, Committee Member

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ABSTRACT

Biological development and progression are managed by a diverse macromolecular group called proteins. Protein structure results from a complex folding process that leads to a final active form. This protein state is susceptible to changes in the surrounding environment and an incorrect structure can be produced. Changes in the protein conformation can lead to the formation of protein aggregates. Adsorption of proteins onto surfaces is utilized in many research analyses, but is capable of irreversibly changing the protein structure and causing aggregation. Albumin is a plasma protein that adsorbs on many different surfaces because the structure easily rearranges. The structure of albumin once adsorbed has been shown to deteriorate; however, outcomes of both stabilization and aggregation have been found.

A dynamic laser light scattering instrument will be utilized to measure the differences in size and determine the amount of aggregation. Our lab has developed a z-axis translating laser light scattering device (ZATLLS) that has been used to measure the sedimentation velocity of several different materials in solution. In this case, bovine serum albumin (BSA) will be adsorbed onto polystyrene particles and the particle settling velocity determined. The settling solution viscosity and density will also be ascertained, so Stoke's law can infer the average aggregate size of each experiment. BSA-coated polystyrene particles displayed a more controlled settling behavior compared to non-coated polystyrene particles. Although the BSA-coated particles had a smaller sedimentation velocity, a larger aggregate size was found due to the greater solution viscosity. Therefore, the ZATLLS instrument can be employed to measure sedimentation velocities of multiple interactions and the aggregation level inferred.

Although most albumin molecules are remarkably similar, there are subtle differences in amino acid residues, length, and charge. Sedimentation velocities for

human serum albumin (HSA) coated polystyrene particles and BSA-coated polystyrene particles only had a small difference. However an almost 50% higher solution viscosity was measured in BSA experiment solutions, and resulted in the slower settling of the larger aggregates compared to HSA-coated particles. Viscosity calibration curves for each albumin species were used to determine the amount of protein desorbed from the particles during the settling process. The larger solution viscosity for BSA-coated particle experiments led to a much larger degree of desorption. HSA was shown to be the more stable albumin species when adsorbed onto polystyrene particles.

Temperature denaturing was performed to aid in the determination of the stability of BSA. Reversible and irreversible conformational changes in BSA were produced at 46ºC and 76ºC respectively. The solutions were cooled to room temperature before adsorption onto polystyrene particles and the sedimentation velocities measured. A 50% difference in average viscosity between the reversibly and irreversibly changed BSA was found. This caused the larger aggregates formed in the 76ºC BSA experiments to have an almost equivalent sedimentation velocity to those in the reversibly denatured BSA experiments. Average aggregate size for reversibly denatured BSA was well within the ranges found for non-denatured BSA. In conclusion, irreversibly denatured BSA formed larger aggregates and was more likely to desorb from the polystyrene particles than reversibly changed BSA.

Dedication

I would like to dedicate my thesis to my mom, Helen, for being the wonderful woman and mom that she is. You are a beautiful person and I love you very much.

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