Investigating the potential physical interaction between Albumin and Fetuin-A plasma proteins

John Mullins, Odutayo Odunuga, PhD.
Department of Chemistry and Biochemistry, Stephen F. Austin State University, Nacogdoches, TX.

Abstract

Serum albumin (SA) is a multifunctional, high abundance protein that is found in many organisms. Bioinformatic analysis of several orthologs of serum albumin reveal a consistent potential interaction with Fetuin-A (AHSG), a glycoprotein also present in the plasma, with established roles in blood glucose regulation and insulin resistance. Potential direct interaction between the two proteins has been suggested, but not yet experimentally investigated. Physical interaction between SA and AHSG will have mechanistic implications on blood sugar regulation and disorders, such as type 2 diabetes. Pull-down assays were used to probe the potential interaction between human serum albumin (HSA) and AHSG. SDS-PAGE analysis and Western blotting were used to detect protein profiles. Analysis of assay data suggests that HSA and AHSG proteins physically interact.

Background

Serum albumin is the most abundant plasma protein in many different organisms. It is a water soluble, globular protein that serves a variety of roles in drug transport, maintenance of oncotic pressure, and blood sugar regulation [1]. Serum Albumin is a single polypeptide chain folded into three domains and six subdomains. It is made up of 75% alpha helices and contains 17 disulfide bonds. The mature human protein (HSA) contains 585 amino acid residues [2]. Bioinformatic analysis using STRING predicted potential interaction of several serum albumin homologs with Alpha-2-Heremans-Schmid Glycoprotein (AHSG, a.k.a. Fetuin-A). AHSG has been implicated in the pathogenesis of insulin resistance and type 2 diabetes. Fetuin-A is a known inhibitor of the insulin receptor tyrosine kinase activity [3]. Fetuin-A is a heterodimeric protein comprising two chains connected by a single disulfide bond. Other studies have also suggested a potential physical interaction between HSA and AHSG, though this concept has never been investigated [4]. These previous studies suggested that SA and AHSG might have a compensatory functional relationship in the plasma. The overarching hypothesis of this project is that serum albumin modulates the steady-state concentrations of variously glycosylated AHSG proteins in the plasma by direct physical interaction in response to changes in blood sugar level. This particular experiment focuses on establishing whether the two proteins physically interact.

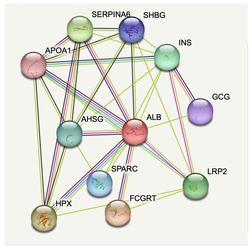

Figure 1. STRING Analysis-Swiss Institute of Bioinformatics

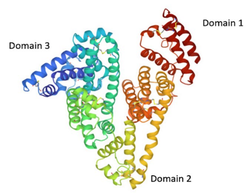

Figure 2. Human Serum Albumin (HSA, PDB 1AO6)

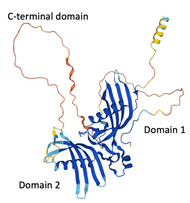

Figure 3. Fetuin-A (AHSG, UniProt P02765)

Materials and Methods

Lyophilized HSA, Purity: >97%, m.w.: 66.5 kDa (Sigma-Aldrich) and lyophilized AHSG, poly-His tag at C-terminus, Purity: >98%, m.w.: 38.2kDa (ACROBiosystems) were reconstituted to stock solutions of 1 mg/mL.

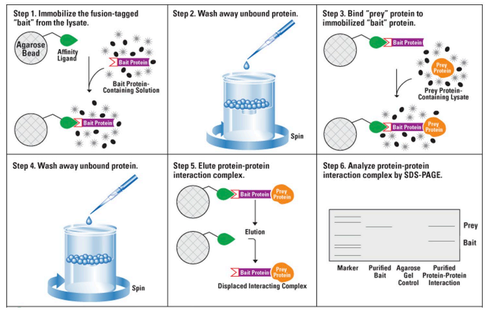

Figure 4. General scheme of a pull-down assay. In this project, AHSG conjugated with a poly-His tag at C-terminus was immobilized and HSA was bound to it. After several wash steps, the complex was eluted and resolved by SDS-PAGE.

(Image from Thermofisher Protein Biology Center)

Pull down assays were performed using Ni-NTA agarose resin (G-Biosciences) and Anti-His affinity resin (GenScript). Varying concentrations of proteins were used, while maintaining a 1:1 mol ratio, based roughly on the size of each protein. 1X PBS binding buffer, pH 7.4, was used for all trials. SDS-PAGE loading buffer was added directly to the Ni-NTA samples. 0.1 M Glycine-HCl pH 2.5, was used to elute protein complex from the Anti-His samples before adding SDS-PAGE loading buffer. Proteins were resolved on 12.5% SDS gels at 200 V for 1 h.

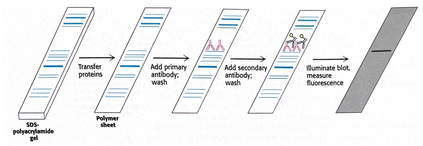

Figure 5. Detection of proteins by Western blotting and chemiluminescence.

Proteins were transferred to nitrocellulose membrane by Western blotting. The membranes were blocked with Azure Chemi Blot blocking buffer overnight at 4°C. The presence of HSA was detected by incubating with rabbit anti-HSA antibody, followed by an HRP-conjugated rabbit anti-human IgG, and visualized by chemiluminescence.

Detection of HSA by SDS-PAGE analysis

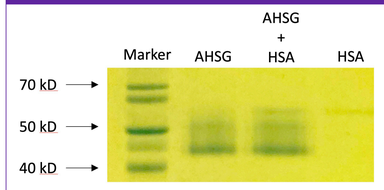

Figure 6. SDS-PAGE analysis of Ni-NTA affinity pull-down assay. 5 µL AHSG and 10 µL HSA were used.

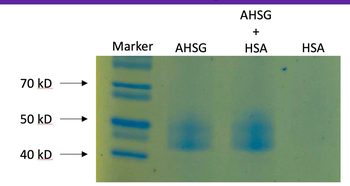

Figure 6. SDS-PAGE analysis of Anti-His affinity pull-down assay. 5 µL AHSG and 10 µL HSA were used.

Analysis of the resolved protein profiles revealed that there was interaction between HSA and AHSG. The presence of the HSA band was observed in all test samples. Control samples showed significant HSA binding to the resin. However this background binding faded as lower concentrations of both proteins were used while the specific signal was still strong. This observation shows that the interaction between HSA and AHSG was specific.

Detection of HSA by Western Blotting

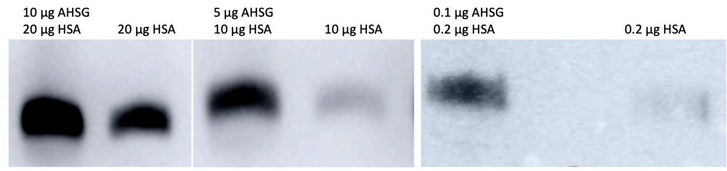

Figure 6. Proteins were transferred to nitrocellulose membrane by Western blotting. Presence of HSA was detected by incubating with rabbit anti-HSA antibody, followed by an HRP-conjugated rabbit anti-human IgG, both diluted at a 1:7000 ratio in blocking buffer and observed by chemiluminescence.

Chemiluminescence revealed presence of HSA after pull-down assays were performed using Anti-His affinity resin. However, the control lanes also contained HSA, meaning non-specific background binding was still occurring. Since this technique has a much lower limit of detection of proteins, the concentrations were diluted 10 times, followed by 100 times before performing the pull-down assays. Background binding was greatly reduced in the 10 times dilution and almost completely removed in the 100 times dilution, while the intensity of the test lanes remained present.

Conclusions and Future Work

Conclusions:

- Bioinformatic analysis predicted a direct physical interaction between HSA and AHSG plasma proteins.
- SDS-PAGE analysis indicated that binding occurs between HSA and AHSG proteins.
- Observed binding between HSA and AHSG was further confirmed by Western blotting followed by chemiluminescence.

Future Work:

- Perform gel-filtration chromatography to detect protein-protein interaction.
- Modify pull-down assay by switching His-tag from AHSG to HSA.
- Perform Surface Plasmon Resonance (SPR) Spectroscopy to detect binding and collect kinetic data.

Contacts

John Mullins
Dept. of Chemistry and Biochemistry
Stephen F. Austin State University
mulllinsja3@jacks.sfasu.edu

Dr. Odutayo Odunuga
Dept. of Chemistry and Biochemistry
Stephen F. Austin State University
odunugaoo@sfasu.edu

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