1-30-2014

A Column Free Protein Purification Procedure using E. coli Single - Stranded DNA Binding Protein (SSB) as an Affinity Tag

Mark Soffe
Utah State University

A Column Free Protein Purification Procedure using *E. coli* Single-Stranded DNA Binding Protein (SSB) as an Affinity Tag

Mark Soffe
Utah State University

Edwin Antony
Utah State University

I. Abstract

Single Stranded DNA Binding (SSB) proteins mediate DNA replication, repair, and recombination in prokaryotic cells. While its most important function is to bind and protect exposed single stranded DNA during these processes, it also recruits other key enzymes onto DNA which make these processes possible. Most bacterial SSB proteins function as homotetramers.

Here we utilize two biochemical properties of the *E.coli* SSB protein to develop a novel procedure to purify proteins using a column-free strategy. 1. Ec-SSB binds to single stranded DNA (ssDNA) with extremely high affinity (K 10^{13} M^{-1}). 2. It is also a unique protein with respect to its purification – it is possible to obtain greater than 95% purity from the total cell lysate without using any sort of column or resin, utilizing polyethylene imine (PEI) and Ammonium Sulfate Precipitation.

Our design uses SSB as an affinity/ solubility tag to enhance the solubility and expression of difficult-to-purify proteins, and allows for the simple, resin-less purification using ammonium sulfate and PEI precipitation. We present our progress on this project. Constructs have been made that include the SSB gene, along with the ability to fuse any gene of interest, as well as a TEV Protease cleavage sequence allowing for proteolytic cleavage after gene expression, if desired. Two genes of interest have been cloned thus far—TEV protease and Rad51. In both cases, the genes of interest expressed very well when attached to SSB.

Figure 1- Subunit Architecture of SSB

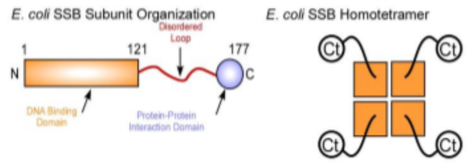
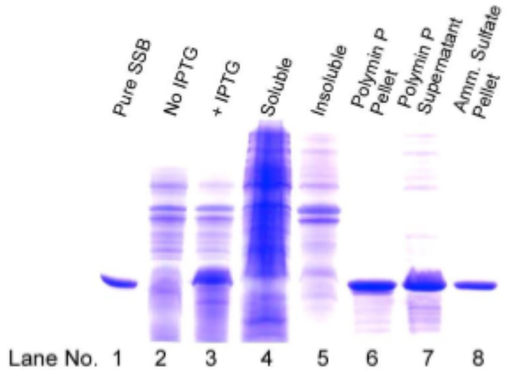


Figure 2- Column Free Purification of SSB Using PEI and Ammonium Sulfate Precipitation



II. Foundation For Project

Ec-SSB can be recombinantly expressed using the BL-21 system and purified without any chromatographic (resin requiring) steps. Cells are lyzed and SSB is highly soluble. Ec-SSB can be selectively precipitated out using Polymin P and ammonium sulfate. The protein is greater than 95% pure. Further purity (if required) can also be achieved by using a ssDNA cellulose column.

III. Experimental Theory

Our idea is to fuse any protein of interest to SSB and when purified using the same procedure as SSB, the SSB-protein complex should be selectively purified as shown above for Ec-SSB (Figure 2).

A site-specific protease site (like TEV) can also be engineered between SSB and the protein of interest and we can simply cleave off the SSB that serves as the affinity tag.

To make this idea even better, we can generate a SSB protein fused to the protease. After cleavage, then all the cleaved SSB along with the SSB-protease tag can be precipitated out leaving our protein of interest in solution.

Figure 3 – Outline of Research Strategy to Test the SSB-based Purification Technique

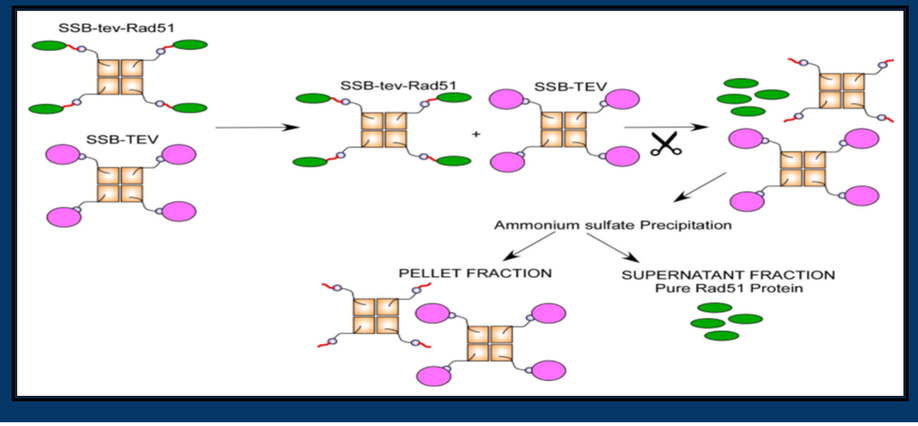


Figure 4 – Steps Involved in Generating the Gene Constructs and Associated Protein Products

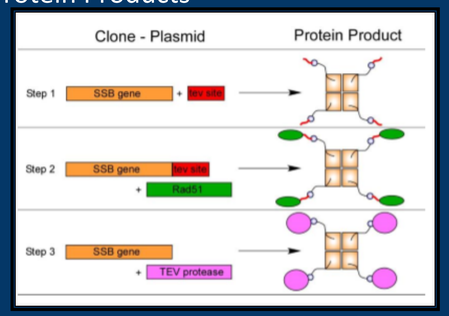
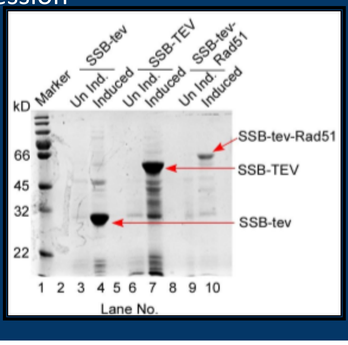


Figure 5 – SDS-PAGE Results of Protein Expression



IV. Results And Future Work

To test our idea, we chose the *S. cerevisiae* Rad51 protein as our target protein of interest. The Tobacco Etch Mosaic Virus protease (TEV) was our choice of protease to cleave Rad51 from SSB.

The recombinant expression constructs were generated and sequenced, showing the integrity of the genetic constructs, and when over-expressed, the protein products showed great expression and solubility.

Future work will include testing the SSB-based purification of the recombinant proteins and optimizing the procedures to maximize the protein yield and solubility.

Study conducted with funding from a USU Undergraduate Research and Creative Opportunity (URCO) Grant and lab assistance from the USU Department of Chemistry and Biochemistry.

Mark Soffe
Utah State University
Chemistry and Biochemistry
m.s.soffe@aggiemail.usu.edu

Please leave this space free for a Poster Number