2018

2018 - Purification and Expression of SKIP - Poster Presentation

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Purification and Expression of SKIP

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ABSTRACT

SKIP (Skeletal Muscle and Kidney-enriched Inositol Phosphatase) is a ~51 kDa inositol polyphosphate 5' phosphatase.

The goal of this project is to purify biologically active, recombinant human SKIP.

A pGST-SKIP construct was created which produces a GST-SKIP chimera, the GST allows for purification and detection. Multiple E. coli strains were transformed with pGST-SKIP. Cultures containing each cell strain + pGST-SKIP were grown to variable cell concentrations and protein expression was induced with a variable concentration of IPTG to determine the ideal conditions for protein expression.

SDS-PAGE and Western Blot analysis concluded that GST-SKIP was present, however, water solubility testing determined that only proteins produced in Rosetta™ 2 pLysS E. coli was soluble at a low temperature. Proteins synthesized in other *E. coli* strains were insoluble, suggesting that the GST-SKIP chimera had misfolded and therefore would be enzymatically inactive.

This poster will present preliminary results of the work previously described.

PURPOSE

The purpose for the SKIP project is to present a manufactured recombinant SKIP and a standardized purification protocol for purifying biologically active SKIP.

BACKGROUND

SKIP is a ~51 kDa inositol polyphosphate 5' phosphatase, found in humans and other mammals, that has been shown to be a negative regulator in insulin signaling and has been shown to inhibit membrane ruffle formation. SKIP is found in particularly high abundance in skeletal muscle, heart, and kidney cells. The SKIP gene encodes a protein with 5 phosphatase activity toward polyphosphate inositol.

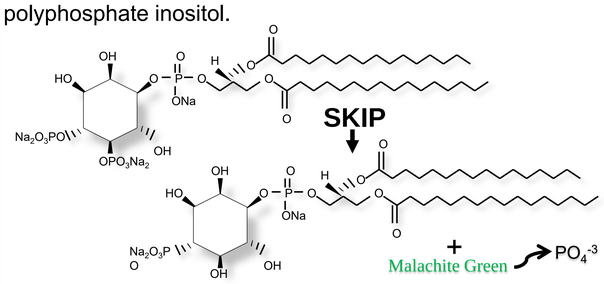


Fig. 1: Reaction of SKIP activity assay. Depicting the phosphate produced when SKIP catalyzes the hydrolysis of a phosphate functional group from the sugar head of a phospholipid phosphoinositide (4,5)P2DC16, which is detected using a malachite green solution.

BACKGROUND CONT.

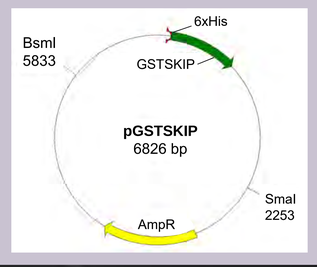
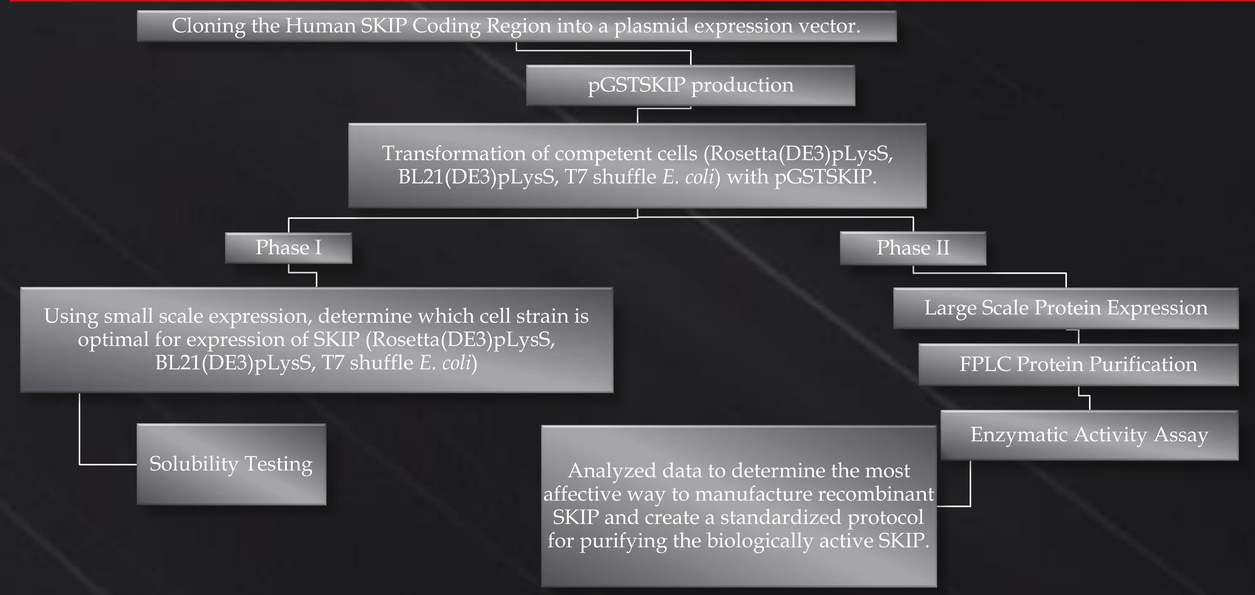


Fig. 2: Expression vector used. A diagram representing pGSTSKIP plasmid map (6826 bp) depicting the two restriction sites used (BsmI & SmaI), the ampicillin resistance gene, and the His/GST region.

Previous phases of the project involved the construction of the plasmid vector pGSTSKIP, which contains the gene for a GST-SKIP chimera, an ampicillin resistance factor, the Lac Operon, and genes for the production of T7 DNA Polymerase. Ampicillin resistance is used for selecting cells which have successfully undergone transformation. The Lac Operon, and genes for T7 DNA Polymerase act as triggers which can be chemically induced to synthesize the inserted GST-SKIP chimera in the presence of IPTG.

METHODS



RESULTS

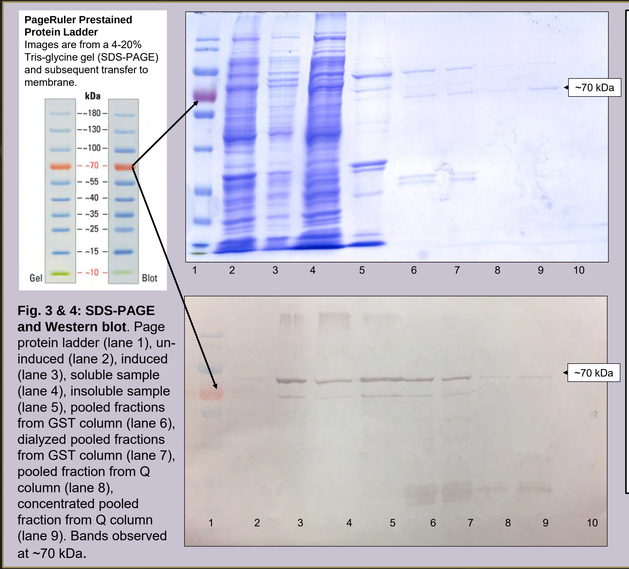


Fig. 3 & 4: SDS-PAGE and Western blot. Page protein ladder (lane 1), un-induced (lane 2), induced (lane 3), soluble sample (lane 4), insoluble sample (lane 5), pooled fractions from GST column (lane 6), dialyzed pooled fractions from GST column (lane 7), pooled fraction from Q column (lane 8), concentrated pooled fraction from Q column (lane 9). Bands observed at ~70 kDa.

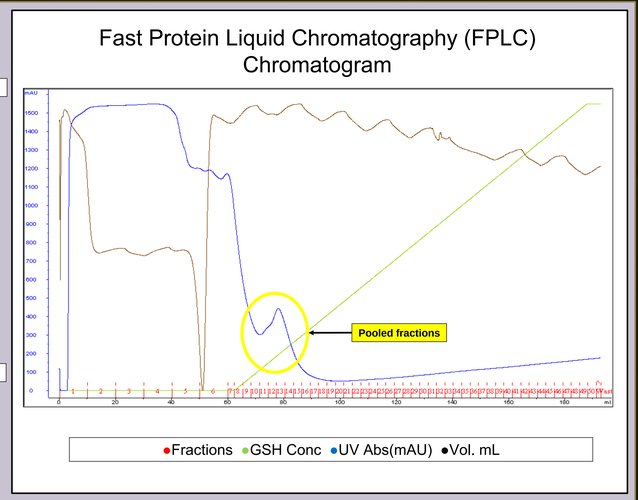


Fig. 5: FPLC chromatogram depicting mAU of UV (blue) and concentration of GSH (green) at each fraction (red) in mL (black). Fractions 9-14 were obtained for dialysis.

CONCLUSIONS

The results gathered show that expression of SKIP was confirmed:

1. SKIP was observed at ~70 kDa on the SDS-PAGE gel from FPLC pooled fractions, before dialysis, after dialysis, and when concentrated by centrifugation.
2. SKIP was also observed at ~70 kDa on the Western blot in the eluate from the Q sepharose column.

FUTURE DIRECTIONS

Future experiments will include determining protein concentration by conducting a Bradford assay and determining SKIP activity using a Malachite Green phosphatase curve.

Additionally, a solution to misfolding that is being explored is the co-expression of GST-SKIP and a heat shock chaperone gene (pGroESL) in Rosetta™ 2 pLysS E. coli. Co-expression would be achieved by transforming the competent cells with both pGSTSKIP, as well as pGroESL. Ideally, the chaperone protein coded for by pGroESL would support in GST-SKIP chimera folding, and yield biologically active SKIP.

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