Expression and Purification of hPEP Inhibitor Peptide

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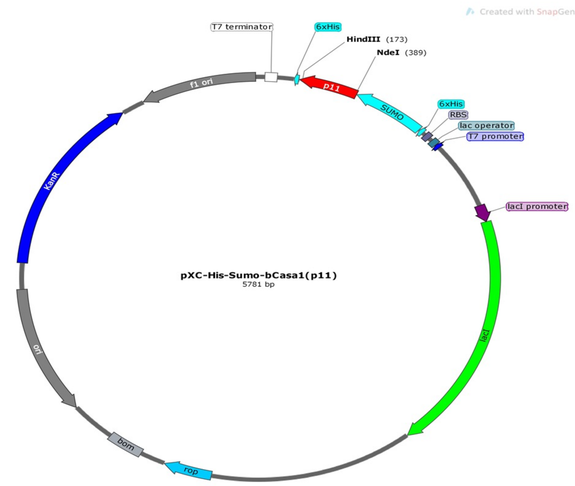
1 11 21 31 48 58 68 78
MKLLILTCLV AVALARPKHP IKHQGLPQEV LNENLLRFFV APFPEVFGKE KVNELSKDIG SESTEDQAME DIKQMEAESI SSSEEIVPNS VEQKHIQKED VPSERYLGLE QLLRLKKYKV PQLEIVPNSA
EERLHSMKEG IHAQQKEPMI GVNQELAYFY PELFRQFYQL DAYPSGAWYY VPLGTQYTDA PSDSDIPNPI GSENSEKTTM PLW

Abstract

The enzyme human prolyl peptidase, or hPEP, has been linked to many processes within the brain, including breaking down neural peptides. A bovine milk protein, α-s1-casein (see above), has been found to inhibit hPEP in colon cancer cells. A 68 amino acid long section (bolded) of α-s1-casein containing the inhibitor region was cloned. The casein fragment expression was optimized for expression of the peptide. The purpose for optimizing expression is to have much of it on hand to utilize in further studies on its inhibitory properties on hPEP. By using this fragment of casein, one can study the structure and function of hPEP by testing different locations of the inhibitor sequence in the peptides to see how exactly it is inhibited.

Methods

- PCR (Figure 1) was used to confirm desired peptide was cloned
- Spectrophotometry was used to monitor protein expression
- The peptide was purified by lysing and centrifugation
- The cleared lysate was incubated with nickel resin to bind the desired peptide-SUMO construct for purification. A step-gradient dilution was performed to isolate the SUMO-peptide fusion
- Sumoase used to cleave peptide from SUMO
- Bradford assay (Figure 2) and gel electrophoresis (Figures 3 & 4) were performed for analysis



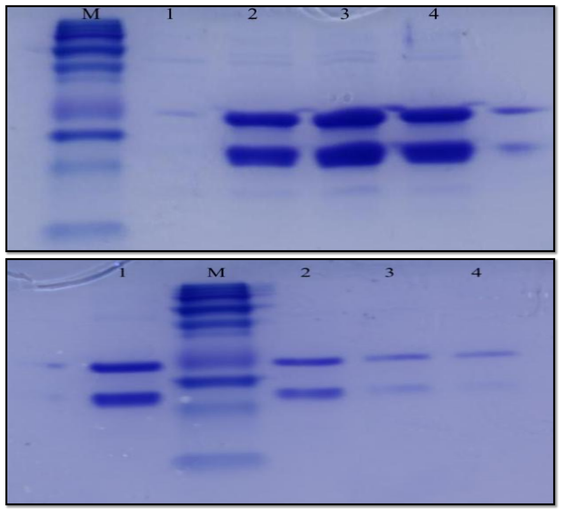


Figure 3

Figure 4

Results

Figure 1

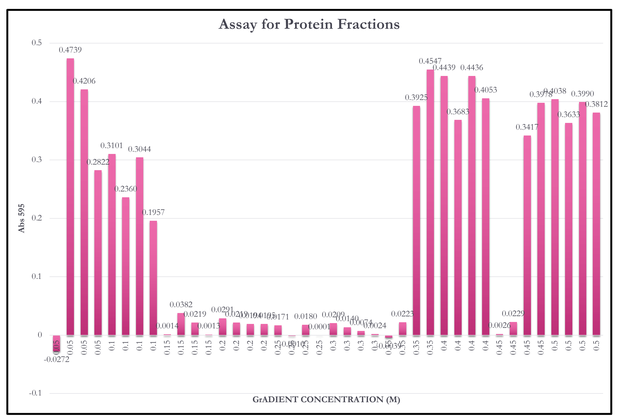


Figure 2

Discussion

Expression of the desired α-s1-casein peptide was observed, however, two bands were observed instead of one as expected. The next step would be to investigate the presence of the two bands and further optimization of peptide purification. Having much of this peptide on hand is important for upcoming projects to learn more about the structure and function of hPEP.

References

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