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Expression and Purification of Inflammatory Cytokine Protein

Brittany Rushing
Boise State University

Katelyn Delamontanya
Boise State University

Terrell Engmann
Boise State University

Hanna Suman
Boise State University

Bri Grantham
Boise State University

See next page for additional authors

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Abstract

Both breast and prostate tumors can be surgically removed when in Stages I, II, and III. Once the tumor undergoes metastasis (stage IV), survival rates drop; and the surgical removal of the primary tumor will no longer be sufficient for treatment. An inflammatory cytokine (IC), a protein that acts as a signal molecule in inflammation, has been identified as a primary signal in metastasis. Therefore, targeting the inflammatory cytokine with a small molecule inhibitor (SMI) will prevent metastasis from occurring. Structure-based drug design requires an accurate measurement of how well the SMI is able to bind to the inflammatory cytokine. This binding requires the expression and purification of the inflammatory protein with high purity and accuracy. The construct designed for the purification consists of the cytokine protein attached to a maltose-binding protein (MBP) and six histidines. The MBP serves to increase solubility; however, it also is electrostatically attracted to the inflammatory cytokine. To ensure efficient separation of these two proteins, a six-histidine tag is attached to the MBP. Ongoing experiments aim to design a protocol to produce pure, bioactive protein.

Authors

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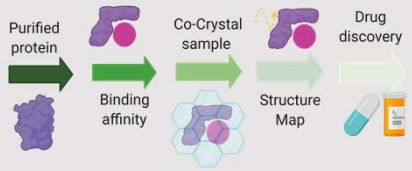
IDAHO INBRE
IDeA Network of Biomedical Research Excellence

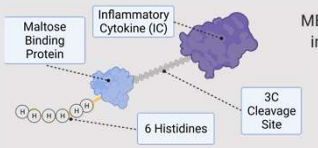
Abstract

Both breast and prostate tumors can be surgically removed when in Stages I, II, and III. Once the tumor undergoes metastasis (stage IV), survival rates drop; and the surgical removal of the primary tumor will no longer be sufficient for treatment. An inflammatory cytokine (IC), a protein that acts as a signal molecule in inflammation, has been identified as a primary signal in metastasis. Therefore, targeting the inflammatory cytokine with a small molecule inhibitor (SMI) will prevent metastasis from occurring. Structure-based drug design requires an accurate measurement of how well the SMI can bind to the inflammatory cytokine. This binding requires the expression and purification of the inflammatory protein with high purity and accuracy. The construct designed for the purification consists of the cytokine protein attached to a maltose-binding protein (MBP) and six histidines. The MBP serves to increase solubility; however, it also is electrostatically attracted to the inflammatory cytokine. To ensure efficient separation of these two proteins, a six-histidine tag is attached to the MBP. Ongoing experiments aim to design a protocol to produce pure, bioactive protein.

Structure-Based Drug Design

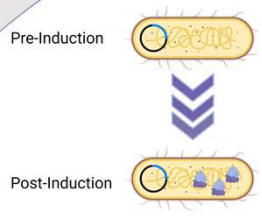


Construct Design

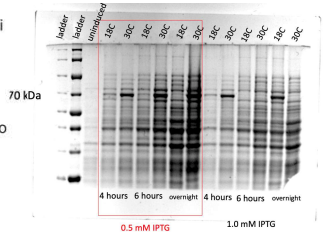


MBP is attached to IC to increase solubility. The 6His tag attached to MBP allows for better separation of IC and MBP

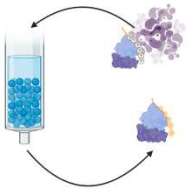
1. Expression



SHuffle cells (E. coli strain) are used to express the inflammatory cytokine complex due to their ability to build disulfide bonds

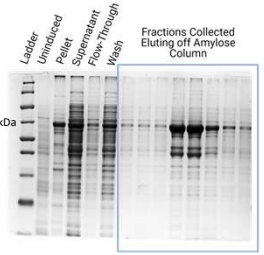


2. Amylose Column

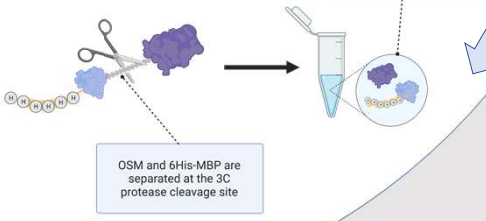


Cell components mixed with inflammatory cytokine complex

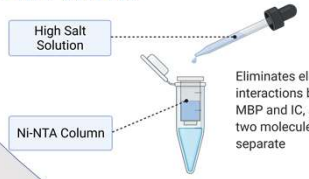
Isolated inflammatory cytokine complex



3. Cleavage

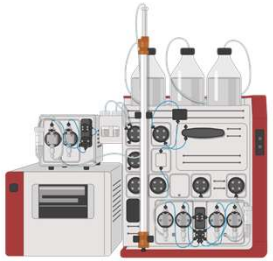


4. Ni-NTA Column / High Salt Wash



Eliminates electrostatic interactions between MBP and IC, allowing the two molecules to separate

4. Size Exclusion Chromatography



OSM and MBP are separated by size, allowing for pure OSM to be obtained

Discussion

Optimization of the expression reaction is shown under "1. Expression." From this, the highest yield of the inflammatory cytokine construct (70 kDa) was found when 0.5 mM IPTG was used for induction and the reaction incubated overnight at 30 °C. The amylose column shows a successful reduction of other cell components and almost complete isolation of the inflammatory cytokine construct.

Future Work

Recent efforts aim to improve 3C cleavage reaction and increase yield after Ni-NTA column. Future work could include using a construct with a TEV cleavage site in place of the 3C cleavage site.

Acknowledgements

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Works Cited

- Figures made using Biorender.com.