An Enzyme Characterization to Produce a Wearable Estrone Biosensor

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Introduction

- A bacterial redox enzyme (Enzyme B) putatively degrades estrone into 4-hydroxyestrone (4-OHE1).
- Estrone is a type of estrogen, which is a steroid hormone found in mainly in postmenopausal women.

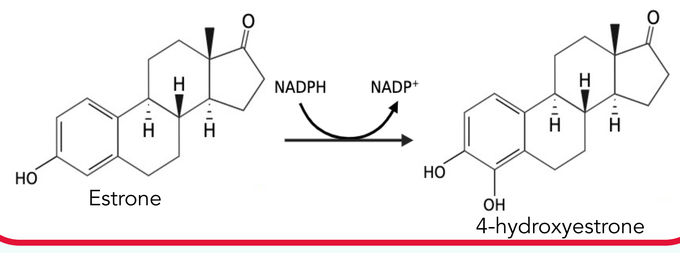

Fig. 1 Estrone degradation reaction catalyzed by Enzyme B. Created with BioRender.com

- The goal of this study is to be able to characterize Enzyme B for its use in developing a wearable estrone biosensor.
- Estrogen imbalance is involved in breast cancer and osteoporosis.
- Here we present the initial cloning and purification of Enzyme B

Methods

Expression and Purification

- Conducted mini prep to isolate a plasmid encoding for Enzyme B and transformed it in *E. coli* chaperone cell lines from Takara Bio.
- Then a large growth was conducted in Terrific Broth (TB) supplemented with iron (II) chloride and 5-ALA (5-aminolevulinic acid), a precursor to build the cofactor for Enzyme B.

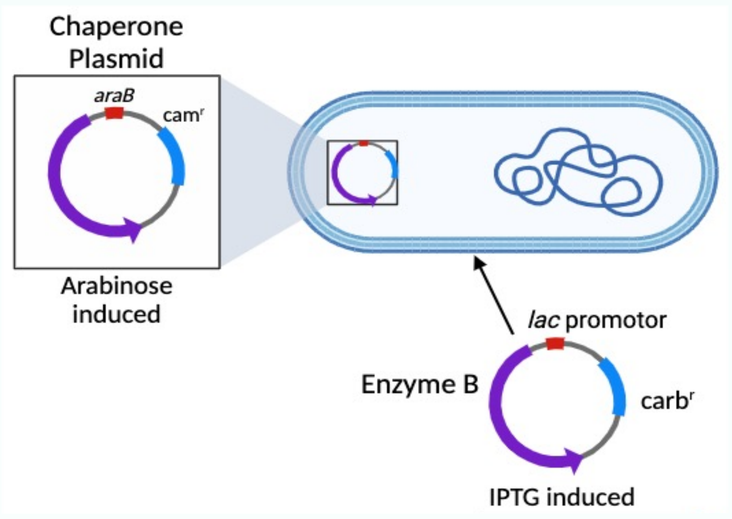

Fig. 2 Transformation of Enzyme B into chaperone cell line. Created with BioRender.com

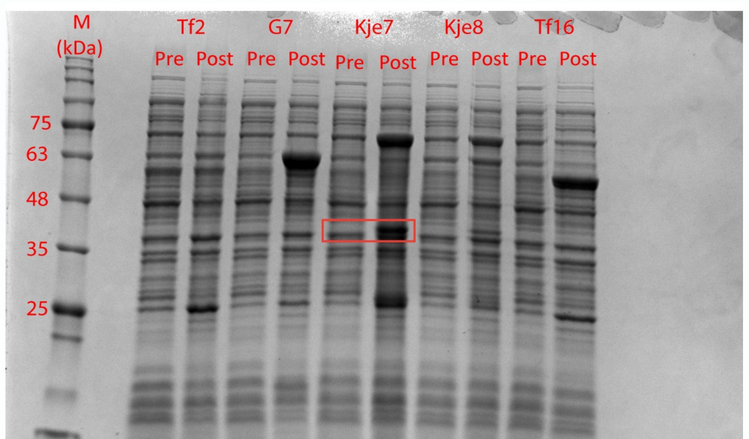

Fig. 3 The pKJE7 chaperone plasmid was successful during the test expression. Therefore, it was used during the large growth and purification process.

Protein Purification

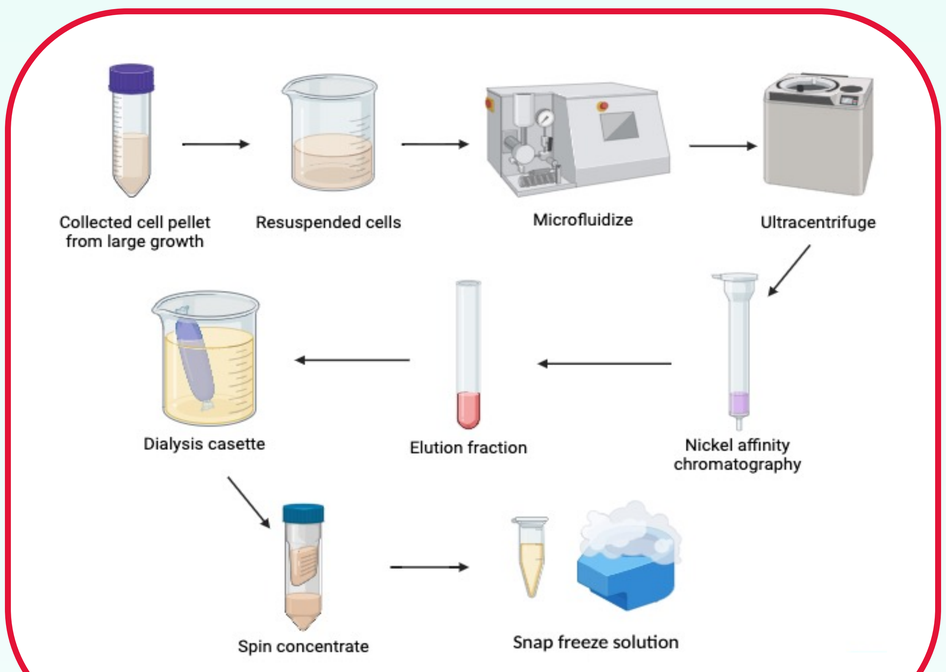

Fig. 4 Purification protocol for Enzyme B. Created with BioRender.com

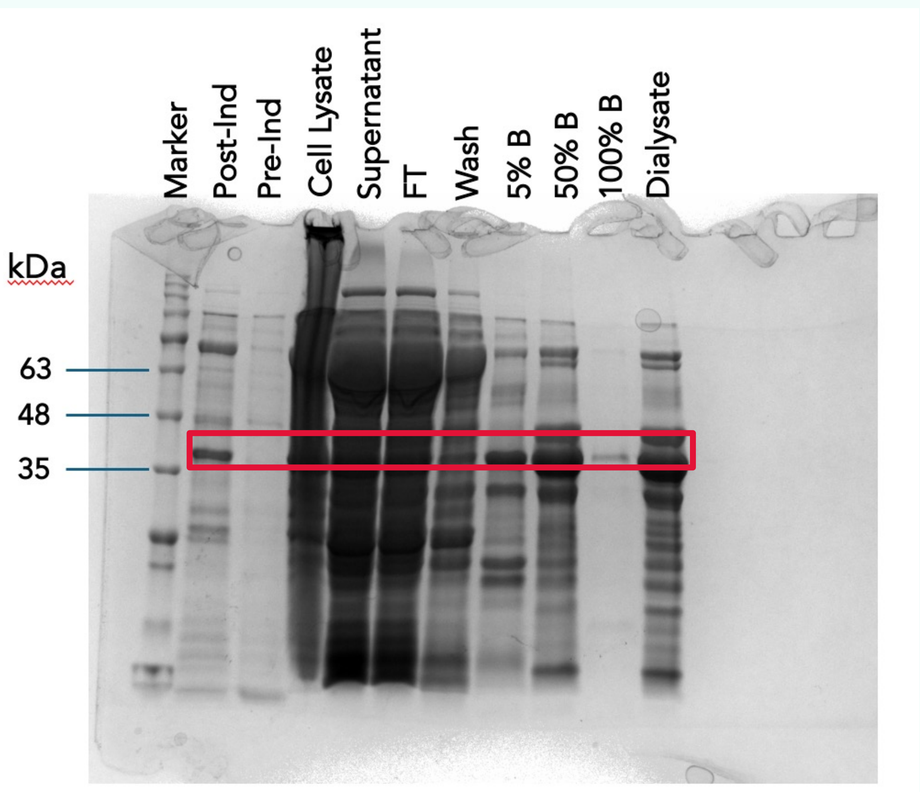

Fig. 5 The protein was successfully purified to approximately 60% purity shown by the sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). The pre and post induction solution were collected during cell growth. The lysate and supernatant were obtained during the purification process. The remaining samples were obtained during the nickel chromatography.

Protein Quantitation

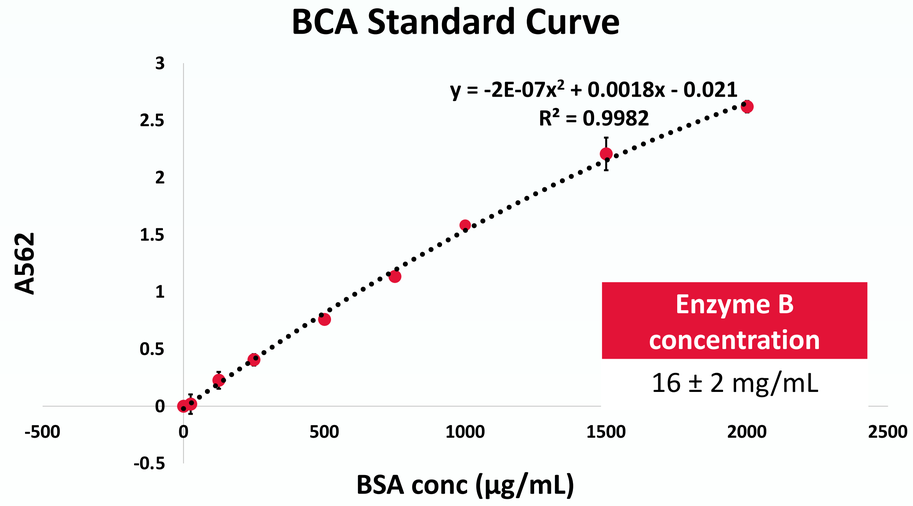

Fig. 6 The calibration curve for the BCA assay allows for quantitation of total protein concentration. A series of Bovine Serum Albumin (BSA) standards were prepared and their absorbances were measured at 562 nm to generate this standard curve and quantify Enzyme B.

Crystallography

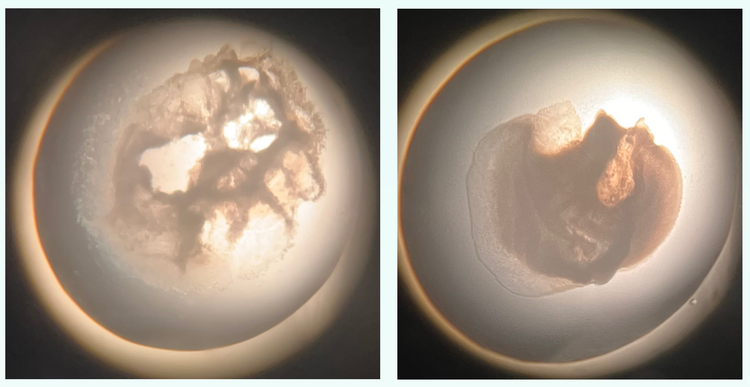

Fig. 7 Enzyme B precipitate in Hampton HT Index sparce matrix screen. Initial crystallization conditions will require optimization to grow diffraction quality crystals. Left: 0.2 M Magnesium chloride hexahydrate, 0.1 M BIS-TRIS pH 6.5, 25% w/v Polyethylene glycol 3,350. Right: 0.1 M Potassium thiocyanate, 30% w/v Polyethylene glycol monomethyl ether 2,000.

Conclusion

- Based on SDS-PAGE our sample contains the protein.
- For future work, a western blot test could be used to definitively show how pure our protein is.
- Further optimization of the purification can be done to grow higher quality crystals.
- We will develop an assay to determine the steady state kinetic parameters for Enzyme B.
- This study could lead to a multiplex sensor for real-time monitoring of estrone and other steroids.

References

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