Expression and Purification of Oxygen Dependent Domains of Hypoxia-Inducible Transcription Factor

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In this project, we express and purify the oxygen dependent domain (ODD) protein of the Hypoxia-Inducible Factor (HIF). Purification is a step toward further studies in ODD interactions as a substrate with other proteins, specifically the Prolyl Hydroxylase domain enzymes (PHDs). This could be useful in studies relating to cancers, cardiac diseases, angiogenesis, and other severe diseases.

## Background
- Hypoxia: state with limited oxygen supply
- PHD and HIF interaction key in oxygen homeostasis
- Helps cell activate gene transcriptions
- HIF is heterodimeric; HIF-1α and HIF-2α
- ODD is a part of HIF-1α
- ODD contains two prolyl residues
- 3 PHDs: PHD1, PHD2, PHD3
- Project focuses on PHD3
- PHD3 production increased in hypoxia
- PHD1, PHD2, down regulated
- Normoxia, ODD undergoes hydroxylation by PHD
- Modified, -OH groups added to prolines
- Hypoxia, ODD not modified
- HIF-1α activates gene transcriptions
- Helps maintain the cell oxygen level
- Hydroxylation by PHD has many affects:
  - Cancer, angiogenesis, metabolism, cell survival, cell invasion, metastasis
  - Associated with cell apoptosis, cardiac diseases, heart diseases
  - ODD as potential target for therapies and cures to some heart disorders
- Activation by mutation or hypoxia
  - Oxidative to glycolytic metabolism
  - Increased glycolysis and lactate
  - Decreased consumption of oxygen
  - Commonly seen in cancer cell

## Methods
### Steps to obtain ODD construct
1. Transformation, GST-ODD fusion protein construct into BL21 competent cells
2. Innoculated 1 L culture with 250 mL starter culture
3. Grew cells at 37°C in 2xTY media (16g Bacto-tryptone, 10g Yeast Extract, 5g NaCl for every 1 liter)
4. OD-1.0, induced with 0.5mM IPTG
5. Continued growth for four hours in 37°C
6. Centrifuged culture for 30 minutes at 5000 rpm to obtain cell pellet

### ODD purification process
7. Resuspended cell pellet in a lysis buffer (50mM Tris-HCl, 150mM NaCl, 0.1% Tween-20, 1mM PMSF, pH 7.5)
8. Used a sonicator to breakups (4 min)
9. Centrifuged at 18000 rpm at 4°C for 45 minutes to bring excess cell parts to pellet
10. Loaded supernatant (containing GST-ODD fusion) through glutathione column
11. Washed column with wash buffer (20mM Tris-HCl, 100mM NaCl, 0.1mM glutathione, pH 7.5)
12. To obtain GST-ODD fusion protein, eluted fusion with elution buffer (20mM Tris-HCl, 50mM NaCl, 5mM glutathione, pH 7.5)
13. Collected five sample fractions, which contained GST-ODD fusion protein

## Results
- GST-ODD fusion protein suspended in lysis buffer (50mM Tris- HCl, 150mM NaCl, 0.1% Tween-20, 1mM PMSF, pH 7.5)
- GST-ODD fusion bound onto glutathione agarose resin
- Used elution buffer (20mM Tris-HCl, 50mM NaCl, 5mM glutathione, pH 7.5) to obtain and isolate protein

## Implications
- Purification and isolation process only partially successful
- Used UV-visible spectrophotometry by a nanodrop to determine protein concentration
- Concentration of GST-ODD fusion protein determined to be 19 mg/mL

## References

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