Immobilization of the Glutamine Binding Protein (QBP) onto the Surface of Transparent Microbeads for use in a Biosensor

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Introduction

- Type 2 diabetes is a major risk factor for cardiovascular disease development (CVD) and affects over 350 million people worldwide[1]
- Circulating glutamine concentration is reduced significantly in patients with type 2 diabetes compared with healthy individuals[2]
- Currently there are no methods for non-invasive measurements of glutamine in human blood
  - This work will look into expressing then using the Glutamine Binding Protein (QBP) immobilized onto the surface of transparent microbeads to non invasively measure glutamine concentrations

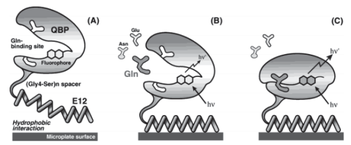

Figure 1. Glutamine Binding Protein. (A) shows QBP without any glutamine bound while (C) shows how the structure of QBP changes when glutamine is bound. Taken from Wada et. al. *J. Am. Chem. Soc.* **2003.** 125, 16228-34

QBP Expression

- QBP was grown from *Escherichia coli.* S179C mutant form
- The protein was extracted through osmotic shock and labeled with the fluorophore Acrylodan.
- Protein was then run through two different types of purification columns, size exclusion (G25) and anion exchange chromatography (DEAE)

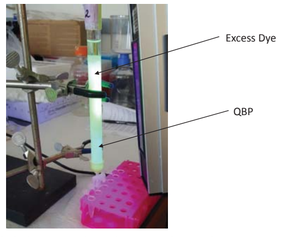

Figure 2. Acrylodan labeled QBP run through a G25 column. The top is excess dye removed from the protein and the bottom is the QBP that is collected and used for testing. UV light is used to see where separation of the two occurs.

QBP Response

- There were two different runs of QBP through purification columns
  - One through size exclusion (G25 only)
  - One through size exclusion and anion exchange (G25 and DEAE)

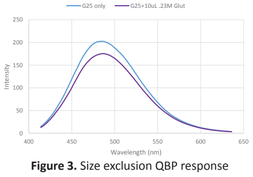

Figure 3. Size exclusion QBP response

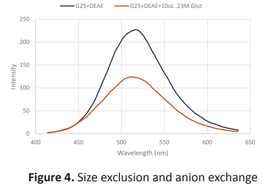

Figure 4. Size exclusion and anion exchange QBP response

- The fluorescence response of both samples were tested when the same concentration (7.7µM) of glutamine was added.
- Varian Spectrophotometer was used
- G25 only had 13.5% response
- G25 and DEAE had 45.5% response
- Samples that were run through both columns were chosen to be used for immobilization

Synthesis of Gellan Beads and Immobilization

Gellan Bead Synthesis

- Gellan beads were synthesized from KELCOGEL® Gellan gum and canola oil
- Beads need to be activated before immobilzation[3]
  - Using organic activation reaction[3] beads were activated using the following reagents

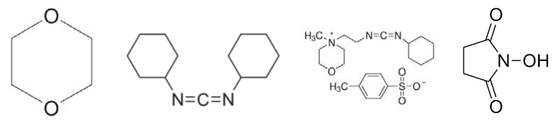

Figure 5. Reagents used organic bead activation. From L-R: 1,4 Dioxane, N,N′-Dicyclohexylcarbodiimide (DCC), 1-cyclohexyl-3-(2-morpholinoethyl) carbodiimide metho-p-toluenesulfonate (CMC), N-hydroxysuccinimide (NHS)

- Beads were washed extensively with the main solvent, dioxane, before activation
- DCC and NHS are used for amino acid coupling which helps attach the protein to the surface of the beads
- CMC is used for coupling ligands
- Beads were allowed to react with a solution of CMC, NHS, and DCC before being combined with the protein

Immobilization

- After beads were activated, QBP was immobilized onto the surface
- Reacted for 2 hours at 4°C and 1.5 hours at 20°C

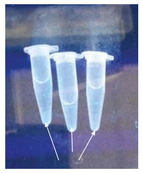

Figure 6. Immobilized QBP on gellan beads bulk of which is where arrows are pointing. Beads are sitting in a PBS buffer pH 7.4

- Immobilized beads are kept at 4°C in order to avoid denaturing of protein
- For 15mL of labeled protein initially run through both purification columns, .375 grams of immobilized beads were obtained

Results

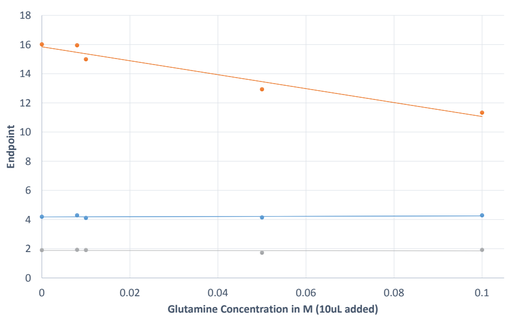

Figure 7. Initial response testing of immobilized protein using endpoint measurements in SpectraMax well plate reader. Grey line is PBS buffer, blue is un-immobilized beads, and orange is immobilized beads. Immobilized protein has equation of *y= -(48±6) x concentration + 15.8±.3* All samples were excited at 390 nm with a cutoff at 495nm. R^2=0.96

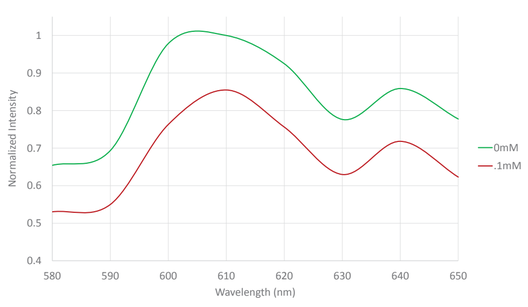

Figure 8. Fluorescence spectrum testing of immobilized protein taken during the initial immobilized testing. Comparing this graph to Figure 3 and 4, the wavelength at which the maximum occurs has shifted over.

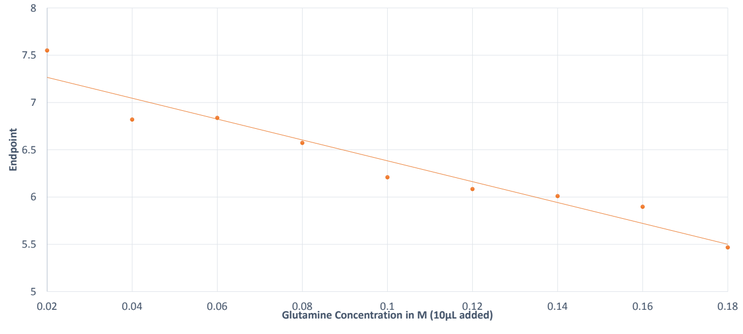

Figure 8. Second testing of immobilized protein using the same instrument and settings. This time no buffer and immobilized beads were measured. Also the concentration range was expanded in order to see where the saturation point occurs. The next measurement (.2M) is where the endpoint response begins to flat line. The line of best fit is *y= (-11±1) x concentration + 7.5 ± .1* R^2=0.93

Conclusions

- Able to produce highly responsive QBP from *E. Coli*
- Immobilization of QBP unto gellan beads was successful as shown by simple UV light shining and response testing in SpectraMax

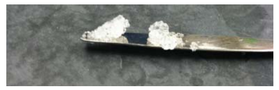

Figure 10. Transparent gellan beads before QBP was immobilized on their surface

- Immobilized beads reach a saturation point after 10µL of .2M is added

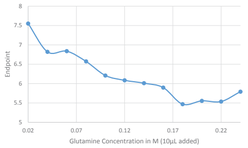

Figure 11. Same data from Figure 8 this time with the measurements past saturation

- Process does not seem to be reversible since beads disintegrate after being tested
- Yield was not as high as desired considering amount of protein initially run through purification column

Future Directions

- Attempt immobilization of QBP onto the surface of Nickel-Nitrilotriacetic acid (Ni-NTA) and Cobalt-Nitrilotriacetic acid (Co-NTA) beads as done previously with the Glucose Binding Protein (GBP)
- Determine how much protein was immobilized unto the gellan beads and devise a method to maximize the amount of protein immobilized unto its surface
  - Collect and measuring supernatant after immobilization
- Use immobilized QBP on gellan beads in a biosensor and test its response

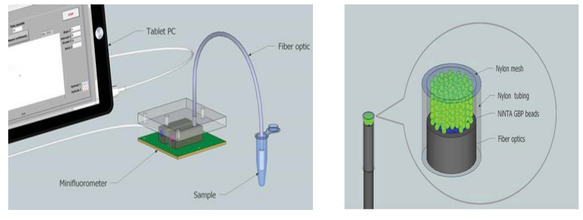

Figure 12. (L) Current glucose bio sensor with immobilized GBP on surface of Ni-NTA beads. (R) Probe of biosensor with immobilized protein kept under the surface of a nylon mesh.

- Test transdermal diffusion of glutamine using the proposed biosensor
- Test aqueous activation of gellan beads and see how much immobilized is obtained from this method

References

- [1]Molfino et. al. *Nutritional Therapy and Metabolism* **2010.** 28, 7-11
  - *"Metabolic effects of glutamine on insulin sensitivity"*
- [2]Mansour et. al. *Nutrition* **2015.** 31, 119-26
  - *"Effect of glutamine supplementation on cardiovascular risk factors in patients with type 2 diabetes"*
- [3]Ferrance et. al. *J. of Chromatography A* **2007.** 1165, 86-92
  - *"Gellan Beads as a transparent media for protein immobilization and affinity capture"*

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