Introduction

E-DNA Biosensors

Mycobacterial infection and mortality rates remain high; an estimated 10 million cases of Tuberculosis emerge each year. However, less studied Mycobacterium species are becoming a growing concern: species of nontuberculous Mycobacterium (NTM). Over the last 30 years, NTM-based lung disease cases have grown to outnumber TB infections in many regions. The “gold standard” for NTM diagnosis involves a weeks-to-months-long microbiological culture-based method. Here, we are developing a novel electrochemical DNA (E-DNA) biosensor to detect NTM for a more efficient diagnostic tool than the current, time-intensive methods.

Previous Work (ManLAM E-DNA Biosensor)

E-DNA-based biosensors have previously shown reliable detection of a myriad of target proteins/drugs down to picomolar concentrations. These sensors hold great promise in efficient point-of-care (POC) clinical diagnostics. Previously, we have developed an E-DNA biosensor using an ssDNA aptamer sequence that changes conformational shape upon binding to the target ManLAM.

Glycopeptidolipids (GPLs)

GPLs are a glycosylated molecule with a tetrapeptide core abundant in many pathogenic NTM species.

Why GPL?

Our lab is in the process of optimizing the ManLAM biosensor; however, engineering a novel E-DNA biosensor for the detection of GPL molecules would increase selectivity of our diagnostic tool. Having a GPL-based biosensor would enable detection below the genus level (i.e. Mycobacterium avium Complex – MAC) due to a variety of serovar chemistry. Additionally, our lab has reported numerous biosensors using peptide-based biomarkers; as GPL has a peptide backbone, it makes for an ideal target.

Methods & Techniques

- Silica column chromatography was used to purify GPL from total lipid extracts of NTM species isolated from clinical samples (obtained from Dr. Jennifer Honda, National Jewish Health).
- Confirmation was done with thin-layer chromatography (TLC) using silica gel 60 plates after testing several solvents.
- Systematic Evolution of Ligands by Exponential Enrichment (SELEX) will be done with GPL isolated to create an aptamer for incorporation into biosensor.

Results and Challenges

- While unable to successfully purify GPL as of yet, TLC plates have shown separation of resuspended lipid extracts.
- Initial results (left) from TLC plates did not show significant GPL identification when using a Chloroform, Methanol and H2O solution (30:8:1).
- After overnight charring with 10% cupric sulfate & 8% phosphoric acid, bands in the TLC plate became apparent.

Further Directions

1. Finish purification of GPL from the total lipid extracts of clinical samples.
2. Run SELEX to form an aptamer sequence for biosensor incorporation.
3. Use Square Wave Voltammetry to analyze and optimize biosensor response to GPL in buffer conditions.
4. Run trials against live microbes as well as serum infected with NTM, through collaboration with Dr. Jenn Honda at National Jewish Health.

References Cited


Acknowledgements

We wish to thank our collaborator Dr. Jenn Honda, National Jewish Health. Research funded by MSU Denver Chemistry & Biochemistry Department and MSU Denver Applied Learning Center.