

Annual Report Summary

Period Covered by the Report: August 1, 2000 - July 31, 2001

Date of Report: April 1 2002

Title: Synthesis and Characterization of an Electrochemical Peptide Nucleic Acid Probe

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Institution: University of South Alabama

Research Category: Small Grants for Exploratory Research (SGER)

Project Period: July 1, 2000 – December 31, 2002

Objective(s) of the Research Project: The objective of this research project is to develop an electrochemical nucleic acid hybridization assay that is sensitive (picomole to femtomole detection level) stable with storage (6 months), portable and easy to use in a simple assay format.

Progress Summary/Accomplishments: The major accomplishment on the project has been the synthesis of the electrochemical probe and positive results from the initial hybridization assays. The initial synthesis to attach ferrocene to a nucleic acid strand was performed using polyadenylic acid (poly(A)). The ferrocenyl poly (A) was characterized using cyclic voltammetry and UV-visible spectroscopy to confirm the incorporation of the ferrocene group into the nucleic acid strand. Hybridization assays using immobilized poly (U) as target and immobilized poly (C) as control showed an increased signal for the target sample as compared to the control sample upon hybridizing with the ferrocenyl poly (A). The poly (U) and poly (C) was immobilized on the bottom of polyethylene containers using irradiation for one minute at 254 nm. A more specific end-attachment of the nucleic acid strand to the solid support is discussed later. The ferrocene redox recycling system using laccase showed no improvement in the voltammetry signal. After numerous attempts to amplify the ferrocene signal without success, the redox recycling step was removed from the protocol. The initial work with the poly (A) demonstrates that a simple and portable electrochemical hybridization assay is feasible. Next, ferrocene was incorporated into the peptide nucleic acid (PNA) detector probe using a slight modification of the poly (A) conditions. The ferrocenyl PNA was characterized using cyclic voltammetry and UV-visible spectroscopy. Another aspect of the project that has been investigated is the synthesis and immobilization of azido-tailed poly (A). The azido-tailed poly (A) was prepared using poly (A) polymerase. The incorporation of the azido groups into the poly (A) strand was confirmed by the absorption spectrum (200 nm to 400 nm) before and after irradiation of a sample with 254 nm light. The irradiated sample showed a decrease in absorption spectrum as compared to the non-irradiated sample. The immobilization of the azido-tailed poly (A) was not successful. This is probably due to the large size to azido label ratio that occurs with the poly (A) molecule. The procedure is being performed using oligonucleotides.

The development of a portable and simple to use hybridization assay with good sensitivity (picomole and lower detection level) is important in the monitoring of seafood safety. Food borne illnesses can occur from eating fish and shellfish contaminated with pathogenic bacteria. Current detection methods are not suitable for rapid on-site monitoring of seafood samples for pathogenic bacterial contamination. Using an electrochemical hybridization assay format can be as portable and as simple as taking a pH reading. Simply lyse the bacterial cell wall and release

the ribosomal RNA, add to container containing capture and detector probe, incubate, rinse, add ferricyanide and read the current using a potentiometer.

Publications/Presentations:

1. A. C. Robinson, S. Jivarajan and **N. F. Campbell**. 2002. Synthesis of Ferrocene Labeled Peptide Nucleic Acid Probe. 223th ACS National Meeting, Undergraduate Division.
2. S. Jivarajan, A. C. Robinson, T. R. Taylor and **N. F. Campbell**. 2001. Electrochemical Detection of Hybridized Ferrocenyl Polyadenylic Acid. ACS Regional Meeting, Undergraduate Division.
3. B. E. Horne, K. M. Ratliff and **N. F. Campbell**. 2000. Synthesis and Electrochemical Characterization of Ferrocene-Labeled Polyadenylic Acid. 220th ACS National Meeting, Undergraduate Division.

Future Activities: The major objective will be the immobilization of the target nucleic acid and the evaluation of the electrochemical hybridization assay format. Immobilization of the target nucleic acid will involve the attachment of an oligonucleotide (complementary sequence to a portion of target molecule) to the solid support followed by the hybridization of target nucleic acid. This process will require 2-3 months to complete and evaluate. The next step will be to optimize the hybridization conditions between the target ribosomal RNA and the ferrocenyl peptide nucleic acid probe (complementary sequence to a different portion of target nucleic acid). Lastly, the hybridization assay format will be evaluated using for specificity using ribosomal RNA from *Salmonella typhimurium* and *Escherichia coli* (control). This portion of the project will require about 6 months for completion.

Supplemental Keywords: Peptide Nucleic Acid Probe, Electrochemical Hybridization Assay, Ribosomal RNA, Ferrocene Nucleic Acid Probe, Electrochemical Nucleic Acid Probe.

Relevant Web Sites: None

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