

Annual Report Summary

Period Covered by the Report: August 1, 2001 - December 31, 2002

Date of Report: January 21, 2003

EPA Agreement Number: 5-21820

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, 2003

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:

I. Synthesis and Characterization of Ferrocenyl PNA Product. An electroactive molecule, ferrocene, was covalently attached to a peptide nucleic acid strand (PNA; base sequence 3'-GCT-TTT-GTG-TAC) using a slight modification of the poly (A) procedures. The ferrocenyl PNA was purified using gel permeation chromatography (Sephadex G-15; fractionation range of the gel is less than 1500 Da and the molar mass of the PNA strand is 3267 Da; the PNA strand eluted in the void volume). The BCA (bicinchoninic acid) assay was performed on all the fractions that eluted from the column to test for the presence of peptide bonds (present in the PNA strands). The fractions that tested positive using the BCA assay were pooled and characterized using cyclic voltammetry and UV-visible spectroscopy. Absorption at a wavelength of 450 nm (λ_{\max} for ferrocene) and voltammetry analysis of the ferrocenyl PNA sample was used to confirm the incorporation of the ferrocene into the PNA strand.

II. Synthesis and Immobilization of Azido-Tailed Oligonucleotide. Another aspect of the project is the synthesis and immobilization of azido-tailed poly (A). As reported in the previous annual report, the azido-tailed poly (A) was prepared using poly (A) polymerase. However, the immobilization of the azido-tailed poly (A) was not successful because of the large size to azido label ratio that occurs with the poly (A) molecule. The procedure was repeated using an oligonucleotide [20 bases total; RNA 12 bases + (rA)₈]. The azido label was incorporated using poly (A) polymerase and 8-azidoadenosine 5'-triphosphate. The oligonucleotide was purified using gel permeation chromatography (Sephadex G-15) and the fractions were analyzed at wavelengths of 260 nm and 280 nm. The incorporation of the azido groups into the oligonucleotide 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. Next, the azido-tailed oligonucleotide complement and azido-tailed oligonucleotide control were placed in polystyrene containers (n = 6 for each sample) and the solvent evaporated overnight at room temperature. All the samples were irradiated with light at a wavelength of 254 nm for 30 seconds. The containers were rinsed to remove any unattached oligonucleotide strands and ultraviolet spectroscopy of the rinses was used to determine percent immobilization.

III. Hybridization and Electrochemical Analysis. The ferrocenyl PNA (3'-GCT-TTT-GTG-TAC) was hybridized to its immobilized oligonucleotide complement (5'-CGA-AAA-CAC-AUC; 1 base mismatch) and its immobilized oligonucleotide control (5'-CGA-AUU-GUC-AUC; 5 bases mismatch). Cyclic voltammetry were performed on each complement and control hybridization sample. The current for the control sample was 23.691 μ A and the current for the complement sample was 64.675 μ A. These results showed an increased current reading for the complement hybridization versus the control sample.

IV. Budget.

Budget Item	Percent Remaining	Amount
Salaries	1%	\$3.05
Benefits	20%	\$969.64
Supplies	45%	\$2,232.62
Other	81%	\$162.75
Indirect	11%	\$780.91
TOTAL	11%	\$4,148.97

V. Relevance and Practical Application. 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.

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.

Future Activities: The last objective of this project is to evaluate the hybridization assay and ferrocenyl probe specificity using ribosomal RNA from *Salmonella typhimurium* and *Escherichia coli* (control). This portion of the project will require about 6- 9 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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