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Determining the Analytical Performance of Various Mutant RNA & DNA Aptamer-Based Biosensors for the Detection of the Aminoglycoside Antibiotic, Gentamycin Sulfate

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ABSTRACT

There is a need for reliable and effective biosensors that can enable, for example, studies of biological systems to answer important biochemical questions and to provide therapeutic monitoring of patients for improved patient care. Electrochemical biosensors that utilize the conformation-switching ability of various aptamers are selective for specific analytes, sensitive to changes in analyte concentration, and can be easily altered for the detection of other analytes of interest. In this project, electrochemical aptamer-based sensors were modified with various mutated RNA and DNA aminoglycoside aptamers to determine the quantitative effects on sensor performance. These sensors were then used for the detection of the aminoglycoside antibiotic, gentamycin sulfate, in buffer and fetal bovine serum using square wave voltammetry (SWV) in order to determine the specificity, selectivity and sensitivity of each of these aminoglycoside aptamers. It was determined that these various aminoglycoside aptamers can detect gentamycin sulfate in both buffer and fetal bovine serum, but with a greater electrochemical signal change and binding affinity for gentamycin sulfate in fetal bovine serum. In order to further characterize the specificity, selectivity and sensitivity of these aminoglycoside aptamers for gentamycin sulfate, repeated trials should be conducted. In order to better predict the behavior of these aminoglycoside aptamers as reliable and effective biosensors in human beings, experiments should be conducted in increasingly complex biological media, such as blood. This project is supported by the National Science Foundation Research Experience for Undergraduates (REU) Award No. CHE-1460653.

BACKGROUND

Electrochemical aptamer-based (E-AB) sensors (Fig. 1) are sensors that are made with redox-labeled, conformation-switching aptamers for specific, reagent-less electrochemical detection.

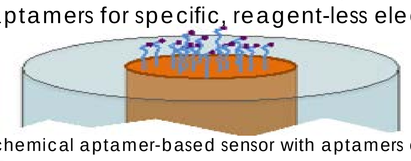

Fig. 1. Electrochemical aptamer-based sensor with aptamers on surface of a gold electrode.

Aptamers are single-stranded DNA or RNA sequences that can bind to selected targets with high affinity and specificity. These aptamers have redox markers, such as methylene blue, on the distal end of the aptamers that participate in electron transfer with the electrode surface. The aptamers bind specifically to target molecules and undergo a conformational change that results in a change in the amount of electron transfer between the redox marker and the electrode surface, as shown in Figure 2. This change in electron transfer can be detected and measured electrochemically as the analyte concentration is varied. The analytical performance of these E-AB sensors depends on the magnitude of the conformation change of the aptamers they are modified with.

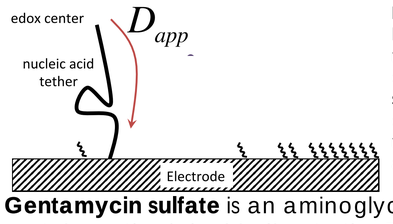

Fig. 2. Process by which a redox-labeled aptamer (nucleic acid tether) undergoes a conformational change upon binding of a specific stimulus or target molecule, changing the amount of electron transfer between the redox center and the surface of the electrode.

Gentamycin sulfate is an aminoglycoside antibiotic that inhibits protein synthesis by binding to the 50S ribosomal subunit of bacteria. **Aminoglycosides** are effective antibiotics that are very potent and have a broad spectrum of uses. Examples of aminoglycoside antibiotics include gentamycin, tobramycin and kanamycin. The structures of these aminoglycosides are shown below. The aminoglycoside aptamers that are studied in this research project are all capable of binding gentamycin sulfate, tobramycin and kanamycin.

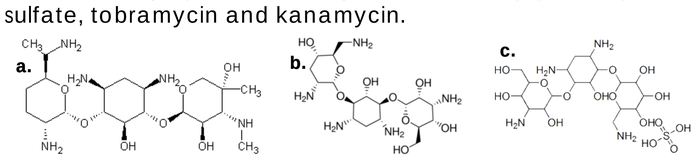

Fig. 3. Chemical structures of the aminoglycoside antibiotics, gentamycin (**a**), tobramycin (**b**) and kanamycin (**c**).

MATERIALS & METHODS

Electrochemical Aptamer-Based (E-AB) Sensor Preparation

Mechanical Polishing

1. 1 μM monocrystalline diamond suspension (Buehler)
2. Alumina oxide slurry (Buehler)
3. Sonication in ethanol for 5 minutes

Electrochemical Cleaning

1. Voltammetric cycling in 0.5 M NaOH
2. Oxidation and reduction in 0.5 M H_2SO_4
3. Potential cycling in 0.5 M H_2SO_4
4. Chloride etching in 0.1 M H_2SO_4/0.01 M KCl
5. Area determination in 0.05 M H_2SO_4

Modification of Electrodes with Aptamers

1. All RNA and DNA aptamers from Table 1 were prepared by reacting 2 μL of aptamer in 4 μL of 10 mM TCEP(Sigma-Aldrich) for 1 h to reduce 5'-disulfide bond
2. Incubation of 200 nM RNA or DNA aptamer solution with electrodes for 1 h
3. Incubation E-AB sensors in a 3 mM solution of 6-mercapto-1-hexanol solution for 1 h to passivate aptamers

Fig. 4. All E-AB sensors were prepared on 2 mm diameter polycrystalline gold electrodes (CH Instruments, Austin, TX) (left).

Electrochemical Measurements

- Electrochemical measurements performed using CH Instruments 620D Electrochemical Workstation (CH Instruments, Austin, TX) using an Ag/AgCl (3 M NaCl) reference electrode and a platinum wire counter electrode
- Square wave voltammetry parameters were: pulse amplitude of 25 mV, varied frequencies depending on the aptamer, step width of 1 mV
- Frequency sweeps were conducted for all aptamers in both Tris buffer and filtered fetal bovine serum
- Concentrations of gentamycin sulfate varied from 100 nM-4 mM
- For measurements conducted in fetal bovine serum, the E-AB sensors were left to equilibrate in serum for 1 h prior to electrochemical measurements.

Table 1. Parent and Mutant Aptamer Sequences Used for E-AB Sensors

Sequence Name	Sequence*
Parent RNA Aptamer	5'-HSC$_6$-GGGACU**UGGUUUAGGUAAUG**AGUCCC-MB-3'
Parent DNA Aptamer	5'-HSC$_6$-GGGACT**TGGTTTAGGTAATG**AGTCCC-MB-3'
Mutant DNA 1 Aptamer	5'-HSC$_6$-GACTATCCGTTGGAT-MB-AA**TGGTTTAGGTAATG**G-3'
Mutant DNA 2 Aptamer	5'-HSC$_6$-GCTGGCCGAGCC**T**-MB-**GGTTAGGTAATG**G-3'
Mutant DNA 3 Aptamer	5'-HSC$_6$-TCCCAATTCGTT-MB-GAATT**TGGTTTAGGTAATG**A-3'
Mutant DNA 4 Aptamer	5'-HSC$_6$-GGCTAGGTGTGACCT-MB-**TGGTTTAGGTAATG**-3'

*Underlined sequence is conserved in all aptamers except for Mutant DNA 2

ANALYSIS

It can be seen that all the aminoglycoside aptamers in Table 1 are capable of binding the aminoglycoside antibiotic, gentamycin sulfate, in both buffer and serum. However, it should be noted that these aptamers, with the exception of Mutant DNA 2, bind to tobramycin with a greater binding affinity than to gentamycin sulfate. The Parent RNA aptamer could not be electrochemically measured in serum due to its instability and susceptibility to degradation in the presence of RNAse. In buffer, the Parent RNA, Parent DNA and Mutant DNA 2 aptamers demonstrated distinctive 'signal off' trends at lower frequencies, whereas all the aptamers demonstrated distinctive 'signal on' trends at higher frequencies. In serum, the Parent DNA aptamer has a distinctive 'signal off' trend at lower frequencies, while the Mutant DNA 2 aptamer has a slight 'signal off' trend at lower frequencies. However, all DNA aptamers have distinctive 'signal on' trends at higher frequencies in serum. For both the frequency sweeps and titration curves, the DNA aptamers generally demonstrated a greater signal change with smaller standard deviations in serum than in buffer. However, all DNA aptamers, except for Mutant DNA 2, had a greater binding affinity to gentamycin sulfate in buffer than in serum. Mutant DNA aptamers 1, 3, and 4 were designed by a specific algorithm to optimize the binding of tobramycin, but those aptamers also optimized the binding of gentamycin sulfate in buffer and serum, demonstrated in the increase in percent signal change in those aptamers as compared to the Parent DNA aptamer. Mutant DNA 2 aptamer was designed to be much less functional as an aminoglycoside aptamer, which was confirmed by the titration curve in buffer. However, in serum, Mutant DNA 2 demonstrated great binding capability with gentamycin sulfate.

RESULTS

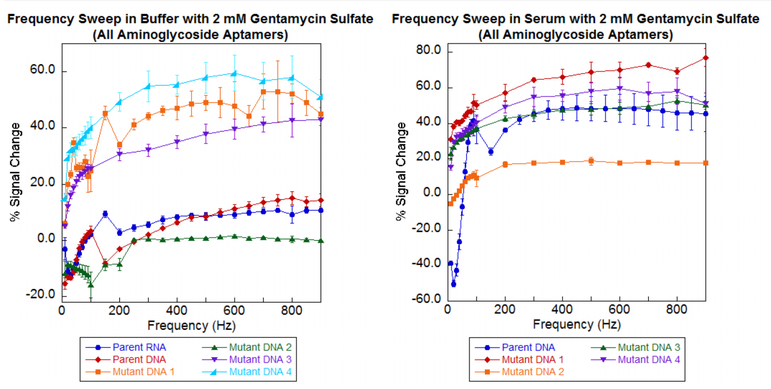

Fig. 5. Frequency sweeps for all aptamers in buffer (*left*) and serum (*right*) with 2 mM gentamycin sulfate to determine optimal frequencies for peak aptamer performance.

Table 2. Optimal Frequency Values and K_d Values for All Aptamers in Buffer and Serum

Aptamer	Optimal Frequency in Buffer (Hz)	Optimal Frequency in Serum (Hz)	K_d Values* in Buffer (μM)	K_d Values* in Buffer (μM)
Parent RNA	30	--	18.093 / *25.244*	--
Parent DNA	800	400	70.392 / *8.8132*	452.25 / *11.779*
Mutant DNA 1	700	700	563.11 / *2.25E+9*	11714 / *2.3242E+9*
Mutant DNA 2	100	700	2.41E+16 / *5.897*	82.393 / *1.4884*
Mutant DNA 3	900	700	679.13 / *4.0541*	2143.4 / *2.063*
Mutant DNA 4	600	700	651 / *47.778*	5849.5 / *6.7915*

*First K_d value corresponds to the K_d value obtained from full titration curves (**a** and **b**) in Fig.6. Second K_d *italicized* corresponds to K_d value obtained from titration curves **c** and **d** in Fig. 6.

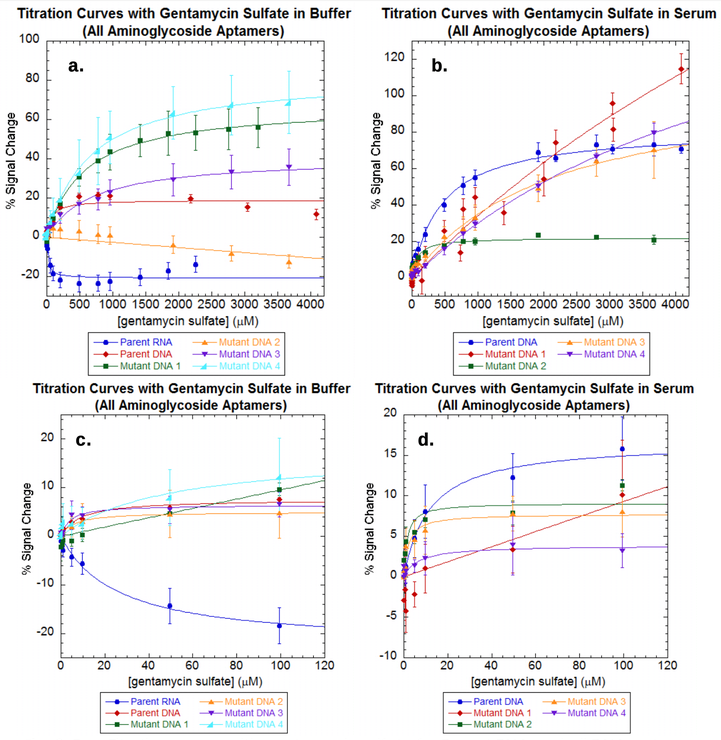

Fig. 6. *Top*: Titration curves with gentamycin sulfate for Parent RNA and all DNA aptamers in buffer (**a**) and for all DNA aptamers in serum (**b**). *Bottom*: Titration curves **a** and **b** zoomed in for gentamycin sulfate concentrations of 0-100 μM (**c** and **d**, respectively).

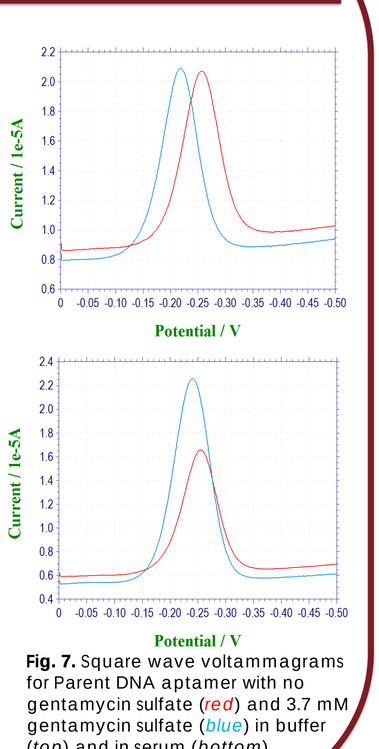

Fig. 7. Square wave voltammagrams for Parent DNA aptamer with no gentamycin sulfate (*red*) and 3.7 mM gentamycin sulfate (*blue*) in buffer (*top*) and in serum (*bottom*).

CONCLUSION

- All aminoglycoside aptamers were capable of binding gentamycin sulfate in both buffer and serum (except for Parent RNA).
  - The DNA aptamers displayed a greater overall percent signal change in serum than in buffer, but had a lower binding affinity to the gentamycin in serum than in buffer.
  - These aptamers have demonstrated good analytical performance in complex media, i.e. serum, meaning that they provide great promise to be used in even more complex media, such as plasma or whole blood, which are the next steps in characterizing these aptamers.
  - Thus, these aptamers provide promise to be used as effective biosensors for studying biological systems and for therapeutic monitoring.

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