The Detection of PETN Using a Bulk Single Wall Carbon Nanotube/Peptide System

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ABSTRACT

The present research work investigates the novel detection of pentaerythritol tetranitrate (PETN), a high powered explosive, using peptide surface functionalized single wall carbon nanotubes. Here, through the use of a phage display technique and enzyme-linked immunosorbent assays (ELISA), a peptide library was tested against PETNH (pentaerythritol trinitrate hemisuccinate) to determine amino acid sequences with affinity toward the aforementioned explosive. Subsequently, 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) was used to link the peptides produced from the phage display to carboxylated single wall carbon nanotubes (SWCNTs). Simultaneously, PETNH was biotinylated with Biotin-PEG4-Hydrazide for tagging purposes, using the aforementioned EDC. Results from HABA/Avidin biotin incorporation assays show that the concentration of tagged PETNH in solution decreases when brought into contact with the functionalized SWCNTs. These liquid detection results suggest that the peptide functionalization on the SWCNTs allows the binding of PETNH. It is expected to use these biologically functionalized carbon nanotubes on the development of a solid state PETN nanosensor.

Keywords: carbon nanotubes, explosive detection, sensors

1 INTRODUCTION

The defense of national security is always an aspect of high priority concern. One important facet of the national defense is the early detection of dangerous agents, such as explosives materials, before acts of violence can be carried out. Certainly, considerable work has been carried out to develop new and improved techniques for the detection of trace amounts of different explosive systems [1-5]. Currently, many spectroscopic techniques such as ion mobility spectroscopy [6], mass spectroscopy [7], and terahertz spectroscopy [8] are used in explosive detection. However, these techniques inherently comprise convoluted sensing approaches as well as portability issues. An additional form of detection that has been used for many years is the use of highly trained sniffer dogs [9]. Some of the major drawbacks for this approach include a high cost for training and inconsistencies due to variable conditions. One method commonly employed in airports is X-ray detection which is plagued with the issue that only explosives consisting of a metal casing or trigger mechanism can be detected [10-11]. This means that through the adaptation or modification of an explosive device, the current x-ray detection mechanism can be avoided. Thus, research into new detection methods that not only combat adaptation, but also provide reliable detection in diverse environmental conditions is of high importance.

One explosive that is raising increasing concern is pentaerythritol tetranitrate (PETN). In military applications, this explosive is used in conjunction with RDX to make semtex explosives [12]. Recently though, homemade bombs with PETN being the sole ingredient have been found and used [13]. One of the features of this explosive is that its vapor pressure is very low. Due to this feature, detection of a pure PETN explosive device requires very high sensitivity detectors.

A viable option for the detection of PETN is the implementation of a peptide structure selective to the aforementioned explosive. Peptides targeting volatile organic compounds such as benzene have been used to functionalize micro cantilever surfaces to be used in detection platform [14]. Here, the peptides assisted not only on creating a sensitive detector, but also by imposing selectivity to the device. Similarly, peptides have been found for targeting TNT [15-16]. When the peptides were implemented in an assay format, the sensor was able to detect very low amounts of the TNT.

The present research aims to use phage display technology to find peptides with affinity towards PETNH (a surrogate of PETN). This is performed via biopanning and enzyme-linked immunosorbent assays (ELISA) of a phage library expressing various peptides. The peptides are then used to covalently functionalize the surface of a carbon nanotube for liquid state detection tests. It is expected to eventually use this technology for creating solid state sensors.

2 EXPERIMENTAL METHODS

The basic flow of the research began with the exposure of immobilized PETNH to an M13KE Ph. D.-7 library, in a biopanning procedure, to isolate the phage, which exhibited specificity to the PETNH. The isolated phage was then quantified by titers, and then each phage was amplified and PEG purified. Next, ELISAs were performed on each of the amplified and purified phage to determine the relative selectivity of each phage to PETNH. Subsequently, the peptides (purchased from NeoBiolab) were sequenced, and then covalently attached to single wall carbon nanotubes (SWCNTs). Finally, these functionalized tubes were tested as sensors against the PETNH in a liquid state.

2.1 Enzyme-linked Immunosorbent Assay(ELISA)

To immobilize PETN for ELISA techniques, a PETN hapten was used. The surrogate used was pentaerythritol trinitrate hemisuccinate (PENTH) and has the chemical structure as seen in Figure 1b.

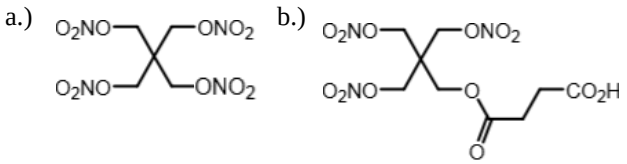

Figure 1: Chemical structure of a.) PETN and b.) PETNH

This hapten was employed because it contains a carboxyl group that allows for its immobilization in amine surface coated microwell plates by a 1-Ethyl-3-(3 dimethylaminopropyl) carbodiimide (EDC) approach while also retaining as much of the original PETN chemical structure as possible.

The basic method began with immobilization of the PETNH in the wells. Then, the wells were blocked by nonfat dry milk in TBS so that phage interact mainly with the immobilized ligand and not with the well walls. Next, phage from the M13KE Ph. D.-7 library, acquired from NeoBiolabs, were allowed to bind to the PETNH. Subsequently, unbound phage were washed away and horseradish peroxidase (HRP)/ Anti-M13 monoclonal conjugate was added. These antibodies bind to the phage which is still present in the wells. Finally, unbound antibodies were washed away and tetramethylbenzidine (TMB) was added. The TMB reacts with the HRP on the antibodies and the resulting colorimetry is used to determine the relative amount of phage bound to the PETNH in each well.

2.2 Biopanning

The purpose of biopanning is to isolate the phage with the highest selectivity to the PETNH. The first step was to allow phage to adsorb to a blocked microwell to remove non-specific phage that would bind to wells. Then the pre-screened phage were transferred to a well where the PETNH had been immobilized. Here, the phage with low and high specificity bind to the PETNH. Next, the unbound phage were removed from the well. Subsequently, the bound phage were eluted. The preceding steps were then repeated, and with each panning round, the phage become more and more specific to the target PETNH.

2.3 Titer

Titers were run at various steps in the research to determine the concentration of phage in solution. A standard protocol supplied by NeoBiolabs was used for all titers.

2.4 Sequencing

The Sequencing procedure performed in this work allowed the identification of the amino acid sequences of the peptides expressed by the phage isolated in the biopanning stage. In this study, a Beckman CEQ2000 DNA Sequencer was used in conjunction with a Wizard® Plus SV Minipreps DNA Purification System

2.5 Peptide Functionalization of the Carbon Nanotubes

To covalently functionalize SWCNTs with the sequenced peptides, SWCNTs with surface carboxyl groups (SWCNT-COOH) were purchased from nanolab. The presence of the carboxyl groups allows for attachment to side chain and n-terminus amine groups present on the peptides. This was done by first adding a 0.5 mg/ml peptide solution to 5 mg of SWCNTs with carboxyl surface groups and mixing. Then, 2 µL of a 100 mg/ml solution of EDC was added to the mixture. This solution was then allowed to rock overnight. Subsequently, the peptide functionalized SWCNTs were pelleted by centrifugation and the unbound peptide was removed.

To determine the amount of peptide bound to the tubes similar-parallel tests were run, where instead of using the pure peptide to functionalize the tubes, the peptide was tagged with biotin-PEG4-hydrazide. Here, the free biotin from the biotinylated peptide, was separated through graphite spin columns from Thermo Scientific. This was performed in order to ensure that the quantified biotin content was related to the tagged biotin. Thus, a mass balance through the system provided the amount of bound peptide on the carbon nanotubes.

2.6 Detection Tests

The first step in the detection test was attaching biotin-PEG4-hydrazide to PETNH. This allowed the concentration of the explosive to be tracked. To remove the unbound free

biotin from the biotinylated PETNH (PETNH-B), SWCNT-COOHs were used as a filter. The concentration of PETNH-B was then quantified by a HABA/Avidin assay. Subsequently, the biotinylated explosive was added to both the peptide functionalized SWCNTs and to the SWCNT-COOHs. The addition of the explosive to the SWCNT-COOHs was performed as a control for the experiment. These solutions were then allowed to rock for 2 hours at room temperature. Subsequently, the unbound explosive was separated from the peptide functionalized SWCNTs by centrifugation and syringe filter, and then used in a HABA/Avidin assay to determine the amount of explosive left.

3 RESULTS AND DISCUSSION

Initially, an ELISA was performed on the M13KE library in order to assess its feasibility for being used on this research work, as well as to determine the presence of phage with affinity towards PETNH. The results of the ELISA test are shown in Figure 2.

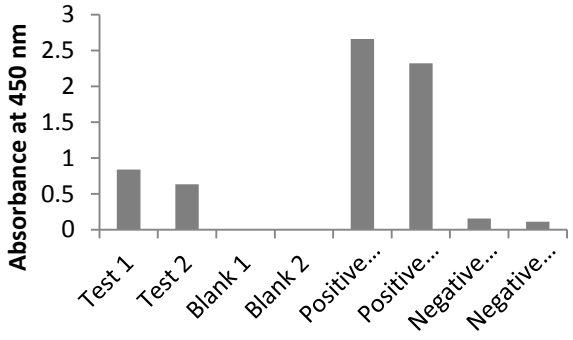

Figure 2: M13KE phage library ELISA results

From the figure, it can be seen that the test wells exceed the absorbance of the negative control wells. This suggests that there are phage contained within the M13KE library that exhibit peptides with affinity towards PETNH.

Since it was determined that the M13KE library was suitable for targeting immobilized PETNH, biopanning was carried out. After the first round of panning, the library reduced from $2.0x10^{11}$pfu/ml to $2.2x10^{7}$pfu/ml. After the third round of panning, the concentration of phage was only 50 pfu/ml. This shows that between the first and third rounds, the concentration decreased by six orders of magnitude and that the specificity of the phage increased with more panning rounds. The plaques from the third round of titering were picked from the plate and then amplified and PEG purified to be used in the ELISA testing.

To determine the selectivity of each isolated phage, ELISAs were run on the immobilized PETNH against each isolated phage solution being used in place of the entire library. The results of each ELISA test are shown in Figure 3.

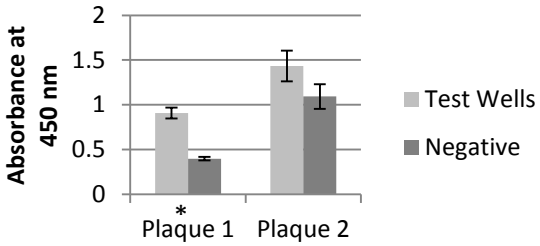

Figure 3: ELISA results comparing the absorbance of the test wells to the negative control wells for isolated phage from plaques 1 and 2. The presence of an asterisk (*) above the plaque indicates that the mean difference is significant ($P \leq 0.05$) via a t-test.

From Figure 3, it can be seen and confirmed by t-test that the absorbance of the test wells containing phage from plaque 1 show a significantly higher absorbance than the negative control wells. Phage from plaque 2 present a visible difference in absorbance, but the overlapping standard error and t-test results suggest that there is not a significant difference between test and negative control wells.

The phage from both plaques were sequenced using a Beckman CEQ2000 DNA Sequencer. Though only phage from plaque 1 showed a significant difference in absorbances between the test and the negative wells, the phage from plaque 2 was also chosen to be sequenced in order to have a comparison between phage showing marked affinity and nonsignificant affinity. The results of the peptide sequencing are presented in Table 1.

Peptide 1	
ggtttgtggacgaatgttgatggtggaggt	
5'3' Frame 1	G L W T N V D
Peptide 2	
ggtttgagtacgtttgatgtgggtggaggt	
3'5' Frame 1	G L S T F D V

Table 1: Sequencing results for peptide 1 and 2. The bolded 30 letter long code is the nucleotide sequence, and the identified amino acid sequences are based on the single letter code (ie T=Threonine, etc.).

From Table 1, it can be seen that although the ELISAs showed differences in affinity, the peptides present some similarities such as the Glycine-Leucine segments in both peptides.

Finally, peptides 1 and 2 were used to functionalize 5 mg of SWCNTs each which were then brought into contact with PETNHB. In parallel, the same amount of PETNHB was added to SWCNT-COOH to act as a control. The results of this test are shown in Table 2.

Surface Functionalization	Detection %
Peptide 1	52.8
Peptide 2	61.8
Carboxyl (no peptide)	28.3

Table 2: The amount of biotinylated PETNH (PETNHB) bound to the functionalized SWCNTs after 2 hours of mixing.

From table 2, it can be seen that more PETNHB was bound to the SWCNTs functionalized by the peptides, than to the plain carboxylated nanotubes (approximately two times the degree of detection). The detection of PETNHB exhibited by the control is most likely due to the explosive surrogate becoming trapped in entangled groups of the SWCNT-COOHs. Another noteworthy aspect presented in the table is that SWCNTs functionalized by peptide 2, resulted in a larger amount of PETNHB detected. Due to this feature, future selectivity tests will be needed to determine if peptide 2 would produce a better sensor than peptide 1 in solid state conditions.

4 CONCLUSION

In this study, peptides with affinity towards a PETN surrogate were investigated. These peptides were found via phage display technology utilizing the M13KE Ph. D.-7 library. The sequences of the two peptides exhibit similarities between them. Both peptides show different levels of affinity to the explosive surrogate, but both show a higher sensing capability than the control. Currently, work is being done to identify more peptides with affinity towards PETN, and the adaptation of the work into a solid state platform.

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