Vapor-Phase Substrate Nitroreductase Reaction and its Application as TNT Electrochemical Gas Sensor

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Abstract

Nitroreductase (NTR) is an enzyme with a specific activity to nitroaromatic reduction. Some nitroaromatic explosives are exploited by terrorists. Among these materials, 2,4,6- trinitrotoluene (TNT) is a major high explosive which makes detection of TNT an important research topic. TNT vapor detection by gas sensor is an approach under investigated for many years. Even though, there is a limited success at present, in part, due to the ultra-low volatility nature of TNT. In this work, we propose an application of the NTR reaction in a hydrogel gas sensor to detect TNT vapor. Firstly, NTR activity in the hydrogel was confirmed by adding TNT and 2,4-dinitrotoluene (DNT) solutions to an NTR-hydrogel sensor following by cyclic voltammetry (CV) experiments. Then, the other CV experiments were carried out with gas phase samples. The CV graphs of NTR-hydrogels detecting vapors containing the explosive substrates and negative controls were compared. Results showed that the signal from the nitroaromatics increased with exposure time where the TNT signal was the highest of all tested compounds. The gas sensor was sensitive enough to detect TNT gas presented in an ambient air at room temperature.

Introduction

Terrorists always employ bomb devices to destroy their targets as part of operations. The device contains a high explosive component to generate the power from chemical chain reactions. Most of explosives are nitro compounds which exhibit such reactions upon oxidation. Within this class of compounds, 2,4,6- trinitrotoluene (TNT) is widely used as a major high-explosive. The bomb device can be hidden in a car, motorcycle, or buried in soil, or a street, to make searching difficult and considered as a high-risk task even by well-trained personnel. High efficiency bomb searching instruments could partially solve this problem, however many of them are unsuitable for fieldwork. For example, they lack portability [1] or are hindered by their sheer size [2]. In this circumstance, highly sensitive but small sensor-based instruments seem to be attractive. Researchers have carried out works on TNT sensors for years where many groups have succeeded [3-5]. Nonetheless, in making TNT sensors for real application, there are points of concern make these sensors less attractive. Some of these sensors are very sensitive but used to detect TNT in solution [6-8]. A typical bomb device contains TNT in solid form covered inside, so detection of TNT has to be conducted outside from TNT vapor. But the gas sensor must be extremely sensitive because TNT

is an ultra-low volatile substance (vapor pressure of 4.8×10^{-6} mmHg at 20 °C) [9]. Some TNT vapor sensors have been reported. For examples, the sensors based on electrical signal change upon TNT vapor absorption on a transducer [10], or a chemosensor which relies on a catalytic decomposition of explosive on thin film [11]. It should be noted that a typical characteristic of TNT gas sensors reported have in common is the detection, which always requires longer time than the detection of the analyte in solution [12,13]. In part, the very low vapor pressure of TNT mentioned means it takes long period allowing TNT concentration to reach a value starts to generate a significant sensor signal. Using preconcentrating materials to trap vapors [14-16] can partially increase TNT concentration, but again it substantially delays detection time [17]. Normally, response time of explosive detection is more than 5 min due to the time

dependent mass transport of TNT to the sensor active sites. Along with sensitivity, the sensor selectivity is an equally important aspect. In real application, TNT detection needs to work properly in the environment where a background interference is abundant. In this regard, the distinctive responding signal from TNT rather than from the background chemicals is preferred. This could be achieved by using functionalized material [18,19] or a chemical reaction more selective to TNT [20] such as nitroreductase (NTR) catalysis to generate the sensor signal. An NTR reaction, whose underlying mechanism involves electron transfer of nitroaromatic, has been exploited in liquid-phase TNT analysis [21-23]. Even though, NTR application in TNT gas sensor has not yet been reported, there is evidence that gas-phase substrate reactions of some other enzymes [24] could be observed under low humidity [25] or alternative solvent environments [26]. Some of the enzymes have even gained enormous relative activity with gas substrates comparing with true liquid-phase substrate activity [27]. In this regard, gas-phase enzymatic reaction has a potential to be applied in ultra-low vapor chemical detection like TNT. In this study, we aimed to determine if we could use the vapor-substrate approach to enhance the NTR activity sufficient for TNT vapor detection. The specificity of NTR reduction to only nitroaromatics would minimize the chance of false-positive signals from other compounds presented in the environment. The different reaction rates of various nitroaromatics could also be used to distinguish TNT from the other substrates and interferences. The experiments on detection of two nitroaromatics [2,4-dinitrotoluene (DNT) and TNT], the non-aromatic explosives [pentaerythritol tetranitrate (PETN)], and the negative control [phloroglucinol vapor] were carried out and compared. The results showed the highest sensor signal response to TNT vapor with a responding time of 10 min. Working conditions and limitations of the sensor were also reported.

Results And Discussion

The NTR-gelatin hydrogel gas sensor will experiment to verify NTR reaction occurrence in the hydrogel and its application for nitroaromatic detection. The current changes from the explosive and other chemicals in solution or vapors were detected by cyclic voltammetry (CV) using a potentiostat (STAT8000, Metrohm Siam, Thailand) controlled by Dropview 8400 software. After soaking the NTR-gelatin hydrogel in 10mM PBS for 1 hr., it was placed on the screen-printed electrode (SPE; Fig. 4b), with gold as the working and reference electrodes and silver as the counter electrode. All CV measurements were carried out with a potential range from -0.8 to +0.8 volts and at a 20 mV/s scan rate. The electrical signal change from an NTR electron transfer reaction in hydrogel as an indication of nitroaromatic presence in vapor. In the first place, NTR without its substrate was added directly to the hydrogel on an electrode followed by CV scans. The CV graph pattern was in agreement with previous studies [22] with the reduction peak at -0.45 Volts (Fig. 1b). This indicated that an NTR reaction in hydrogel media did indeed happen. When nitroaromatic solutions were added to the NTR-hydrogel, the CV graphs showed current increasing with reaction time where the TNT signal was higher than the DNT signal (Fig. 1). In line with the previous result, the reaction of hydrogel in TNT-NTR was faster than that in the DNT-NTR [21]. As a preliminary study, we were able to distinguish TNT signal from the DNT by following nitroaromatic-NTR activity in solution. To confirm the NTR-hydrogel activity with the nitroaromatic vapor, the CV experiment was subsequently set up.

In this experiment, the measurements were conducted by using the gas detection system (See Fig. 4b.). The test compounds comprise: 1) two nitroaromatics (TNT and DNT), and 2) the negative controls including the non-aromatic high explosive (PETN) and a chemical having a substructure like TNT (phloroglucinol). In congruence with gas experiment, the change in the current corresponding to the enzyme activity of NTR was observed in substrate in solution at -0.45 V (See Fig. 1). The signal from TNT vapor, which increased dramatically within 10 min before saturation at 25 min, was the highest of all tested compounds (See Fig. 2) while the other aromatic, DNT, increased gradually. At the first 25 min, the DNT signal rose at a slow rate and was lower when compared to the TNT, PETN, phloroglucinol, or the ambient air signals. After that, the DNT signal rose significantly and became greater than those of the negative controls. Notably, all the negative control signals have lower than initial measurement. According to these, the NTR-hydrogel sensor holds great promise in high-sensitive and selective to detect TNT and DNT. The rationale underlying the observed sensitivity of NTR-hydrogel sensor towards TNT and DNT was credited to detection time (TNT = 10 min; DNT at least 35 min). Furthermore, this work gains new insights into the sensitivity of NTR-hydrogel sensor detection within 70 min before it became too dry and lost its sensitivity.

Another experiment was carried out to see the NTR reaction in hydrogel at different quantity of TNT. This experiment to investigate the effect of NTR reaction was conducted on TNT quantity at 1 g, 5 g and 10 g. Similarly, it was used represent the experiment at different concentrations. The results in Fig. 3 show the current change of the NTR-gelatin hydrogel gas sensors increasing with TNT quantity. The signal from TNT vapor will increased when quantity of TNT more.

Conclusion

We have demonstrated an application of the NTR reaction with nitroaromatics to detect TNT, a low-volatile explosive compound by a hydrogel gas sensor. Specificity to nitroaromatics and different reaction rates to particular substrates enabled the sensor to detect nitroaromatic explosives and distinguish TNT from DNT and the negative controls. We showed in the first place that in solution NTR reactions with the nitroaromatics, TNT and DNT could be observed by monitoring the reduction peak in CV. Then, a gas phase experiment was carried out from which the changes in the signal after passing nitroaromatics through were higher than the negative controls. The sensor was sensitive and capable of detecting TNT vapor after 10 minutes. The DNT signal was lower than the TNT signal but significantly higher than the negative controls after 25 minutes. The data from the literature reported the TNT vapor pressure as 9.15 ppb and DNT as 411 ppb at 25°C which could be assumed as the detection limits for our sensor. The working period of the hydrogel sensor was approximately 1 hour after which the loss of water from the gel hindered NTR functionality. Nonetheless, our work could be considered as an achievement where the enzymatic reaction can be applied as a sensitive rapid method for TNT vapor detection compared with the results from other groups [28,29]. However, it should be noted that the higher gain from TNT than DNT in the gas phase measurement was unexpected. When TNT or DNT solutions were added to the hydrogel before the CV scan, both had comparable signal levels where the TNT signal increased marginally faster. However, throughout the gas-phase experiment, the TNT vapor resulted in a higher signal than DNT. This

observation was unexpected considering TNT vapor pressure is several times lower than DNT. We hypothesize that the higher TNT current was the result of the better hydrogel absorptivity of TNT vapor, and further study on this should be conducted.

Materials And Methods

Chemicals. The explosive materials, TNT and PETN were provided by the Royal Thai Army (RTA); DNT was purchased from the Tokyo Chemical Industry (TCI) and phloroglucinol was purchased from Merck, Germany. Gelatin was purchased from McGarrett, Bangkok, Thailand. NTR (≥ 100 units/mg) from *Escherichia coli*, and NADH were purchased from Sigma–Aldrich. The redox active reagent was NTR/NADH in pH 7.4 of phosphate buffered saline (PBS).

Gel preparation. The gelatin solution was prepared by mixing 5 g gelatin with 40 mL of 10 mM PBS at pH 7.4 (0.8% water content). The mixture was heated to 70°C for 20 min and left to cool down to room temperature. Subsequently, an NTR-gelatin hydrogel in a small batch was prepared by adding 7.1 μL of NTR (350 $\mu\text{g/mL}$) and 3.8 μL of NADH (16 mM) to 2.0 mL gelatin mixture (NTR 0.05 nmol: NADH 15 nmol). The mixture was transferred to a 40×10×3 mm acrylic mold in a refrigerator to set before cutting into 1×1 cm² pieces (each piece of gel contained NTR and NADH 0.94 and 12 μg , respectively). Then, the gel was soaked in 10 mM PBS and kept in a refrigerator for 1 h. The averaged water content of each piece was 53%. Finally, it was washed thoroughly with deionized water before used (Fig. 4a).

Gas detection system. A gas detecting system was assembled according to the diagram as shown in Fig. 4b. A pump carried in ambient air for transfer a sample into the chamber at 1,000 mL/min flowrate. The measurement was carried out in a temperature and humidity control room (25 °C with 50% relative humidity). After mixture of air and sample vapor was delivered to a measuring chamber, the sensor signal was monitored. The electrochemical signal was investigated by the NTR-gelatin hydrogel sensor which was placed on a screen-printed gold electrode (DRP-220BT, Metrohm Siam, Thailand) connected to the potentiostat.

Declarations

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Author contributions

T.P., W.S., and C.S. conceived the research. T.P., W.S., and C.S. designed the experiments. T.P. and C.T. performed all experiments. T.P., W.S., C.T., and C.S. wrote and revised the manuscript. All the authors

discussed the results and commented on the manuscript.

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Figures

Figure 1

The current change from 2 fg DNT and TNT solutions dropped on NTR hydrogel at various time (a) with CV diagrams of TNT solution at the corresponding time above (b).

Figure 2

The current change from test compounds as a result of NTR reaction in hydrogel at various time (a) and CV diagram of TNT vapor at the corresponding time above (b).

Figure 3

The current change from different quantity of TNT as a result of NTR reaction in hydrogel at various time

Figure 4

The diagram depicts preparation of NTR gelatin-hydrogel (a) and the gas flow system used for explosive vapors detection (b).