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RESEARCH PAPER

Aptamer-Based Microcantilever Sensor for *O*-ethyl S-[2(diisopropylamino)ethyl] methylphosphonothiolate, Sarin Detection and Kinetic Analysis

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Abstract: A novel method for *O*-ethyl S-[2(diisopropylamino)ethyl] methylphosphonothiolate (VX), sarin detection and kinetic analysis based on piezoresistive microcantilever aptasensor was proposed, where VX and sarin aptamers were immobilized on the microcantilever surface by biotin-avidin binding system. A linear relationship between the response voltage and the concentration of VX in the range of 2–60 μg L^{-1} was obtained, the linear regression equation was $\Delta U_e = 0.886C - 1.039$ ($n = 5$, $R = 0.984$, $p < 0.001$) and the detection limit was 2 μg L^{-1} ($S/N \geq 3$). A linear relationship between the response voltage and the concentration of sarin in the range of 10–60 μg L^{-1} was obtained, the linear regression equation was $\Delta U_e = 0.716C - 2.304$ ($n = 5$, $R = 0.996$, $p < 0.001$) and the detection limit was 10 μg L^{-1} ($S/N \geq 3$). The sensor showed no response for O-butyl methylphosphonochloridate, a structural analog of VX and sarin, indicating a high specificity and good selectivity. On this basis, a reaction kinetic model based on receptor-ligand binding and the relationship with response voltage was established. Response voltage (ΔU_e) and response time (t_0) obtained from the fitting equation on different concentrations of VX and sarin fitted well with the measured values.

Key Words: Microcantilever; Aptasensor; *O*-ethyl S-[2(diisopropylamino)ethyl] methylphosphonothiolate; Sarin; Detection; Kinetic analysis

1 Introduction

S-[2(diisopropylamino)ethyl] methylphosphonothiolate (VX) and sarin are nerve agents with the characteristics of high toxicity and quickly killing. The common detection methods for VX and sarin are chromatography, chromatography-mass spectrometry and spectroscopy, etc. All these methods exhibit high sensitivity but also have a lot of shortcomings, such as requirement of sophisticated instruments, high cost, tedious pre-treatment procedure, long detection time and so on[1–4]. Currently used enzyme biosensors can detect phosphorus agents quickly and sensitively, and can be used for the detection of complicated samples on-spot. However, enzyme biosensors usually use cholinesterase as the sensitive material based on the principle of inhibition of enzymatic activity, which may respond to a lot of phosphorus agents and carbamate. So it could not distinguish different types of agents[5].

Aptamer has the features of easy preparation, high affinity, high specificity and good stability, and has attracted much attention as a new recognition element[5,6]. Piezoresistive microcantilever integrates piezoresistive materials into microcantilever, which converts the curvature of microcantilever into a voltage signal directly by Wheatstone bridge. Due to its simple read-out way, easy integration, low cost and small volume, piezoresistive microcantilever has an excellent prospect in the field test of biological and chemical molecules[6–8]. However, there is no report on the detection of VX and sarin by aptasensor or piezoresistive microcantilever sensor. Because the conventional enzyme biosensor could not specifically recognize VX and sarin, one goal of this study was to establish a specific detection method for VX and sarin which used aptamer as the sensing material. The study was also aimed at establishing a kinetic model to piezoresistive microcantilever aptasensor of VX and sarin, thus provided theoretical basis for data processing and eliminating nonspecific signals in the detection.

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2 Experimental

2.1 Instruments and reagents

Piezoresisitive microcantilever detection platform was built by the Institute of Microelectronics, Peking University and our lab (the size of the piezoresisitive microcantilever chip was roughly 200 μm × 50 μm × 1 μm). The methanol stock solution of VX, sarin and VX aptamer (5'-Bio-TCG CAA GAC GGA CAG AAG GTT TTT ATT TTA TCT TTG ATT ACT GTT TTT TTG TTT AGT TGT GTT GGT GGA GCG ATT TGT-3'), GB aptamer (5'-Bio-TCG CAA GAC GGA CAG AAG TTG GGA CTG CCA CTT TGT GTT TTG GTT ATA GTA CTT ATT TGC GTT GGT GGA GCG ATT TGT-3') were prepared by our lab. Biotin-NHS ester, 3,3,-dithiodipropionic acid (DDPA), 1-ethyl-3-(3-dimethyl-aminopropyl)-carbodiimide hydrochloride (EDC), *N*-Hydro-xysuccinimide (NHS), avidin, cholamine were purchased from Sigma-Aldrich; bovine serum albumin (BSA) was purchased from Sinopharm (Shanghai China). All other reagents were of analytical reagent grade and used without further purification. PBS solution (pH 7.4, 0.01 M) and other aqueous solution were prepared with double distilled water (DDW).

2.2 Fabrication of the piezoresistive microcantilever aptasensor

The microcantilever chip was dipped into the DDPA solution (5 g L^{-1}) at room temperature for 1 h. Then, a mixture of 5 g L^{-1} EDC and 5 g L^{-1} NHS (3:1, *V/V*) was used to activate the carboxyl group on the microcantilever surface. After that, 20 μL of avidin solution (100 mg L^{-1}) was added to the modified surface to react with the activated NHS-ester. Then, 20 μL of cholamine solution (1 M) was added on the chip surface and incubated at room temperature for 1 h to block excess active group. The modified chip was washed with distilled water before use and then incubated with 2 μM of biotinylated VX (or sarin) aptamer for 2 h, followed by washing with distilled water to remove the nonspecific adsorption.

2.3 VX and sarin detection

The aptamer-modified chip was dipped into the 0.01 M PBS solution until the output voltage reached a stable value. After that, different concentrations of VX (or sarin) solutions were added to the sensor respectively and the response voltages of the sensor were recorded. As a control, 0.01 M PBS solution and 200 μg L^{-1} O-butyl methyl phosphonic chloride were detected.

2.4 Kinetic model for the detection of VX and sarin by microcantilever aptasensor

A reaction kinetic model based on receptor-ligand binding and the relationship with response voltage was established. Then the model was used to fit the measured data of VX and sarin, and analyze the relationship between the measured values and the fitting values of response voltage (ΔU_e) and response time (t_0).

2.5 Test of VX and sarin in simulated samples

About 5 μL of 320 mg L^{-1} VX (or sarin) standard substance was respectively added into 1.0 g of soil sample, 1.0 mL of cucumber juice and 1.0 mL of river water. The obtained mixtures were then supplemented with dilution solution to a final volume of 8.0 mL to get simulated sample with a final VX (or sarin) concentration of 20 μg L^{-1}. River water and cucumber juice samples were tested directly. The soil sample was centrifuged at 5000 *g* for 20 min, and the supernatants were tested. Recovery and standard deviations were calculated.

3 Results and discussion

3.1 Determination of VX and sarin

As shown in Fig.1, when the concentrations of VX were 60, 30 and 20 μg L^{-1}, the response voltage was 55.8, 21.5 and 12.5 μV respectively. When the concentrations of VX were 4 and 2 μg L^{-1}, the response voltage decreased to 4.5 μV and 3.1 μV, respectively. If the concentration of VX further decreased to 1 μg L^{-1}, the response voltage was close to the noise level (about 1 μV). So 2 μg L^{-1} was set as the detection limit of VX ($S/N \geq 3$). A linear relationship between the response voltage and the concentration of VX in the range of 2–60 μg L^{-1} was obtained, with the linear regression equation of $\Delta U_e = 0.886C - 1.039$ ($n = 5$, $R = 0.984$, $p < 0.001$). In the same way, a linear relationship between the response voltage and the concentration of sarin in the range of 10–60 μg L^{-1} was also obtained. The linear regression equation was $\Delta U_e = 0.716C - 2.304$ ($n = 5$, $R = 0.996$, $p < 0.001$) and the detection limit was 10 μg L^{-1} ($S/N \geqslant 3$).

In this study, O-butyl methylphosphonochloridate (200 ng mL^{-1}) was used as a disturbing control and PBS was served as a blank control. The sensor showed no response for both control samples although the O-butyl methylphosphono-chloridate was a structural analogue of VX and sarin, which indicated a good specificity of this sensor. 20 μg L^{-1} of VX and 10 μg L^{-1} of sarin were tested using our sensor system. The response voltages were (13.7 ± 1.08) μV and (5.0 ± 0.35) μV ($n = 3$), with the RSD of 7.9% and 7.0%, respectively, indicating the good repeatability of the sensor.

3.2 Kinetic model built for the detection of VX and sarin by microcantilever aptasensor