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Determination of organophosphate pesticides at a carbon nanotube/organophosphorus hydrolase electrochemical biosensor

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Abstract

An amperometric biosensor for oganophosphorus (OP) pesticides based on a carbon nanotube (CNT)-modified transducer and an organophosphorus hydrolase (OPH) biocatalyst is described. A bilayer approach with the OPH layer atop of the CNT film was used for preparing the CNT/OPH biosensor. The CNT layer leads to a greatly improved anodic detection of the enzymatically generated *p*-nitrophenol product, including higher sensitivity and stability. The sensor performance was optimized with respect to the surface modification and operating conditions. Under the optimal conditions the biosensor was used to measure as low as 0.15 μM paraoxon and 0.8 μM methyl parathion with sensitivities of 25 and 6 nA/μM, respectively.

Keywords: OPH; CNT; Biosensors; Oganophosphorus compounds; Nitrophenol

1. Introduction

Oganophosphorus (OP) compounds are among the most toxic substances and are thus commonly used as pesticides, insecticides and chemical warfare agents. Early detection of OP neurotoxins is important for protecting water resources and food supplies, in the defense against terrorist activity, and for monitoring detoxification processes [1]. Accordingly, there are growing demands for field-deployable devices for reliable on-site monitoring of OP compounds. Common laboratory-based analytical methods for determining OP compounds include primarily gas and liquid chromatography [2].

Biosensors based on the inhibition of acetylcholine esterase (AChE) have been widely used for the detection of OP compounds. Such inhibition biosensors are not selective, are indirect and slow. A preferred direct biosensing route for detecting OP neurotoxins involves the biocatalytic activity of organophosphorus hydrolase (OPH) [3]. Several OPH-based amperometric, potentiometric, or optical biosensing devices have been described [4,5]. Amperometric OPH electrodes commonly rely on monitoring the oxidation of the *p*-nitrophenol product of the enzyme reaction. Improved anodic detection of the *p*-nitrophenol is highly desired to address the high-overvoltage and surface-fouling limitations associated with such transduction reaction.

This article reports on the use of carbon nanotube (CNT)-based amperometric transducer for improving the amperometric biosensing of OP compounds. The discovery of carbon nanotubes in 1991 [6] has triggered considerable research activity owing to the unique chemical and physical properties

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of these materials. Electrochemical biosensors, particularly enzyme electrodes, have greatly benefited from the ability of CNT-based transducers to promote the electron-transfer reactions of enzymatically generated species such as hydrogen peroxide [7,8] or NADH [9], and from the resistance to surface fouling of such transducers. CNT-based transducers have thus been shown useful for enhancing the performance of enzyme electrodes for monitoring glucose [8,10] and ethanol [11], but have not been combined with OPH-based biosensors. The accelerated oxidation of hydrogen peroxide at CNT transducers has been exploited recently for improved AChE-based inhibition biosensing of OP compounds [12]. The ability of CNT-modified electrodes to promote the oxidation of phenolic compounds (including the *p*-nitrophenol product of the OPH reaction) and to minimize surface fouling associated with such oxidation processes [13] paves the way to the new OPH-CNT amperometric biosensor. The optimization and advantages of the CNT-based OPH amperometric biosensor are reported in the following sections.

2. Experimental

2.1. Apparatus

Amperometric experiments were performed with a Bioanalytical Systems (BAS) CV-27 voltammograph, in connection with a BAS X-Y recorder. Cyclic voltammograms were recorded with the Autolab PGSTAT10 Electrochemical Analyzer (Eco Chemie BV, Utrecht, Netherlands). Modified and unmodified glassy carbon (GC) disks (CH Instruments, 3 mm diameter) served as the working electrodes, with the Ag/AgCl (3 M NaCl) electrode and platinum wire acting as the reference and counter electrodes, respectively. The three electrodes were inserted into a 15 mL electrochemical cell through holes in its Teflon cover. A magnetic stirrer provided the convective transport during the amperometric measurements.

The flow-injection amperometric (FIA) system consisted of an Alitea (U1/48R) peristaltic pump (Sweden), an injection valve (Rainin Model 501) with a 50 μL sample loop, interconnecting Teflon tubing and a large-volume wall-jet detector. The three electrodes mentioned above were introduced into the large-volume wall-jet flow cell through the holes in its Teflon cover. The solution inlet into the cell was kept 1 mm away from the center of the working electrode disk.

2.2. Chemicals

All solutions were prepared from double-distilled water. Stock solutions of paraoxon and methyl parathion (both 100 μM from Sulpelco) were prepared in acetonitrile (EM science) and diluted as required in phosphate buffer (0.05 M; pH 7.4) based on KH_2PO_4 and K_2HPO_4, Sigma. Organophosphorus hydrolase (OPH; 2700 IU/mg of protein, 3.4 mg of protein/mL; activity measured using paraoxon as substrate) was produced and purified according to the method described by Mulchandani et al. [15]. The 9180 U/mL activity enzyme stock solution was prepared by mixing 3.4 mg of lysate in 1 mL deionized water. The stock solution (1 mM) of *p*-nitrophenol (Aldrich) was prepared in phosphate buffer (0.05 M; pH 7.4) and diluted as required in the same medium.

Single-wall carbon nanotubes (SWNT), with ca. 90% purity, were purchased from Nanostructured and Amorphous Materials Inc. (o.d. 1–2 nm; Los Alamos, USA). Multi-wall carbon nanotubes (MWNT), prepared by chemical vapor deposition (MWNT-CVD), with ca. 95% purity, were obtained from NanoLab Inc. (1–5 μm length, 20–40 nm o.d., Brighton, MA), whilst MWNT, prepared using the ARC discharge method (MWNT-ARC), were purchased from Bucky, USA (Bu-201, Houston, TX, USA). The Nafion (5 wt.% in lower aliphatic alcohols) was purchased from Aldrich. A 0.05 M phosphate buffer (pH 7.4) served as the supporting electrolyte.

2.3. Electrode preparation and modification

The initial CNT layer was cast according to an earlier procedure [7]. In brief, 100 μL of a 5% Nafion solution were mixed with 900 μL of phosphate buffer (pH 7.4). Then 5 mg of MWNT was subsequently added to this mixture and the solution was sonicated for 30 min. A 20 μL aliquot of this CNT solution (in phosphate-buffer/Nafion) was cast on a cleaned (polished and sonicated) GC electrode. The coating was air dried at room temperature for 1 h.

For biosensing experiments, the enzyme was immobilized by casting a 10 μL solution of OPH (50 IU/mL) in Nafion (0.5% in ethanol) onto the modified (10 μL solution of CNT) or unmodified GC electrodes, and allowing the solvent to evaporate. The enzyme-modified electrode was dried at room temperature and was kept in a refrigerator (at 4 °C) until use.

2.4. Procedure

Flow-injection amperometric analysis was performed using a phosphate buffer (0.05 M, pH 7.4) carrier solution. A potential of +0.85 V was applied at a flow rate of 1.0 mL/min and the transient currents were allowed to decay to a steady-state value. Cyclic voltammetric measurements were performed under batch conditions. The voltammogram was continuously recorded between −0.2 and +1.2 V at a scan rate of 50 mV/s. All measurements were performed at room temperature.

3. Results and discussion

3.1. Anodic detection of p-nitrophenol at CNT-modified electrodes

The OPH-based amperometric biosensing of OP pesticides relies on the anodic detection of the enzymatically

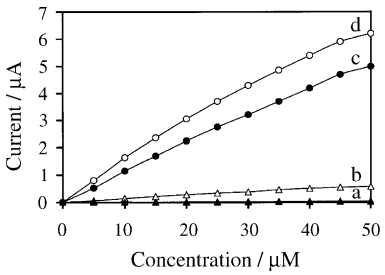

Fig. 1. Calibration plots resulting from amperometric measurement of 5 μM successive additions of *p*-nitrophenol at various electrodes: (a) bare glassy carbon (GC); (b) ARC produced multi-wall carbon nanotube (MWNT-ARC); (c) chemical vapor deposition produced single-wall carbon nanotube (SWNT-CVD); (d) chemical vapor deposition produced multi-wall carbon nanotube (MWNT-CVD)-modified GC electrodes. Electrolyte, phosphate buffer (0.05 M, pH 7.4); stirring rate, ∼300 rpm; operating potential, +0.85 V. Data points are average of duplicate measurements.

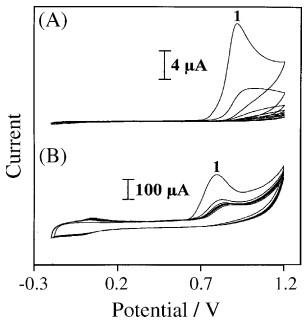

Fig. 3. Cyclic voltammograms showing 10 repetitive scans of 1 mM *p*-nitrophenol at unmodified (A), MWNT-CVD-modified (B) GC electrodes. The first scans in (A) and (B) are indicated as 1. Scan rate, 50 mV/s. Other conditions as in Fig. 1.

liberated *p*-nitrophenol [4]. CNT-modified electrodes have been shown extremely useful for improving the sensitivity and stability of oxidative measurements of phenolic compounds [13]. Since the electrochemical reactivity of CNT is strongly dependent upon their structure and preparation process [9,14,16,17], we have examined first the anodic detection of *p*-nitrophenol at different CNT-modified electrodes.

Fig. 1 compares calibration plots for *p*-nitrophenol (over the 5–50 μM range) at CVD (c, d) and ARC (b) produced CNT-modified electrodes. Also shown is the response of the bare GC electrode (a). Both the SW- and MW-CVD-CNT coated surfaces offer a dramatic enhancement of the sensitivity compared to the ARC-CNT and bare electrodes (c, d versus b, a, respectively). The higher sensitivity of the CVD-CNT-modified electrode reflects differences in the density of edge-plane-like defects that lead to higher electrochemical reactivity as was recently described by Banks et al. [18] and Lawrence et al. [14].

CNT surface modifiers have also shown useful for addressing surface-fouling problems associated with the oxidation of phenolic compounds [13]. Accordingly, we examined whether CNT-modified electrodes can impart higher stability onto anodic measurements of *p*-nitrophenol. Fig. 2 compares the amperometric response for 0.1 mM *p*-nitrophenol

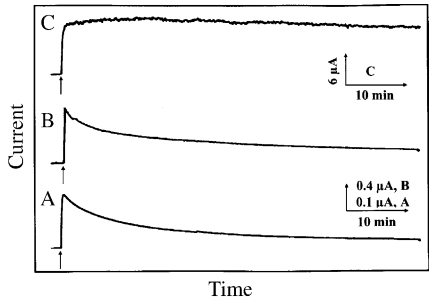

Fig. 2. Current–time response for 100 μM *p*-nitrophenol at unmodified (A), MWNT-ARC- (B) and MWNT-CVD-modified (C) GC electrodes. Other conditions as in Fig. 1.

as recorded (over a continuous 60 min period) at the bare GC surface (A) and ARC (B) and CVD (C) CNT-modified electrodes. The bare electrode displays a rapid decay of the signal, with 50 and 87% current diminutions following 10 and 60 min, respectively. The ARC-CNT displays some resistance to surface fouling with 48 and 75% signal diminutions following 10 and 60 min, respectively. In contrast, a highly stable response, with no apparent loss in sensitivity, is observed using the CVD-CNT-modified electrode. The enhanced stability is likely to reflect a different reaction mechanism [13] and/or the greater surface area of the CNT layer [19,20]. The higher sensitivity of the CVD-produced CNT-modified electrode is also indicated from the largely different current scales. All subsequent work employed the CVD-MWNT-modified electrode.

Further insights into the improved sensitivity and stability observed in the *p*-nitrophenol measurements of Figs. 1 and 2 can be obtained in cyclic voltammetric experiments. Fig. 3 displays repetitive cyclic voltammograms for 1 mM *p*-nitrophenol solution recorded at the bare (A) and CNT-modified (B) GC electrodes. Both electrodes display a well-defined oxidation peak during the first scan (1), with peak potentials of 0.90 V (A) and 0.77 V (B). The CNT-coated electrode displays a substantially larger anodic signal, as indicated from the largely different (X25) current scales (B versus A). Apparently, the substantial enhancement of the *p*-nitrophenol response at the CNT-modified electrode reflects both its larger surface area and electrocatalytic activity. The lowering of the overvoltage is relatively small compared to that reported for hydrogen peroxide [7] or NADH [9] in connection to oxidase or dehydrogenase biosensors, respectively. Note also the largely different degrees of surface passivation at the two electrodes. The current signal of the bare GC electrode decreases rapidly upon repetitive scans and completely disappears after the fifth scan. In contrast, the CNT-modified electrode displays a rapid current diminution during the first 2–3 cycles, and a stable signal thereafter. Notice also the larger background-current envelop of the CNT electrode and the appearance of a new redox peak (around 0.0 V) during successive scans. Such behavior of the CNT-modified elec-

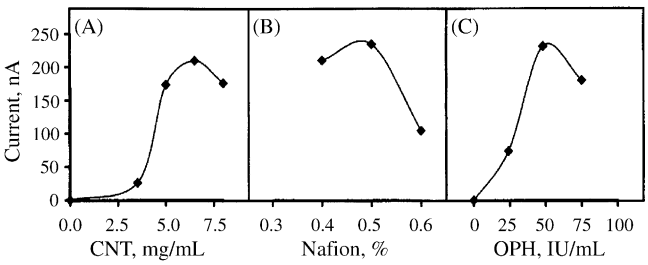

Fig. 4. Effect of the surface loading of MWNT-CVD (A), Nafion (B) and OPH (C) upon the flow-injection response to 50 μM paraoxon. Surface loading values when held constant are 5 mg/mL MWNT-CVD, 0.5% Nafion and 50 IU/mL OPH. Flow rate, 1 mL/min. Other conditions as in Fig. 1.

trode is in good agreement with the anodic behavior of other phenolic compounds at such surfaces [13].

3.2. Optimization and characterization of the OPH-CNT amperometric biosensor

A bilayer approach with the OPH layer atop of the CNT film was used for preparing the CNT/OPH biosensor. Both the CNT and OPH were dispersed in a Nafion solution, in view of the suitability of this perfluorosulfonated polymer for dispersing CNT and for electrochemical biosensor work [7]. Fig. 4 examines the influence of key variables related to the preparation of the OPH amperometric biosensor. The response for the paraoxon increases slowly with the CNT surface loading up to 3 mg/mL, then more rapidly, and starts to decrease above 6 mg/mL CNT (A). The signal increases slightly upon raising the percentage of Nafion in the CNT casting solution between 0.4 and 0.5% and decreases rapidly thereafter (B). The response increases sharply with the OPH loading up to 50 IU/mL and decreases thereafter (C).

Fig. 5 depicts hydrodynamic voltammograms for 10 μM paraoxon at the OPH/CNT- (A) and OPH-modified (B) GC electrodes. At both biosensors, the response starts above +0.75 V. The CNT-containing enzyme electrode displays a fast increase in the current up to +0.9 V with a slower one thereafter. A substantially smaller response is observed at the 'CNT-free' OPH biosensor, with the current rises slowly up to +0.9 V and starts to level off thereafter. All the subsequent amperometric detection was carried out at a potential of +0.85 V that offered the most favorable signal-to-noise characteristics.

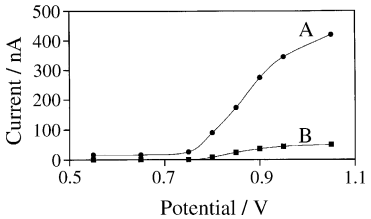

Fig. 5. Hydrodynamic voltammograms for 10 μM paraoxon at the OPH/MWNT-CVD- (A) and OPH-modified (B) GC electrodes. Surface loading, 5 mg/mL MWNT-CVD, 0.5% Nafion and 50 IU/mL OPH. Other conditions as in Fig. 4.

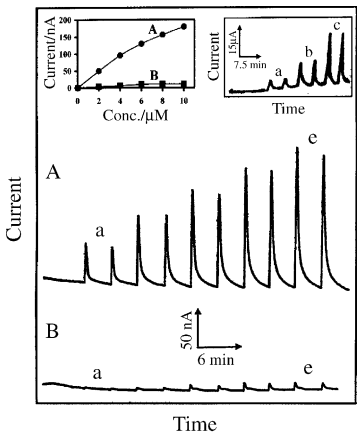

Fig. 6. Current–time FIA signals for successive additions of paraoxon in 2 μM steps (a–e) at the OPH/CVD-MWNT-modified (A) and OPH-modified (B) GC electrodes. Also shown (left inset) is the resulting calibration plots and FIA-amperometric response (right inset) for 250 nm (a), 500 nm (b) and 1000 nM (c) paraoxon. Operating potential for the right inset, +0.90 V; other conditions as in Fig. 5.

Fig. 6 compares flow-injection amperometric signals at the OPH (B) and OPH/CNT (A) electrochemical detectors for increasing levels of paraoxon in 2 μM steps (a–e). The OPH electrode does not permit convenient quantitation at these levels that are close to its detection limit. In contrast, well-defined peaks – with favorable signal-to-noise (S/N) characteristics – are observed at the OPH/CNT detector. The rapid increase in the current is coupled to a slower decay. The resulting calibration plot (of data point averages of the duplicate peaks; A in the left inset) is linear up to 4 μM, with a slight curvature thereafter. The sensitivity in the linear portion corresponds to 25 nA/μM. Also shown (in the right inset) are flow-injection signals at the OPH/CNT detector for 250 nm (a), 500 nm (b) and 1000 nm (c) paraoxon. These indicate convenient measurements at the nanomolar level, with a detection limit around 150 nM (based on S/N = 3).

Similar improvements in the sensitivity (versus the 'CNT-free' biosensor) are observed in Fig. 7 for analogous flow-injection measurements of methyl parathion. The corre-

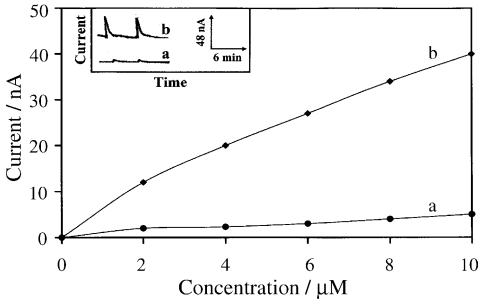

Fig. 7. Calibration plot for methyl parathion over the 2–10 μM range at the OPH (a) and CNT/OPH (b) biosensor detectors. Also shown (inset) are FIA-amperometric signals to 6 μM methyl parathion at the OPH (a) and CNT/OPH (b) detectors. Other conditions as in Fig. 5.

sponding calibration plots indicate ca. 10-fold greater signals over the 2–10 μM range (b versus a). The response is linear up to 2 μM, with a sensitivity of 6 nA/μM. Typical response peaks (for 6 μM methyl parathion) from this series are also shown in Fig. 7 (inset). The favorable S/N characteristics of the response at OPH/CNT detector indicate a detection limit of around 800 nM. Apparently, the biosensor is less sensitive to methyl parathion compared to paraoxon. The lower sensitivity of the biosensor towards methyl parathion compared to paraoxon is consistent with the reported class selectivity of OPH [21–23]. The coupling of the catalytic action of CNT with screen-printed OPH biosensors, that offer lower detection limits than glassy-carbon/OPH devices [4], should lead to further improvements in the detectability.

4. Conclusions

We have demonstrated the use of CNT for a greatly enhanced amperometric biosensing of OP compounds. The CNT-based transducer leads to a highly sensitive and stable detection of the enzymatically (OPH) liberated *p*-nitrophenol. Such coupling of OPH-based biorecognition and amperometric transduction on CNT transducers is advantageous over AChE-based CNT biosensors that lack specificity towards OP compounds and require addition of the substrate and an incubation period. The new OPH/CNT biosensor when coupled with hand-held (battery-operated) instruments thus offer great promise for rapid field screening of OP pesticides and nerve agents in natural water resources and food supplies, thereby providing the necessary warning/alarm and related proactive action. The use of OPH amperometric biosensors for direct (including remote) measurements in untreated natural water samples has been demonstrated [24]. Such on-site applications would greatly benefit from the use of disposable OPH/CNT-coated screen-printed electrodes. Potential interferences from easily oxidizable species should be considered in such real-life applications; the Nafion coating should alleviate such electroactive interferences.

Acknowledgements

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References

[1] S. Chapalamadugu, G.S. Chaudry, Crit. Rev. Biotechnol. 12 (1992) 357.
[2] J. Sherma, Anal. Chem. 65 (1993) 40R.
[3] A. Mulchandani, W. Chen, P. Mulchandani, J. Wang, K. Rogers, Biosens. Bioelec. 16 (2001) 225.
[4] A. Mulchandani, P. Mulchandani, W. Chen, J. Wang, L. Chen, Anal. Chem. 71 (1999) 2246.
[5] A. Mulchandani, S. Pan, W. Chen, Biotechnol. Progr. 15 (1999) 130.
[6] S. Iijima, Nature 354 (1991) 56.
[7] J. Wang, M. Musameh, Y. Lin, J. Am. Chem. Soc. 125 (2003) 2408.
[8] M.D. Rubianes, G.A. Rivas, Electrochem. Commun. 5 (2003) 689.
[9] M. Musameh, J. Wang, A. Merkoci, Y. Lin, Electochem. Commun. 4 (2002) 743.
[10] S. Hrapovic, Y. Liu, K. Male, J.H. Luong, Anal. Chem. 76 (2004) 1083.
[11] J. Wang, M. Musameh, Anal. Lett. 36 (2003) 2041.
[12] Y. Lin, F. Lu, J. Wang, Electroanalysis 16 (2004) 145.
[13] J. Wang, R.P. Deo, M. Musameh, Electroanalysis 15 (2003) 1830.
[14] N. Lawrence, R. P. Deo, J. Wang, Electroanalysis, in press.
[15] P. Mulchandani, A. Mulchandani, I. Kneva, W. Chen, Biosens. Bioelec. 14 (1999) 77.
[16] M. Terrones, Annu. Rev. Mater. Res. 33 (2003) 419.
[17] Z. Xu, X. Chen, X. Qu, S. Dong, Electroanalysis 16 (2004) 684.
[18] C.E. Banks, R.R. Moore, T.J. Davies, R.G. Compton, Electrochem. Commun. 16 (2004) 1804.
[19] J. Li, A. Cassell, L. Delzeit, J. Han, M. Meyyappan, J. Phys. Chem. B 106 (2002) 9299.
[20] N. Lawrence, R.P. Deo, J. Wang, Anal. Chim. Acta 517 (2004) 131–137.
[21] P. Mulchandani, W. Chen, A. Mulchandani, Environ. Sci. Technol. 35 (2001) 2562.
[22] A. Mulchandani, P. Mulchandani, I. Kaneva, W. Chen, Anal. Chem. 70 (1998) 4140.
[23] J. Wang, R. Krause, K. Block, M. Musameh, A. Mulchandani, M.J. Schoning, Biosens. Bioelec. 18 (2003) 255.
[24] J. Wang, L. Chen, A. Mulchandani, P. Mulchandani, W. Chen, Electroanalysis 11 (1999) 866.