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Organophosphorus hydrolase multilayer modified microcantilevers for organophosphorus detection

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Abstract

We report a biosensor based on organophosphorus hydrolase (OPH) multilayer modified microcantilever (MCL) for detection of organophosphorus compounds (OPs). The assay is based on substrate-dependent bending of the OPH functionalized MCLs. The cantilever bending amplitude at equilibrium was a function of the concentration of paraoxon with the dynamic range extending from 10^{-7} to 10^{-3} M. The lower detection limit of approximately 10^{-7} M for paraoxon was an order of magnitude better than the OPH-based potentiometric and optical biosensors based on pH modulation. There was a good intra-sensor and an acceptable inter-sensor reproducibility as evidenced by the standard errors of 5% and 15%, respectively. OPs measured using this technique included parathion and diisopropyl fluorophosphate (DFP) in the order of paraoxon > DFP > parathion. The conformational change of the OPH was most likely the main origin of MCL bending.

Keywords: Organophosphorus hydrolase; Microcantilever; Biosensor; Sensor; MEMS; Nanomechanical; Organophosphorus compounds; Nerve agent

1. Introduction

All nerve agents belong to the family of organophosphorus (OP) compounds, which are among the most toxic of known substances. The toxicity of these compounds arises from their irreversible binding to acetylcholinesterase that is essential to nerve impulse responses (Tammelin, 1957). Besides nerve gases, many pesticides also belong to the organophosphorus compound group. In an effort to feed the growing world population, the agriculture industry has increasingly taken the assistance of pesticides to increase the crop yield by fending off pest infestation. Their widespread use in agriculture may contaminate drinking water.

The threats posed by nerve gases and other OPs have made them important targets for detection. Analytical methods employed have included gas and liquid chromatographic methods with a variety of detection systems, e.g., mass spectrometry (Nassar et al., 2000), and atomic emission spectroscopy (Stuff et al., 1999). However, most of these methods require instrumentation that is emphatically non-portable, quite expensive, and very complex, which is not well suited to the *field* analysis of multiple samples.

There are numerous optical and electrochemical methods (Janata, 1990; Mulchandani et al., 1998a,b; Bachels and Schafer, 1998) used for detecting and identifying OP agents. More novel methods include ion mobility spectrometry (Steiner et al., 2002), immunoassays (Erhard et al., 1993), fiber optic microsphere array (Epstein et al., 2002), and surface acoustic wave (SAW) technology (Paddle, 1996; McGill et al., 1999), etc. Many of these methods are excellent for the purpose of real-time in-field analysis. However, the cost of most of these systems makes them impractical for placement in multiple sites.

Recent advances in the field of micro-electro-mechanical systems (MEMS) and their uses offer unique opportunities in the design of cost-effective analytical methods. In 1994, it was realized that microcantilevers (MCLs) could be made extremely sensitive to chemical and physical changes (Chen et al., 1994; Gimzewski et al., 1994; Thundat et al., 1994). MCLs can be mass produced through a typical lithography process,

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and can be readily integrated into a micro-electro-mechanical system (MEMS). To date, extremely sensitive chemical vapor sensors based on MCLs have been demonstrated using selective coatings on the cantilever. Examples include molecular beam (Bachels and Schafer, 1998), alcohol (Lang et al., 1999), mercury (Thundat et al., 1995a,b), mercaptans (Fritz et al., 2000), relative humidity (Thundat et al., 1995a,b), Cs^+ (Ji et al., 2000), DNA (Hansen et al., 2001), antigen-antibody interactions (Zhang and Ji, 2004), etc. Requirements such as sensitivity, small size, low cost, and simultaneous detection of multiple species make the MCL sensor approach very attractive.

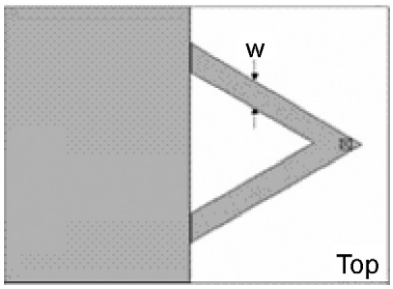
The unique characteristic of MCLs is their ability to deflect due to molecular adsorption- or binding-induced change in surface tension. These binding processes cause changes in surface stress on the MCL, which produces the upward or downward bending of the MCL. By recording the deflection magnitude or thermodynamics of the MCL, the concentration of target biological or chemical species can be measured. This characteristic of MCLs qualifies them for detecting species both in air and solution. From molecular point of view, the binding or adsorption result in electrostatic repulsion (Fritz et al., 2000) or attraction (Yang et al., 2003), steric effects (Berger et al., 1998), and intermolecular interactions (Tang et al., 2004) between molecules on the cantilever surfaces that alter the surface stresses on the cantilever. This is achieved by confining the adsorption to one side of the MCL. The key to MCL sensor development is to choose appropriate coatings for identification of specific chemical species.

Cu^{2+} complexes and acetylcholinesterase (AChE) (Rosenberry, 1975) have been used for selective and sensitive recognition of OPs. These recognition agents are generally used to develop one-time-use, disposable sensors for environmental characterization. These sensors cannot be readily regenerated due to their strong complexation with the phosphonyl group.

Organophosphorus hydrolase (OPH), on the other hand, can be used for continuous monitoring of OPs in the environment. Biosensors based on OPH have been reported recently for the detection of pesticides and nerve agents (Lai et al., 1994; Mulchandani et al., 1998a,b; Constantine et al., 2003a; Lee et al., 2003). The foundation of these devices is the hydrolysis of OPs, producing two protons, catalyzed in a highly specific manner by OPH:

$$\text{OP} + \text{H}_2\text{O} \xrightarrow{\text{OPH}} \text{OP-acid} + \text{R-OH(or R-SH)}. \quad (1)$$

For example, methyl paraoxon can be hydrolyzed by OPH to dimethyl phosphate and *p*-nitrophenol while releasing two protons. The resulting decrease in pH has been monitored and correlated to OPs concentration for constructing a potentiometric enzyme electrode. Other biosensors based on the detection of *p*-nitrophenol by amperometry and fiber optics have also been reported.



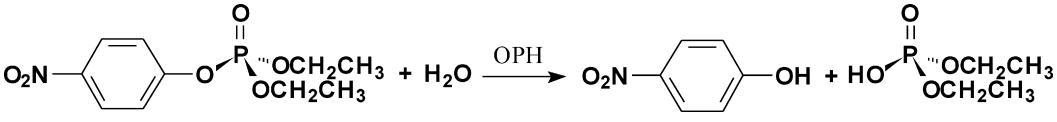
Scheme 1. Top view of MCL (Veeco Instruments, Santa Barbara, CA) used in these experiments.

Molecular recognition agent can be assembled or covalently linked to one side of the microcantilevers. Modification of the MCLs has been realized by self-assembled monolayers (SAMs) (Ji et al., 2000; Fritz et al., 2000), polymers (Lang et al., 1999; Battiston et al., 2001; Raiteri et al., 2001), sol–gels (Fagan et al., 2000), and hydrogels (Bashir et al., 2002; Zhang et al., 2003; Liu and Ji, 2004), etc. Recently, we introduced the nanoassembled layer-by-layer (LBL) approach for MCL modification (Yan et al., 2005). The layer-by-layer technique, which was developed in 1993 (Lvov et al., 1993), allows the formation of ultrathin, organized films on any surfaces through alternate adsorption of oppositely charged components, such as linear polyions and enzymes, primarily via electrostatic attraction (Lvov et al., 1995; Decher, 1997; Yoo et al., 1998; Dubas and Schlenoff, 1999). It is a simple modification process with nanoscale control of the film thickness. The required component could be positioned at a desired location in the film with nanometer precision. In this paper, we report an OPH multilayer modified MCL for the detection of OPs. The principle of detection is based on the deflection of the MCL due to the change in the film network mesh size upon the hydrolysis of OPs by the immobilized OPH. Unlike the earlier MCL-based sensors for OPs that were single use the OPH-based MCL are reusable.

2. Methods

2.1. Materials

In our experiments, we used commercially available silicon MCLs (Veeco Instruments, Santa Barbara, CA). The dimensions of the V-shaped silicon MCLs were 180 μm in length, 25 μm in leg width, and 1 μm in thickness. One side of these cantilevers was covered with a thin film of chromium (3 nm) and followed by a 20-nm layer of gold, both deposited by e-beam evaporation. Since gold film does not stick well to SiO_2, the thin chromium layer was used to improve adhesion. On the uncoated side of the commercial microcantilever was silicon with a 12–19-Å-thick naturally grown SiO_2 layer, which is called "native oxide". The shape of the MCL used in this work is shown in Scheme 1.



Wild type OPH was prepared as described previously (Mulchandani et al., 1998a,b). Sodium salt of 2-mercaptoethane sulfonic acid (MES), PSS (M_w = 70,000, powder), paraoxon, parathion, and diisopropyl fluorophosphates (DFP) were used as received from Sigma–Aldrich. Polyethyleneimine (PEI, 14%, M_w = 25,000, ρ = 1.043) was a gift from Max Planck Institute, Germany. A 10^{-2} M MES solution was prepared in ethanol. All other solutions were prepared in a 0.01 M pH 7 phosphate buffer (an additional 50 μM $CoCl_2$ in OPH solution).

2.2. Deflection measurement

The deflection experiments were performed in a flow-through glass cell (Digital Instruments, Santa Barbara, CA) similar to those used in atomic force microscopy (AFM). The MCL was immersed in the 0.01 M phosphate buffer (pH 7.0) solution. For continuous flow-through experiments, initially, the buffer solution was circulated through the cell using a syringe pump. A schematic diagram of the apparatus used in this study was previously reported (Ji et al., 2001). A constant flow rate was maintained during each experiment. Experimental solutions containing different concentrations of paraoxon were injected directly into the flowing fluid stream via a low-pressure injection port sample loop arrangement with a loop volume of 2.0 ml. This arrangement allows for continuous exposure of the cantilever to the desired solution without disturbing the flow cell or changing the flow rate. Since the volume of the glass cell, including the tubing, was only 0.3 ml, a relatively fast replacement of the liquid in contact with the cantilever was achieved. Microcantilever deflection measurements were determined using the optical beam deflection method. The bending of the cantilever was measured by monitoring the position of a laser beam reflected from the gold-coated side of the cantilever onto a four-quadrant AFM photodiode. We define bending toward the gold side as "bending up" and towards silicon side as "bending down". In case adsorption occurs on the gold surface, in general, the downward bending is caused by repulsion or expansion of molecules on the gold surface, which is so called compressive stress; vice versa, the upward bending is caused by attraction or contraction of molecules on the gold surface, which is called tensile surface stress. The cantilever was immersed in the buffer solution until a baseline was obtained and the voltage of the position-sensitive detector was set as background corresponding to 0 nm.

Cantilever based detection systems are prone to drift problems. We observed similar drifting problems in these experiments. The drifting problem, however, was alleviated after equilibrating the prepared cantilevers in the buffer solutions for 1 h. Typically, a good baseline (<4 nm noise) was obtained in one to two hours after equilibrating the cantilevers in the buffer solutions. In these experiments, we injected the sample after a good baseline was obtained.

2.3. MCL layer-by-layer OPH surface immobilization process

The electric charge of a polyelectrolyte in a solution depends on the isoelectric point (p*I*) of the polyelectrolyte and the solvent. At pH 7.0, the OPH is a positively charged polyelectrolyte (the p*I* of OPH is 8.0) (Constantine et al., 2003a).

Since microcantilever bending is generated from adsorption-induced surface stress from one side of the microcantilever, the key to surface modification technology is to control the formation of multilayers on only one surface of the microcantilever by choosing appropriate surface materials. In a typical multilayer formation procedure, the aimed target is alternately dipped into a polycation and polyanion solution, and the process is repeated several times for multilayer formation. When this procedure was applied, multilayer nanoassembly film formation was found on both sides of the cantilever. Recently, we reported a modified multilayer growing process (Yan et al., 2004) taking advantage of hydrophobic/lipophobic properties of the perfluorocarbon materials. In this method, (tridecafluoro-1,1,2,2-tetrahydrooctyl)triethoxysilane (TTS) was used to develop a thin perfluorocarbon monolayer on a silicon surface using a typical silicon surface modification procedure, and the polymeric multilayers were found only on the gold surface of the MCL.

The modified LbL procedure specific for MCL surface modification used in this experiment was as following: (A) A monolayer of MES was self-assembled on the gold surface of a MCL by immersing it in a 5 mM MES aqueous solution for 12 h, and then rinsing with deionized (DI) water three times. (B) The MCL was immersed in a 1.5 mg/mL of PEI solution for 10 min and then rinsed with flowing water at a flow rate equal or faster than 1 mL/s for 1 min. The MCL was then immersed in the 3.0 mg/mL oppositely charged polyelectrolyte (PSS in these experiments) for 10 min, followed with another rinsing with flowing water. (C) This cycle was repeated several times until a desired number of multilayers were reached.

This modified multilayer formation was applied to all the MCLs used in these experiments. In the case of enzyme-modified MCL, the cantilever was first coated with three bilayers of PEI/PSS to provide a solid base followed by three bilayers of OPH/PSS. The enzyme monolayer was formed by immersing the MCL in an OPH enzyme solution (4 mg/ML of OPH) for 10 min followed by rinsing in flowing DI water. The process was continued until three bilayers of OPH/PSS were formed on top of the PEI/PSS base. Each PEI/PSS bilayer was about 1–2 nm in thickness (Lvov et al., 1995). Although OPH/PSS bilayer thickness was not determined, based on the dimension of the OPH it is expected that the thickness was approximately 8 nm. Thus, we assume the thickness of the $(OPH/PSS)_3$ layer was approximately 25 nm.

3. Results and discussions

3.1. Deflection profiles of MCLs upon exposure to paraoxon

As shown in Fig. 1, repeat exposure of a MCL to a 10^{-3} M solution of paraoxon caused similar deflection amplitudes. The standard error for the three measurements was within 5% demonstrating good sample-to-sample reproducibility. Exposure of a 10^{-3} M solution of paraoxon to five different MCLs prepared under the same conditions also caused similar deflection ampli-

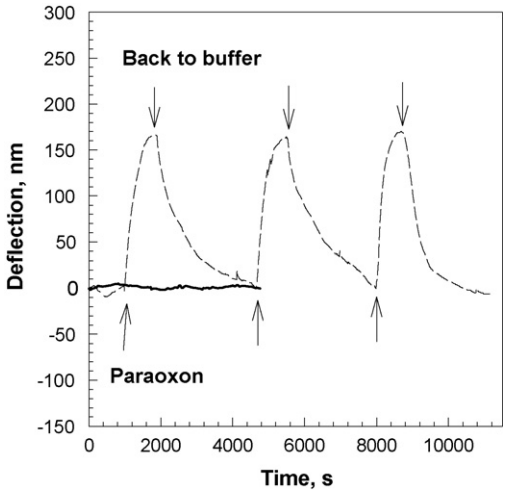


Fig. 1. Three replicates (dashed line) of bending responses as a function of time for a $(OPH/PSS)_3$ multilayer modified MCL following injection of a 10^{-3} M paraoxon in 0.01 M phosphate buffer (the injection point is indicated with arrows) at 4.5 mL/h flow rate. Control experiments were performed by exposing a $(PSS/PEI)_6$ modified MCL to a 10^{-3} M solution of paraoxon (solid line).

tudes and the standard error was within 15% indicating an acceptable MCL-to-MCL reproducibility. The MCLs bent up showing the multilayer film shrunk upon exposure to paraoxon. Control experiments were performed by exposing a $(PSS/PEI)_6$ modified MCL to a 10^{-3} M solution of paraoxon. No deflection of the cantilever was observed (also shown in Fig. 1).

3.2. Relationship between deflection amplitudes and the concentrations of paraoxon

Fig. 2 shows the bending response of an OPH multilayer modified MCL to various concentrations of paraoxon. The MCL

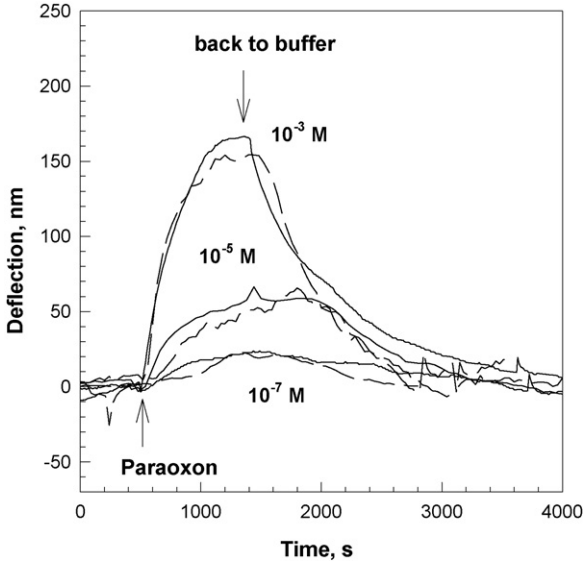


Fig. 2. Dynamics of bending amplitude for a freshly-made $(PSS/OPH)_3$ modified MCL (solid line) and a $(PSS/OPH)_3$ multilayer modified MCL (dashed line) after storing in a dry state in the refrigerator at 4 °C for 2 months as a function of paraoxon concentration in 0.01 M phosphate buffer (the injection point is indicated with arrows).

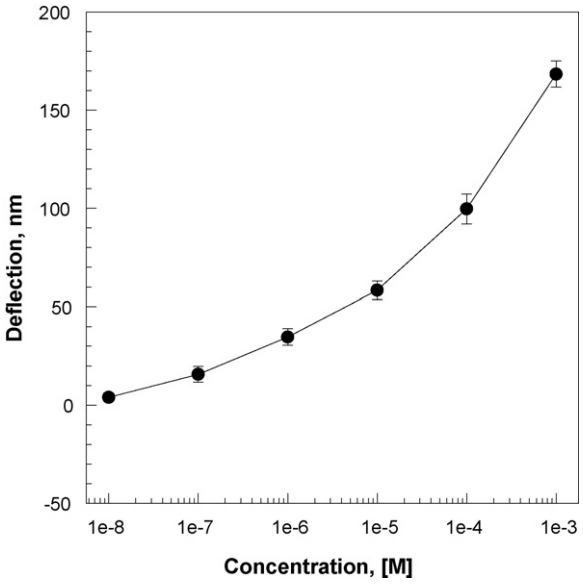


Fig. 3. Correlation between maximum bending amplitudes of a $(PSS/OPH)_3$ modified MCL and paraoxon concentration in a 0.01 M phosphate buffer.

deflection was fully reversible and increased as the concentrations of paraoxon increased. The maximum deflection was a function of the paraoxon concentration and had a wide dynamic range (Fig. 3). The detection limit was approximately 10^{-7} M. This is an order of magnitude better than the OPH-based potentiometric and optical biosensors that also measured the pH modulation (Mulchandani et al., 2001).

3.3. Stability of the OPH-modified MCL

The OPH-multilayer modified MCLs demonstrated excellent stability for more than 2 months when stored in a dry state at 4 °C as evidenced by the similar profiles and bending amplitude (Fig. 2). However, the OPH activity was lost in less than 2 weeks when the multilayer film modified MCL was stored in the buffer solution at room temperature (data not shown).

3.4. Thermodynamic and equilibrium study of multilayer shrinking

Recently, Thundat and coworkers (Subramanian et al., 2002) and our group (Yan et al., 2005) have concluded that the enthalpy change of the enzymatic reaction does not contribute to the deflection of the MCLs. The possible contributions to the MCL bending include pH change and the conformational change of the enzymes. In order to interpret the mechanism of the enzymatic reaction induced stress on the MCLs at the molecular level, we studied the thermodynamics and kinetics of the MCL response to paraoxon catalyzed paraoxon hydrolysis and correlated it with changes in the bending response of the MCL. The following provides the details of this investigation.

3.4.1. OPH amount in OPH/PSS bilayer-modified MCL

The MCLs used in these experiments were V-shaped SiO_2 microcantilevers that have dimensions of 180 μm × 38 μm ×

1 μm and a layer of 20 nm gold on one surface. The MCL surface area was 1.12×10^{-2} mm^2. Assuming the thickness of the $(OPH/PSS)_3$ multilayer film was approximately 25 nm, the volume of the $(OPH/PSS)_3$ film was approximately 2.8×10^{-13} L. Based on the molecular weight of 68.8 kDa for OPH dimer and the reported density of 267 ng/0.586 cm^2 or 0.456 μg/cm^2 corresponding to 6.63×10^{-12} mol/cm^2 or 7.43×10^{-16} mol on the MCL surface for the OPH/PSS layers (Lee et al., 2003), the concentration of the OPH in the multilayer film was calculated to be 2.65×10^{-3} M.

3.4.2. OPH catalyzed paraoxon hydrolysis reaction velocity

Consider the OPH-catalyzed reaction to follow Michaelis–Menten model represented by the following equation (Lee et al., 2003):

$$\text{velocity}\,(\text{m s}^{-1}) = \frac{V_{\max}[\text{OP}]}{[\text{OP}] + K_{\text{m}}^{\text{OP}}} = \frac{k_{\text{cat}}[\text{enzyme}][\text{OP}]}{[\text{OP}] + K_{\text{m}}^{\text{OP}}}, \quad (2)$$

where $V_{\max}$ is the maximum reaction rate (i.e. at saturation) and K_m is the substrate concentration at which half the maximum velocity rate occurs. $V_{\max}$ can be expressed as a function of the total enzyme concentration and the irreversible forward rate of reaction (k_{cat}). Additionally, we assume that at equilibrium the paraoxon concentration inside the multilayer film was constant and was equal to the concentration of paraoxon injected; this assumption was reasonable because of the very small film thickness (~25 nm), the high diffusion constant of paraoxon, and the fast liquid flow rate (0.4 mm/s) over the MCL surface. Further, based on the report by Lee et al. (2003) that the kinetic parameters of OPH in multilayers were close to those in solutions, we used the values of kinetic parameters for OPH in solution, $k_{\text{cat}} = 1.5 \times 10^4$ s^{-1} and $K_{\text{m}} = 0.12$ mM reported by diSioudi et al. (1999). Solving Eq. (2) with the above assumptions the theoretical/predicted OPH catalyzed paraoxon hydrolysis velocity in the OPH/PSS bilayer of the MCL as a function of paraoxon concentration was determined (Fig. 4).

3.4.3. pH versus paraoxon concentration profile

The OPH catalyzed hydrolysis of paraoxon produces *p*-nitrophenol and *O*,*O*-diethyl phosphoric acid, that are responsible in lowering the pH inside the multilayer film and changing the structure of the multilayer film. The formation rate of *O*,*O*-diethyl phosphoric acid or *p*-nitrophenol on the MCL surface can be expressed as

$$\frac{\partial P}{\partial t} = V - D_{\text{P}}\frac{\partial^2 P}{\partial x^2}, \quad (3)$$

where P is the concentration of products, D_{P} the diffusion constant of phosphoric acid or *p*-nitrophenol in the $(OPH/PSS)_3$ multilayer film, and V is the reaction rate described above. At steady state, $\partial P/\partial t = 0$, therefore

$$D_{\text{P}}\frac{\partial^2 P}{\partial x^2} = V. \quad (4)$$

The diffusivity of organic compounds through the multilayers (10^{-8} cm^2/s) is generally two orders of magnitude smaller than in water (Rani et al., 2004; Walhout and Burden, 2004). For instance, the diffusion constants for ascorbic acid (D_{A}) in multilayer film is 4.7×10^{-8} cm^2/s compared to 5.8×10^{-6} cm^2/s in water (Walhout and Burden, 2004). No diffusion constant of *O*,*O*-diethyl phosphoric acid and *p*-nitrophenol in the OPH/PSS multilayer film has been reported, however, these constants can be estimated using the Stokes–Einstein equation (Einstein, 1905):

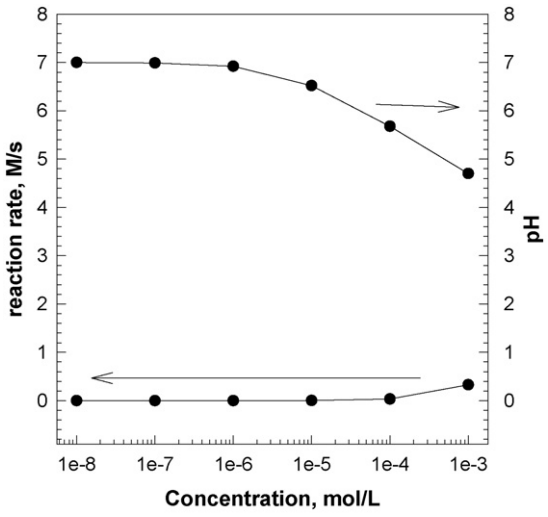


Fig. 4. The calculated/predicted paraoxon hydrolysis rate (*y*-axis on the left) according to Eq. (2) in a $(PSS/OPH)_3$ film on the modified MCL and the resultant pH (*y*-axis on the right) in the $(OPH/PSS)_3$ multilayer film on the modified MCL as a function of paraoxon concentration.

$$\frac{D_1}{D_2} = \left(\frac{M_2}{M_1}\right)^{1/3}. \quad (5)$$

According to this equation, similar molecular weight compounds, such as ascorbic acid ($M_{\text{A}} = 176$ g/mol), *O*,*O*-diethyl phosphoric acid ($M_{\text{P}} = 154$ g/mol), and nitrophenol ($M_{\text{N}} = 139$ g/mol), will have a similar diffusion constant. Using Eq. (5), the diffusion constant of *O*,*O*-diethyl phosphoric acid (D_{P}) and 4-nitrophenol (D_{N}) were calculated to be 5.14×10^{-8} and 5.05×10^{-8} cm^2/s, respectively.

Solving Eq. (4) gave the concentration of diethyl phosphoric acid and 4-nitrophenol in the multilayer film on the MCL surface at equilibrium as a function of the paraoxon concentration injected. The pK_{a} of diethyl phosphoric acid and 4-nitrophenol are 2.0 and 7.0, respectively. The concentration of protons (in the form of pH) versus the concentration of paraoxon injected was then calculated and is shown in Fig. 4.

3.4.4. Effect of pH on cantilever bending

In order to determine the effect of pH on the MCL bending, $(OPH/PSS)_3$ multilayer modified MCLs were exposed to different pH 0.01 M phosphate buffer. As shown in Fig. 5, changing the MCL environment pH from 7 to 4 produced ~30 nm deflection only. This deflection is much less than the amplitude of the MCL bending upon exposure to paraoxon (Fig. 3), suggesting that pH change was not the only contribution to MCL bending.

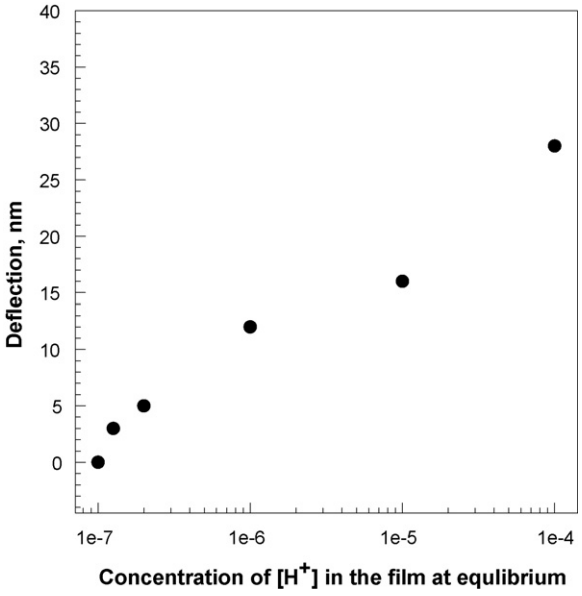


Fig. 5. Maximum bending responses for a $(OPH/PSS)_3$ modified MCL after injection of a 0.01 M phosphate buffer with different $[H^+]$ (pH) at 4.5 mL/h flow rate. The MCL was pre-equilibrated in a 0.01 M buffer solution with pH 7.0.

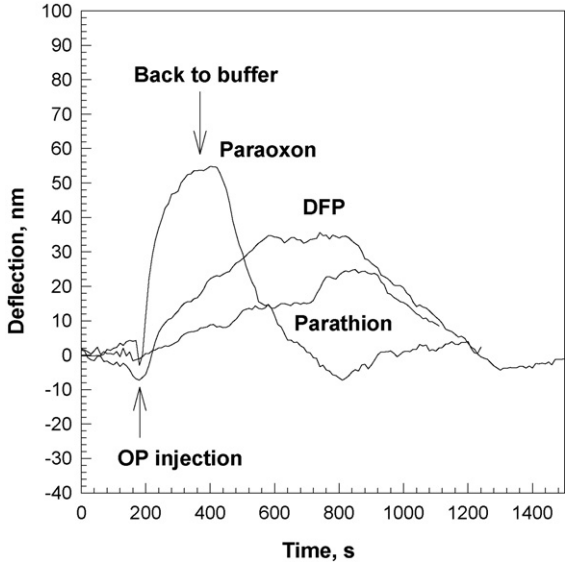


Fig. 6. Bending responses as a function of time for a $(OPH/PSS)_3$ modified MCL upon exposure to 10^{-3} M paraoxon, parathion, and DFP at 40 mL/h flow rate. At this flow rate, the OPs flux through the fluid cell in 3 min. The MCL may not reach it maximum bending in this time scale.

3.5. Conformational change of the OPH enzyme

OPH is a dimeric bacterial protein with a molecular weight of approximately 70 kDa. The corresponding dimensions of the dimer are 90 Å × 56 Å × 40 Å (Lei et al., 2002).

Since the OPs in these experiments were electrically neutral, the possible bending mechanism based on the absorption-induced electrostatic repulsion or attraction between OPs and OPs with OPH or PSS can be ruled out. Steric effects and intermolecular interactions between OPs were too weak to contribute to the surface stresses changes on the cantilevers because of the long-distance between two OPH-bound OPs.

Recently, the conformational changes of OPH in multilayer film, upon exposure to paraoxon have been investigated by Leblanc and coworkers (Constantine et al., 2003a,b,c,d; Zheng et al., 2004; Ji et al., 2005). Using circular dichromism (CD) measurements, it was determined that in presence of paraoxon the β-strand of OPH increased from 3.37% to 17.33%, that was attributed to the binding of paraoxon with OPH.

Based on the above results, it is more likely that the conformational change of OPH upon binding of paraoxon and the subsequent interactions between OPHs and OPH with PSS plays a major role in the multilayer film volume change and the consequent bending response of the MCLs.

3.6. Response to other OPs

It's known that wild-type OPH has higher selectivity towards paraoxon than other OPs, such as parathion and DFP. For instance, the k_{cat}/K_M value of OPH for DFP is four order of magnitude lower than that for paraoxon (diSioudi et al., 1999). MCLs' responses to parathion and DFP were measured and the results showed the expected smaller amplitude and slower bending rate response of the OPH-multilayer modified MCLs (Fig. 6) than that of paraoxon. It was also noticed that after the parathion or DFP were fluxed out from the fluid cell, the MCL kept bending up for the next 400 s before bending back. One possible explanation of this phenomenon is a relatively slower post-binding conformational change of the OPH, supporting the proposed OPH conformational change of induced mechanism for MCL bending. It is possible to obtain the binding constants of these OPs with OPH using MCL bending dynamics. These phenomena merit further investigation in the future.

4. Conclusions

OPH was immobilized on the MCL surface using layer-by-layer nanoassembly technology for OPs detection. The detection limit for paraoxon was approximately 10^{-7} M. This is an order of magnitude better than the OPH-based potentiometric and optical biosensors that measured the pH modulation. The multilayer modified MCLs responded to paraoxon, parathion, and DFP at different bending amplitude and bending rate. The bending mechanism investigation suggests that the conformational change of the OPH may be the primary contributor of the MCL bending. The results also suggest that research in molecular biology to increase the first binding constant of the OP to OPH could be a new strategy to improve the sensitivity of enzyme based biosensors. This strategy would make it possible to develop a sensor with both high sensitivity and continuous monitoring of target molecules.

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