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Short communication

Potentiometric sensor for dipicolinic acid

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Abstract

A potentiometric chemosensor for selective determination of dipicolinic acid (2,6-pyridinedicarboxylic acid, DPA) was developed based on the surface imprinting technique coupled with a nanoscale transducer: an indium tin oxide (ITO)-coated glass plate. The sensor fabrication conditions, optimal recognition condition, as well as selectivity, sensitivity, and stability of the DPA sensor have been investigated. The DPA sensor could recognize DPA from 3,5-pyridinedicarboxylic acid. Potentiometric measurements demonstrated selective detection of DPA in a concentration range of 1.5×10^{-6} to 0.0194 M. The response time of DPA sensor for 4×10^{-4} M DPA was 25 s. The potentiometric response of the DPA sensor to DPA is at 90% of its initial magnitude after 550 times measurement. The viability of such a modified ITO electrode in the presence of other inorganic, organic, and biological materials was probed.

Keywords: Dipicolinic acid; Spores; Sensor; Potentiometry; Surface imprinted polymer

1. Introduction

The rapid identification of *Bacillus anthracis* spores is of importance because of its potential use as a biological warfare agent (Barnaby, 1997). In consideration of emergency response plans for such an attack with biological weapons, the development of an inexpensive, rapid, and sensitive field portable sensor is extremely valuable for military and civilian use. Dipicolinic acid (2,6-pyridinedicarboxylic acid, DPA) is a major constituent of bacterial endospores (including *B. anthracis* spores), comprising 5–14% of their dry weight after extraction (Murrell, 1969). The analysis of DPA is of importance in studies of sporulation, germination, and spore structure (Setlow, 2003), and its presence is considered diagnostic for the bacterial endospores. Several methods such as spectrophotometry (Janssen et al., 1958; Vullev and Jones, 2000), ultraviolet spectrophotometry (Lewis, 1967; Scott and Ellar, 1978), Fourier transform infrared spectroscopy (Goodacre et al., 2000), liquid chromatography (Warth, 1979; Paulus, 1981) luminescence (Pellegrino et al., 1999; Fell et al., 2001; Rosen et al., 1997; Scholl et al., 2000), electron monochromator mass spectrometry (Beverly et al., 2000), fluorescence (Hindle and Hall, 1999), pyrolysis–gas chromatography/ion mobility spectrometry (Snyder et al., 1996) and pyrolysis mass spectrometry (Goodacre et al., 2000; Beverly et al., 1996) have been applied for DPA detection but they use large and expensive instruments and require sophisticated, often extensive analysis procedures.

Molecular imprinting technique (Sellergren, 2001) has been widely adopted to construct robust and specific molecular recognition sites for sensor application. Meanwhile, the proton sensitive indium tin oxide (ITO) has been proved to be a valuable transducer with high sensitivity for a sensor (Zhou et al., 2003). Previously, we have reported the detection of chiral amino acids, as well as methylphosphonic acid with surface imprinted ITO electrodes (Zhou et al., 2003, 2004). A mixed monolayer of polysiloxane and template molecules was formed on the ITO glass surface. After the removal of the template, the sensor showed molecular recognition ability towards the target molecule, which was used as template during surface imprinting process. The sensitivity of ITO to proton was investigated at different pH ranges and the response revealed discontinuous slopes (e.g. 30 mV/decade in a pH range of 3.5–5 and 180 mV/decade over 2.5–3.5) with the change of the pH and showed an unusually steep slope for monovalent proton compared to other electrodes, which exhibit slopes of 59 mV/decade (Zhou et al., 2003). The thermal behavior of

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the monolayers prepared by the above method was shown to result in an unusual mechanical, chemical, and electrical stability (Cohen et al., 1986). Heating the film only caused slight disorientation of the chain in octadecylsiloxane (ODS) and no sharp melting point was observed for temperature up to 140 °C (Cohen et al., 1986). The demonstration of many of the predicted advantages of the integration of surface imprinting and the transduction process has encouraged the exploration of the same principle for sensing of other analytes.

In this work, the above novel concept will be extended to detect DPA, the biomarker of endospores. The viability of this DPA sensor in the presence of potential environmental constituents is also presented. Since the atmosphere contains appreciable quantities of inorganic, organic, and biological materials, samples from all three of those categories were examined.

2. Experimental

2.1. Materials

Chloroform and carbon tetrachloride were distilled over CaH_2. Other chemicals were used without further purification. All the substances used in these experiments and their symbols are as following: dipicolinic acid, $NaNO_3$ (nan), NH_4NO_3 (amn), $(NH_4)_2SO_4$ (ams), $CaCO_3$ (cac), sodium benzoate (nab), and L-tyrosine (tyr) were purchased from Aldrich (Milwaukee, WI). L-Glucose (glu), D(+)-malic acid (mal), nutrient broth (nub), DL-tryptophan (trp), tryptone (nbt), riboflavin (rib), D-phenylalanine (phe), β-NAD (nad), *Bacillus subtilis* in nutrient broth (ebs, ATCC no. 9372), *Micrococcus luteus* in enriched nutrient broth (eml, ATCC no. 4698), yeast *Candida utiliz* (cuy, ATCC no. 9226), lyophilized *B. subtilis* (lbs, ATCC no. 6633), lyophilized *Aerobacter aerogenes* Type I (laa), lyophilized *Azotobacter vinelandii* (lav, ATCC no. 12518), and lyophilized *Pseudomonas fluorescens* Type IV (lpf, ATCC no. 12633) were from Sigma (St. Louis, MO). Defatted German cockroach (gci), nondefatted Eastern cottonwood pollen (ecp), nondefatted cultivated wheat pollen (cwp), nondefatted desert ragweed pollen (drp), defatted *Aspergillus flavus* mold (afm), and defatted *Cladosporium herbarum* mold (chm) were obtained from Greer Laboratories (Lenier, NC). These materials were chosen as substances that might be present in a sample collected from the atmosphere. Unless otherwise specified, all solutions and suspensions were prepared from water purified using a Millipore system (resistivity: 18.2 MΩ cm). A sample of nutrient broth with tryptone, a standard microbiological growth medium, was made with 8 g/L nutrient broth and 10 g/L tryptone in distilled sterile water. The ITO-coated glass plates (CG-50IN-CUV) used as indicator electrodes were obtained from Delta Technologies, Ltd. (Stillwater, MN).

2.2. Sensor construction

DPA (template) and octadecyltrichlorosilane (OTS, $C_{18}H_{37}SiCl_3$) were coadsorbed on the polar solid surface of the ITO glass plate (effective surface area about 0.7 cm × 4 cm) from a $CHCl_3/CCl_4$ solution (2:3 v/v) at 0 °C for a period of time. Then, the electrode was rinsed with 30× 1 mL $CHCl_3$. An ODS modified electrode without the DPA cavity was also prepared as a control.

2.3. Experimental setup and measurement

All measurements were made in 50 mL of 0.1 M phosphate buffered saline (PBS: pH 7.2)/0.15 M NaCl in a 100 mL working volume electrochemical cell, equipped with a magnetic stirrer. The two-electrode system consisted of an Ag/AgCl (saturated KCl) reference electrode and the DPA ODS/ITO sensor or the control as the working electrode. The potentials of the DPA ODS/ITO indicator electrodes were measured against the Ag/AgCl reference electrode with an Orion 920A potentiometer. Thereafter, the potential responses of the sensor were defined as the difference between the electrode potential with and without DPA in solution (i.e. $\Delta E = E_1 - E_0$, where E_0 and E_1 are the electrode potentials before and after DPA addition, respectively). The potential responses of the sensor as a function of pH were determined using the same two-electrode system described above but with one more pH electrode to record the changes in the pH of the bulk solution.

3. Results and discussion

3.1. The influence of template

A mixed monolayer of DPA template molecules and a silane component was assembled on the polar surface of the ITO glass when glass plate was immersed in a mixture solution of DPA and OTS. The hydrophobic layer of organosiloxane groups was covalently bound onto the ITO electrode in the presence of the DPA. The physically adsorbed DPA was removed by repeated extractions with $CHCl_3$. After the extraction, the imprints from the template were generated in the polysiloxane monolayer on ITO surface demonstrating structural features. Such created imprints are assumed to possess specific geometrical features are complementary to undissociated DPA. The DPA ODS/ITO electrode (with template) when applied to DPA solution, had greater potential responses than control. This suggests that the surface imprinted electrode recognizes DPA by offering an access for DPA molecules to the ITO substrate. Meanwhile, the control (without template) also produced some potentiometric responses possibly due to porosity of polysiloxane, which was consistent with our previous work (Zhou et al., 2003, 2004). Such a response