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Short communication

Biochemical gas sensor (bio-sniffer) for ultrahigh-sensitive gaseous formaldehyde monitoring☆

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ABSTRACT

An ultrahigh-sensitive fiber-optic biochemical gas sensor (bio-sniffer) for continuous monitoring of indoor formaldehyde was constructed and tested. The bio-sniffer measures gaseous formaldehyde as fluorescence of nicotinamide adenine dinucleotide (NADH), which is the product of formaldehyde dehydrogenase (FALDH) reaction. The bio-sniffer device was constructed by attaching a flow cell with a FALDH immobilized membrane onto a fiber-optic NADH measurement system. The NADH measurement system utilizes an ultraviolet-light emitting diode (UV-LED) with peak emission of 335 nm as an excitation light source. The excitation light was introduced to an optical fiber probe, and fluorescence emission of neighboring NADH, which was produced by applying formaldehyde vapor to the FALDH membrane, was concentrically measured with a photomultiplier tube. Assessment of the bio-sniffer was carried out using a standard gas generator. Response, calibration range and selectivity to other chemical substances were investigated. Circulating phosphate buffer, which contained NAD^+, available for continuous monitoring of formaldehyde vapor. The calibration range of the bio-sniffer was 2.5 ppb to 10 ppm, which covers the guideline value of the World Health Organization (80 ppb). High selectivity to other gaseous substances due to specific activity of FALDH was also confirmed. Considering its high sensitivity, a possible application of the bio-sniffer is continuous indoor formaldehyde monitoring to provide healthy residential atmosphere.

1. Introduction

Formaldehyde (FA) is a reactive and flammable chemical, which is well known as one of the volatile organic compounds (VOCs). FA was upgraded in classification from group 2A (probably carcinogenic to humans) to group 1 (carcinogenic to humans) by International Agency for Research on Cancer (IARC) in 2004 (Cogliano et al., 2005). The major experiences induced by FA exposure (in short term) are itching or burning sensations in the eyes (Andersen and Molhave, 1983), coughing and throat irritations (Kulle et al., 1987). Health damages for long-term exposure to FA are also reported. A combination of respiratory disease, allergic dermatitis and other ailments, so-called sick building syndrome (SBS), is associated with chronic exposure to low-level FA (Gamble, 1983; Maibach, 1983; Beall and Ulsamer, 1984). Risk of nasal cavity carcinoma increases with high-level, long-term occupational exposure (Vaughan et al., 1986a,b; Beane Freeman et al., 2009). For this reason, guideline level of FA exposure is set to 80 ppb by the World Health Organization (WHO). Nonetheless, FA is widely used for many industrial purposes. It is typically used in pressed-wood products, adhesives and coatings. Outgassing from resins in furniture, wallpaper or paints can be the major indoor FA emission sources. FA emissions in residential atmosphere cause SBS even in the level lower than 80 ppb. For instance, risk of asthma increases in children exposed to FA level of 49 ppb (Rumchev et al., 2002). FA is also used as formalin (saturated solution of formaldehyde, 37% by mass) in medical laboratories for its disinfection and preservative efficacy. Local FA level, in particular, becomes several parts per million in a pathology laboratory, which consumes large amount of formalin.

Accurate determination of environmental FA and control of indoor FA level can be effective approaches to prevent people from health damages. Since formaldehyde emission depends

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on temperature and humidity (Myers, 1985), indoor FA level shows large fluctuation throughout the day. This indicates that continuous monitoring of indoor FA is also requested for this purpose. Previously, relationships between indoor FA levels and health hazards were investigated by analyzing passive filters or sampling devices using high-performance liquid chromatography (HPLC) (Levin et al., 1985) or gas chromatography (GC) (NIOSH, 1994). Although chromatographic methods provide analytical determination of ppb to sub-ppb FA, they are too time-consuming for monitoring purposes. Semiconductor gas sensors based on gas-sensitive NiO films (Dirksen et al., 2001; Lee et al., 2007) are preferred indoor FA monitoring devices for their stability, reduced cost and fast response. Although these sorts of FA sensors have been improved in structures and materials thus far (Lv et al., 2008), they still suffer from several problems in sensitivity and selectivity. Restriction in selectivity is a fundamental problem in solid-state gas sensors based on changes in electrical conductivity of the device following adsorption of gaseous substances (Tonoike, 1988; Chen and Colbow, 1992; Maekawa et al., 1992).

On the other hand, enzyme-based biosensors allow highly specific determination of various chemical substances with a simple and low-cost system. These sorts of sensors or measurement techniques are widely used in the fields of food industry, agriculture, biomedical or environmental analysis (Hart and Wring, 1997; Mello and Kubota, 2002; Velasco-Garcia and Mottram, 2003; Farré and Barcelo, 2003). A nicotinamide adenine dinucleotide (NAD^+)-dependent biosensor, in particular (Svensson et al., 2005; Achmann et al., 2008; Demkiv et al., 2008), is a promising candidate for indoor FA monitoring. An NAD^+-dependent biosensor measures redox ratio of NAD^+/NADH to determine the concentrations of target substrates. Fluorescent approach is one of the useful methods for NADH determination (Duysens and Amesz, 1957; Harrison and Chance, 1970; Farabegoli et al., 2003). Formaldehyde dehydrogenase (FALDH) can be a key enzyme for highly selective FA assay (Vianello et al., 1996; Kiba et al., 1999; Ben Ali et al., 2006). Since enzymes recognize substrate by their steric structure, they lose their activities under dry conditions. In the previous study, we reported a biochemical gas sensor (bio-sniffer) that utilizes a flow cell for circulating buffer solution to prevent enzyme from deactivation (Mitsubayashi et al., 2006; Kudo et al., 2010). Biochemical recognition system allows highly selective and highly sensitive gas monitoring with a simplified structure. On the other hand, we also developed a fiber-optic NADH measurement system using an ultraviolet-light emitting diode (UV-LED) with a peak emission at 335 nm (Kudo et al., 2009). A simplified FA monitoring system with high selectivity, which is suitable for indoor FA monitoring, can be expected by combining these methods.

In this study, a highly sensitive fiber-optic bio-sniffer using FALDH immobilized membrane for gaseous FA monitoring was developed. The bio-sniffer measures FA concentration as fluorescence of NADH. In order to obtain sufficient sensitivity for indoor FA monitoring, a photomultiplier tube (PMT) was employed as a fluorescence detector. The UV-LED based excitation system enabled the bio-sniffer to be a simplified and miniaturized gas sensor for its low power consumption and low heat generation. LEDs also provided advantages in security and controllability compared with general UV lamps. This paper reports the construction, characteristics and possible applications of the fiber-optic bio-sniffer.

2. Experimental

2.1. Reagents

FALDH immobilized membrane was prepared by immobilizing FALDH (EC 1.2.1.1, 1 units/mg, solid, from *Pseudomonas* sp., Funakoshi Co., Ltd., Tokyo, Japan) with an activity of 1 unit/mg of protein onto a hydrophilic PTFE (H-PTFE) membrane filter (Porosity: 80%, Pore size: 0.2 μm, JGWP14225, Millipore Co., USA) as well as our previous paper (Kudo et al., 2008). 2-methacryloyloxyethyl phosphorylcholine (MPC) copolymerized with 2-ethylhexyl methacrylate (EHMA) was used for enzyme immobilization. MPC-co-EHMA (PMEH) was synthesized using free radical-polymerization method. NADH (β-nicotinamide adenine dinucleotide, reduced from disodium salt, Oriental Yeast Co., Ltd., Japan) was prepared for characterization of the NADH measurement system. Phosphate buffer solution (PB, pH 8.0, 80 mmol/L) containing 20 mmol/L NAD^+ (β-NAD^+: Oriental Yeast Co., Ltd., Japan) was prepared in order to wet the enzyme-immobilized membrane during gas measurement.

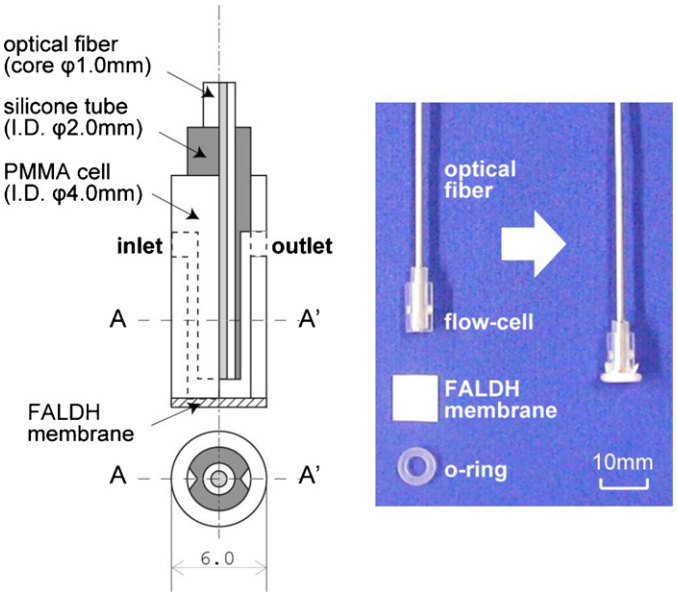

Fig. 1. Structure of flow cell with FALDH membrane (left hand) and the photograph of the optode (right hand).

2.2. Construction of the fiber-optic FALDH bio-sniffer

Above all, a fiber-optic NADH measurement system was constructed. A UV-LED (UVTOP® BL335, λ = 335 nm, Sensor Electronic Technology Inc., USA) was employed as an excitation light source. Emission of the UV-LED was coupled into a branched optical fiber (BIF600-UV/VIS, Ocean Optics Inc., USA) using custom-fabricated UV-LED power supply (KLV Co., Ltd., Japan) with an adjustable optical fiber connector. A PMT (C9692, Hamamatsu Photonics Co., Ltd., Japan) was connected to the other branched terminal. An optical fiber probe (F1000-900, core diameter: 1.0 mm, flat end, Ocean Optics Inc., USA) was connected to the assembled terminal of the branched optical fiber. In order to reduce background level, excitation light and fluorescence were filtered using band-pass filters (330–350 nm and 490–510 nm), respectively. Thus, fluorescence of NADH at the neighborhood of the optical fiber probe could be measured coaxially by the PMT.

A formaldehyde-sensitive optode was constructed by attaching a flow cell with FALDH immobilized membrane onto the optical fiber probe. The flow cell was fabricated by assembling a silicone tube (Ø2 mm) and a cylindrical PMMA cell (Ø4 mm) with inlet/outlet as shown in Fig. 1. FALDH immobilized membrane was prepared by curing a mixture of PMEH solution (1 μL/cm^2) and FALDH (50 units/cm^2), which was spread on the H-PTFE membrane filter, for 180 min under 4 °C. The FALDH membrane was cut into 1 cm × 1 cm and tightly fixed onto the end of the PMMA tube using a silicone O-ring. A FALDH bio-sniffer was obtained by connecting