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Highly sensitive and rapid gas biosensor for formaldehyde based on an enzymatic cycling system

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ABSTRACT

An enzymatic cycling system for detecting formaldehyde in water and indoor environments has been developed. The system is based on both the dehydrogenation of formaldehyde and the reduction of nicotinamide adenine dinucleotide (NAD^+) in the presence of formaldehyde dehydrogenase. NADH generated in the reaction then reduces 4-[3-(2-methoxy-4-nitrophenyl)-2-(4-nitrophenyl)-2H-5-tetrazolio]-l,3-benzene disulfonate sodium salt (WST-8) to yellow WST-8 formazan in the presence of enzyme diaphorase. The quantity of formaldehyde can be detected by the color change resulting from the formation of WST-8 formazan. The assay solution was optimized with respect to the pH, type of buffer, and enzyme and WST-8 concentrations. The limit of detection for formaldehyde in water was found to be 10 ppb after 3 min. The assay solution also displayed a highly selective response to formaldehyde over other pollutant gases present in indoor air. In addition, formaldehyde gas was detected after bubbling prepared formaldehyde/air mixtures with known formaldehyde concentrations in assay solutions. The limit of detection for formaldehyde gas was 1.5 ppb after 5 min. This system can quickly and accurately detect low concentration of formaldehyde in water and gas.

1. Introduction

Formaldehyde, the simplest aldehyde, is a colorless gaseous organic compound that is used as a preservative in cosmetics and in the production of resins in wood products, vinyl flooring, and other plastics. The International Agency for Research on Cancer classified formaldehyde as a Group-1 carcinogen based on evidence in humans of nasopharyngeal cancer and studies on the carcinogenicity of auramine in humans and animals [1]. Exposure to formaldehyde can cause many potential health risks, such as central nervous system damage, immune system disorders, asthma, and nasopharyngeal cancer [2–4]. According to the World Health Organization (WHO) guidelines for residential indoor air quality, the formaldehyde exposure limit is defined as 80 ppb [5]. This criterion is insufficient for asthma among young children, however, because the risk of asthma increases due to the chronic aspiration of formaldehyde even at levels below 50 ppb [6].

In 2012, relatively high concentrations of formaldehyde (>80 ppb) were detected in drinking water at water purification plants in Japan. The high concentrations of formaldehyde were attributed to the presence of hexamethylenetetramine in industrial waste water, which reacted with chlorine used as a disinfectant. The detection led to the suspension of water supply, affecting many residents that receive water from these plants.

This example demonstrates why the analysis of ppb levels of formaldehyde in gases and liquids is an important technology for atmospheric environmental measurement and control. High-performance liquid chromatography (HPLC) and gas chromatography–mass spectrometry (GC-MS) are accurate qualitative and quantitative analytical methods for detecting low concentrations of formaldehyde [7]. However, HPLC and GC-MS cannot be performed onsite, and often these methods involve lengthy analysis times.

Detector tubes, chemical sensors and optical sensors are the most widely used methods for detecting formaldehyde [8–11]. These devices have high portability, but lack rapidity and selectivity [12]. Biosensors based on enzyme-catalyzed dissolution have improved rapidity and selectivity [13–19], and biosensors for detecting formaldehyde have been based on the reaction of a nicotinamide adenine dinucleotide (NAD^+)-dependent dehydrogenase enzyme using different mediators for NADH electro-oxidation, either in batch reactors or under flow conditions [20–22]. The

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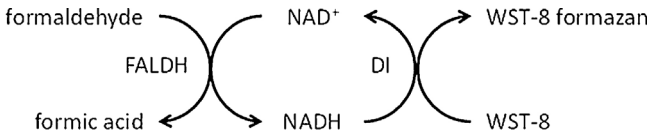

Scheme 1. Mechanism of the enzymatic cycling reaction used for detecting formaldehyde.

concentration of formaldehyde can be determined from the quantity of NADH produced, which can be measured based on its absorbance at 339 nm or fluorescence at 470 nm [23–25]. However, the sensitivity of such methods is insufficient due to the low extinction coefficient and weak fluorescence of NADH [26].

In contrast, enzymatic cycling systems are highly sensitive. In such systems, the target metabolite is regenerated via two enzymatic reactions occurring in the opposite directions. In addition, the quantity of the target metabolite can be detected using colorimetric analysis. For example, tetrazolium salts produce highly colored formazan dyes upon NADH reduction, and thus enzymatic cycling system assays using these salts are based on the colorimetric quantification of NADH. In particular, 4-[3-(2-methoxy-4-nitrophenyl)-2-(4-nitrophenyl)-2H-5-tetrazolio]-l,3-benzene disulfonate sodium salt (WST-8) is a water soluble tetrazolium salt with high stability and high sensitivity [27]. A WST-8 cell proliferation assay based on an enzymatic cycling system has been developed for monitoring cell proliferation and cytotoxicity using a spectrophotometer [28]. Colorimetric assays based on enzymatic cycling systems have also been applied to detect minor components in drugs and food substances because of their greater sensitivity and accuracy compared to NADH fluorescent methods [29,30].

Herein is described a highly sensitive and accurate formaldehyde biosensor based on an enzymatic cycling system. This sensor can detect formaldehyde in both water and indoor air. The mechanism of the assay is shown in Scheme 1. NAD^+ is hydrogenated to NADH, while formaldehyde is simultaneously oxidized to formic acid by formaldehyde dehydrogenase (FALDH). The NADH is then dehydrogenated to NAD^+ during the enzymatic cycling reaction of diaphorase (DI), which employs the tetrazolium salt WST-8 to produce water-soluble, yellow WST-8 formazan. The concentration of formaldehyde can be calculated based on the change in the absorbance of WST-8 formazan at 460 nm.

2. Experimental

2.1. Reagents and chemicals

WST-8 was purchased from Dojindo Laboratories (Kumamoto, Japan). FALDH (FALDH, EC 1.2.1.46, 1 unit/mg protein, solid, from *Pseudomonas sp.*) and DI (EC 1.6.99, 30 units/mg protein, from *Clostridium sp.*) were purchased from Toyobo (Osaka, Japan). NAD^+ was obtained from Wako Pure Chemical Industries (Osaka, Japan). Ethylenediaminetetraacetic acid disodium salt dihydrate (EDTA) was obtained from Sigma–Aldrich (St Louis, MO). All the reagents were of analytical-reagent grade and were used without further purification. All solutions were prepared with water purified using a Milli-Q system (Millipore, Tokyo, Japan).

2.2. Preparation of assay solutions and the analytical method for formaldehyde in water

First, to determine the pH dependence of the assay solution, the change in the conversion ratio of WST-8 was examined at pH values ranging from 7.0 to 10.0 using 100 mM phosphate (pH 7.0, 7.5, and 8.0), 100 mM tris–HCl (pH 8.0, 8.5, and 9.0), 100 mM borate (pH 8.0, 8.5, and 9.0), and 100 mM carbonate (pH 9.5 and 10.0) buffer solutions. The formaldehyde concentration in these solutions was fixed at 0.033 mM, which corresponds to a formalin concentration of 1 ppm. The assay solutions were prepared by dissolving NAD^+ (0.3 mM), WST-8 (0.3 mM), EDTA (0.1 mM), DI (1 U/ml), and FALDH (1 U/ml) in different buffer solutions.

To optimize the concentration of DI, solutions containing DI concentrations ranging from 0 to 1.0 U/ml were prepared by dissolving NAD^+ (0.3 mM), WST-8 (1 mM), EDTA (0.1 mM), and FALDH (1 U/ml) in the 100 mM phosphate buffer solution (pH 8.0). The effect of DI concentration on the absorbance of the solutions was then determined.

To optimize the concentration of FALDH, solutions containing FALDH concentrations ranging from 0 to 1.0 U/ml were prepared by dissolving NAD^+ (0.3 mM), WST-8 (1 mM), EDTA (0.1 mM), and DI (1 U/ml) in the 100 mM phosphate buffer solution (pH 8.0). The effect of FALDH concentration on the absorbance of the solutions was then determined.

To observe the effect of WST-8 on the absorbance, solutions with WST-8 concentrations ranging from 0 to 1.0 mM were prepared by dissolving NAD^+ (0.3 mM), EDTA (0.1 mM), FALDH (1 U/ml), and DI (1 U/ml) in the 100 mM phosphate buffer solution (pH 8.0).

A calibration curve was prepared and the selectivity for formaldehyde was also evaluated using assay solution consisting of NAD^+ (0.3 mM), WST-8 (1 mM), EDTA (0.1 mM), and DI (1 U/ml) in the 100 mM phosphate buffer solution (pH 8.0). Each data point was an average of five measurements. To establish the calibration curve, solutions with formaldehyde concentrations ranging from 5 to 500 ppb were prepared and their absorbances were determined. To evaluate the selectivity of the biosensor, the absorbance of solutions containing 1 ppm of different organic solvents were measured.

In all the experiments, the reaction mixture was incubated at 25 °C for 3 min. During this process, the change in absorbance was measured at 460 nm using spectroscopic system. The cell folder was connected to the spectrometer and the light source through 100 μm optical fibers. The light source was a tungsten halogen lamp (LS-1, Ocean Optics) optimized for the visible-NIR (VIS–NIR) range (360–2500 nm). The absorbance was measured using a UV–Visible spectrophotometer (USB2000, Ocean Optics).

2.3. Detection of formaldehyde gas using the enzymatic cycling system

To evaluate the performance of the enzymatic cycling system for detecting formaldehyde in gas samples, a vapor generation system was fabricated to provide variable formaldehyde gas concentrations. Dry air (humidity < 1%) generated using a clean air unit (P4-QD10, IAC) was employed as the base gas in the sensing system. Air samples with low concentrations of formaldehyde (5–80 ppb) were obtained using a standard gas generation system (Permeater PD-1B-2, GASTEC) based on the diffusion method.

The detection of formaldehyde gas by the sensor was investigated using an assay solution consisting of NAD^+ (0.3 mM), WST-8 (1 mM), EDTA (0.1 mM), and DI (1 U/ml) dissolved in the 100 mM phosphate buffer solution (pH 8.0). Fig. 1 shows the schematic diagram of the bubbling system used to introduce the formaldehyde/air gas mixture in the assay solution. The flow rate of the sample gas was controlled using a mass flow controller (MODEL8500, KOFLOC). After bubbling the sample gas for 5 min, the assay solution was transferred to a quartz cell for spectroscopic analysis. Each data point represents the average of 5 measurements.