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Fiber-Optic Bio-sniffer (Biochemical Gas Sensor) Using Reverse Reaction of Alcohol Dehydrogenase for Exhaled Acetaldehyde

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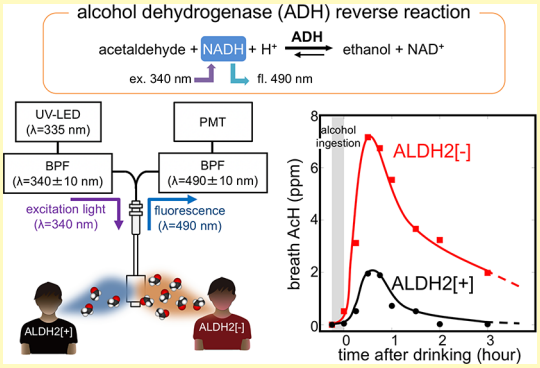
Supporting Information

ABSTRACT: Volatile organic compounds (VOCs) exhaled in breath have huge potential as indicators of diseases and metabolisms. Application of breath analysis for disease screening and metabolism assessment is expected since breath samples can be noninvasively collected and measured. In this research, a highly sensitive and selective biochemical gas sensor (bio-sniffer) for gaseous acetaldehyde (AcH) was developed. In the AcH bio-sniffer, a reverse reaction of alcohol dehydrogenase (ADH) was employed for reducing AcH to ethanol and simultaneously consuming a coenzyme, reduced form of nicotinamide adenine dinucleotide (NADH). The concentration of AcH can be quantified by fluorescence detection of NADH that was consumed by reverse reaction of ADH. The AcH bio-sniffer was composed of an ultraviolet light-emitting diode (UV-LED) as an excitation light source, a photomultiplier tube (PMT) as a fluorescence detector, and an optical fiber probe, and these three components were connected with a bifurcated optical fiber. A gas-sensing region of the fiber probe was developed with a flow-cell and an ADH-immobilized membrane. In the experiment, after optimization of the enzyme reaction conditions, the selectivity and dynamic range of the AcH bio-sniffer were investigated. The AcH bio-sniffer showed a short measurement time (within 2 min) and a broad dynamic range for determination of gaseous AcH, 0.02−10 ppm, which encompassed a typical AcH concentration in exhaled breath (1.2−6.0 ppm). Also, the AcH bio-sniffer exhibited a high selectivity to gaseous AcH based on the specificity of ADH. The sensor outputs were observed only from AcH-contained standard gaseous samples. Finally, the AcH bio-sniffer was applied to measure the concentration of AcH in exhaled breath from healthy subjects after ingestion of alcohol. As a result, a significant difference of AcH concentration between subjects with different aldehyde dehydrogenase type 2 (ALDH2) phenotypes was observed. The AcH bio-sniffer can be used for breath measurement, and further, an application of breath analysis-based disease screening or metabolism assessment can be expected due to the versatility of its detection principle, which allows it to measure other VOCs by using NADH-dependent dehydrogenases.



KEYWORDS: *gas sensor, biosensor, acetaldehyde, NADH, alcohol dehydrogenase, fiber-optic, fluorescence*

Quantitative assessment of the condition of diseases and metabolisms is important in medical fields. The relationship between the concentration of volatile organic compounds (VOCs) in biological samples such as blood,[1] urine,[2] saliva,[3] tear,[4] breath,[5] and diseases or metabolisms has been reported in numerous studies. Especially, analysis of VOCs in breath and skin gas which can be collected noninvasively[6−8] is expected to become a disease screening and metabolism assessment method with low burdens on subjects. Acetaldehyde (AcH, CH_3CHO) has high volatility, and it is an intermediate substance of ethanol metabolism produced by alcohol dehydrogenase (ADH) mainly in the liver after drinking.[9] Even in short term exposure, AcH is a harmful substance causing various symptoms such as alcohol flushing, vomiting, and headache. Besides, animal experiments revealed that long-term exposure to AcH increases the risk of damage to DNA and induces tumors.[10] For example, Garaycoechea et al. reported that AcH causes mutational damage of DNA in hematopoietic stem cells.[11] The American Conference of Governmental Industrial Hygienists (ACGIH) set the threshold of gaseous AcH concentration in working and living environments below 25 ppm. It is well-known that the racial

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difference of metabolic ability to oxidize AcH to acetic acid by aldehyde dehydrogenase type 2 (ALDH2) is extensive, and about 40% of Asians show a lower enzyme activity of ALDH2 (ALDH2[−]) than the others (ALDH2[+]).[12] The previous paper reported that the blood AcH concentrations that were measured by the headspace gas chromatographic method for Japanese flushers and nonflushers were 30 μM and 3−5 μM, respectively, when the mean blood ethanol concentrations of them were 10 mM.[13] Some studies reported that people with ALDH2[−] might have a high risk of carcinogenesis such as pharynx and tongue cancer due to an influence of AcH generated from drinking alcohol.[14−16] Ideally, people should understand the gene polymorphism of ALDH2 and monitor the blood AcH level when drinking alcoholic beverages to prevent diseases. The step-by-step mechanism between alcohol ingestion and the development of symptoms and lesions is poorly understood in alcohol-related diseases such as alcoholic myopathy and alcoholic cardiomyopathy.[17,18] AcH appears to have an important role in the etiology of the disease. Besides, measurement of AcH is useful for assessment of the curative effect of alcohol deterrent drugs such as disulfiram, and screening for a new drug to provide aversion therapy treatment of alcoholics.[19] However, a complicated experimental procedure and special precautions are required to measure the concentration of AcH in blood directly. The blood ethanol concentration is 1000−10000 times higher than blood AcH during metabolizing ethanol; therefore, depending on the treatment and assay procedures, more or less artifactual formation of AcH could occur. Also, the rapid disappearance of AcH in human blood because of interaction between acetate and AcH was demonstrated. Breath AcH measurements have been developed in order to avoid the methodological issues regarding blood AcH determination.[20] Part of the AcH in blood is released from the body as exhalation with a partition coefficient of breath:blood = 1:109,[21] and thereby the blood AcH concentration can be estimated noninvasively and conveniently by measuring breath AcH concentration. The concentrations of blood AcH estimated from breath AcH concentrations were 2−4, 4−8, and 8−12 μM when the blood ethanol concentrations were 10, 20, and 30 mM, respectively.[20] These values are in good agreement with the above-mentioned blood AcH concentrations that were measured directly. The technique for quantifying gaseous AcH contained in breath and skin gas has been developed using GC-MS,[22] SIFT-MS,[23] PTR-MS,[24] etc. However, these devices were bulky and expensive, and time-consuming. On the other hand, small, inexpensive, and real-time gas sensors using semiconductors are developed, but there was a challenge in selectivity.[25] Due to the fact that breath contains many compounds, high selectivity of the sensor is necessary for an accurate measurement. Previously we have developed biochemical gas sensors (bio-sniffer) with high sensitivity and selectivity that are capable of real-time analysis of VOCs in breath by using nicotinamide adenine dinucleotide (NAD)-dependent enzymatic reaction.[26−29] Also, as reported in a previous paper, there are ADH and aldehyde dehydrogenases (ALDHs) such as NAD-dependent enzymes capable of catalyzing AcH, but it is possible to prepare a sensor with high sensitivity when ADH was used.[30] Based on the previous research, we developed a gas-imaging system (sniff-cam) that visualized the concentration distribution of AcH in the gas-phase.[31] The AcH sniff-cam was a specialized system for focusing on the distribution measurement of gaseous AcH, and it cannot measure gaseous AcH continuously. Also, the dynamic range of the AcH sniff-cam was 100 ppb to 10 ppm, which did not encompass the breath AcH concentration at rest. In this study, we developed an AcH bio-sniffer utilizing a reverse reaction of ADH to achieve the highly sensitive and selective continuous measurement of AcH in the gas-phase. Then we measured the gaseous AcH contained in breath after drinking using the AcH bio-sniffer. Furthermore, the metabolic statuses of AcH during alcohol metabolism in ALDH2[−] and [+] subjects were compared.

EXPERIMENTAL SECTION

Materials and reagents. ADH (E.C. 1.1.1.1, from *Saccharomyces cerevisiae*, 369 unit/mg solid, Cat. No. A7011) and ALDH (E.C. 1.2.1.5 from *yeast*, 20 unit/mg solid, Cat. No. 10171832001) were purchased from Sigma-Aldrich (USA) and Roche diagnostics (Germany), respectively. A poly[2-methacryloyloxyethyl phosphorylcholine (MPC)-*co*-2-ethylhexyl methacrylate (EHMA)] (PMEH) that was synthesized by a free radical polymerization method[32] as an enzyme immobilization material and hydrophilic polytetrafluoroethylene (H-PTFE, the porosity of 80%, the pore size of 0.2 μm, the thickness of 80 μm, ommnipore membrane filters, Cat. No. HGWP14425, Millipore, USA) as a substrate of enzyme immobilization were used. Oxidized (NAD^+, Cat. No. 44005700) and the reduced form (NADH, Cat. No. 44327000) of NAD were from Oriental Yeast (Japan). Buffer chemicals including acetic acid (99.7%, Cat. No. 017-00256), sodium acetate (98.5%, Cat. No. 192-01075), potassium dihydrogen phosphate (99.5%, Cat. No. 169-04245), dipotassium hydrogen phosphate (99.0%, Cat. No. 164-04295), 2-amino-2-hydroxymethyl-1,3-propanediol, (99.9%, Cat. No. 013-16385), hydrochloric acid (35%, Cat. No. 083-03485), sodium hydrogen carbonate (99.5%, Cat. No. 191-01305), and sodium carbonate (99.8%, Cat. No. 199-01585) were purchased from Wako (Japan). All of the buffer solutions were prepared with ultrapure water obtained with a Mill-Q purification system from Millipore (USA). Standard gaseous AcH was prepared with a gas generator (Permeater, Cat. No. PD-1B-2, Gastec, Japan) with an AcH permeation tube (P-tube, Cat. No. P-92-1, Gastec, Japan). A standard gas used for investigating the selectivity of the AcH bio-sniffer was prepared with ethanol (99.5%, Cat. No. 14033-00, KANTO KAGAKU, Japan), methanol (99.8%, Cat. No. 131-01826, Wako, Japan), acetone (99.5%, Cat. No. 016-00346, Wako, Japan), and 2-propanol (99.7%, Cat. No. 166-04836, Wako, Japan) injected in a diffusion tube (D-tube, Cat. No.3200, Gastec, Japan). The alcoholic beverages that were used for the measurement of breath AcH after drinking were purchased at a local store in Japan.

Construction of the AcH bio-sniffer. ADH can catalyze AcH to ethanol and simultaneously oxidize NADH to NAD^+. The enzymatic reaction of ADH is shown in the following equation:

$$\mathrm{AcH} + \mathrm{NADH} + \mathrm{H}^+ \underset{\text{pH 8.5−9.5}}{\overset{\text{ADH, pH 6.0−7.0}}{\rightleftharpoons}} \text{ethanol} + \mathrm{NAD}^+ \quad (1)$$

NADH has a fluorescence property that it emits visible fluorescence at a wavelength of 490 nm when it absorbs ultraviolet (UV) light at a wavelength of 340 nm. Since the fluorescence intensity of NADH correlates with its concentration, quantitative determination of AcH is possible by measuring the decreasing fluorescence intensity from NADH. In this study, ADH was immobilized on an H-PTFE membrane with PMEH by physical entrapment.[33] ADH (60 units/cm^2) was dissolved in phosphate buffer solution (PB, pH 7.0, 0.1 M) and mixed with PMEH solution (10% w/w in ethanol, 10 μL/cm^2). Then, the mixture was spread homogeneously onto a cleaned H-PTFE membrane (2 cm × 2 cm) surface and cured in a refrigerator at 4 °C for 3 h. Afterward, the superfluous enzyme was flushed out by PB. Figure 1 shows the experimental setup. A flow-cell with an enzyme-immobilized membrane as a gas−liquid diaphragm was employed to measure the gaseous AcH. The flow-cell was fabricated by combining different diameters of tubular poly(methyl methacrylate) as illustrated Figure 1. A liquid phase flow-cell was connected to a reservoir of a buffer solution with coenzyme (coenzyme solution) with a silicone

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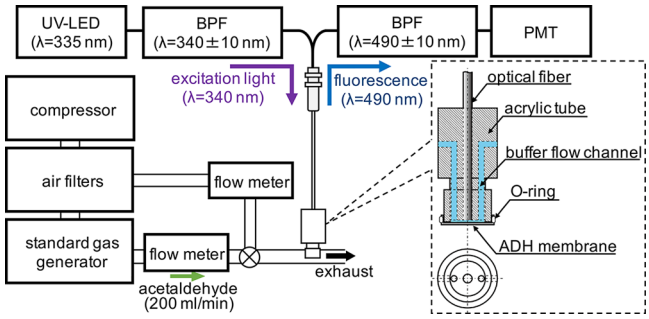

Figure 1. Experimental setup of the AcH bio-sniffer including a UV-LED, PMT, and optical fiber probe. Inset drawing shows the structure of the flow-cell.

tube (outer diameter: 2 mm, inner diameter: 1 mm, ASONE, Japan). The coenzyme solution in the flow-cell was supplied by a pump (SP-21-32, FLOM, Japan) with a degasifier (BG-32-02, FLOM, Japan). Fresh coenzyme was supplied to the enzyme-immobilized membrane, and the products that were generated by enzymatic reaction were removed simultaneously by flowing the coenzyme solution using the flow-cell. Therefore, continuous measurement of gaseous AcH was possible. The bifurcated optical fiber (BIF600-UV/vis, core size of 600 ± 10 μm, Ocean Optics, USA) was used to integrate the excitation light source that was composed of a UV-light emitting diode (UV-LED, λ of 335 nm, Sensor Electronic, USA) with a stabilized DC power source (GS200, Yokogawa, Japan) and a photomultiplier tube (PMT, Hamamatsu Photonics, Japan) as a fluorescence detector to construct the AcH bio-sniffer. The excitation light and the fluorescence detector were connected to the bifurcated optical fiber end, and the common end was assembled with an optical fiber probe (F1000-ANGLE90, Ocean Optics, USA) with the flow-cell. Two bandpass filters were used on the UV-LED (BPF_{ex}, 340 ± 10 nm, MX0340, Asahi spectra, Japan) and PMT side (BPF_{fl}, 490 ± 10 nm, MX0490, Asahi spectra, Japan) for eliminating unwanted light.

Characterization of the AcH bio-sniffer. To generate standard gaseous AcH, clean and dry air obtained from an air compressor (SLP-15EBD, Anest Iwata, Japan), which was connected with an activated carbon filter (3001-17201, GL-Science, Japan) and a silica-gel air dryer (3001-17111, GL-Science, Japan), was supplied to a gas generator. Gaseous AcH was generated by a P-tube that was a standard method of National Bureau of Standards in the USA. Generated gaseous AcH and dry air were loaded to the AcH bio-sniffer through the polytetrafluoroethylene tube (F-8006-017, the outer diameter of 6 mm, the inner diameter of 4 mm, FLON industry, Japan) that showed excellent chemical resistant and low adhesion properties. The flow rates of gaseous AcH and dry air were controlled at 200 mL/min by a flow meter with a needle valve (RK1250, KOFLOC, Japan).

The flow rate of coenzyme solution in the flow-cell affected the outputs and the responsiveness of the AcH bio-sniffer. Therefore, the flow rate of the coenzyme solution at 1.0, 1.5, 2.0, 2.5, and 3.0 mL/min controlled by the pump was evaluated. The measured AcH concentration was set at 1000 ppb, and the change in intensity of the fluorescence and the time of reaching to 90% response were observed.

For investigating the reproducibility of the AcH bio-sniffer, a cyclic experiment was performed. First, the clean air was loaded for 100 s, and then 1000 ppb of gaseous AcH was measured for 225 s. Finally, dry air was loaded again for 100 s. This cycle was repeated ten times, and the coefficient of variation (C.V.) was evaluated.

The effect of buffer pH of coenzyme solution was evaluated for optimizing the reaction condition of ADH. In the experiment, various buffers with different pH values were used as coenzyme solutions: acetate buffer (AB, pH 4.0, 5.0 and 5.5, 0.1 M), Tris-HCl buffer (Tris, pH 6.0, 6.5, 7.0, 7.5, 8.0 at 0.1 M), potassium phosphate buffer (K−K_2, pH 5.5, 6.0, 6.5, 7.0, 7.5, 8.0 at 0.1 M), carbonate−-bicarbonate buffer (CB, pH 8.0, 9.0, 10.0 at 0.1 M). The output obtained with each buffer was measured by the following steps: The dry air was loaded, and then the loading of dry air was stopped, followed by starting to apply 1000 ppb of gaseous AcH. Finally, supplying of AcH was stopped, and dry air was loaded again.

To measure AcH in exhaled breath, dynamic range and selectivity were investigated. In the experiment, NADH solution was adjusted to 100 μM (in K−K_2 at pH 6.5, 0.1 M) and supplied to the flow-cell at a flow rate of 1.5 mL/min, and various concentrations of standard gaseous AcH (10, 20, 30, 60, 120, 250, 500, 1000, 2500, 5000, 10000 ppb) were loaded to the AcH bio-sniffer at a gas flow rate of 200 mL/min. The same experiment took place with the AcH bio-sniffer using ALDH as a reference. One hundred units of ALDH was immobilized on a H-PTFE membrane by the same method of immobilization of ADH. The immobilized amount of ALDH was decided by referencing our previous reports.[34] In the experiment of an ALDH-mediated AcH bio-sniffer, an NAD^+ solution (in PB, at pH 8.0, 0.1M) adjusted to 100 μM was supplied to the flow-cell at a flow rate of 1.5 mL/min, and various concentrations of standard gaseous AcH (60, 120, 250, 500, 1000, 2500, 5000, 10000 ppb) were loaded to the ALDH-mediated bio-sniffer. The quantitative and 90% response times of ADH-mediated and ALDH-mediated bio-sniffer were calculated and compared. The selectivity of the ADH-mediated AcH bio-sniffer was evaluated by typical chemical substances in breath after drinking: AcH (3 ppm), ethanol (100 ppm), methanol (0.1 ppm), 2-propanol (0.1 ppm), and acetone (0.6 ppm).[35,36] Also, a mixture gas of AcH (3 ppm) and ethanol (100 ppm) was measured to evaluate the influence of ethanol on the sensor output. Gaseous ethanol, methanol, 2-propanol, and acetone were generated by the standard gas generator using a D-tube, respectively, and the gas flow rate was 200 mL/min. The measurement steps of each characterization experiment were the same as the investigation of the pH effect.

Measurement of breath AcH after drinking. The temporal change of breath AcH concentration obtained from ALDH2[+] and ALDH2[−] subjects after drinking was measured. Different concentrations of AcH in exhaled breath were attributed to alcohol metabolism by ADH type 1B (ADH1B) and ALDH2 in the liver. This experiment was conducted based on the authorization of the Human Investigations Committee of the Institute of Biomaterials and Bioengineering, Tokyo Medical and Dental University, authorization code 2012-6 updated in 2015, acting up to Declaration of Helsinki. All the participating subjects in this study complied with the following requirements—no smoking, medicine taking, alcoholic beverage drinking within 72 h and without illness. In the experiment after 4 h from the last eating, each subject slowly administrated the alcoholic beverage within 15 min, and the total ingested ethanol was 0.4 g/kg body weight. The protocol exhaled breath collection was referenced by the study of Bikov et al.[37] Briefly, the subjects at a rest state were asked to do a deep inhalation through the nose and to hold the breath for 15 s. Then the first 3 s of the exhaling air was discarded, and the rest of the breath was collected using a 2-L gas-sampling bag (2-9981-03, As one, Japan). This protocol was necessary because the first exhaled breath was supposed to contain the dead space air, and that might affect the result.[38] The gas-sampling bag that was filled with the collected sample was connected to a diaphragm air pump (DSA-2-12, Denso Sangyo, Japan) and delivered to the AcH bio-sniffer for measurement. The breath sample was loaded at the flow rate of 200 mL/min, the as same as standard AcH gas. The breath sampling and measurement were performed at before drinking and 0, 15, 30, 45, 60, 120, and 180 min after drinking. Note that each sample that was measured by the AcH bio-sniffer was measured by gas detection tube for AcH (Cat. No. 92L, GASTEC, Japan) as a standard comparison to confirm the reliability of the results.

RESULTS AND DISCUSSION

Characteristics of the AcH bio-sniffer. The effect of the flow rate of the coenzyme solution was evaluated and optimized. The lower flow rate induced a high signal output, but a long time to reach the steady state (Figure S-1). 1.5 mL/min was chosen for the successive experimental condition, considering the balance of signal intensity and response time.

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ACS Sens. 2018, 3, 425−431

The reproducibility is one of the critical factors for continuous measurement. The result of the 10 times cyclic test of 1000 ppb gaseous AcH is shown in Figure 2. ΔI was

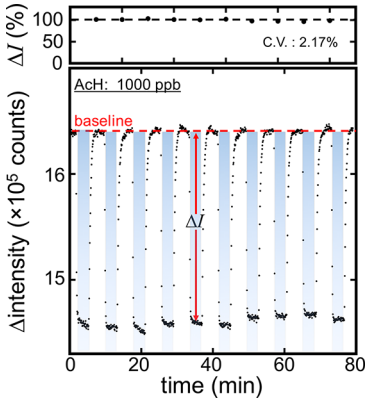

Figure 2. Reproducibility of the AcH bio-sniffer. The upper graph shows relative outputs that were defined as the average of ΔI being 100%. ΔI was defined as the output of each measurement that was calculated by the difference between baseline and average of 75−85 s after loading the gaseous AcH in the lower graph. The C.V. value was 2.17% for ten times of 1000 ppb AcH cyclic measurements.

defined as an output of each measurement that was calculated by the difference between the baseline and average of 75−85 s after loading of the gaseous AcH. The upper graph shows the relative output. The ADH-immobilized membrane of the AcH bio-sniffer was disposable for measurement of biological samples from a single subject. Therefore, the lifetime and stability have to be maintained for an acceptable measurement term (e.g., 1 h) without a physical and mental burden on the subject. The C.V. was 2.17% (n = 10) in 80 min. The high reproducibility of the AcH bio-sniffer could be explained because of the stable immobilization of ADH in the H-PTFE membrane. This result suggested that the AcH bio-sniffer has a potential of continuous measurement of breath samples.

Next, the effect of the pH value of the circulated buffer solution was investigated. Buffers and pH value affect the activity of ADH, the fluorescence intensity of NADH, and the stability of the bio-sniffer. Figure 3 describes the effect of buffer pH on the relative ΔI that was defined as K−K_2 at pH 6.5 and 100%. ADH has reversibility on the reaction, and the forward reaction that oxidizes ethanol to produce AcH predominates in the alkaline environment (pH 8.5−9.5). In contrast, the reverse reaction of ADH that uses the AcH bio-sniffer showed the

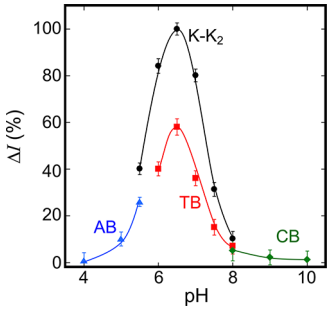

Figure 3. Dependence of ADH activities to gaseous AcH on the buffer pH: (▲) acetate buffer, AB; (●) potassium phosphate buffer, K−K_2; (♦) carbonate-bicarbonate buffer, CB; (■) Tris-HCl buffer, TB.

highest activity at pH 6.5. Also, ADH did not react to 1000 ppb of ethanol when pH-optimized coenzyme solution (pH 6.5 with 0.1 mM of NADH) was used. These results indicate that the ADH-mediated AcH bio-sniffer can measure the AcH without the influence of the forward reaction of ADH.

Then, the responsiveness and dynamic range of the AcH bio-sniffer were evaluated. AcH bio-sniffer using the forward reaction of ALDH was also used as a contrast. Note that the ALDH bio-sniffer was not optimized for the gaseous AcH, and the pH of coenzyme solution was decided based on pre-experiments with AcH solution.[30] Figure 4a shows a typical

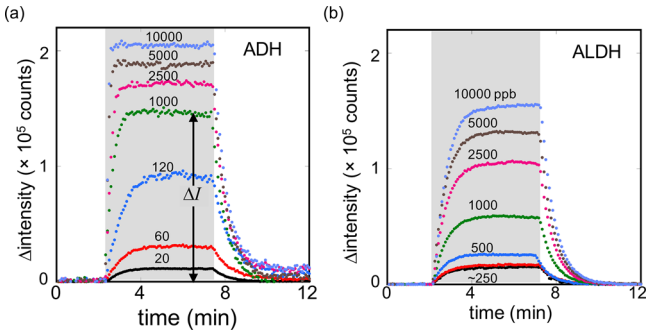

Figure 4. Responses of (a) ADH-mediated and (b) ALDH-mediated AcH bio-sniffer to gaseous AcH at different concentrations (20−10000 ppb). The ΔI was determined by averaging the fluorescence intensity from 5−6 min.

response of the ADH-mediated AcH bio-sniffer against the various concentrations of gaseous AcH. The fluorescence intensity decreased from the baseline as shown in Figure 2 when gaseous AcH was loaded. In this graph, the amount of change from the baseline was defined as Δintensity and displayed in absolute value. Also, Figure 4b shows typical responses of ALDH-mediated AcH bio-sniffer. The 90% response time (T_{90}), which was defined as the time from AcH loading to ΔI reaching 90%, tended to be short for both ADH and ALDH when the concentration of AcH was high (Figure S-2). However, an apparent difference of T_{90} between ADH-mediated (35−100 s) and ALDH-mediated (80−100 s) was observed.

Figure 5a displays a comparison of calibration curves between ADH-mediated and ALDH-mediated AcH bio-sniffers. The ADH-mediated bio-sniffer showed high sensitivity and

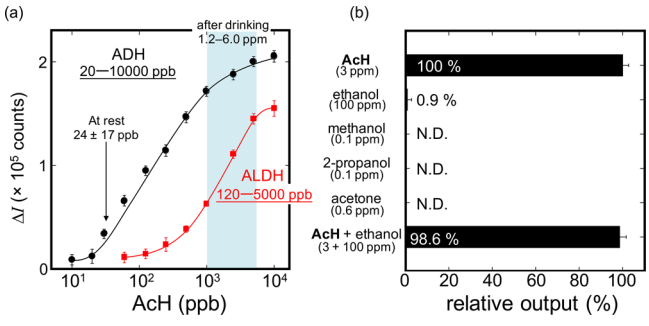

Figure 5. (a) Comparison of the dynamic range of the AcH bio-sniffer with ADH (●) and ALDH (■). The typical concentration of AcH after drinking is 1.2 ± 0.8−6.0 ± 3.0 ppm. (b) Selectivity of the AcH bio-sniffer against the typical components present in breath at a typical concentration after drinking.

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wide calibration curve range. The calibration range for AcH was 20−10000 ppb (correlation coefficient (R) = 0.997) and 120−5000 ppb (R = 0.999) obtained from ADH-mediated and ALDH-mediated bio-sniffer, respectively. The dynamic range of ALDH-mediated bio-sniffer was almost the same as that of the previous research.[34] The calibration curves were described by the following equations:

$$\text{ADH} - \text{mediated } \Delta I \ (\times 10^5 counts) = \left(\frac{-46.7 + (2.1 + 46.7)}{1 - \left(\frac{1664.4}{AcH\ conc.\ [ppb]} \right)^{1.0}} \right)^{0.009} \quad (2)$$

$$\text{ALDH} - \text{mediated } \Delta I \ (\times 10^5 counts) = \left(\frac{-0.01 + (1.7 - 0.01)}{1 - \left(\frac{3699.5}{AcH\ conc.\ [ppb]} \right)^{2.2}} \right)^{0.33} \quad (3)$$

The concentration of breath AcH after drinking was 0.2−9 ppm, which was higher than that at rest condition (24 ± 17 ppb).[23] The ADH showed higher ΔI than the ALDH at each concentration. In particular, the difference was significant in the region of low concentration; by using ADH-mediated bio-sniffer, the output was 6.7-fold at 250 ppb, 5.7-fold at 500 ppb, and 2.9-fold at 1000 ppb higher than those of ALDH-mediated bio-sniffer. Therefore, it was considered that the ADH-mediated bio-sniffer was more suitable to breath AcH measurement because of its superior responsiveness and sensitivity. Figure 5b shows the selectivity of AcH bio-sniffer against the typical breath components. The output of gaseous AcH at 3 ppm that was mean concentration after drinking for ALDH2[+] and ALDH2[−] subjects was defined as 100%, and the others were normalized by the AcH value. The AcH bio-sniffer reacted only in AcH-contained samples. It validated a high selectivity of the AcH bio-sniffer to AcH that was based on the substrate specificity of the ADH.

Assessment of AcH in breath changing over time after drinking. Figure 6 shows the time course of breath AcH concentrations of ALDH2[+] and ALDH2[−] subjects after drinking. As a result, a clear difference originated from the phenotype of ALDH2 was observed. For both samples, the concentration peaked 30 min after drinking, and ALDH2[−] showed 3.6-fold higher concentration (7.2 ppm) than that of ALDH2[+] (2.0 ppm) (Figure S-3). The average breath AcH concentrations of ALDH2[+] and ALDH2[−] after drinking were reported as 1.2 ± 1.0 ppm and 6.9 ± 2.5 ppm, respectively.[39] These results are in good agreement with previous research. Figure S-4 displays a comparison of AcH concentration in exhaled breath that was sampled and analyzed from ALDH2[+] and ALDH2[−] subjects. The detection tube can be used easily, but it required reading out the concentration of AcH from the scale gauge; therefore, the resolution of the detection tube was limited based on visual acuity. Nevertheless, the high correlation between two methods was confirmed by the Pearson correlation coefficient statistic method (correlation coefficient R = 0.994, probability $p < 0.001$) as shown in Figure S-5; these results indicated the reliability of the result that was obtained from the AcH bio-sniffer.

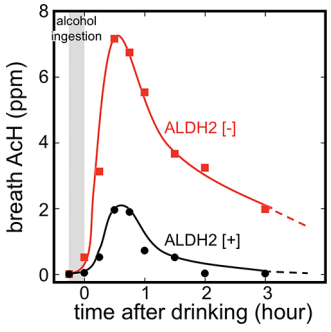

Figure 6. Time course of concentration of breath AcH after drinking. About 3-fold difference between ALDH2[+] subject (●) and ALDH2[−] subject (■) at the concentration of breath AcH after drinking was observed.

CONCLUSION

In this study, an AcH bio-sniffer for assessment of the intermediate of ethanol metabolism by measuring breath AcH was developed. A bifurcated optical fiber was used to integrate a UV-LED and a PMT to compose the main part of the bio-sniffer. An optical fiber probe equipped with a flow-cell and an ADH-immobilized membrane constituted the gas-sensing region. The calibration range of the AcH bio-sniffer was from 20 ppb to 10 ppm, which encompassed the concentrations of breath AcH in a healthy person at a rest condition and after drinking. Besides, a high reproducibility demonstrated the potential for real-time breath monitoring. Measurement of AcH in breath after drinking was achieved, and significant differences between ALDH2[+] and ALDH2[−] subjects were distinguished. In the future, a miniaturized AcH bio-sniffer could provide small, low cost, easy, and rapid instruments for point-of-care, disease screening, remote medicine, and home care.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acssensors.7b00865.

Effect of flow-rate of coenzyme solution in flow-cell; relationship between AcH concentration and response time of the AcH bio-sniffer; typical responses in measurement of AcH in breath; comparison of concentrations of AcH in exhaled breath measured by the AcH bio-sniffer and detection tubes; and correlation of AcH concentration measured by the AcH bio-sniffer and detection tubes. (PDF)

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Notes

The authors declare no competing financial interest.

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ACS Sens. 2018, 3, 425−431