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Short communication

Tonometric biosensor with a differential pressure sensor for chemo-mechanical measurement of glucose

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ABSTRACT

A tonometric biosensor for glucose was constructed using a chemo-mechanical reaction unit and a differential pressure sensor. The reaction unit was fabricated by using both liquid and gas cells separated by an enzyme diaphragm membrane, in which glucose oxidase was immobilized onto the single (gas cell) side of the dialysis membrane. By applying glucose solution (0, 25.0, 50.0, 100, 150 and 200 mmol/l) into the liquid cell of the chemo-mechanical reaction unit, the pressure in the gas cell decreased continuously with a steady de-pressure slope because the oxygen consumption in the gas cell was induced by the glucose oxidase (GOD) enzyme reaction at the enzyme side of the porous diaphragm membrane. The steady de-pressure slope in the gas cell showed the linear relationship with the glucose concentration in the liquid cell between 25.0 and 200.0 mmol/l (correlation coefficient of 0.998).

A substrate regeneration cycle coupling GOD with L-ascorbic acid (AsA: 0, 1.0, 3.0, 10.0 and 50.0 mmol/l; as reducing reagent system) was applied to the chemo-mechanical reaction unit in order to amplify the output signal of the tonometric biosensor. 3.0 mmol/l concentration of AsA could optimally amplify the sensor signal more than 2.5 times in comparison with that of non-AsA reagent.

1. Introduction

Many kinds of biosensor have been developed by using electrochemical devices and chemical luminescence optical (luminescence and fluorescence) probes for detecting enzymatic products and other substrate consumptions (Sulak et al., 2006; Lin and Chen, 2006; Kurita et al., 2006; Law et al., 2003; Wolfbeis and Li, 1993; Gunasingham and Tan, 1992). Calorimetric devices such as thermister have been also used for the measurement of the thermal change by the reaction heat (exothermal and endothermal) at those enzymatic reactions (Rajkumar et al., 2008; Ramanathan and Danielsson, 2001; Urban et al., 1991). In addition, mass changes caused by intermolecular reactions can be assessed by Quartz Crystal Microbalance (QCM) or Surface Acoustic Wave (SAW) devices (Briand et al., 2007; Fatoyinbo et al., 2007; Ogi et al., 2007; Gronewold et al., 2005; Joseph et al., 2005; Martin et al., 2004). However, a chemo-mechanical conversion device for biosensor and biological measurements has not been reported. The chemo-mechanical type bio-sensors and -devices could contribute for a development of human mimetic systems and fusion medical devices between living matter and artifact (such as intelligent artificial organs), not only for sensing and analysis, but also for direct chemo-mechanical driving and regulation.

In living organism, motor proteins (i.e. actin/myosin (muscle molecule), kinesin, dynein, flagellum motor, etc.) have a direct function of chemo-mechanical energy conversion and transfer with high efficiency using ATP (adenosine tri-phosphate) chemical substance. Since some biocatalyst could catalyze chemical reactions with volume change at room temperature and pressure (i.e. catalase with hydrogen peroxide), mechanical force such as pressure increase would be provided directly from chemical energy (Sand et al., 2003; Mitsubayashi et al., 2003). In this paper, a novel tonometric biosensor was constructed by using the chemo-mechanical reaction unit with an asymmetric enzyme immobilized membrane. Glucose was selected as model analyte because of typical biological energy substance in living being and ecological/environmental substance. A substrate regeneration cycle has been also applied to the tonometric sensor in order to amplify the chemo-mechanical conversion signal, (Hasebe, 1998; Hasebe et al., 1995; Uchiyama et al., 1994).

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2. Experimental

2.1. Chemo-mechanical reaction unit for tonometric biosensing

Fig. 1 illustrates a schematic diagram of a chemo-mechanical reaction unit and a biochemical depressurization principle with glucose solution. The reaction unit in tonometric glucose sensor consisted of upper gaseous- and lower liquid-cells separated by an asymmetric GOD (glucose oxidase) immobilized membrane (Mitsubayashi et al., 2003).

The reaction unit was fabricated as follows. The GOD enzyme ("Amano"AM, EC 1.1.3.4, 250 u/mg; AMANO enzyme Inc., Nagoya, Japan) was mixed with photocrosslinkable polyvinyl alcohol containing stilbazolium groups (PVA-SbQ, type: SPP-H-13 (Bio), Toyo Gosei Co. Ltd., Tokyo, Japan) in a weight ratio of 1:100 (Ichimura, 1984; Mitsubayashi and Hashimoto, 2000; Iguchi et al., 2007; Kudo et al., 2008). The GOD mixture was coated onto a single side of a hydrophilic dialysis membrane (part no. 157-0144-02, thickness: 15 μm, Technicon Chemicals Co., S.A., Oecq, Belgium) and was desiccated in the dark place below 2 °C for 1 h. And then, the membrane was irradiated with a fluorescent light for 30 min (Mitsubayashi et al., 1994). The asymmetric GOD immobilized membrane was functioned as the diaphragm of the gaseous- and the liquid cells in the reaction unit (dialysis flow-cell, FA-1, volume 1.0 ml, Sanplatec Co., Tokyo, Japan). The enzyme side of the asymmetric GOD membrane was faced to the gaseous cell of the chemo-mechanical reaction unit.

The phosphate buffer solution (50 mmol/l, pH 7.0) with varying concentration of glucose could flow through inlet tube connector to the liquid cell in the reaction unit. The gaseous cell was filled with air with 20.9% oxygen. In this system, glucose molecule was possible to permeate through the micro-pores of the hydrophilic diaphragm membrane and reach to the GOD enzyme side. The GOD enzyme reaction with glucose molecule would be induced by the oxygen consumption at the gaseous side, thus resulting in a depressurization in the gaseous cell (as illustrated in Fig. 1).

2.2. Experimental set-up of tonometric biosensor system

The experimental set-up of the tonometric biosensor system consisted of the chemo-mechanical reaction cell and a differential pressure sensor. The tonometric system of glucose measurement was constructed by connecting the outlet tube of the gaseous cell in the chemo-mechanical reaction unit with the tube port of the differential pressure sensor (CS7040A, range: 0–2500 Pa, Toyoda Machine Works Co., Kariya, Japan). The characteristics of the tonometric sensor system for glucose solution were evaluated using a batch-flow measurement system. After the application of phosphate buffer (50.0 mmol/l, pH 7.0) for 3 min, various concentrations of glucose solution in a carrier reservoir were flowed into the liquid cell with a constant flow rate of 3.0 ml/min by means of a peristaltic pump (Model: MP-3N, Tokyo Rikakikai Co., Ltd., Tokyo, Japan) for 6 min. From the viewpoint of hydrodynamic (water pressure, atmosphere pressure, pulsating pressure by the peristaltic pump, etc.), all of the equipments and their open-tube edges were adjusted to their positions and heights.

The output signal of the differential pressure sensor was then monitored graphically on the computer-display via an analog-to-digital converter (ADC-16, PICO Technology Co., Ltd., UK), and saved on a hard-disk for later analyses of the sensor behavior (signal response, detection limit, calibration range, signal amplification, etc.). When not in use, the chemo-mechanical reaction unit with phosphate buffer solution filling the liquid cell was stored below 10 °C.

In order to amplify the output signal of the tonometric biosensor, a substrate regeneration cycle was applied for the glucose measurement by coupling the GOD with the reducing reagent system of L-ascorbic acid (AsA) (Hasebe et al., 1993, 1995; Hasebe, 1998; Uchiyama et al., 1994; Mitsubayashi and Hashimoto, 2002). Varying concentrations of AsA (0, 1.0, 3.0, 10.0 and 50.0 mmol/l) in the sample solution were prepared to investigate the effect of the reducing reagent on the signal amplification of the tonometric sensor system.

3. Results and discussion

3.1. Evaluation of the tonometric biosensor for glucose

The characteristics of the tonometric biosensor with the chemo-mechanical reaction unit were evaluated with the batch-flow measurement system to varying concentrations of glucose with and without AsA solution. Fig. 2 shows typical response curves of the tonometric biosensor to glucose solution (0, 25.0, 50.0, 100, 150 and 200 mmol/l) without AsA. After showing steady-state pressure for 3 min with phosphate buffer, the pressure in the gaseous cell of the reaction unit decreased gradually following the reach of standard glucose solution into the liquid cell to give a steady-state depressurization slope. Glucose molecule would be expected to be diffused into the hydrophilic porous membrane, and reached to the GOD immobilized layer of the diaphragm. The oxygen consumption with the GOD enzyme reaction could induce the continuous active depressurization because the gaseous cell is the enclosed space without any open edges to the outer atmosphere.

The slope of pressure change to the application of glucose solution (0, 25.0, 50.0, 100.0, 150.0 and 200 mmol/l) was calculated

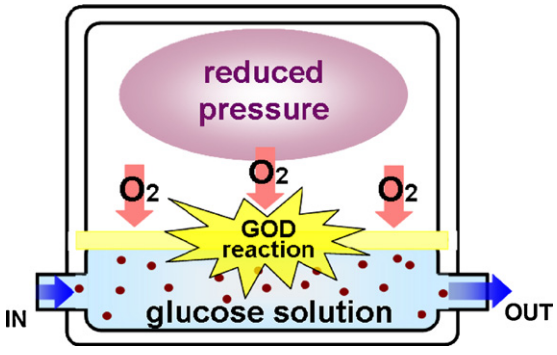

Fig. 1. Schematic diagram of chemo-mechanical reaction unit with GOD-diaphragm membrane for tonometric glucose sensor.

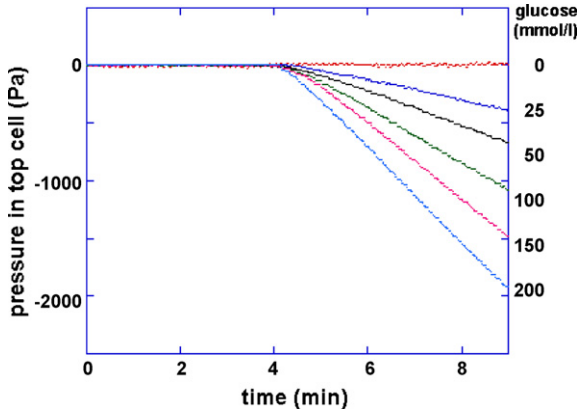

Fig. 2. Typical responses of the tonometric biosensor to varying concentration of glucose (0, 25.0, 50.0, 100.0, 150.0 and 200 mmol/l).

by a moving average method with 30 points because of normalizing the pressure change. The pressure slope decreased rapidly for 2 min to equalize the glucose concentration in the liquid cell and reached the steady slope value. The steady slope was related to the concentration of glucose (25.0–200 mmol/l). Therefore, phosphate buffer solution without glucose did not induce any pressure change in the gaseous cell of the chemo-mechanical reaction unit. However, the application of glucose solution induced the pressure decrease in the gaseous cell because glucose molecule would penetrate the diaphragm membrane and reach to the GOD enzyme side area (Mitsubayashi et al., 2003). The GOD enzyme reaction was occurred at the enzyme membrane, especially gaseous side. The oxygen consumption was occurred by the GOD enzyme reaction, thus inducing the continuous depressurization in the gaseous cell.

The relationship between the glucose concentration and the steady slope of pressure change 5 min after applying glucose solution to the liquid cell was evaluated. The steady slope of pressure was related to the concentration of glucose in the liquid cell over the range 25.0–200.0 mmol/l (correlation coefficient of 0.998) with a good reproducibility (C.V.: 3.6%, $n = 5$, 50.0 mmol/l glucose) over multiple measurements and the coefficient of variation of 14.7% ($n = 5$, 50.0 mmol/l glucose) across multiple sensors, deduced from regression analysis by a method of least squares according to the following equation:

$$\text{pressure slope (Pa/s)} = -0.839 - 0.031\,[\text{glucose (mmol/l)}]$$

The maximum concentration for the tonometric sensor was 200 mmol/l because the dynamic range of the differential pressure sensor is from 0 to 2500 Pa. The tonometric biosensor would be used for measuring the high concentration of glucose by using the pressure sensor with the wide dynamic range. In this study, the minimum concentration for glucose tonometric measurement was 5.0 mmol/l with unstable pressure change. The sensor sensitivity would be dependent on many factors such as substrate molecular size, diffusivity, external pressure, diaphragm property (permeability, thickness, porous size and polarity). The lower sensitivity will be improved by optimizing those parameters.

The substrate regeneration cycle was also applied as one of the unique techniques for the improvement of the sensor sensitivity in this study. In order to amplify the depressurization signal and to improve the sensitivity of the tonometric biosensor, the substrate regeneration cycle was applied for the glucose measurement by coupling the GOD with the reducing reagent system of AsA. The effect of the AsA concentration (0, 1.0, 3.0, 10.0 and 50.0 mmol/l) in buffer solution on the steady-state slope for the measurement of glucose is shown in Fig. 3. The signal effect of the AsA concentration on the tonometric signal was evaluated on the batch-flow measurement of 5.0 mmol/l glucose solution. As Fig. 3 illustrates, the sensor outputs were increased by the addition of AsA into the buffer solution. The maximum output was obtained at 3.0 mmol/l of AsA concentration with the signal amplification of more than 2.5 times in comparison with that of non-AsA solution (Mitsubayashi and Hashimoto, 2003; Mitsubayashi et al., 2006; Saito et al., 2007; Minamide et al., 2005a,b). However, the tonometric output decreased over 3.0 mmol/l of AsA concentration and was independent on the AsA concentration of 10 mmol/l or more.

A flocculant production was also observed in the liquid cell at the high concentration of AsA (Hasebe et al., 1993, 1995; Hasebe, 1998; Uchiyama et al., 1994; Mitsubayashi and Hashimoto, 2002). The reason of the signal decrease at high concentration of AsA is considered to be a decrease of the substrate diffusibility at the porous membrane with the flocculant production.

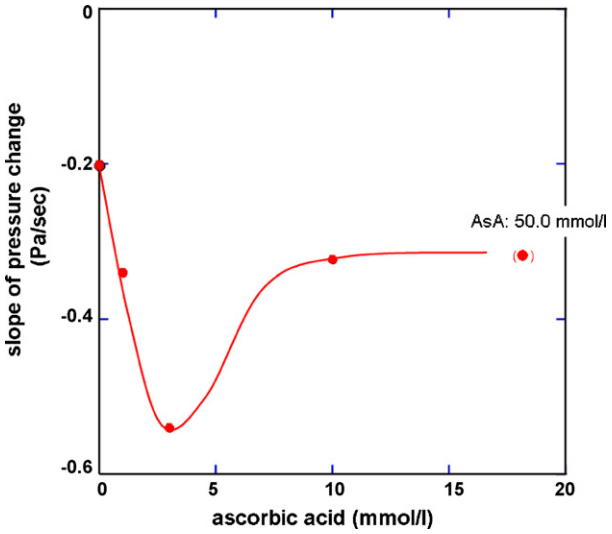

Fig. 3. Effect of the concentration of ascorbic acid on the slope of the pressure change in the tonometric glucose sensor (AsA: 0, 1.0, 3.0, 10.0 and 50.0 mmol/l).

4. Conclusions

The tonometric glucose biosensor was fabricated using the chemo-mechanical reaction unit with the GOD diaphragm membrane and the differential pressure sensor. By applying glucose solution into the liquid cell of the chemo-mechanical reaction unit, the pressure in the gaseous cell successfully decreased with the steady de-pressure slope because of the oxygen consumption by GOD enzyme reaction. The calibration range of the tonometric biosensor for glucose was from 25.0 to 200.0 mmol/l (correlation coefficient of 0.998). The substrate regeneration cycle caused by coupling GOD with AsA as reducing reagent system successfully amplified the tonometric biosensor output. 3.0 mmol/l concentration of AsA was the optimum concentration for the amplification of the sensor output.

The potential applications of the tonometric biosensor and device include not only chemo-mechanical biosensors, but also movable and visible biosensors and intelligent biomimetic machines. We will report the smart organic machines in the near future.

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References

Briand, E., Salmain, M., Compère, C., Pradier, C.M., 2007. Biosens. Bioelectron. 22, 2884–2890.
Fatoyinbo, H.O., Hoettges, K.F., Reddy, S.M., Hughes, M.P., 2007. Biosens. Bioelectron. 23, 225–232.
Gronewold, T.M.A., Glass, S., Quandt, E., Famulok, M., 2005. Biosens. Bioelectron. 20, 2044–2052.
Gunasingham, H., Tan, C.H., 1992. Biosens. Bioelectron. 7, 353–359.
Hasebe, Y., Takamori, K., Uchiyama, S., 1993. Anal. Chim. Acta 282, 363–367.
Hasebe, Y., Oshima, K., Takise, O., Uchiyama, S., 1995. Talanta 42, 2079–2085.
Hasebe, Y., 1998. Chem. Sens. 14 (3), 116–123.
Ichimura, K., 1984. J. Polym. Sci. 22, 2817–2828.
Iguchi, S., Kudo, H., Saito, T., Ogawa, M., Saito, H., Otsuka, K., Funakubo, A., Mitsubayashi, K., 2007. Biomed. Microdevices 9, 603–609.

Joseph, S., Marten, T., Gronewold, A., Schlensog, M.D., Olbrich, C., Quandt, E., Famulok, M., Schirner, M., 2005. Biosens. Bioelectron. 20, 1829–1835.
Kudo, H., Goto, T., Saito, T., Saito, H., Otsuka, K., Mitsubayashi, K., 2008. Microchim. Acta 160, 421–426.
Kurita, R., Yabumoto, N., Niwa, O., 2006. Biosens. Bioelectron. 21, 1649–1653.
Law, K.A., Warrington, R.J., McGurk, A., Higson, S.P.J., 2003. Biosens. Bioelectron. 18, 579–585.
Lin, K.C., Chen, S.M., 2006. Biosens. Bioelectron. 21, 1737–1745.
Martin, F., Newton, M.I., McHale, G., Melzak, K.A., Gizeli, E., 2004. Biosens. Bioelectron. 19, 627–632.
Minamide, T., Mitsubayashi, K., Jaffrezic-Renault, N., Hibi, K., Endo, H., Saito, H., 2005a. Analyst 130, 1490–1494.
Minamide, T., Mitsubayashi, K., Saito, H., 2005b. Sens. Actuators B 108, 639–645.
Mitsubayashi, K., Suzuki, M., Tamiya, E., Karube, I., 1994. Anal. Chim. Acta 289, 27–34.
Mitsubayashi, K., Hashimoto, Y., 2000. Electrochemistry 68 (11), 901–903.
Mitsubayashi, K., Hashimoto, Y., 2002. IEEE Sens. 2, 133–139.
Mitsubayashi, K., Wakabayashi, Y., Okamoto, T., 2003. Abstracts of 83rd Jpn Chem Meeting, p. 1147.
Mitsubayashi, K., Hashimoto, Y., 2003. Biosens. Bioelectron. 19, 193–198.
Mitsubayashi, K., Minamide, T., Otsuka, K., Kudo, H., Saito, H., 2006. Anal. Chim. Acta 573-574, 75–80.
Ogi, H., Motohisa, K., Hatanaka, K., Ohmori, T., Hirao, M., Nishiyama, M., 2007. Biosens. Bioelectron. 22, 3238–3242.
Rajkumar, R., Katterle, M., Warsinke, A., Möhwald, H., Scheller, F.W., 2008. Biosens. Bioelectron. 23, 1195–1199.
Ramanathan, K., Danielsson, B., 2001. Biosens. Bioelectron. 16, 417–423.
Saito, H., Kaneko, Y., Hashimoto, Y., Shirai, T., Kudo, H., Otsuka, K., Mitsubayashi, K., 2007. Sens. Actuators B 123, 877–881.
Sand, T.T., Zielinski, J.E., Arthur, C., Bradley, D., Wie, S., 2003. Biosens. Bioelectron. 18, 797–804.
Sulak, M.T., Gökdoğan, O., Gülce, A., Gülce, H., 2006. Biosens. Bioelectron. 21, 1719–1726.
Uchiyama, S., Shimizu, H., Hasebe, Y., 1994. Anal. Chem. 66, 1873–1876.
Urban, G., Kamper, H., Jachimowicz, A., Kohl, F., Kuttner, H., Olcaytug, F., Goiser, P., Pittner, F., Schalkhammer, T., Mann-Buxbaum, E., 1991. Biosens. Bioelectron. 6, 275–280.
Wolfbeis, O.S., Li, H., 1993. Biosens. Bioelectron. 8, 161–216.