



An enzyme-modified capillary as a platform for simultaneous fluorometric detection of D-glucose and L- lactate

Atsushi Shoji^{a,b,*}, Yusuke Takahashi^a, Saki Osato^a, Masao Sugawara^a

^a Department of Chemistry, College of Humanities and Sciences, Nihon University, Sakurajousui, Setagaya, Tokyo, 156-8550, Japan

^b School of Pharmacy, Tokyo University of Pharmacy and Life Science, 1432-1 Horinouchi, Hachioji, Tokyo, 192-0392, Japan

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ABSTRACT

The preparation of a glass capillary patterned with lipid layers on which lactate dehydrogenase (LDH) and glucose dehydrogenase (GDH) were regionally adsorbed and its application for simultaneous detection of D-glucose and L-lactate in human serum is described. A lipid layer was formed on the surface of BSA-unabsorbed octadecyltrichlorosilane (OTS) inner wall of a glass capillary. The electrostatic charge of the lipid layer was a key factor for adsorbing the enzymes on the lipid layer. The fluorescence intensities were observed at each enzyme site in the presence of diaphorase (DIA), β -nicotinamide-adenine dinucleotide oxidized (NAD), resazurin, D-glucose and L-lactate. The fluorescence intensities at each enzyme site increased with an increase in the concentration of D-glucose and L-lactate with the detection limits of 32 μ M and 4.9 μ M, respectively.

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1. Introduction

Biosensors are analytical tools for clinical diagnosis [1,2], food industry [3,4], environment monitoring [5,6]. For designing biosensors, a glass capillary has some attractive properties, such as high surface area to volume ratio, small sample size, and low cost. In addition, a glass capillary can easily be adapted to flow systems. Therefore, capillary-based enzyme biosensors [7–12], capillary-based enzyme immunoassay [13,14] and capillary-based DNA sensors [15,16] have been reported. Many researchers have detected marker molecules, which are produced by enzymatic reaction, at the outlet of the capillary after elution in a flow system [7–13]. On the other hand, detection of an enzymatic product in a capillary has been reported [14–16]. Gao *et al.* has detected marker molecules inside a capillary using a general fluorophotometry with a capillary holder [13,14]. In our previous paper, we developed a capillary enzyme sensor for simultaneous detection of D-glucose and galactose inside a capillary [17]. The immobilization of glucose oxidase and galactose oxidase at different regions of the inner wall of a capillary was achieved by the photocrosslinking technique using biotin-4-fluorescein to design the capillary-based sensor.

The immobilization of biomolecules is important for the design of capillary-based biosensors. In most of capillary-based biosen-

sors, covalent coupling and avidin-biotin binding were used for the immobilization. These strategies often lead to the loss of the biomolecules activity [18,19]. On the other hand, immobilization by adsorption through van der Waals' force and electrostatic and/or hydrophobic interaction are believed to cause little or no biomolecules inactivation, and its procedure is simple and easy [18,19].

Phospholipid mono- and bilayers are attractive absorbent materials because the charge of lipid layers can be controlled by changing lipid composition. Further, adsorption of biomolecules onto a lipid layer does not affect their activity significantly because of high biocompatibility. However, only a few studies have been reported about the development of capillary-based enzyme biosensors using lipid layers as an adsorbent material [20].

In the present study, we investigate the preparation of an enzyme-modified glass capillary, where the inside wall of a glass capillary is patterned with lipid and bovine serum albumin (BSA). Glucose dehydrogenase (GDH) and L-lactate dehydrogenase (LDH) are immobilized at different regions of the capillary patterned with lipid and BSA by physical adsorption. Monitoring D-glucose and L-lactate is important as a diagnostic tool to detect ischemic events and monitor therapeutic interventions [21,22]. Capillaries patterned with supported lipid bilayers have been reported for separation of proteins by several authors [23–26], however, the development of a fluorometric capillary biosensor has not been reported yet.

* Corresponding author at: Tokyo University of Pharmacy and Life Science, 1432-1 Horinouchi, Hachioji, Tokyo, 192-0392, Japan.

E-mail address: ashoji@toyaku.ac.jp (A. Shoji).

2. Experimental

2.1. Reagents

L- α -Phosphatidylcholine (PC, purity >99%, 50 mg/ml chloroform solution), 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine-N-(cap biotinyl) (sodium salt) (powder, B-cap-PE), L- α -phosphatidylethanolamine (PE, 10 mg/ml chloroform solution), L- α -phosphatidylserine (PS, 10 mg/ml chloroform solution), 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine-N-carboxyfluorescein (CFPE, 1 mg/ml chloroform solution) and 1,2-dioleoyl-*sn*-glycero-3-ethylphosphocholine (EPC, 10 mg/ml chloroform solution) were purchased from Avanti Polar Lipids (Alabaster, AL, USA). Octadecyltrichlorosilane (OTS) and 3-mercaptopropyl-trimethylsilane (MTS) were obtained from Shin-Etsu Chemical (Tokyo, Japan). Human serum albumin (HSA) and bovine serum albumin (BSA) were obtained from Sigma Chemical Co. (St Louis, MO). *N*-(11-Maleimidoundecanoyloxy)succinimide (KMUS) was obtained from Dojindo Laboratories (Kumamoto, Japan). Dimethyl sulfoxide (DMSO, dehydrated), sodium dihydrogenphosphate (NaH_2PO_4 , dehydrate), toluene (super dehydrated), glucose dehydrogenase (GDH), L-lactate dehydrogenase (LDH), diaphorase from colistridium kluysteri (DIA), β -nicotinamide-adenine dinucleotide oxidized (NAD), resazurin sodium salt, D-glucose, sodium L-lactate, chloroform and sodium chloride were purchased from Wako Pure Chemicals. Fluorescein-isothiocyanate (FITC) and 5/6-carboxy-tetramethyl-rhodamine succinimidyl ester (NHS-rhodamine) were obtained from Thermo Fisher Scientific Inc. (Waltham, MA). All other chemicals used were of analytical reagent grade. Milli-Q water (Millipore reagent water system, Bedford, MA, USA) was used throughout the experiments.

2.2. Apparatus

All fluorometric images of capillaries were obtained with a FluorImager 595 (Molecular Dynamics, Sunnyvale, CA, USA). A Denki Kagaku Keiki (Tokyo, Japan) glass electrode pH meter (model IOL30) was used for pH measurements.

2.3. Preparation of FITC-modified LDH and rhodamine-modified GDH

Lactate dehydrogenase (LDH) or glucose dehydrogenase (GDH) was dissolved in 0.02 M $\text{NaH}_2\text{PO}_4/\text{NaOH}$ solution (pH 7.4) (a PB solution) to give a 1.0 mg/ml solution. A 0.30 ml portion of the LDH solution or the GDH solution was mixed with 3.7 μl of 1 mM FITC or 1 mM rhodamine in dimethyl sulfoxide (DMSO, anhydrous). Then, the mixture was incubated for 1 h at room temperature. Unreacted label reagents were removed and replaced by a PB solution using Dye Removal Columns (Thermo Scientific). The LDH and GDH, each labeled with FITC and NHS-rhodamine, are abbreviated as FITC-LDH, FITC-GDH and rhodamine-GDH, respectively.

2.4. Preparation of liposomes

A 164 μl portion of a chloroform solution containing 2.5 mg PC, 0.34 mg/ml PE and 0.8 mg/ml EPC or 2.5 mg PC, 0.34 mg/ml PE and 0.8 mg/ml CFPE was dried to a lipid film and subjected to high vacuum. Each lipid film was hydrated in a 208 μl portion of a PB solution by vortex mixing for 10 min, followed by bath sonication for 10 min. The mixture was centrifuged at 12,000 g at 4 °C for 5 min. The precipitates were washed 4 times with a PB solution. Finally, the suspension was centrifuged (12,000 g). Liposomes of PC and CFPE (99:1, w/w) and PC and PE (99:1, w/w) were also prepared in the same manner as above.

2.5. Modification of the inner wall of a glass capillary with octadecyltrichlorosilane (OTS)

Borosilicate glass capillaries (1.5 mm o.d. and 0.86 mm i.d., Harvard Apparatus Ltd., Kent, UK) were washed with 1.0 M NaOH overnight and then thoroughly washed with Milli-Q water by sonication. A very small portion (approximately 18 μl) of octadecyltrichlorosilane (OTS) was sucked up by capillary action into the glass capillary and incubated for 1 h at room temperature. Then, the OTS solution was removed with Kimwipe paper. The capillaries were washed 15 times with toluene by sucking up and aspirating toluene with Kimwipe paper. The OTS-modified capillary is abbreviated as an OTS-capillary.

2.6. Patterning of the inner wall of an OTS-capillary with BSA and lipid

A 0.1 mg/ml BSA in a PB solution was partially injected into three different sections of an OTS-capillary, i.e., middle part and one-fourth parts of the capillary from both ends, with a micropipette (Scheme 1a). The capillary was incubated for 1 h. The inner wall of the capillary was washed 10 times with a 1 ml portion of a PB solution to remove excess BSA molecules. Then, a liposome suspension was run through the BSA-capillary for 30 min at a flow rate of 5 $\mu\text{l min}^{-1}$ using an ESP-64 micro syringe pump (Eicom, Kyoto, Japan), followed by washing with 1 M NaCl in a PB solution for 15 min at the same flow rate (Scheme 1b). This allowed us to form a lipid-patterned capillary, which was used for adsorption of enzymes. The capillary is abbreviated as a lipid(PC:PE:EPC)-modified capillary.

The adsorption of BSA on the inner surface of an OTS-capillary was confirmed by observing an increase in the fluorescence intensity arising from FITC-labeled BSA (Fig. S1). This capillary is abbreviated as a BSA(non-patterned)-capillary.

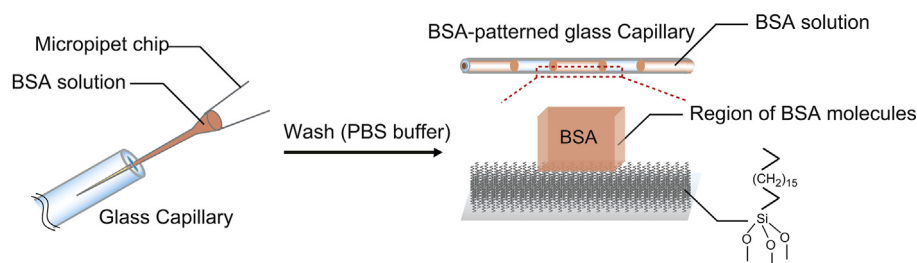
2.7. Adsorption of LDH and GDH on a lipid-modified capillary

Adsorption of LDH and GDH on the inside wall of a lipid (PC:PE:EPC)-modified capillary was performed according to the procedure described schematically in Scheme 2. First, a 3 μl portion of a 0.01 mg/ml LDH in a PB solution was sucked into roughly one-third length of a lipid-modified capillary from one end with a syringe connected to the capillary through a silicone tube. The capillary was incubated for 5 min. Then, the capillary was connected to an ESP-64 micro syringe pump and a PB solution containing 150 mM NaCl was run from the other end at a flow rate of 5 $\mu\text{l/min}$ for 12 min (Scheme 2a). Next, a 3- μl portion of 0.1 mg/ml GDH in a PB solution was sucked from the other end into roughly one-third length of the capillary. After incubation for 5 min, excess enzymes were washed out by running a PB solution containing 150 mM NaCl at a flow rate of 5 $\mu\text{l/min}$ for 12 min (Scheme 2b). Thus, GDH and LDH were immobilized stepwise on two different places of the capillary. The capillary is abbreviated as an enzyme (LDH and GDH)-modified capillary.

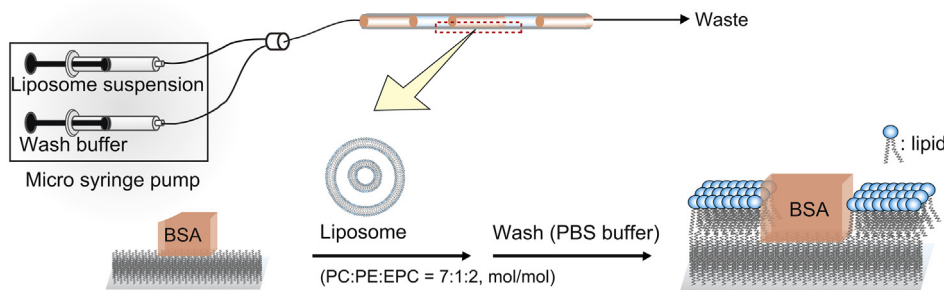
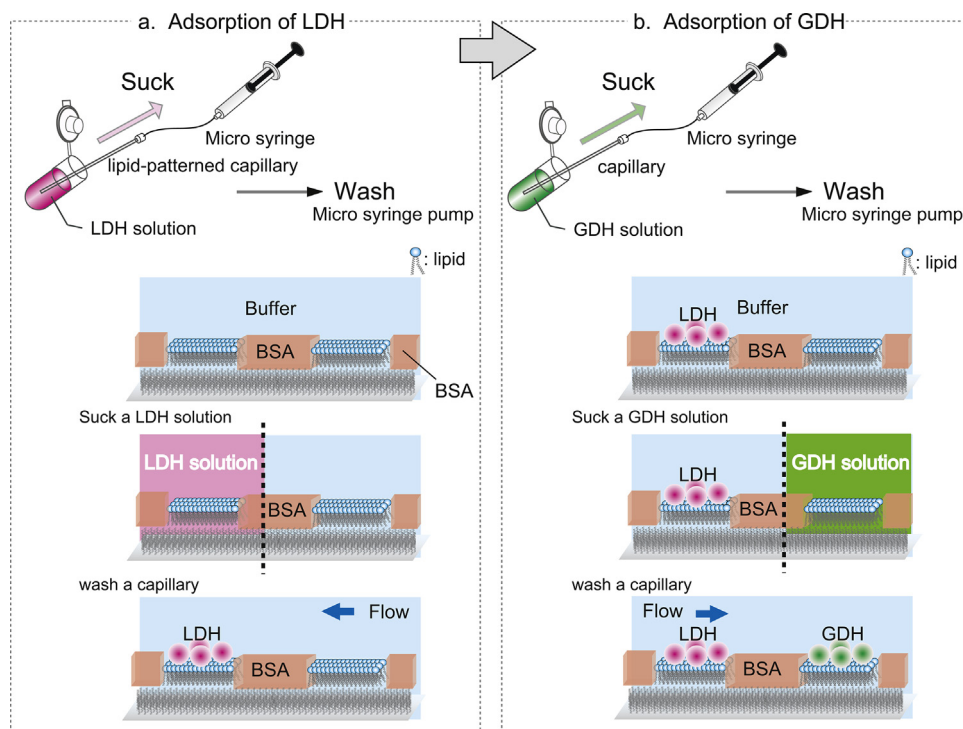
2.8. Simultaneous quantification of D-glucose and L-lactate

To maintain the adsorption of enzymes on the lipid surface through an electrostatic interaction, a PB solution without NaCl was used in the enzymatic reaction. A PB solution containing 0.1 mg/ml diaphorase (DIA), 1 mM NAD, 40 μM resazurin and analytes (D-glucose, L-lactate or their mixture) of known concentration was sampled into an enzyme (LDH and GDH)-modified capillary. After incubation for 10 min, the fluorometric images (ex. 514 nm, em. 590 nm) of the enzyme-modified capillary were captured with a FluorImager 595 (Molecular Dynamics, Sunnyvale, CA). The mean fluorescence intensity for the whole area except for the edge of

a) the method of preparing BSA patterned capillary



b) the method of preparing lipid patterned capillary

**Scheme 1.** Schematic illustration for the preparation of a lipid (PC:PE:EPC)-modified capillary.**Scheme 2.** Schematic illustration for the preparation of an enzyme (LDH and GDH)-modified capillary.

each fluorescence spot was obtained with Imager Quant version 5.0 and plotted against the initial concentration of D-glucose and L-lactate. Changing the display value of the captured image can alter the scanned images, but the mean fluorescence intensity for the initial image was not altered by this procedure. Also, the region of interest (ROI) defined for obtaining mean fluorescence intensity did not affect the magnitude of mean fluorescence intensity, as far as ROI was set within the patterned area.

3. Result and discussion

3.1. Formation of lipid layers inside a capillary

Phospholipid mono- and bilayers have been reported to be formed onto solid substrates such as glass, quartz, mica and silicon [27–39] if liposomes are adsorbed on the surface of the substrate and they undergo rupture spontaneously [30,31]. We investigated the formation of a lipid layer on the inside surfaces of glass-, OTS-

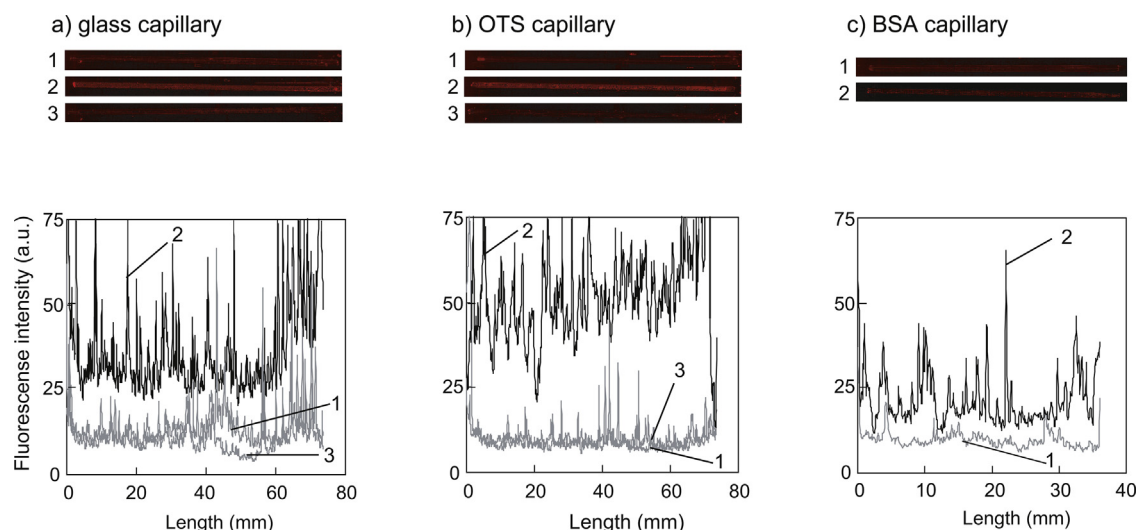


Fig. 1. Fluorescence images (upper) and intensity profiles (bottom) of (a) a glass capillary, (b) an OTS-capillary and (c) a BSA(non-patterned)-capillary. The BSA-capillary was prepared by treating an OTS-capillary with 0.1 mg/ml BSA in a PB solution. Intensity profile (bottom), (1) a capillary without any treatment, (2) a capillary treated with liposomes composed of PC and CFPE (99 : 1, w/w) and (3) a capillary treated with liposomes and 0.5% triton X-100. Ex. 488 nm and em. 530 nm.

and BSA(not patterned)-capillaries. Liposomes composed of PC and CFPE (99:1, w/w) were perfused into a capillary for 30 min, and residual liposomes were washed out with a PB solution containing 1 M NaCl. Then, the fluorometric images of the capillary were captured. As shown in Fig. 1, an increase in the fluorescence intensity was observed at the whole area of the glass- and OTS-capillaries (Fig. 1a and b), but the fluorescence intensity of the OTS-capillary was significantly larger than that of the glass capillary. When the glass- and OTS-capillaries were treated with 5% triton X-100, the fluorescence intensities decreased down to those before a flow of liposomes, indicating that lipid layers formed on the inside wall of the capillary was washed out with triton X-100. On the other hand, when a BSA-capillary was used, no significant increases in the fluorescence intensity were observed with or without a flow of the liposomes composed of PC and CFPE (Fig. 1c). This shows that lipid layers are not formed on the surface of BSA molecules. Therefore, the sectional region that separates BSA and lipid molecules on an OTS-capillary can be formed by patterning a capillary with lipid and BSA.

3.2. Regional formation of lipid membranes on the inner wall of an OTS-capillary

The results obtained above suggest that BSA molecules can be used as a masking reagent for patterning an OTS-capillary with lipid. To investigate the potential of this approach, an OTS-capillary was modified with BSA and lipid (a mixture of PC and CFPE), according to the procedure shown in Scheme 2, where a mixture of PC and CFPE without EPC was used as lipid. The fluorometric images obtained after washing the capillary with a PB solution containing NaCl are shown in Fig. 2. Patterned regions are clearly seen as a bright image at distance around 5.2–12 mm and 25–30 mm, respectively, confirming that the inside wall of an OTS-capillary was successfully patterned with BSA and lipid. Thus, the molecular assembly that separates BSA and lipid layers can be formed within an OTS-capillary.

3.3. Adsorption of enzymes on lipid capillaries

The potential of lipid layers as an adsorbent support of enzymes was investigated for 5 kinds of enzymes. Each enzyme (1 mg/ml) was perfused into a lipid (PC and PE)-capillary, and the incubated

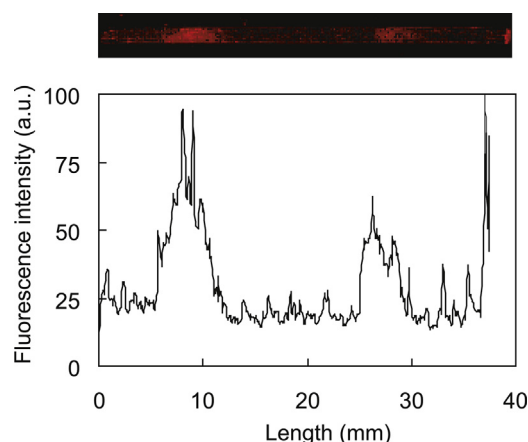


Fig. 2. A fluorescence image (upper) and an intensity profile (bottom) of an OTS-capillary patterned with BSA and lipid at two different positions. A PB solution containing liposomes composed of PC and CFPE (99 : 1, w/w) was pumped into a OTS-capillary at a flow rate of 5 ml/min for 30 min and the capillary was washed with 1 M NaCl in a PB solution for 15 min at the same flow rate. Ex. 488 nm and em. 530 nm.

Table 1

The adsorption of enzymes on the charged lipid layer in capillaries.

liposome composition	charge	enzyme (pI)
PC : PE : EPC (7:1:2, mol/mol)	positive	LDH (4.7), GDH (5.0), GOD (4.2) ¹ , LOD (4.6) ²
PC : PE : PS (7:1:2, mol/mol)	negative	GalOD (12) ³

¹ reference [36], ² reference [37], ³ reference [38].

Abbreviations: LDH, lactate dehydrogenase; GDH, glucose dehydrogenase; GOD, glucose oxidase; LOD, Lactate oxidase; GalOD, galactose oxidase.

capillary was washed with a PB solution containing 150 mM NaCl. The adsorption of each enzyme was detected by qualitative analysis of an enzymatic product, i.e., coloration or fluorescence emission (Fig. S2). The results obtained are summarized in Table 1, together with pI values. The negatively charged enzymes, i.e., LDH, GDH, glucose oxidase and L-lactate oxidase at pH 7.4, were adsorbed onto the lipid layers containing positively charged EPC, but they were not adsorbed on the negatively charged lipid layer containing PS. On the other hand, galactose oxidase was adsorbed onto the lipid

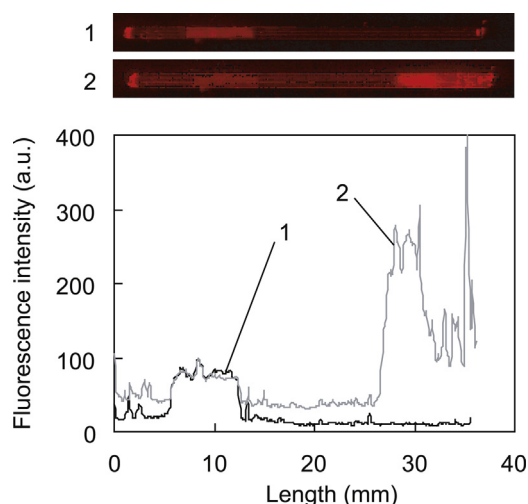


Fig. 3. Fluorescence images (upper) and intensity profiles (bottom) of enzyme (LDH and GDH)-patterned capillaries. FITC-LDH and rhodamine-GDH were adsorbed on different positions of lipid (PC:PE)-patterned capillaries. (1) Ex. 488 nm and em. 530 nm (FITC). (2) Ex. 514 nm and em. 570 nm (rhodamine).

layer containing PS. These results indicate that the charges of lipid layers play an important role in adsorbing the enzymes on lipid (PC and PE)-capillaries at physiological pH values. Further, the extent of adsorbed enzymes can be controlled by their concentrations, which are sampled into the lipid-layer capillary (Fig. S3).

Considering the above results, we performed the adsorption of LDH and GDH on a lipid (PC:PE)-capillary by incubating one place with a FITC-LDH solution and another place with a Rho-GDH solution (Scheme 2). The fluorescence images and intensity profiles of the capillary are shown in Fig. 3. The fluorescence emission at 530 nm corresponding to FITC-LDH was observed at distance from 5.0 to 12 mm (profile 1), while the emission at 570 nm corresponding to Rho-GDH was observed at distance from 27 mm to 31 mm (profile 2). The weak emission at 570 nm observed at distance from 5.4 to 12 mm is ascribable to FITC-LDH. Thus, LDH and GDH were adsorbed at different positions of a lipid (PC:PE)-capillary by treating the capillary with the enzymes in sequence.

3.4. Design of an enzyme (LDH and GDH)-capillary

An enzyme-based sensing system was designed using an enzyme capillary, where glucose dehydrogenase (GDH) and lactate dehydrogenase (LDH) were used as the sensing elements for D-glucose and L-lactate. The enzymatic activities of these enzymes are known to increase in the presence of lipid bilayers [32,33]. The isoelectric points of GDH and LDH are known to be at pH 4.7–4.8 [34] and pH 5.0 [35], respectively. First, we investigated the effect of the fraction of EPC on the fluorescence intensity. The lipid-modified capillaries were prepared by treating OTS-capillaries with a PB solution containing liposomes composed of PC, PE and EPC at different fractions. After washing the capillary, a 0.1 mg/ml FITC-LDH solution or a 0.1 mg/ml FITC-GDH solution was sucked into each capillary, excess enzyme was washed out with a PB solution. The effect of the fraction of EPC in a lipid mixture (PC, PE and EPC) on the degree of adsorption of FITC-LDH and FITC-GDH is shown in Fig. 4. The extent of adsorption of each enzyme increased in the presence of EPC and the amount of LDH adsorbed was the largest at 20% EPC.

Next, we examined the time course of fluorescence intensity. The time course of fluorescence images of an enzyme (LDH and GDH)-modified capillary in response to D-glucose and L-lactate are shown in Fig. 5a. The fluorescence emission caused by each enzymatic reaction was observed at the places where the respective

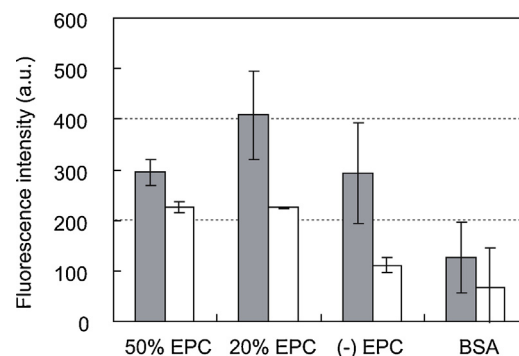


Fig. 4. Effect of cationic lipid (EPC) on the adsorption of FITC-LDH (closed bar) and FITC-GDH (open bar).

A PB solution containing liposomes composed of PC, PE and EPC was pumped into OTS-capillaries. Then, FITC-LDH and FITC-GDH were adsorbed according to Scheme 2. Column BSA indicates the fluorescence intensity caused by the absorption of the enzymes on BSA (not patterned)-capillaries. Ex. 488 nm, Em. 530 nm. Error bars indicate mean $\pm$ standard deviation.

enzymes were adsorbed. As shown in Fig. 5b, as time elapsed, the fluorescence intensity at the respective regions increased due to an increase in the resorufin amount produced by the enzymatic reaction inside the capillary. The fluorescence intensity in response to L-lactate and D-glucose increased with time till 50 min (Fig. 5c). Further, the lower extent of LDH and GDH modification on each lipid area led to a decrease in the obtained fluorescence intensities (Fig. S5), but the relationships between the fluorescence intensities and time were linear for both cases of D-glucose and L-lactate (Fig. 5). These suggested that desorption of the modified enzymes did not occur significantly, resulting in the successful measurements for D-glucose and L-lactate. However, we noticed that a fluorescence region gradually broadened with incubation time. To investigate the effect of broadening of a fluorescence band along with a lipid (PC and PE)-modified capillary, a 9 μ l portion of a 0.1 mg/ml FITC-GDH or FITC-LDH in a PB solution was sampled into roughly one-third length of the capillary from one end with a syringe, and the capillary was incubated for 5 min and 30 min (Fig. S3). For the case of 5 min incubation, the fluorescence region ascribable to FITC-GDH was clearly observed. However, when the capillary was incubated for 30 min, the fluorescence intensity lowered and an additional region appeared at distance around 25–30 (Fig. S4). This indicates that FITC-GDH diffused along the capillary during the incubation period. The similar results were obtained for FITC-LDH. Consequently, the incubation time of the capillary was determined to be 10 min. The fluorescence intensities ascribable to D-glucose and L-lactate using an enzyme (LDH and GDH)-modified capillary (Fig. S6) were almost the same as those using the enzyme capillaries where LDH or GDH was modified at the whole area of the inner wall. This indicated that non-specific binding between the enzymes and BSA rather than diffusion contributed the broadening of a fluorescence band of resorfin when the enzyme (LDH and GDH)-modified capillary was incubated for 10 min.

3.5. Concentration dependence and selectivity

The responses of an enzyme (LDH and GDH)-modified capillary to D-glucose and L-lactate in their mixture are shown in Fig. 6. The fluorescence intensity of the LDH site at 10 min increased with an increase in the concentration of L-lactate ranging from 1.0 to 20 μ M with the detection limit of 4.9 μ M ($S/N=3$, $n=3$) and that of the GDH site at 10 min increased with an increase in the D-glucose concentration ranging from 60 to 200 μ M. The detection limit was 32 μ M ($S/N=3$, $n=3$). Thus, simultaneous quantification of D-glucose and L-lactate in a mixture was achieved with the

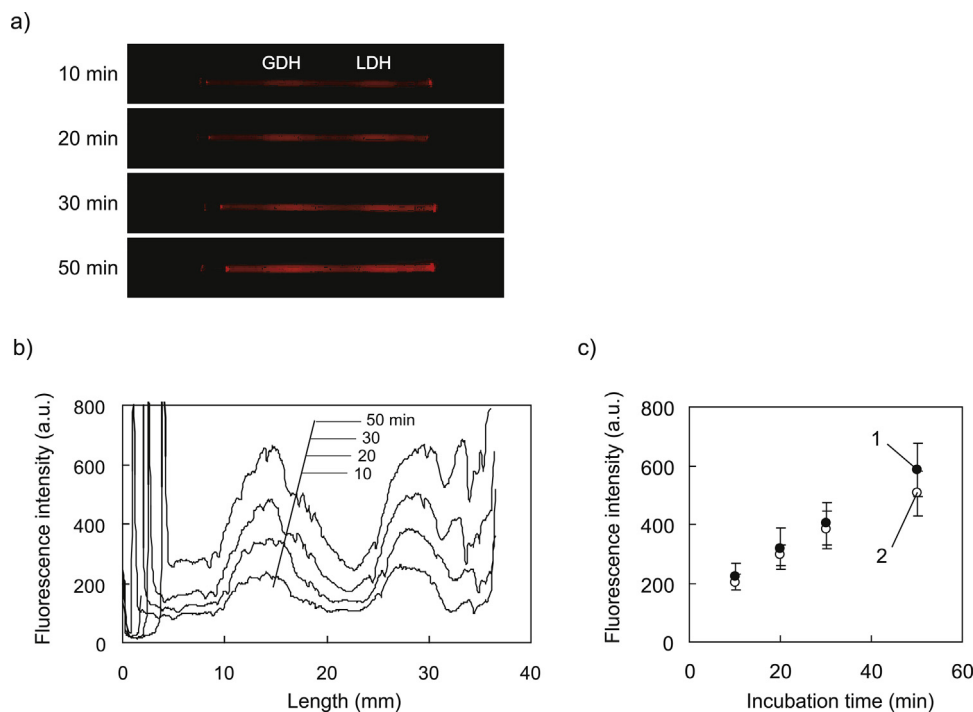


Fig. 5. Time courses of enzymatic reaction with an enzyme (LDH and GDH)-patterned capillary after flowing a PB solution containing 0.1 mg/ml DIA, 1 mM NAD, 40 μ M resazurin, 60 μ M D-glucose and 10 μ M L-lactate. The enzyme (LDH and GDH)-patterned capillary was prepared with 0.01 mg/ml LDH and 0.1 mg/ml GDH solutions according to Scheme 2. Ex. 517 nm and em. 590 nm. Error bars indicate mean $\pm$ standard deviation. (a) Fluorescence image, (b) fluorescence profile and (c) fluorescence intensity. For the case of (c), the open circle (1) indicates the fluorescence intensity caused by the enzymatic reaction of LDH and the closed circle indicates that of GDH.

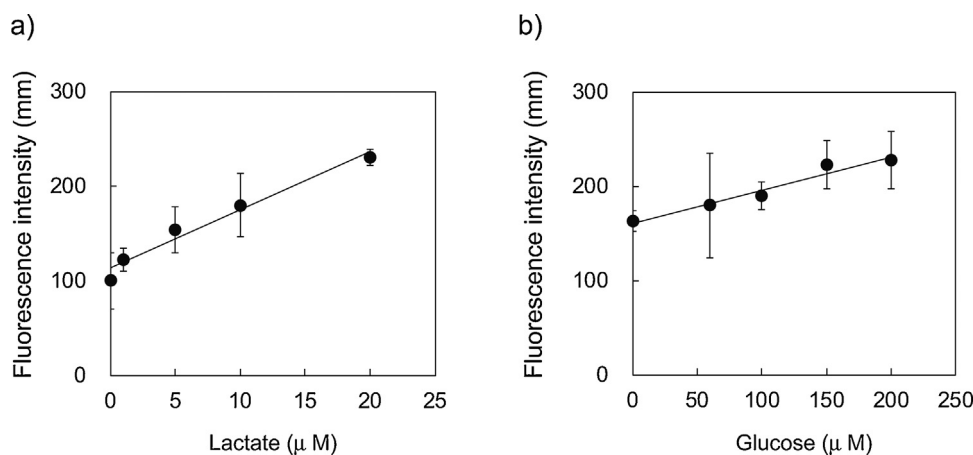


Fig. 6. Concentration dependence for L-lactate in the presence of 60 μ M D-glucose (left) and for D-glucose in the presence of 10 μ M L-lactate (right) with an enzyme (LDH and GDH)-patterned capillary. The enzyme-patterned capillaries were incubated with a PB solution containing 0.1 mg/ml DIA, 1 mM NAD, 40 μ M resazurin, D-glucose and L-lactate for 10 min. Ex. 517 nm and em. 590 nm. Error bars indicate mean $\pm$ standard deviation.

double enzyme-modified capillary. The limits of quantification of L-lactate and D-glucose were 16 μ M ($S/N=10$, $n=3$) and 100 μ M ($S/N=10$, $n=3$), respectively. The fluorescence intensities obtained from the intra-day measurements for D-glucose and L-lactate were almost the same level as that from the inter-day measurements (Fig. S7). In addition, there were no difference in standard deviation between intra- and inter-day. The concentration dependences for D-glucose and L-lactate were obtained using different the enzyme (LDH and GDH) modified capillaries. Therefore, the error bars in Fig. 6 included the lot-to-lot errors

The interference of human serum albumin (HSA) and γ -globulin on the assay of D-glucose and L-lactate was investigated. The presence of 0.25 mg/ml HSA and 6.1 μ g/ml γ -globulin did not have a significant influence on the fluorescence intensity caused by the

enzymatic reactions (Fig. S8). This indicates that the presence of HSA and γ -globulin do not affect the quantification of D-glucose and L-lactate.

3.6. Simultaneous determination for D-glucose and L-lactate in human serum

The D-glucose and L-lactate levels in human serum have been reported to be in the range from 3.8 mM to 5.6 mM [39,40], and 0.22 mM to 2.9 mM [41,42], respectively. After human blood serum was 20 times diluted with a phosphate solution (pH 7.4), the determination of D-glucose and L-lactate in the diluted human blood serum was performed. The determination of D-glucose and L-lactate in the diluted human serum showed that

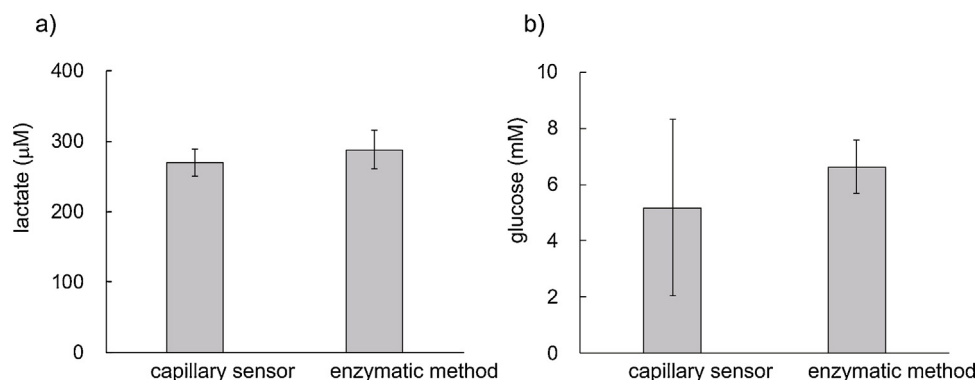


Fig. 7. Simultaneous assay of D-glucose and L-lactate in diluted human serum.

The enzyme(LDH and GDH)-patterned capillaries were incubated with a PB solution containing 0.1 mg/ml DIA, 1 mM NAD, 40 μM resazurin, 20 times diluted human serum for 10 min. Ex. 517 nm and em. 590 nm. In the enzymatic method, a PB solution containing 10 $\mu\text{g/ml}$ glucose oxidase or L-lactate oxidase, 0.1 mg/ml horseradish peroxidase, 100 mM DA-64 and 120 times diluted human serum was incubated for 40 min and absorbance at 724 nm was measured. These measurements were repeated three times for a human serum sample. Error bars indicate mean $\pm$ standard deviation. (a) L-lactate. (b) D-glucose.

the concentration of D-glucose and L-lactate were 5.2 ± 3.1 mM and 0.27 ± 0.02 mM, respectively. The present results were compared with those obtained by the conventional absorptiometry. The D-glucose and L-lactate levels in diluted human serum by both methods were in fairly good agreement (Fig. 7). The larger error bars for glucose detection was due to the differences of the extent of modified GDH between the prepared capillaries. The reproducibility of GDH extent appeared to be lower than that of LDH, considering that the error bars of the fluorescence intensities of FITC-GDH was larger (Fig. S4).

4. Conclusion

We demonstrated that the usefulness of lipid membranes as absorbent material of LDH and GDH for the design of a capillary-based biosensor. The lipid membrane as an absorbent material has the potential of immobilizing various kinds of proteins by changing electrostatic charges of lipid membranes. The adsorption of glucose dehydrogenase and lactate dehydrogenase was successfully achieved through the regional formation of the lipid membranes on the inner wall of an OTS-capillary. The present capillary system allowed us to quantify L-lactate and D-glucose simultaneously in diluted human serum. Compared with the capillary enzyme sensor utilizing covalent bond [17], the preparation of the enzyme (LDH and GDH) modified capillary was simple, and the sensitivity and accuracy was enough to measure L-lactate and D-glucose simultaneously in diluted human serum. The enzyme (LDH and GDH)-modified capillary is suitable to be used as a disposable sensor for measuring D-glucose and L-lactate. The small size and low cost are advantageous to its use. The present approach may work an analytical tool for clinical diagnosis, food industry, environment monitoring with simple procedures.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.jpba.2018.09.028>.

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