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Stimuli-responsive hydrogel–silver nanoparticles composite for development of localized surface plasmon resonance-based optical biosensor

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ABSTRACT

In this paper, the development of a localized surface plasmon resonance (LSPR)-based optical enzyme biosensor using stimuli-responsive hydrogel–silver nanoparticles composite is described. This optical enzyme biosensor was constructed by immobilizing glucose oxidase (GOx) into the stimuli-responsive hydrogel. When a sample solution such as glucose was applied to the surface of this optical enzyme biosensor, the interparticle distances of the silver nanoparticles present in the stimuli-responsive hydrogel were increased, and thus the absorbance strength of the LSPR was decreased. Furthermore, hydrogen peroxide, which was produced by the enzymatic reaction, induced the degradation of highly clustered silver nanoparticles by the decomposition of hydrogen peroxide. Hence, a drastic LSPR absorbance change, which depends on the glucose concentrations, could be observed. On the basis of the abovementioned mechanism, the characterization of the LSPR-based optical enzyme biosensor was carried out. It was found that the LSPR-based optical enzyme biosensor could be used to specifically determine glucose concentrations. Furthermore, the detection limit of this biosensor was 10 pM. Therefore, this LSPR-based optical enzyme biosensor has the potential to be applied in cost-effective, highly simplified, and highly sensitive test kits for medical applications.

1. Introduction

Nanotechnology and nanomaterials are novel and exciting fields of research [1]. Furthermore, the inherently small size and the unusual optical, magnetic, catalytic, and mechanical properties of nanoparticles, which are not found in bulk materials, permit the development of novel devices and applications in diverse fields such as electronics, optics, and biotechnology [2]. Therefore, the characteristics of nanomaterials such as nanoparticles, nanotubes, and nanofibers have been theoretically studied in detail [3,4].

Nanomaterials have unique characteristics due to their quantum size effects. Based on these characteristics, nanomaterials were used as quantum dots (QDs) [5] and noble

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metal nanoparticles such as gold and silver [6,7] for several applications. One of the earliest applications using nanomaterials that had been realized was the development of chemical sensors and biosensors [8–11]. Remarkable progress has been made in the development of optical sensors and their utilization in environmental applications, biotechnology, medicine, drug screening, and food control.

In particular, the localized surface plasmon resonance (LSPR)-based optical chemical sensor and biosensor, which is excited using noble metal nanomaterials such as gold and silver, has been expected to realize the highly sensitive detection of target molecules in medical applications [12–16]. LSPR is well known to be excited on nanostructured noble metals such as gold and silver. Noble metals have intrigued people for centuries because of their optical properties, which include the display a bright glow in various attractive colors. As described by the Mie theory, these properties are strongly dependent on the size, shape, interparticle distance, and the local environment of the noble metal nanostructures [17]. Briefly, as the size of a metal structure decreases from the bulk-scale to the nano-scale, the movement of electrons through the internal metal framework is restricted. As a result, metal nanoparticles display specific extinction bands in the UV–vis spectra when the incident light resonates with the conduction band electrons on their surfaces. These charge density oscillations are simply defined as LSPR [18].

Using these specific characteristics of LSPR, LSPR-based optical biosensors can detect an immediate increase in the thickness of a biomolecular layer on the surface of a sensitive element that is caused by the reaction between the experimental solution and the receptor layer immobilized on the surface. Hence, using these characteristics of LSPR, optical biosensors that are more convenient to use can be developed. Previously, we achieved the label-free detection of antigen–antibody reactions [19,20] and peptide nucleic acid (PNA)–DNA and DNA–DNA hybridizations using LSPR-based optical biosensors [21,22]. As a result, the high capability of the LSPR-based optical biosensor could be observed. Based on these characteristics of the LSPR-based optical biosensor, we fabricated a multi-array, LSPR-based optical biosensor for application in the multiple detection of antigen–antibody reactions [23]. With this background, we aimed to develop the LSPR-based optical enzyme biosensor.

To date, the applications of enzyme biosensors using nanomaterials have been strenuously investigated based on electrochemical detection principles [24,25]. Electrochemical detection principles involving enzyme biosensors using nanomaterials are well-established principles and it could be used for the high-sensitive detection of target molecules. However, the detection of target molecules using electrochemical detection principles involves complex, expensive apparatuses. Therefore, there is a demand for a more convenient and cost-effective enzyme biosensor using nanomaterials. Hence, we targeted the development of the LSPR-based optical enzyme biosensor for this requirement.

In addition, for the development of LSPR-based optical enzyme biosensors, we focused on the use of stimuli-responsive hydrogels for the fabrication of enzyme biosensors [26,27]. The volume changes of stimuli-responsive hydrogels in response to marked changes in the environmental conditions were reported previously [28,29]. The stimuli-responsive hydrogels are water-swollen, cross-linked networks of hydrogels that exhibit reversible change in the volume in response to specific stimuli. Based on these features, the constituent hydrogels of the stimuli-responsive hydrogels incorporate molecular recognition elements such as enzymes. When the recognition elements react with specific chemical substances, the monomer units bearing the recognition elements undergo a change in either the solubility or the charge state. Changes in the solubility will cause the swelling or shrinking of the hydrogel network. Alternatively, changes in the charge state result in changes in the interactions between the charged groups and alterations in the concentration of electrolytes inside the hydrogel. In either case, either effect results in changes in the hydrogel volume. On the basis of this mechanism using stimuli-responsive hydrogels or polymers, several research groups fabricated biosensors and chemical sensors for medical and environmental applications [30–34]. Therefore, these materials are suitable for the fabrication of enzyme biosensors that can be used to develop LSPR-based biosensors that are more convenient to use.

From the abovementioned interesting characteristics of noble metal nanoparticles and stimuli-responsive hydrogels, in this study, we combined these materials for the biosensor fabrication. The stimuli-responsive hydrogels contain molecular recognition elements such as enzymes that specifically react with the analyte. This recognition event causes the stimuli-responsive hydrogels to swell owing to an increased osmotic pressure. The swelling of stimuli-responsive hydrogels increases the mean internal distance between the noble metal particles, and thus decreases the LSPR absorbance strength (Fig. 1).

In this report, we fabricated an LSPR-based optical enzyme biosensor using stimuli-responsive hydrogel–silver nanoparticles composite. The characteristics of this enzyme biosensor were evaluated by using a UV–vis spectrophotometer. Simultaneously, we evaluated the selectivity and calibration characteristics of this LSPR-based optical enzyme biosensor.

2. Experimental

2.1. Materials

Polyvinylpyrrolidone (PVP)-coated silver nanoparticles (diameter: 20 nm, 0.19%, w/v) for preparing the enzyme biosensor were obtained from Tanaka Kikinzoku Kogyo Inc. (Kanagawa, Japan). The slide glass substrate (S-1111, 76 mm × 26 mm, thickness: 0.8–1.0 mm) was purchased from Matsunami Glass Ind. Ltd. (Osaka, Japan). Acrylamide-HG (AAm), *N*,*N*′-methylenebis(acrylamide)-HG (bis-AAm), *N*,*N*,*N*′,*N*′-tetramethylethylenediamine (TEMED) for electrophoresis and sodium hydroxide, sodium chloride, glucose oxidase (GOx, E.C.1.1.3.4: from *Aspergillus niger*, 20000 units/g solid), and *N*-hydroxysuccinimide (NHS) for peptide synthesis were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). 2,2-diethoxyacetophenone (DEAP), which acts as a photoinitiator for the photopolymerization of the hydrogel,

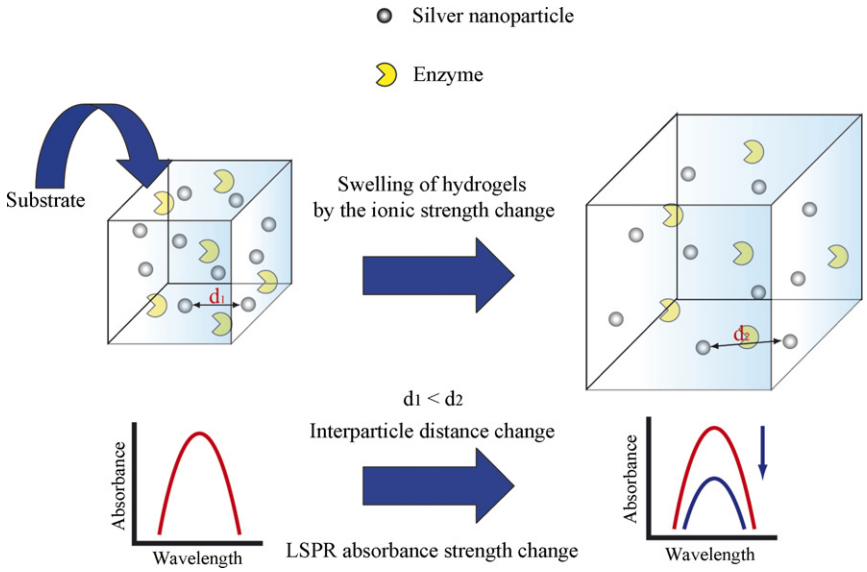

Fig. 1 – Schematic illustration of detection principle of LSPR-based optical enzyme biosensor using stimuli-responsive hydrogel–silver nanoparticles composite.

was purchased from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC), which is used for the activation of carboxyl groups, was purchased from Dojindo (Kumamoto, Japan). For the characterization of the LSPR-based optical enzyme biosensor, D-glucose, sucrose, mannitol and sodium chloride (NaCl) were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Milli-Q water (18.3 MΩ cm) from Millipore (Billerica, USA) was used for all the experiments.

2.2. *Apparatus*

The spectrophotometer (DU® 800, wavelength range: 190–1100 nm) that was used for the evaluation of the optical characteristics of the LSPR-based optical enzyme biosensor was purchased from Beckman Coulter (CA, USA). For the fabrication of the enzyme biosensor, a transilluminator (TM-20, wavelength: 302 nm) was purchased from Funakoshi Co., Ltd. (Tokyo, Japan).

2.3. *Fabrication of LSPR-based optical enzyme biosensor using stimuli-responsive hydrogel–silver nanoparticles composite*

The fabrication of the LSPR-based optical enzyme biosensor using stimuli-responsive hydrogel–silver nanoparticles composite is shown in Fig. 2. A polymerization mixture solution consisting of AAm (9.0%, w/v), bis-AAm (1.0%, w/v), PVP-coated silver nanoparticles (0.01%, w/v), and DEAP (0.1%, v/v) was prepared. The mixture solution was introduced onto the slide glass substrate surface. Photopolymerization was performed using the transilluminator for 5 min at room temperature (RT). The photopolymerized stimuli-responsive hydrogel–silver nanoparticles composite were washed with Milli-Q water. During the preparation of the stimuli-responsive hydrogel–silver nanoparticles composite, a silicon rubber sheet was attached to the slide glass substrate surface for holding the polymerization mixture solution. Finally, the glass substrate was stored in the refrigerator before use.

For immobilization of the enzymes, the photopolymerized stimuli-responsive hydrogel–silver nanoparticles composite (1.0 cm × 1.0 cm × 1.0 mm) were hydrolyzed at RT for 90 min by immersing them in sodium hydroxide solution (0.3 N) in 10% (v/v) aqueous TEMED in order to convert the amides to carboxylates. While washing with Milli-Q water, the hydrolyzed stimuli-responsive hydrogel–silver nanoparticles composite swelled drastically. Therefore, the hydrolyzed stimuli-responsive hydrogel–silver nanoparticles composite were then extensively washed with Milli-Q water and immersed into a mixture of 100 mM EDC solution and 100 mM NHS solution for 90 min at RT. After washing with Milli-Q water and phosphate buffer (20 mM, pH 7.4), immobilization of the enzyme was carried out. An enzyme solution (100 U/mL) was prepared by diluting the enzyme with phosphate buffer. Subsequently, the stimuli-responsive hydrogel–silver nanoparticles composite were placed in the enzyme solution. Immobilization was then allowed to proceed for 90 min, after which the enzyme-immobilized stimuli-responsive hydrogel–silver nanoparticles composite were incubated overnight in phosphate buffer to remove the excess enzyme.

2.4. *Evaluation of optical characteristics of the LSPR-based optical enzyme biosensor using stimuli-responsive hydrogel–silver nanoparticles composite*

The experimental procedure for the evaluation of the optical characteristics of LSPR-based optical enzyme biosensor is

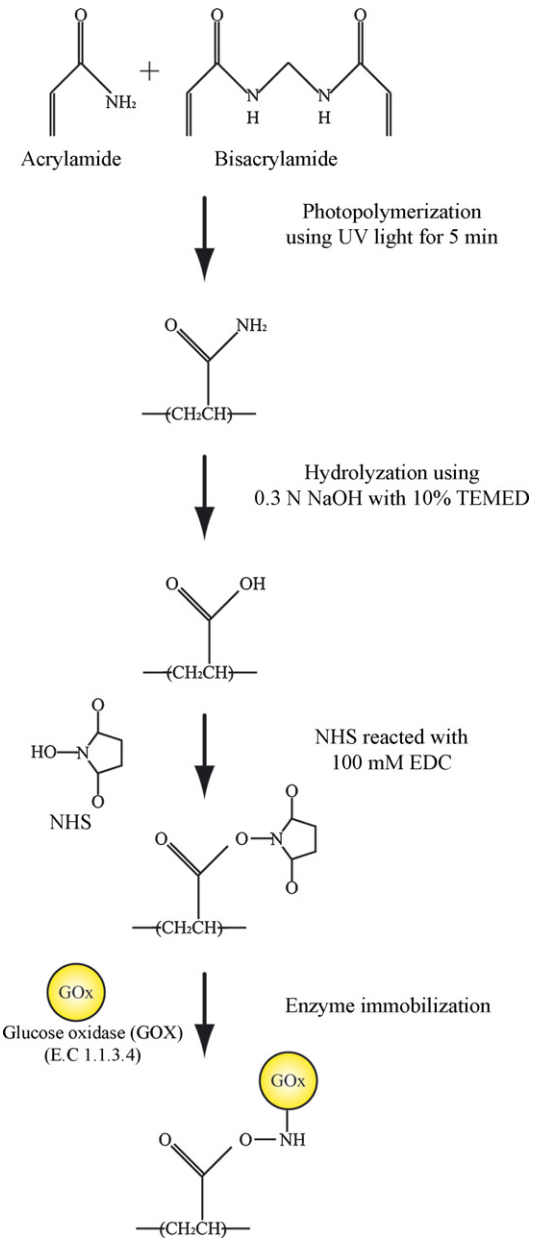

Fig. 2 – Fabrication procedure of LSPR-based optical enzyme biosensor using stimuli-responsive hydrogel–silver nanoparticles composite.

shown in Fig. 3. All the absorbance spectra were recorded from 300 to 800 nm on the UV–vis spectrophotometer at RT. Under these experimental conditions, the optical characteristics of the LSPR-based optical enzyme biosensor were investigated.

In addition, for the characterization of the sensing capability of this biosensor, different kinds of sample solutions (glucose, sucrose, mannitol, phosphate buffer and milli-Q water) were introduced onto the biosensor surface before the evaluation of the optical characteristics was carried out. Simultaneously, changes in the optical characteristics due to the swelling of the stimuli-responsive hydrogel resulting from the enzymatic reaction after changes in the optical characteristics with time (0, 2.5, 5, 10, 20, 30, 60 min) were studied,

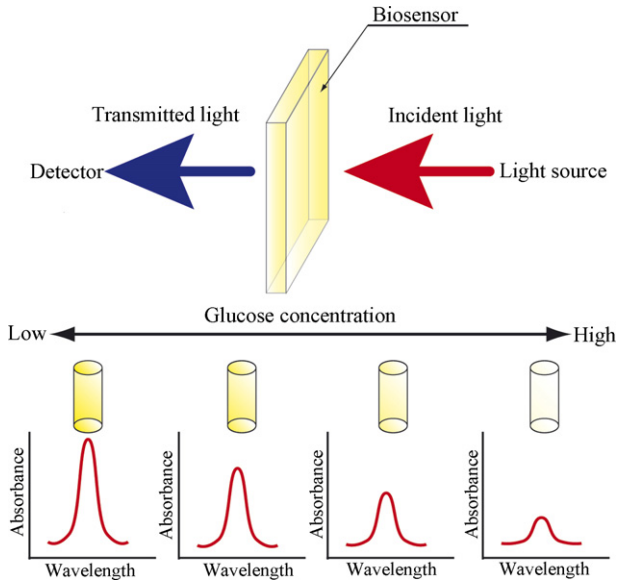

Fig. 3 – Schematic illustration of detection principle of LSPR-based optical enzyme biosensor using stimuli-responsive hydrogel–silver nanoparticles composite.

and evaluation of the calibration characteristics by introducing different concentrations of glucose solutions was carried out.

3. Results and discussions

3.1. *Characteristics of LSPR-based optical enzyme biosensor using stimuli-responsive hydrogel–silver nanoparticles composite*

The evaluation of the optical characteristics was carried out using the LSPR-based optical enzyme biosensor. Fig. 4a shows the transmittance of the biosensor from UV to visible wavelength. The changes in the absorbance strength of the biosensor are shown in Fig. 4a. In addition, the absorbance strength dependencies of the silver nanoparticle concentrations (0.025, 0.01%, w/v) were monitored at 408 nm. Based on these results, the silver nanoparticle concentrations were affected to the absorbance strength by the LSPR absorption. Furthermore, there are difficulties in the evaluating the optical characteristics of the stimuli-responsive hydrogel–silver nanoparticles composite for higher concentrations of silver nanoparticles by using a conventional spectrophotometer. As a result, in this study, a 0.01% (w/v) of silver nanoparticle was used for the fabrication of the LSPR-based optical enzyme biosensor.

Fig. 4b shows a photograph of the LSPR-based optical enzyme biosensor fabricated using different concentrations of silver nanoparticles. The complementary color (yellow) of LSPR absorbance by the silver nanoparticles could be observed. In addition, differences in the color strengths arising from variations in the silver nanoparticle concentrations could be observed with the naked eye. Hence, in accordance with Fig. 4a, the silver nanoparticle concentrations were found to

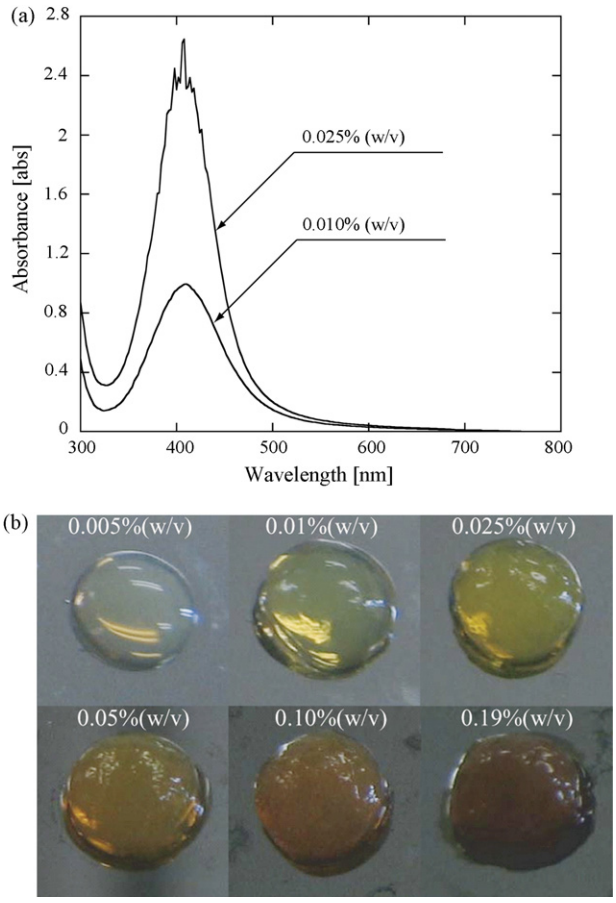

Fig. 4 – (a) Optical characteristics of LSPR-based optical enzyme biosensor using stimuli-responsive hydrogel-silver nanoparticles composite fabricated using different concentrations of silver nanoparticles. (b) Photograph of LSPR–based optical enzyme biosensor fabricated using different concentrations of silver nanoparticles.

affect the optical characteristics of the LSPR-based optical enzyme biosensor.

3.2. *Evaluation of calibration characteristics for glucose using LSPR-based optical enzyme biosensor*

For the evaluation of the specificity of the LSPR-based optical enzyme biosensor, different kinds of sample solutions (glucose, sucrose, mannitol, phosphate buffer, and milli-Q water) were used in this experiment. For the evaluation of the changes in the optical characteristics, 1000 μL of different kinds of 1 mM sample solutions were introduced onto the LSPR-based optical enzyme biosensor surface.

Fig. 5 shows the changes in optical characteristics of the LSPR-based optical enzyme biosensor with time in the visible region (300–800 nm). When the milli-Q water, phosphate buffer, mannitol, sucrose, and glucose solutions were introduced onto the biosensor surfaces after the introduction of the sample solutions, changes in the optical characteristics, which were related to the specificity of GOx, could be observed. When the glucose solution was introduced onto the biosensor, a change in the LSPR absorbance strength due to the swelling of the stimuli-responsive hydrogel could be observed (Fig. 5a). However, during the introduction of other sample solutions, the LSPR absorbance strength decrease could not be observed (Fig. 5b).

The swelling of the hydrogel results from the formation of a reduced flavin adenine dinucleotide (FAD) anion and gluconic acid upon glucose turnover. Theoretically, the enzymatic reaction of GOx can be expressed as shown below:

$$\mathrm{GOx(ox) + Glucose \rightarrow GOx^{-}(red) + gluconicacid + H^{+}}$$

$$\mathrm{H^{+} + GOx^{-}(red) + O_2 \rightarrow H_2O_2 + GOx(ox)}$$

The oxidized FAD is uncharged at neutral pH. However, the reduced FAD is anionic at neutral pH. Furthermore, the reduced FAD is reoxidized by O_2. As a result, the GOx conversion of glucose to gluconic acid reduced the FAD prosthetic group, which became negatively charged. Furthermore, gluconic acid which produced by the enzymatic reaction is also negatively charged. Hence, the formation of these covalently bound hydrogel anions resulted in a Donnan potential;

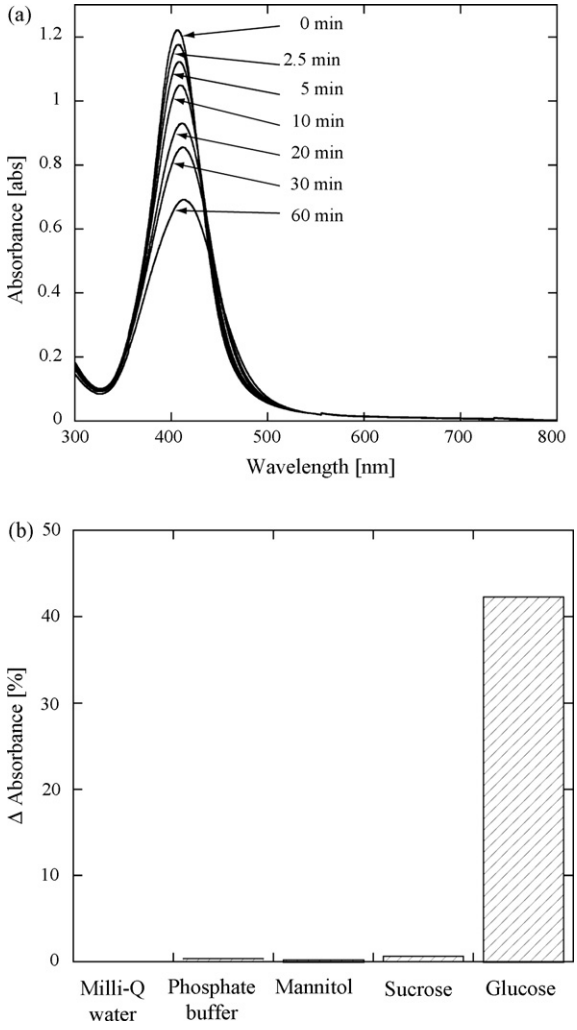

Fig. 5 – (a) Change in optical characteristics with time for 1 mM Glucose. (b) Selectivity of GOx immobilized LSPR-based optical biosensor.

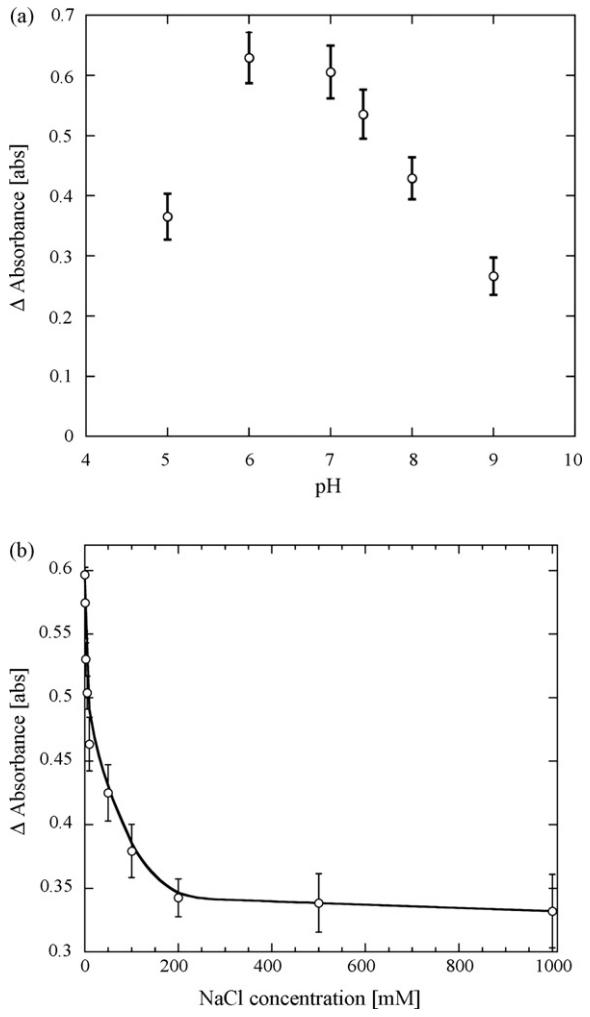

Fig. 6 – (a) pH efficiency of LSPR-based optical biosensor using stimuli-responsive hydrogel–silver nanoparticle composite. (b) NaCl concentration dependency of LSPR-based optical biosensor.

this potential gave rise to an osmotic pressure that caused the hydrogel to swell, which decreased the LSPR absorbance strength.

Furthermore, in the LSPR-based optical enzyme biosensor, hydrogen peroxide, which was produced by the enzymatic reaction between GOx and glucose, also contributed to the LSPR absorbance change. The catalytic ability of silver for the decomposition of hydrogen peroxide has been reported [35]. In this enzyme biosensor, the catalytic decomposition of hydrogen peroxide induces the degradation of highly clustered silver nanoparticles. Hence, a remarkable change in the LSPR absorbance strength could be observed.

In addition, the pH efficiency on the activity of immobilized GOx in stimuli-responsive hydrogel–silver nanoparticles composite was evaluated. The pH efficiency in phosphate buffer at RT at different pH values (5.0–9.0) is shown in Fig. 6a

In the pH efficiency evaluation, the pH values for optimum activity of the immobilized GOx are around pH 6.0–7.4. However, in the different pH, the LSPR absorption strength changes were drastically decreased. From these results, the pH affects the enzyme activity and swelling kinetics of stimuli-responsive hydrogels. Hence, in this study, every experiment was carried out at pH 7.4 for medical applications. Simultaneously, the salt concentration efficiency was also evaluated. The swelling kinetics of stimuli-responsive hydrogel affect in ionic strength of surrounding media. Therefore, in order to evaluate the ionic strength capability, determination of LSPR absorbance strength changes for 1 mM glucose in different concentration (0–1000 mM) of NaCl contained phosphate buffer were carried out. The salt concentration efficiency is shown in Fig. 6b. As a result, the LSPR absorbance strength was decreased with the increment of NaCl concentrations. However, the LSPR-absorbance strength changes were not inhibited completely due to the degradation of silver nanoparticles by the enzymatic reaction.

Based on the abovementioned mechanism of the LSPR-based optical enzyme biosensor and experimental conditions, the evaluation of the calibration characteristics was carried out. For this purpose, different concentrations of glucose solutions were introduced onto the LSPR-based optical enzyme biosensor.In the experiments, different concentrations of glu-

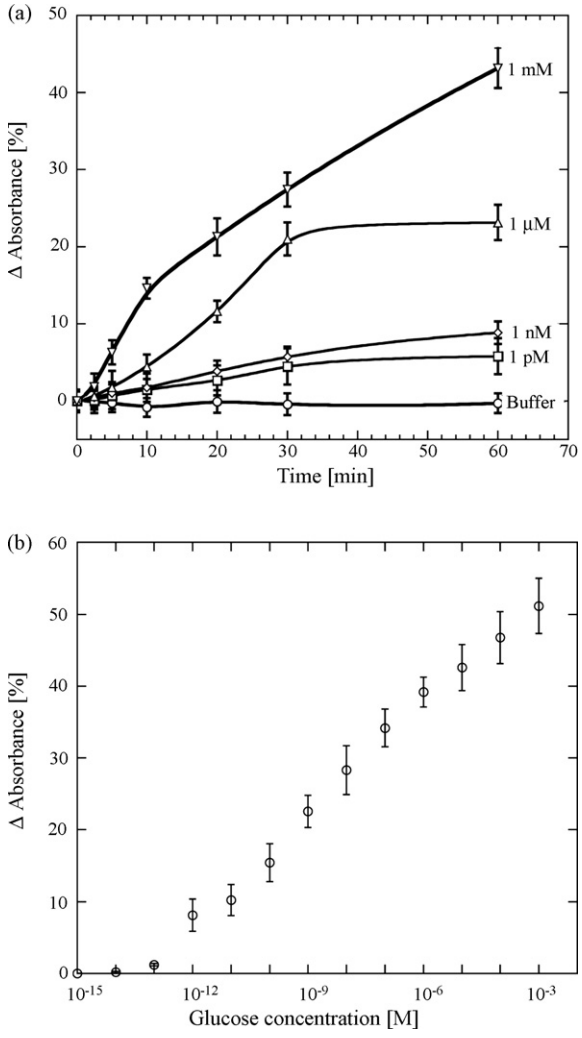

Fig. 7 – Calibration characteristics of LSPR-based optical enzyme biosensor: (a) time dependence and (b) calibration characteristics.

cose solutions that diluted were with phosphate buffer were introduced onto the LSPR-based optical enzyme biosensor for 10 min; subsequently, the evaluation of the optical characteristics was carried out. The calibration characteristics of the biosensor are shown in Fig. 7. Using the biosensor, the change in the LSPR absorbance strength was observed to be related with the glucose concentrations (Fig. 7a). Furthermore, the detection limit of this biosensor was 10 pM (Fig. 7b). Based on the abovementioned characteristics of the LSPR-based optical enzyme biosensor, our method using stimuli-responsive hydrogel–silver nanoparticles composite is a promising candidate for the low-cost and high-sensitive detection of target molecules because there is no necessity of expensive apparatuses. Furthermore, such a high sensitivity and selectivity toward the target molecules indicated that the LSPR-based optical enzyme biosensor reported in this study is also applicable to noninvasive biomonitoring.

4. Conclusions

We developed an LSPR-based optical enzyme biosensor using stimuli-responsive hydrogel–silver nanoparticles composite for the highly sensitive determination of glucose concentrations. The LSPR absorbance strength resulting from the swelling of the stimuli-responsive hydrogel and the rapid degradation of highly clustered silver nanoparticles were observed. Furthermore, the determination of the target molecule concentration is possible by observing the LSPR absorbance strength. Hence, using this detection principle, the LSPR-based optical enzyme biosensor, which uses the stimuli-responsive hydrogel and silver nanoparticles, has the potential to act as a noninvasive glucose biosensor to determine blood glucose levels without using blood samples. In previous reports of glucose concentrations in saliva and tears, the detection range of this biosensor covers the nondiabetic patient's glucose concentration. Therefore, based on these characteristics, this biosensor has the potential to be applied as a simplified test kit for noninvasive biomonitoring.

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