SPR sensor signal amplification based on dye-doped polymer particles

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Abstract

A novel amplification method was prepared based on dye-doped polymer particles for SPR signals originating from antigen–antibody (BSA–anti-BSA) interactions. Coloring the particles enhances the change of SPR signals based on the change of the imaginary part of the refractive index. The signal amplification effect in the reflectance mode was 31-fold stronger compared to the effect obtained with white latex particles and the combined amplification effect due to the presence of polymer particles and the colorant was over 100-fold compared to non-amplified SPR signals originating from BSA–anti-BSA interactions. The amplification method based on the dye-doped particles is widely applicable for the analysis of antibody–antigen interactions and DNA interactions at low concentrations.

Keywords: Surface plasmon resonance; Protein–protein interaction; Amplification; Polymer particle; BSA; Absorption-based SPR; Colored-particle

1. Introduction

SPR sensors are widely used for analyzing interactions of biological molecules and supramolecules, such as DNA–DNA interactions and antigen–antibody interactions [1–5].

Conventional SPR sensors are based on the measurement of changes of dielectric constants/refractive index on the surface of thin gold layers. They are less sensitive to low molecular weight compounds and rare substances in biological samples. Thus, research has been focused on amplification methods for SPR sensors [6–14].

As an amplification method for SPR sensors, a Au colloid based sandwich method was developed in 1999 [7]. In the following several years, several methods based on metal particles were reported [6,8 for review]. Metal colloids successfully amplified SPR signals, however, they resulted in larger non-specific adsorptions of molecules to the sensor surface, leading to increased background signal and decrease repeatability.

Latex particle based amplification methods were developed prior to the Au colloid technique [9–11]. Polymer particles resulted in lower non-specific adsorption, however, the sensitivity was less than obtained with Au colloids. Recently, our co-workers successfully enhanced SPR signals from DNA interaction with hydrogel-microspheres [12,13].

Due to the drawback of Au colloids pointed out above, polymer particles that have low non-specific adsorption would be the method of choice for SPR signal amplification in practical analysis. However, the polymer particles developed so far, do not feature high-sensitivities.

On the other hand, we have proposed and developed an absorption-based SPR sensing technique as one method of signal amplification based on a dye. Ion-optode membranes [14–16] were used as SPR-sensing layers. Based on the change of the color of a membrane-incorporated indicator dye, small molecules were successfully measured due to the change of the imaginary part of the surface refractive index [17–20].

Here, we propose a novel approach to combine the two SPR-signal amplification methods by the application of dye-doped polymer particles, expecting high signal amplification based on a change of the real part of the refractive index due to the presence of the polymer particles and a change of the imaginary part of the refractive index induced by the dye.
The basic theory, a simulation of the change of an SPR curve with dye concentrations based on a four-layer model, and the amplification demonstration with white and dye-doped particles in a BSA–anti-BSA system are compared and discussed in this paper.

2. Experimental

2.1. SPR apparatus

A SPR sensor (SPR-20, DKK Co., Ltd, Tokyo, Japan) equipped with a LED as a light source (630 nm) and a CCD camera (XC-77, Sony Co., Tokyo, Japan) was used in this experiment. The prepared sensor chips (vide infra) were placed on the high-RI glass prism \((n = 1.79)\) with a hemisphere shape. The backside of the sensor chip was adhered to the prism using matching oil \((n = 1.79, \text{Cargille Laboratories, Inc.)}\) while the sensing layer on the front side of the sensor chip faced toward a flow cell via a spacer made from silicone rubber. SPR signals were recorded and analyzed on a computer through an imaging board connected to the CCD camera. The reflectivity was calculated by dividing the intensity of the p-polarized light by that of the s-polarized light in order to reduce the effect of intensity fluctuations arising from the light source.

2.2. Buffer and reagents

The highest-grade commercially available reagents were used for the preparation of the test buffer solutions. The distilled and deionized water used had a resistivity of greater than \(1.5 \times 10^5 \Omega \text{cm at } 25 \degree C\). Phosphate buffer (Wako, pH 7.4, 10 mM) was used as running buffer. Glycine–HCl buffer (Wako, pH 1.4, 10 mM) was used as the regenerating buffer. A SPR sensor (SPR-20, DKK Co., Ltd, Tokyo, Japan) equipped with a LED as a light source (630 nm) and a CCD camera (XC-77, Sony Co., Tokyo, Japan) was used in this experiment. The prepared sensor chips were placed on the high-RI glass prism \((n = 1.79)\) with a hemisphere shape.

2.3. Immobilization of antibody to gold surface

Carboxyl-terminal self-assembled monolayers were prepared by immersing a gold chip in 2 mM ethyl-3-(3-dimethylaminopropyl) carbodimide solution in ethanol. The carboxyl residue was activated by immersing the chip in 0.4 M EDC and 0.1 M NHS in water for 15 min. Then the chip was immersed in anti-BSA (200 μg/ml) in sodium acetate buffer (10 mM, pH 4.5), washed with water and used.

2.4. Labeling of the antigen with polymer particles

Polymer particles (1%), BSA (2 mg/ml), and EDC (20 mg) were incubated in 10 ml of buffer (10 mM phosphate, pH 6) for 15 min, then 0.1 M NaOH (600 ml) was added and stirred for a few minutes. Non-reacted activated carboxyl residue was blocked by the addition of 100 mM glycine. The particles were purified by centrifugal separation.

2.5. Field emission scanning electron microscopy (FE-SEM)

The size and the shape of the polymer particles was observed by FE-SEM (S-4700, Hitachi, Japan). The colloid solution was dropped onto a cover glass and dried in air, followed by sputtering of a 1–2 nm Au layer before observation by FE-SEM.

2.6. Simulation of SPR curve

A simulation of the SPR curve change based on absorption of the dye was performed in order to estimate the effect of the dye [17–20].

For this purpose, a four-layer model \((i, j; 1, \text{gold}; 2, \text{sensing layer}; 3, \text{glass}; 4, \text{water})\) applying the Fresnel equation was used. The reflectance of the incident light is given by the Fresnel equation for p-polarized light as follows

\[
R = \left\{ \frac{\text{Abs} \gamma_{31}(\theta, \lambda) + \gamma_{14}(\theta, \lambda, \text{con}) \exp(2K_{12}(\nu, \lambda)d)}{1 + \gamma_{31}(\theta, \lambda)\gamma_{14}(\theta, \lambda, \text{con}) \exp(2K_{12}(\nu, \lambda)d)} \right\}
\]

(1)

\[
\gamma_{14}(\theta, \lambda, \text{con}) = \frac{\gamma_{12}(\theta, \lambda, \text{con}) + \gamma_{24}(\theta, \lambda, \text{con}) \exp(i2K_{23}(\nu, \lambda, \text{con}))}{1 + \gamma_{12}(\theta, \lambda, \text{con})\gamma_{24}(\theta, \lambda, \text{con}) \exp(i2K_{23}(\nu, \lambda, \text{con}))}
\]

(2)

where the horizontal wave number of the incident light is given in

\[
K_{x}(\theta, \lambda, \text{con}) = n \frac{\alpha(\lambda)}{c} \sin \theta
\]

(3)

The vertical wave number of the incident light

\[
K_{y}(\theta, \lambda, \text{con}) = \left\{ \varepsilon(\lambda) \frac{\alpha(\lambda)^2}{c^2} - K_{x}(\theta, \lambda)^2 \right\}^{1/2}
\]

(4)

\[
K_{z}(\theta, \lambda, \text{con}) = \left\{ \varepsilon(\lambda, \text{con}) \frac{\alpha(\lambda)^2}{c^2} - K_{x}(\theta, \lambda)^2 \right\}^{1/2}
\]

(5)

\[
K_{\nu}(\theta, \lambda) = \left\{ \varepsilon(\lambda) \frac{\alpha(\lambda)^2}{c^2} - K_{x}(\theta, \lambda)^2 \right\}^{1/2}
\]

(6)

\[
K_{\nu}(\theta, \lambda) = \left\{ \varepsilon(\lambda, \text{con}) \frac{\alpha(\lambda)^2}{c^2} - K_{x}(\theta, \lambda)^2 \right\}^{1/2}
\]

(7)
and the reflectance of the electronic field:

$$\gamma_{21}(\theta, \lambda, \text{con}) = \frac{\epsilon_2(\lambda, \text{con})K_{21}(\theta, \lambda) - \epsilon_3K_{22}(\theta, \lambda, \text{con})}{\epsilon_2(\lambda, \text{con})K_{21}(\theta, \lambda) + \epsilon_4K_{22}(\theta, \lambda, \text{con})} \quad (8)$$

$$\gamma_{12}(\theta, \lambda, \text{con}) = \frac{\epsilon_1(\lambda)K_{22}(\theta, \lambda, \text{con}) - \epsilon_3(\lambda, \text{con})K_{11}(\theta, \lambda)}{\epsilon_1(\lambda)K_{22}(\theta, \lambda, \text{con}) + \epsilon_4K_{11}(\theta, \lambda)} \quad (9)$$

$$\gamma_{31}(\theta, \lambda) = \frac{\epsilon_3K_{11}(\theta, \lambda) - \epsilon_4K_{33}(\theta, \lambda)}{\epsilon_3K_{11}(\theta, \lambda) + \epsilon_4K_{33}(\theta, \lambda)} \quad (10)$$

where $\lambda_{ij}$ is the reflectance amplitude given by Fresnel formulas for p-polarization for the i-j interface, $\theta$ is the incident angle of the p-polarized light, con is the dye concentration; $\epsilon_i$ and $K_{ij}$ are the dielectric constant of layer i and the wave-vector component perpendicular to the interface, respectively; $Kx$ is the component of the incident wave-vector parallel to the interface; $n$ is the refractive index of the prism; $d$ is the thickness of the metallic film, $t$ the thickness of the sensing layer, $\omega$ is the angular frequency of the incident light, $\omega_0$ is the angular frequency of the incident light at lambda max of the dye, and $\lambda$ is the wavelength of the incident light.

The dielectric constants of each layer are described by

$$\epsilon_1(\lambda) = 1 - \frac{\lambda^2c\lambda}{2\pi^2(c^2 + \lambda^2)}$$

$$n = 1.79, \quad \epsilon_3 = n^2, \quad \epsilon_4 = 1.33^2$$

with

$$\epsilon_2(\lambda, \text{con}) = 1.4^2 + N_{\omega 0}(\text{con}) \frac{e^2}{m_e \omega_0} \times \frac{1}{\omega - \omega(\lambda)^2 - i\omega(\lambda)\Gamma}, \quad N_{\omega 0}(\text{con})$$

$$= \text{con} \times 10^3 N_A, \quad \omega_0 = \frac{2\pi c}{\lambda_{\text{max}}}, \quad \Gamma = \frac{2\pi c}{\delta \lambda}$$

where $N_A$ is the Avogadro constant; $m_e$ is the mass of the electron; $\varepsilon_0$ is the permittivity of vacuum; $\lambda_{\text{max}}$ is the wavelength of maximum absorption of the dye; and $\delta \lambda$ is the full width wavelength at half-maximum of the absorption spectrum; $f$ is the oscillator strength of the dye. The parameters used in the calculations are: $c = 2.998 \times 10^8 \text{ m/s}$, $e = 1.602 \times 10^{-19} \text{ C}$, $m_e = 9.19 \times 10^{-31} \text{ kg}$, $N_A = 6.02 \times 10^{23} \text{ mol}^{-1}$, $\lambda_{\text{max}} = 600, 630, 660 \text{ nm}$, $\delta \lambda = 109 \text{ nm}$, $f = 1$, $d = 50 \text{ nm}$, $t = 1.48 \mu\text{m}$.

### 3. Result and discussion

#### 3.1. General design of experimental setup

The BSA-anti-BSA interaction was selected as a well-characterized model example of antigen–antibody interactions in order to evaluate the novel SPR signal amplification technique. A schematic figure of the experimental setup is shown in Fig. 1.

The sensing device consisted like in any conventional SPR sensor of a prism with a thin gold layer coated by a sensing film. The sensing film was a self-assembled monolayer (SAM) with immobilized anti-BSA. The SPR signal originating from analyte BSA labeled with dye-doped particles was compared to

![Fig. 1. Schematic representation of the experimental setup for the signal amplification with dye-doped particles.](Image)

![Fig. 2. SPR curves simulated for latex particles containing dye molecules at wavelength of incident light (a) 600, (b) 630, (c) 660 nm.](Image)
the signal from non-labeled BSA and from BSA labeled with white (without dye) polymer particles.

In this case, the BSA–anti-BSA interaction and the presence of the polymer particles induce a change of the real part of the refractive index, while the change of the imaginary part of the refractive index is due to the presence of the dye (that is linked to the light absorption according to the Kramers–Kronig equations). It was expected that this particular combination would strongly enhance the sensitivity of the sensing method.

3.2. Simulation of SPR response

SPR curves change their shape depending on the wavelength of incident light and the light-absorption (the change of the imaginary part of the refractive index).

Fig. 2 shows SPR curves simulated for different incident light wavelengths: (a) 600, (b) 630 and (c) 660 nm.

In the case where the incident light of a wavelength close to the absorption maximum of the dye (630 nm) is used, a broadening of the SPR curves can be observed. The changes of the SPR curves are maximal when using incident light of 660 nm wavelength.

Three situations can be distinguished, depending on the wavelength of incident light $\omega$ and on the absorption maxima of the dye ($\omega_0$):

1. $\omega < \omega_0$ the resonance angle shifts to larger values with increasing dye concentrations;
2. $\omega = \omega_0$ the resonance angle does not shift with the change of the dye concentrations;
3. $\omega > \omega_0$ the resonance angle shifts to smaller values with increasing dye concentrations.

This behavior can be explained from solving the equation of the sensing layer

$$n_s \approx \sqrt{\varepsilon_s} \left( 1 + [Ab] \frac{10^3 N_A e^2 f}{2 \varepsilon_s m_e \varepsilon_0} \frac{\omega_0^2 - \omega^2}{(\omega_0 - \omega)^2} \right)$$

$$\kappa_s \approx [Ab] \frac{10^3 N_A e^2 f}{2 \sqrt{\varepsilon_s m_e \varepsilon_0} (\omega_0^2 - \omega^2)^2 + (\omega \gamma)^2}$$

The second term of this equation is dependent on the correlation of $\omega$ and $\omega_0$.

Thus, it is the basic behavior of the absorption-based changes of the SPR curves in the simulation and we can predict and consider the behavior of SPR curve with dyes.

3.3. SPR amplification effect of the dye-doped polymer particles

Fig. 3 shows experimental SPR curves in detection of BSA–anti-BSA interactions with BSA alone, white-polymer labeled BSA and dye-doped polymer labeled BSA.

Significantly stronger upper shifts of the SPR curves can be observed, measured with the dye-doped particles in
comparison to the results obtained with unlabeled BSA. This result is attributed to the light-absorption of the dye and the corresponding change of the imaginary part of the refractive index. The experimentally observed data corresponds with the prediction based on the simulation.

There is a slight difference in the experimental curve of a 0.02° shift in resonance angle compared to the simulated response curve. Since the simulation assumed absorption changes only, the observed difference is assumed to result from a change of the real part of the refractive index induced by the polymer particles.

Slightly increased upper shifts of the SPR curves measured with white particles are observed in comparison to the results obtained with unlabeled BSA. This is considered to be caused by the change of the real part of the refractive index induced by the polymer particles alone.

Fig. 4 shows the calibration curves based on the reflectance change. While labeling with white particles resulted in a 4-fold increased response compared to unlabeled BSA, the result with dye-doped particles was a 124-fold enhancement of the sensitivity, thus successfully demonstrating the enhancement effect of dye-doped polymer particles on SPR signals.

3.4. Observation of particles by FE-SEM

Fig. 5 shows a SEM image of the particles ((a) white and (b) dye-doped). They are homogeneously sized around 200 nm. Thus, we can confirm that the changes of SPR signals are not due to variations in particle size and shape.

4. Conclusions

This paper demonstrates for the first time an SPR signal amplification method based on dye-doped polymer particles. The amplification based on the particle itself is 4-fold, the effect of the dye is 31-fold, and the combined amplification factor is 124-fold.

The amplification method based on the dye-doped particles is widely applicable for the analysis of antibody–antigen interactions and DNA interactions at low concentrations. The method is also applicable for use with any commercially available SPR sensing system, by adapting the wavelength of the dye.

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