Localized Surface Plasmon Resonance-Based Label-Free Biosensor for Highly Sensitive Detection of Dopamine

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Localized surface plasmon resonance (LSPR) is the phenomenon that is observed on specific metal nanoparticles (NPs) like Au, Ag which can be used for sensitive detection for many kinds of biomaterials. Dopamine (DA) is a typical neurotransmitter considered as indicator of some neural diseases. Due to its small size, it is very difficult to detect DA at low concentrations directly and sensitively with conventional sensing techniques. In this research, we propose a DA detection sensor based on LSPR phenomenon. Electrochemical deposition technique was used to make LSPR substrates, where Au NPs were electrochemically deposited on ITO glasses and these substrates showed optical characteristic of LSPR phenomenon. Different concentrations of DA solution were deposited on antibody immobilized LSPR substrates. With additions of increasing concentrations of DA, LSPR peak intensity was increased linearly. These results could be applied to many fields of clinical trials for diseases caused by small molecules.

Keywords: Dopamine, Nanopattern, Localized Surface Plasmon Resonance, Biosensor.

1. INTRODUCTION

DA is a kind of neurotransmitter which related to neural functions. DA is related to many important functions that consist of voluntary working memory, movement, cognition, and learning. Therefore, decreasing or increasing of DA concentration in body causes some serious neural disease,¹,² such as Parkinson’s diseases³ or schizophrenia.⁴ These diseases bring patients to un-ignorable pain and economic loss. Therefore, early detection of abnormal body DA concentration is very important.

DA has very small molecule that has low molecular weight (153.18 g/mol) so it is hard to detect DA precisely. Until now, to detect DA, ELISA,⁵ SPR,⁶ voltammetric,⁷ electrical⁸ methods have been researched. However, these methods are only able to detect DA concentration up to 0.01 µM. In general, healthy people possess 0.01~0.1 µM of DA,⁹ these methods are not appropriate to detect disease caused by DA concentration lower than normal range. Therefore more precise method for DA detection is required for diagnosing neural diseases.

LSPR is a phenomenon that occurs when light incidents on metal NP. When light incidents on metal NP, free electrons in metal NP make resonance oscillation as plasmon, a quantized particle with electric field of light. At specific wavelength, LSPR is most strong and this wavelength is LSPR wavelength and it is different according to metal NP’s shape, size and matter.¹⁰,¹¹ This property could be measured by spectrometer and it is very sensitive so absorbance peak at LSPR wavelength changes with small change of NP’s surface condition. Recently, many detection techniques are being explored with this property.¹²–¹⁴

In this study, highly sensitive DA detection based on LSPR phenomenon of Au nanopattern was developed. Au nanopattern was fabricated by electrochemical deposition on ITO glass¹⁵ (Fig. 1(a)) and DA antibodies were
immobilized on Au nanopattern to make specificity for DA. After that, different concentration of DA solution was deposited on antibody immobilized Au nanopatterns and peak change at LSPR wavelength was analyzed. Figure 1(b) is the schematic diagram of DA detection based on LSPR phenomenon.

2. EXPERIMENTAL DETAILS

2.1. Materials
HAuCl₄·3H₂O (Gold(III) chloride trihydrate), MHA (6-Mercaptohexanoic acid), PBS (Phosphate buffered saline), GABA (γ-aminobutyric acid) and DA (3-Hydroxytryamine hydrochloride) were purchased from Sigma-Aldrich (USA). 2×2 cm ITO glass was purchased from Gmac (Korea). DA antibody (ab1001) was purchased from Abcam (UK). PEG (Polyethylene glycol, MW: 200) was purchased from Yakuri (Japan). EDC (1-ethyl-3-[3-dimethylamino-propyl]carbodiimide), NHS (N-hydroxysuccinimide) and hydroxylamine·HCl were purchased from Thermo Scientific (USA).

2.2. Fabrication of Au Nanopattern on ITO Glass
HAuCl₄·3H₂O and PEG were dissolved in the distilled water. The mixed solution had concentration of 2 mM HAuCl₄·3H₂O and 20 μl/ml PEG. ITO glass was purchased from Gmac (Korea). DA antibody (ab1001) was purchased from Abcam (UK). PEG (Polyethylene glycol, MW: 200) was purchased from Yakuri (Japan). EDC (1-ethyl-3-[3-dimethylamino-propyl]carbodiimide), NHS (N-hydroxysuccinimide) and hydroxylamine·HCl were purchased from Thermo Scientific (USA).

2.3. Fabrication of Bio-Surface and DA Detection Based on LSPR
Au nanopatterned ITO glasses were immersed into 10 mM MHA/ethyl-alcohol solution for overnight and washed with distilled water and ethyl-alcohol. 50 mM EDC and 100 mM NHS solution were applied to the Au nanopatterned ITO glasses to activate the carboxyl group of MHA. Active MHA acts as linker between Au NP and antibody against DA. Then, 5 μg/ml of DA antibody was applied for 2 hours and washed with distilled water and buffer solution. To block nonspecific binding, 10 mM hydroxylamine·HCl/PBS solution was used. Finally, different concentrations of DA were applied for the detection. All the LSPR measurements were done by UV/Vis spectrometer V-530 (Jasco, Japan).

3. RESULTS AND DISCUSSION

3.1. Optimization of Nanopatterning Condition
To investigate the optimum conditions to form nanopattern without any artifacts, different concentrations of PEG was used to develop nanopattern on the ITO glass. Figures 2(a)–(e) demonstrates the fabricated nanopattern surface with different concentrations of PEG range from 10 μl/ml to 50 μl/ml. Among these, only 20 μl/ml PEG
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Figure 2. SEM images of fabricated nanopattern on the ITO glass with (a) 10 μl/ml, (b) 20 μl/ml, (c) 30 μl/ml, (d) 40 μl/ml, (e) 50 μl/ml of PEG concentration. (f) The absorbance change relate to the antibody and antigen interaction.

solution developed highly uniformed NPs within average diameter of 35 nm. To verify the advantage of this uniformed nanopattern, same concentration of DA (10 μM) sample was applied to the antibody against DA immobilized substrate, to observe the change on the absorbance.

Comparing the changes in the absorbance, the substrate made with 20 μl/ml PEG solution has showed the highest increment (Fig. 2(b)). This result could be related to the uniformed NP size of fabricated pattern on ITO glass. Since, these uniformed NP prevents LSPR peak dispersion on wavelength, strong and sharp increment of absorbance peak could be measured.

3.2. Investigation of Biosurface Based on LSPR

Linker (MHA), blocking agent (Hydroxylamine·HCl), DA antibody and DA layer was developed by step-by-step immobilization on the surface of Au nanopattern. As expected, the absorbance of LSPR peak was increased according to adsorption of materials on Au nanopattern surface. Figure 3 shows LSPR spectra of each layer. It is clearly observed that, for larger molecules, such as DA antibody shows a large peak change. It is also evident that molecules size is also a factor for peak change. Since, the blocking agent, hydroxylamine·HCl, has very small molecular size, the LSPR peak change between DA antibody and blocking stage is not significant.

3.3. Quantitative Determinations of DA Based on LSPR Phenomenon

Different concentration of DA ranging from 0.001 μM to 100 μM was applied to the DA antibody immobilized nanopatterned ITO glass substrate for LSPR measurement. The nanopatterned ITO glasses were fabricated with 20 μl/ml PEG solution. LSPR absorbance peak was increased by DA adsorption and the quantity of change was increased upon increase in concentration of DA (Fig. 4(a)). Calibration curve for DA in Figure 2(b) shows the linear relation with $R^2 = 0.9848$. To clarify specificity for DA, same experiments were repeated with same range concentration of GABA solution as negative control. As a result, negligible and irregular absorbance change was found, which means there no such remarkable non-specific binding between GABA and DA antibodies was observed.
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Figure 4. (a) LSPR peak absorbance spectrum with different concentration of DA antibody (—), 1 nM DA (•••••), 10 nM DA (−−−−), 100 nM DA (−−−−−), 1 μM DA (−−−−−), 10 μM DA (−−−−−), 100 μM DA (−−−−−). (b) Calibration curve based on LSPR peak changes corresponding to different concentrations of DA (—) and negative control GABA (−−).  

4. CONCLUSION

In this study, highly sensitive detection method for DA based on LSPR phenomenon was developed. This is the first attempt to detect DA with the LSPR method and the developed label-free detecting system was so much easy and simple. This system could be alternative for current diagnosis system of three steps which consists the stages of extraction, isolation and quantification. Moreover, most importantly, we got the low detection limit (1 nM). In contrast to conventional sensing methods, this detection limit of 1 nM means enough sensitivity to detect disease caused by low DA concentration.

With these results, we assume that LSPR detection method could be used to estimate other small molecules by antigen-antibody reaction. In advance, it suggests the way to establish a fine detection method in the field of clinical tests, early diagnosis.

Acknowledgment: This work was supported by the Original Technology Research Program for Brain Science through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (2009-0093907)/Korea Institute for Advancement in Technology (KIAT) through the Workforce Development Program in Strategic Technology/The Graduate School of Specialization for Biotechnology Program of the Ministry of Knowledge Economy (MKE) (No. C-7010-1102-0001).

References and Notes

Received: 20 May 2012. Accepted: 1 March 2013.