Ultramicroscopy 109 (2009) 894–898

Contents lists available at ScienceDirect

Ultramicroscopy

journal homepage: www.elsevier.com/locate/ultramic

Effects of dopamine concentration on energy transfer between dendrimer–QD and dye-labeled antibody

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ARTICLE INFO

PACS:
42.50.Fx
47.54.Fj
61.46.Df
68.37.Hk
78.66.Qn

Keywords:
Dendrimer
Quantum dot
ELISA
FRET
AFM

ABSTRACT

The Unified Parkinson's Disease Rating Scale (UPDRS) is currently used to assess Parkinson's disease, and is a key method for determining the progression of disease based on the gross findings of patients. However, this method cannot quantify the extent of disease of patients, which means the administration of drugs cannot be determined on a real-time basis. Thalamotomy also causes discomfort and pain to the patients, and adversely affects treatment as it is performed following the onset of symptoms. Accordingly, the dopamine concentration, which is one of the key factors in determining this disease, needs to be detected quantitatively at ordinary times. Hence, the development of a bio-kit or a bio-sensor is essential for effectively prescribing the correct dopamine concentration in a customizable manner. In this study, the effect of dopamine level on this phenomenon was observed using the Forster resonance energy transfer (FRET) phenomenon generated between a donor and acceptor. By confirming the photoluminescence (PL) and lifetime data, it was demonstrated that the degree of energy transfer increased with increasing dopamine concentration. To apply this phenomenon to an optical sensor, a glass surface was modified with a quantum dot (QD)-encapsulated dendrimer, and analyzed using the contact angle and ATR-FTIR. The topology of surface was determined by an atomic force microscope (AFM).

1. Introduction

Dopamine is a key neurotransmitter in the brain, and used to determine the presence and extent of Parkinson's disease. Primary symptoms, such as balance impairment (stiffness or tremor) and gait disturbance, can occur in cases with a lower dopamine concentration. Senile dementia can develop in cases where these symptoms have worsened. Currently, the treatments are determined using thalamotomy, electrical stimulation due to deep brain stimulation (DBS), or dopaminergic drug administration. Once the symptoms have developed, the treatment must be administered using electrical stimulation. Periodical surgery due to causes such as battery replacement, frequent drug administration, and a daily dose of administration ranging from 500 to 1000 mg, cause considerable discomfort to the patients [1–3]. Moreover, this disease generally occurs in the elderly and shows an accelerated age of onset [4]. The criteria based on Unified Parkinson's Disease Rating Scale (UPDRS) are generally applied to determine the symptoms of patients with Parkinson's disease. This scale is also based on a determination of the gross findings, and cannot determine precisely the disease course or the time point of prescription [5]. Therefore, it is imperative that the dopamine concentration in Parkinson's disease patients be monitored on a real-time basis. Nano-biotechnology, which has attracted increasing interest, is an appropriate potential method for meeting these requirements. A bio-sensor is an alternative method that can provide patients with treatment in a timely manner by monitoring the disease status of the patients on a periodical basis. Currently, many ongoing studies have been carried out to determine the clinical utility of a bio-sensor designed using the Forster resonance energy transfer (FRET) and surface plasmon resonance (SPR) phenomena [6,7]. Of these, the FRET phenomenon is where energy is transferred when the release wavelength of the donor overlaps with the absorption wavelength of an acceptor in the condition that two fluorescent materials are located within a short distance from each other (>10 nm). Kim et al. demonstrated that energy transfer occurred based on the changes in the FRET phenomenon induced by the avidin concentration. This phenomenon has been applied in fields such as protein or DNA screening [8,9]. Therefore, quantification of the changes in the FRET phenomenon might indicate the dopamine concentration. It is also expected that an analysis of the concentration of neurotransmitters within the brain using this technique will be helpful in treating senile dementia in both a timely and a rational manner. In this study, enzyme-linked immunosorbant assay (ELISA) was used. This method involves

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doi:10.1016/j.ultramic.2009.03.012

more complex processes than the direct method. The indirect method with a higher sensitivity is also used in cases in which the dopamine concentration is lower. The labeled conjugate antibody method employs the specific reactions between the antigen and antibody. Therefore, attempts have been made to measure the dopamine concentration through the detection of immunofluorescent materials. The antibody bridge technique is such a method for determining if the dopamine concentration affects the FRET phenomena of labeled secondary antibody and dendrimer–quantum dot (QD). Various functional groups of the dendrimer are appropriate for fixing the antibody to a QD [10].

2. Experimental

2.1. Reagents and chemicals

Cadmium nitrate tetrahydrate (99.999%), sodium sulfide (anhydrous), 3-aminopropyl trimethoxysilane (APTMS, 97%), glutaraldehyde (50% water solution), dopamine chloride, sodium metabisulfite (SMB; 97+%, ACS reagent), and PAMAM dendrimer (G5; 5 wt% solution in methanol) were obtained from Sigma-Aldrich (USA). H_2O_2 (30% w/v solution) was purchased from Junsei (Japan) and 0.05 M phosphate buffer solution (PBS) was prepared with NaH_2PO_4 (anhydrous, Kanto Chemical Co. Inc., Japan) and $Na_2HPO_4 \cdot 7H_2O$ (98–102%, Alfa Aesar, USA). Normal goat serum (NGS) from GIBCO (Invitrogen Corporation, USA) and bovine serum albumine (BSA) from Jackson Immunoresearch Laboratory (USA) were used. Triton X-100 was obtained from Amresco (USA), and 1 M Tris–HCl (pH 7.4) was purchased from Bioneer (Korea). AlexaFluor 488 goat anti-rabbit IgG antibody was obtained from Invitrogen (USA), and rabbit anti-dopamine polyclonal antibody was purchased from Chemicon International Inc. (USA).

2.2. Analytical methods

The optical properties of CdS QDs were determined by measuring the absorbance and emission wavelength using photoluminescence (PL) spectroscopy (Photon Technology International, USA). The lifetime was confirmed with Easy Life (Photon Technology International, USA). The surface was modified to assist in the fixation of the dendrimer–CdS QD to the glass plate. The properties of the surface when the surface was changed using APTMS, glutaraldehyde, and CdS-quantum-dot-encapsulated dendrimer were measured after rinsing the glass plate using the contact angle (Kruss, DSA 100). The liquids used herein were HPLC water and dichloromethane. The volume of liquid used in all tests was 6 μl. The structural changes to the glass surface were confirmed by ATR-FTIR (Bruker, Germany). At this time, the resolution was 2 cm^{-1} and the number of scans was 1064. An atomic force microscope (AFM, XE-150, Park systems, Korea) was used to determine the topology, average surface roughness (Ra), and root mean square roughness (RMS, Rq). The scanning rate for the measurements was 1 Hz and the image was obtained at a scale of $5 \times 5\,\mu m^2$. The change in FRET due to bonding between the dendrimer–CdS QD and antibody, and that between the antibody and antigen was confirmed by ELISA. The intensity was measured by PL. The FRET phenomenon was demonstrated by confirming the lifetime.

2.3. Preparation of dendrimer–CdS quantum dot

The preparation of CdS QDs connected to the PAMAM dendrimers was performed using the methods reported by Lakowicz et al. [11]. QDs bound to the 0–5th generation dendrimer were prepared. The luminescence efficiency of the 5th generation dendrimer was the highest. The following experiment was performed. Firstly, 0.114 mM of the PAMAM dendrimer was dissolved in methanol. The temperature was maintained at 10 °C in a nitrogen gas atmosphere. 2 mM of Cd^{2+} and S^{2-} precursor were mixed, and added ten times at a volume of 0.5 ml at 2-min intervals. A total of 5 ml was infused. The reaction was also performed for 30 min and the specimen was stored at −10 °C. The solvent was changed by removing methanol by vacuum evaporation. The same amount of pH 7.4 PBS was placed and then stirred.

2.4. Dendrimer–CdS-quantum-dot-modified glass

The organic substance present on the glass surface was removed by 2 h treatment with Piranha solution (50% H_2SO_4: 30% H_2O_2 = 3:1, v/v) at 80 °C. Subsequently, ethanol was mixed with 3% (v/v) 3-aminopropyl trimethoxysilane, into which a glass substrate was placed at room temperature for 3 h. After the reaction, the substrate was rinsed with distilled water and dried with a nitrogen gun. An appropriate amount of sodium phosphate monobasic was mixed with sodium phosphate dibasic in 0.05 M PBS at pH 6.8; 5% (v/v) glutaraldehyde was added to this solution and the reaction was performed at room temperature for 1 h. The aldehyde group was prepared and reacted with 2 mM of the dendrimer connected to the QDs. Thus, the modified glass surface was prepared.

2.5. Antibody-binding activity of dopamine immobilized on dendrimer–CdS

Fig. 1 shows the FRET phenomenon to confirm energy transfer between the CdS-encapsulated PAMAM dendrimer and AlexaFluor-488-labeled antibody. ELISA was used to confirm the effect of dopamine on energy transfer through antibody–antigen conjugation. A 96-well plate was used [12,13]. Firstly, 10 mM of glutaraldehyde was added to a pH 7.4 PBS solution; 0.08 ml of the CdS-quantum-dot-encapsulated PAMAM dendrimer (G5) was then placed, and the mixture was stirred for 30 min. 0.05 mol/L of NaBH4 was added to effectively combine the amine-terminated

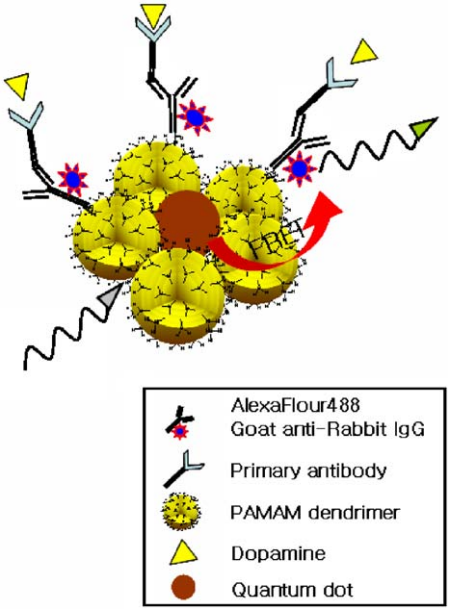


Fig. 1. Scheme of FRET phenomena of dendrimer–QD with AlexaFluor 488.

dendrimer with the amine group of the secondary antibody. Subsequently, AlexaFluor 488 goat anti-rabbit antibody at various concentrations was added. The resulting mixture was stirred in the dark for 1 h; 1% sodium metabisulfite was placed in pH 7.4 Tris–buffer. 0.5% Triton X-100 and 1% NGS were then added; 10 μl rabbit anti-dopamine polyclonal antibody was added as the primary antibody and stirred. The reaction was performed at room temperature for 16 h to allow the primary antibody to combine with the secondary antibody. Finally, 5% glutaraldehyde (50 wt%), 1% SMB, and 1% BSA were added sequentially to 0.1 M of pH 7.4 PBS solution. Dopamine at concentrations ranging from 0 to 10 mM was used to determine the minimum detectable concentration.

3. Results and discussion

3.1. Preparation of dendrimer–CdS quantum dot

CdS QDs, which were prepared using the PAMAM dendrimer (G5), had approximately 100 times higher luminous efficiency than the lower (the 0–3rd) generation [11]. The PAMAM dendrimer had a similar size to the QD, and surrounded the QD. The particle size varied depending on the number of generations. It was the most significant factor affecting the space for the growth of QDs. In addition, as shown in the ligands, the effect of the dendrimer size could be considered in a similar manner to that of empty space surrounding both. It could be compared with the empty space of a vessel containing large and small beads [14,15]. Fig. 2 shows a plot of an overlapped absorption peak of AlexaFluor488 goat anti-rabbit IgG with the emission peak of CdS QD bound to the PAMAM dendrimer (G5). The optical properties of the two fluorescent materials were assessed in order to determine if the FRET phenomenon can be used. If these two fluorescent materials are located within a certain range of distance (<10 mm in general), they are eligible for energy transfer that occurs within the overlapped domain. Two fluorescent materials were used as the donor and acceptor, each emitting and absorbing at a wavelength of 470 and 495 nm, respectively.

3.2. Characteristics of dendrimer-immobilized glass

The dendrimer was fixed to a glass substrate and the changes to the surface were determined with the aim of manufacturing a kit or a bio-medically measurable sensor. The wettability was determined from the contact angle. More than 90 measurements were obtained and the results were averaged. Firstly, the organic substance on the glass surface was removed completely. This converted the surface to hydrophilic hydroxyl group with high surface energy, a low contact angle of 7.0°, and high wettability. However, in cases where the surface was modified with APTMS and glutaraldehyde, the contact angle was 60.7° and 53.8°, respectively. This indicates a low surface energy with a higher contact angle and lower wettability. Fig. 3 shows the changes in topology and surface bonding structures through ATR-FTIR and AFM. Fig. 3(a) shows the ATR-FTIR measurements of a bare glass and Fig. 3(b) shows the treatment of APTMS. This illustrates the stretching vibration of the amine group at 3779 and 3689 cm^{-1}. As shown in Fig. 3(c), the peak was not observed within this domain in cases where glutaraldehyde was bonded. This is because APTMS with a primary amine group has two N–H bonds and shows two peaks. On the other hand, it is converted to the tertiary amine in cases where the amine group is bonded to an aldehyde group and forms a Schiff base. A C = N bond is then formed and the specific peak due to the N–H stretch vibration disappears. In cases where the dendrimer was bound to the CdS QDs, peaks were observed at 3801, 3689, and 3557 cm^{-1}. These peaks were shifted by the primary and secondary amine group, where the amine groups of the dendrimer interact with the CdS QDs [16]. The topology images were confirmed by the changes due to surface reformation. In addition, Fig. 3(a) shows an image of bare glass and indicates that the organic substance was mostly removed by 2-h rinsing at 80 °C using a Piranha solution. Fig. 3(b) shows that the surface roughness increased when APTMS was processed. However, Fig. 3(c) shows a decrease in surface roughness when the surface was treated with glutaraldehyde. This suggests that the aldehyde group located on both sides of glutaraldehyde was bound to the bilateral amine groups of APTMS in the area adjacent to the aldehyde group because APTMS has a higher density, which causes a sub-reaction. In the area where aldehyde group was fixed to the unilateral side, fixation could be obtained by bonding the amine group of the CdS–dendrimer. This was confirmed on the topology image, as shown in (d).

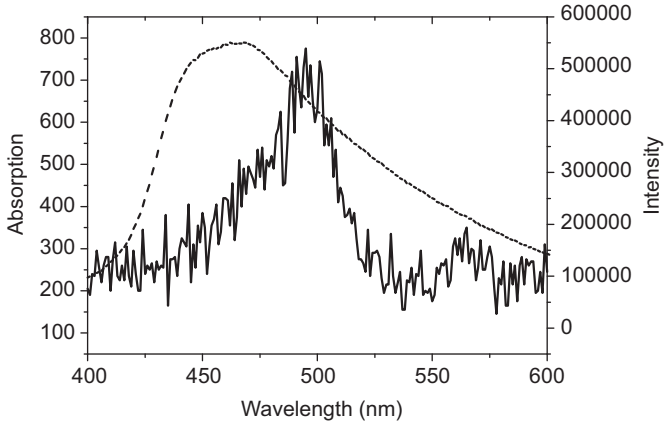


Fig. 2. Comparison of PL data. Emission of PAMAM dendrimer(G5)–CdS (dotted line) and absorption of AlexaFluor 488 goat anti-rabbit IgG (solid line).

3.3. Change of optical density according to concentration of dopamine

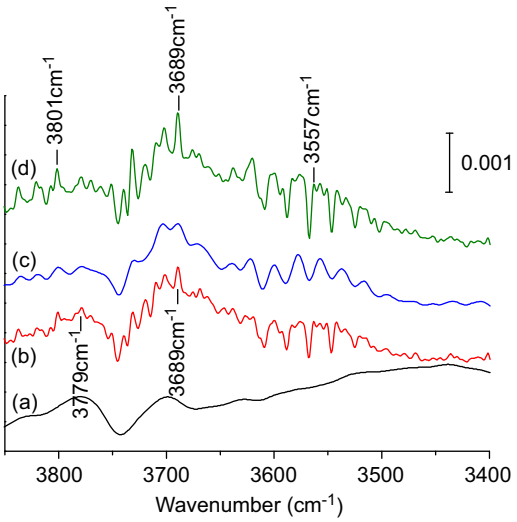
The optical density was measured at a single wavelength of 350 nm to confirm the occurrence of the FRET phenomenon between CdS QDs and AlexaFluor 488. R_0 (Forster distance) was obtained from the PL measurement, where the emission and absorption peaks of the donor and acceptor were used. When the donor was bound to the acceptor, the distance between the donor and acceptor as well as the energy transfer efficiency were measured by applying the intensity value and lifetime. The equations for FRET efficiency are as follows:

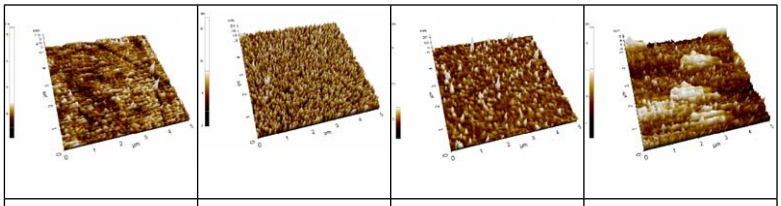
$$R_0 = 0.2108(K^2 n^{-4} \Phi_D J(\lambda))^{1/\delta} \quad (1)$$

where R_0 is the Forster distance in angstroms. Here Φ_D represents the quantum yield of the donor in the absence of an acceptor, and n represents the refractive index of the media. K_2 is the orientation factor of the donor and acceptor, and is generally considered to be 2/3. $J(\lambda)$ represents the degree of overlap of the emission peak of the donor with the absorption peak of the acceptor. The following equations were used to obtain the energy transfer efficiency using the lifetime and intensity value as follows:

$$E = 1 - \frac{\tau_{DA}}{\tau_D} \quad (2)$$

$$E = 1 - \frac{F_{DA}}{F_D} \quad (3)$$





	(a)	(b)	(c)	(d)
R_a	0.463	2.456	1.619	2.365
R_q	0.605	3.037	2.073	3.119

※R_a and R_q scale is nm

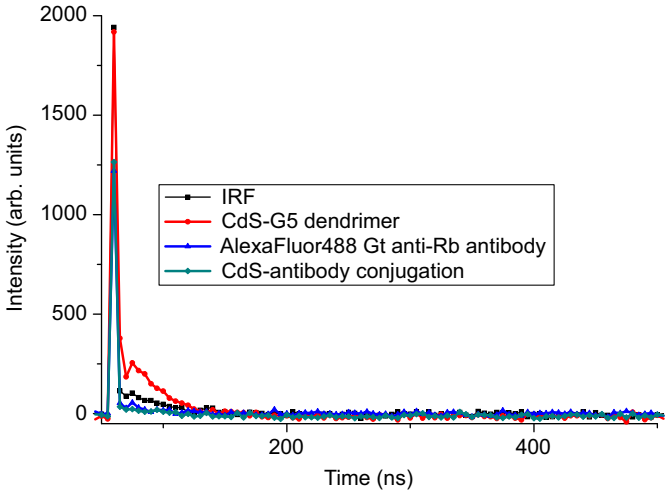
Fig. 3. ATR-FTIR data and AFM topology image of surface-modified glass: (a) bare glass, (b) APTMS modified on the glass, (c) APTMS–glutaraldehyde, and (d) APTMS–glutaraldehyde–dendrimer–QD.

E represents the efficiency of energy transfer; τ_D and τ_{DA} represent the lifetime of the donor and acceptor, respectively. Under the same conditions, the fluorescence intensity is expressed as F_D and F_{DA}, and they are consistent with energy transfer efficiency when they are entered into (2) and (3). R_0, which was obtained from earlier sessions, was entered into (4) and r, the distance between donor and acceptor, could be obtained [8,17]:

$$E = \frac{1}{1 + (r + R_0)^{\delta}} \tag{4}$$

Fig. 4 shows lifetime data, which was useful in obtaining reliable data with a $\varkappa^2$ (chi-square) value ranging from 0.9 to 2 based on the instrument response function (IRF); τ_D and τ_{DA} were set to 10.64 and 0.4116 ns and entered into (2).

The intensity value was then entered into F_D and F_{DA}. The result showed that the efficiency of energy transfer was 0.96. Therefore, the distance between the donor and the acceptor was calculated by Eq. (4) to be 26 Å. As shown in Fig. 5, the rabbit anti-dopamine polyclonal antibody, as the primary antibody, and goat anti-rabbit antibody, as the secondary antibody, were conjugated. This also confirmed the effect of dopamine on the FRET phenomenon using ELISA. The concentration-dependent changes were measured as a function of time. The energy transfer increased gradually according to the progress of the antigen–antibody reaction of dopamine. The intensity showed a significant increase 30 min after initiating the reactions. Thereafter, the rate of increase decreased. This suggests that the antigen–antibody reactions occur mainly within the shortest range of time. Fig. 6



	Fluorescence decay time (ns)	x_2 (chi-square)
Dendrimer(G5)-CdS (in PBS pH 7.4)	10.64	1.023
AlexaFluor 488 Gt anti-Rb antibody	0.3181	1.485
CdS-AlexaFluor 488 conjugation	0.4116	0.9145

Fig. 4. Lifetime data of dendrimer–QD and AlexaFluor 488 Gt anti-Rb antibody.

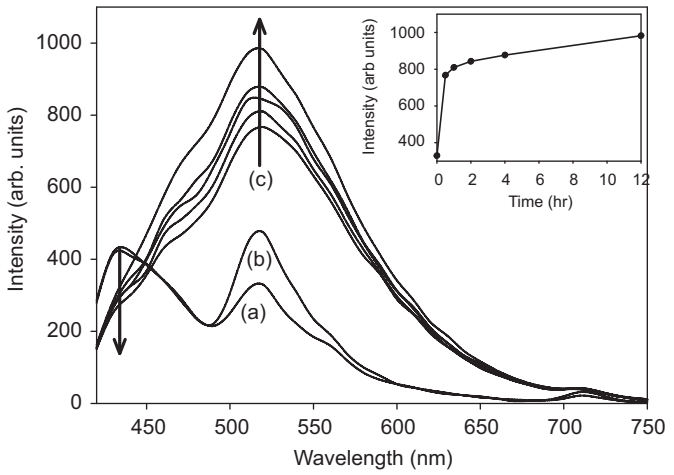


Fig. 5. ELISA data of FRET between PAMAM dendrimer(G5)–CdS and AlexaFluor-488-conjugated antibody: (a) dendrimer–QD and AlexaFluor 488 antibody conjugation, (b) dendrimer–QD–secondary antibody and primary antibody conjugation, and (c) the effect of anti-dopamine–dopamine conjugation (10 mM dopamine) on the elapsed time (30 min, 1, 2, 4, and 12 h). Inner graph is change of optical density at 520 nm.

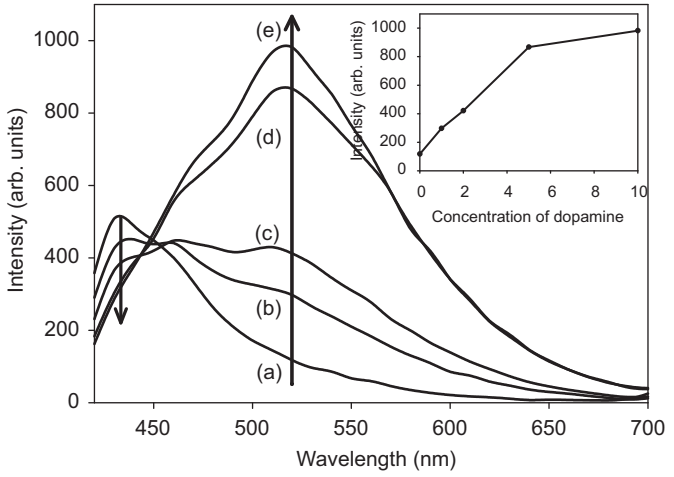


Fig. 6. Optical density change as a function of dopamine concentration in FRET phenomena between dendrimer–CdS quantum dot and AlexaFluor-488-labeled antibody: (a) 0, (b) 1, (c) 2, (d) 5, and (e) 10 mM. Inner graph is change of optical density at 520 nm.

shows the concentration-dependent changes in intensity at concentrations ranging from 0 to 10 mM. The intensity of the emission peak of AlexaFluor 488 increased with increasing dopamine concentration. An internal graph showed that the dopamine concentration increased to 5 mM in a homogeneous manner. There is effective energy transfer between the donor and acceptor because the emission peak of the donor decreases synchronously. The degree of antigen–antibody reaction will also increase with increasing dopamine concentration, which may be the cause of the increased intensity. Besides, non-covalent bonds, such as hydrogen bonds, ionic bond, and Van der Waals interactions form when the antigenic determinant located on the F_{ab} fraction is bound to an epitope. This can trigger changes in the chemical structure of the antibody, which attempts to maintain the most stable status.

4. Conclusion

This study examined the changes in the FRET phenomenon depending on the dopamine concentration by applying dendrimer-QD and AlexaFluor 488 to immunological methods. These results showed that various functional groups on the dendrimer can be used to fix antibodies. Using Schiff base-forming reactions, the amine group and aldehyde group were fixed to the dendrimer. A homogeneous gap was maintained so that FRET phenomenon might occur between the CdS QD and AlexaFluor 488. The valid gap was found to be 26 Å based on the PL and lifetime data. The intensity also increased with increasing dopamine concentration. This shows that the degree of energy transferred to AlexaFluor 488 increases in proportion to the dopamine concentration. In addition, the change in the hinge region, which is susceptible to structural changes, affects the gap between the donor and acceptor when the chemical structure is changed in response to an antigen–antibody reaction. In this study, the dendrimer–QD was fixed to a glass plate. Optical fibers can also be used. This system is expected to form the basis of bio-kits or bio-sensors, which can be used to diagnose a range of diseases.

Acknowledgments

This research was supported by the Korea Science and Engineering Foundation (KOSEF) grant funded by the Korea government (MOST) (2008-2005729). This research was supported by the Kyungwon University Research Fund in 2009.

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