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Detection of Neurotransmitters Via Quantum Dots

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Quantum dots have been used for the detection of gamma amino butyric acid (GABA), one of chief inhibitory neurotransmitters. Quantum dots (QDs) with carboxyl functional group have been covalently bonded to goat anti-rabbit IgG labeled with Alexa Fluor, and their fluorescence spectra change has been studied. By addition of Alexa Fluor, immunocomplex was formed and the local distance between QD and Alexa Fluor became closer and fluorescence resonance energy transfer (FRET) process from QDs to dyes could be observed. The FRET efficiency was obtained based on their spectra intensity ratio, and this was further confirmed by the lifetime measurement. By addition of rabbit polyclonal with GABA, the FRET efficiency was decreased which means the local distance between QDs and Alexa Flour was increased. The dependence of FRET efficiency on GABA concentration shows that QDs combined with fluorescent dyes can be used for the detection of neurotransmitters.

Keywords: antibody; FRET; GABA; neurotransmitters; quantum dot; sensor

INTRODUCTION

Recently quantum dots have been widely used in biological assays including fluorescent biological labeling, drug delivery, diagnosis of tumor cell, tissue imaging, and nucleic acid probing [1,2]. Quantum dots are nanometer sized semiconductors which have peculiar optical properties such as intense fluorescence with narrow emission, broad excitation band, and high resistance to photo bleaching. QDs also show a wide range of size-tunable colors and stability under aqueous biological environment. By surface modification, QDs can bind to

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various materials such as gold, glass, proteins, enzyme, and antibody. Fluorescence resonance energy transfer (FRET) is one of few tools available for measuring nanometer scale distance changes in vivo [3]. FRET is a phenomenon which describes the energy transfer between two fluorophores. When the distance between two fluorophores is close, the fluorescence energy from donor fluorophore can be transferred to acceptor fluorophore, and used to excite it. The FRET efficiency is strongly dependent on the donor- acceptor distance, and can be determined by measuring the decrease of fluorescence intensity of donor in the presence of acceptor. It can be also determined by measuring the lifetime of donor in the presence of acceptor.

The following equation is used for determining FRET efficiency:

$$E = 1 - \frac{I_{DA}}{I_D} \tag{1}$$

where I_{DA} and I_D are the donor fluorescence intensities with and without an acceptor, respectively.

Similar equation can be used for determining FRET efficiency:

$$E = 1 - \frac{\tau_{DA}}{\tau_D} \tag{2}$$

where τ_{DA}, τ_D are the donor lifetime with and without an acceptor, respectively.

The efficiency is also related with donor to acceptor separation distance:

$$E = \frac{1}{1 + (r/R_0)^6} \tag{3}$$

where r is the donor-to-acceptor separation distance and R_0 is the Förster distance of this pair of donor and acceptor at which the FRET efficiency is 50% [4,6].

FRET has extensive application of biopolymer interaction, immunoassay, and biochemical detection. FRET immunosensor utilizes the switching of fluorescence emission wavelength according to the distance change between two fluorophores [5,7,8]. The distance change is due to the conformational change of antibody as it binds to antigen. γ-aminobutyric acid (GABA) is one of chief inhibitory neurotransmitters in the central nervous system. This neurotransmitter regulates the excitability of most neurons in brain and has been an important mediator for physiological events. The altered level of

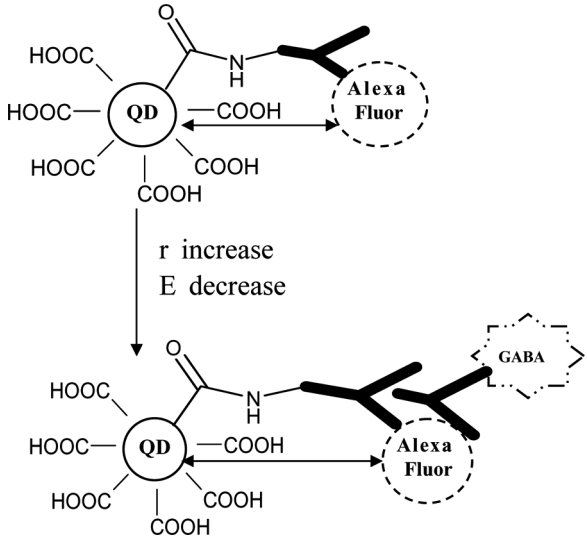

FIGURE 1 The detection of GABA via FRET.

GABA is associated with various neurological and psychiatric disorders. Localized proton magnetic resonance spectroscopy (MRS) provides regional measurement of GABA levels in vivo, but this method is complicated due to the low concentration of GABA and suffers from overlapping resonance signals by other substances such as creatine, glutathione, and other macromolecules. In this paper a new approach has been developed to detect GABA by using FRET technique which is described in Figure 1. Quantum dots (QDs) with carboxyl functional group have been covalently bonded to goat anti-rabbit IgG labeled with Alexa Fluor, and FRET process from QDs to dyes was observed. The FRET efficiency was varied by addition of rabbit polyclonal with GABA due to local distance change induced by the conformational change of antibody.

EXPERIMENTS

Materials and Reagents

Qdot® 525 ITK™ carboxyl quantum dots (8 μM solution), Alexa Fluor® 594 goat anti-rabbit IgG (H+L) (highly cross-adsorbed, 2 mg/ml), and goat serum (NGS) were purchased from Invitrogen Company. The rabbit polyclonal to GABA was obtained from Abcam Company. The albumin bovine (BSA) was purchased from Bio Basic

Inc. 10X PBS (10X phosphate-buffered Saline) and Tris-HCl (pH = 7.4) were obtained from Welgene Inc. and Bioneer corporation, respectively. N-hydroxysuccinimide (NHS, 98%), N-(3-Dimethyl aminopropyl)-N′-ethylcarbodiimide hydrochloride (EDC, protein seq. grade), γ-Aminobutyric acid (GABA, SigmaUltra minimum, 99%), Glutaric dialdehyde (glutaraldehyde, 50 wt% solution in water), Triton X-100 (for molecular biology) and Sodium metabisulfite (SMB, ReagentPlus) were purchased from SigmaAldrich.

Instrumental Analysis

PL spectra were obtained by the Spectra MAX GEMINI XPS (PTI), and lifetime was measured using EasyLife II (PTI).

Preparation of QDs-IgG(Alexa Fluor)

QDs with carboxyl group on the surface can be conjugated to amine compound of IgG by using EDC and NHS as coupling agent. Reaction buffer was made by mixing 5 ml of 0.1 M PBS, 100 μl of 2% NGS, and 15 μl of 0.3% Triton X-100. 0.05 g of EDC and 0.03 g of NHS was added. 2 μl of QDs were mixed with 1080 μl of reaction buffer and various amount of IgG-Alexa Fluor (0, 30, 40 μl) were added. The mixture was incubated for 1 h at room temperature. PL spectra and lifetime for the samples were obtained with excitation wave length of 340 nm. The excitation pulse width for the lifetime measurement was 1.5 ns. FRET efficiency was determined using maximum intensity ratio in PL spectra and lifetime data. 0.1 μl of QDs was mixed with 303 μl of reaction buffer with various concentration of IgG-Alexa Fluor, and PL spectra were obtained.

Detection of GABA by FRET

Anti-GABA buffer was prepared by mixing 4915 μl of 50 mM Tris buffer, 0.01 g 1% SMB, 25 μl of 0.5% Triton X-100, 50 μl of 1% NGS, and 10 μl of rabbit anti-GABA. GABA buffer was prepared by mixing 4500 μl of 0.1 M PBS, 0.001 g of GABA, 500 μl of glutaraldehyde, 0.01 g of SMB, and 0.01 g of BSA. 0.4 μl of QDs and 2 μl of IgG-Alexa Fluor with 320 μl of reaction buffer were mixed and various amounts of anti-GABA buffer and GABA buffer (1:1 mixture) were added. The mixture was incubated for 1 h at room temperature. PL spectra with various concentration of GABA were obtained with excitation wave length of 340 nm. The FRET efficiency was determined by maximum PL intensity ratio.

RESULT AND DISCUSSIONS

Figure 2 shows the fluorescence emission spectra from solutions of QDs-goat anti-rabbit IgG labeled with Alexa Fluor. The figure shows that as the concentration of Alexa Fluor was increased, the emission intensity from QDs was decreased, and emission intensity from Alexa Fluor was increased. As the spectrum change is due to FRET, the point curve shows the emission spectra from donor sample (QDs) while the real line and dashed line show the emission spectra at different concentration of acceptor (Alexa Fluor). The FRET efficiency was obtained using (Eq. 1). By comparing the maximum intensity ratio, the FRET efficiency from samples with IgG(Alexa Fluor) concentration of 56 μg/ml and 74 μg/ml was obtained to be 0.61 and 0.68, respectively.

The FRET process from QDs and Alexa Fluor was further confirmed by measuring lifetime data and calculate the FRET efficiency. Figure 3 shows the lifetime decay curve for QDs-IgG(Alexa Fluor). The concentration of IgG(Alexa Fluor) was varied from 0 to 74 μg/ml. Lifetime was defined as the time width at 50% intensity. FRET calculator provided by the PTI Instruments can give various FRET parameters including Förster distance, FRET efficiency, and local distance between donor and acceptor (r_{DA}), and rate of energy transfer (k_{ET}).

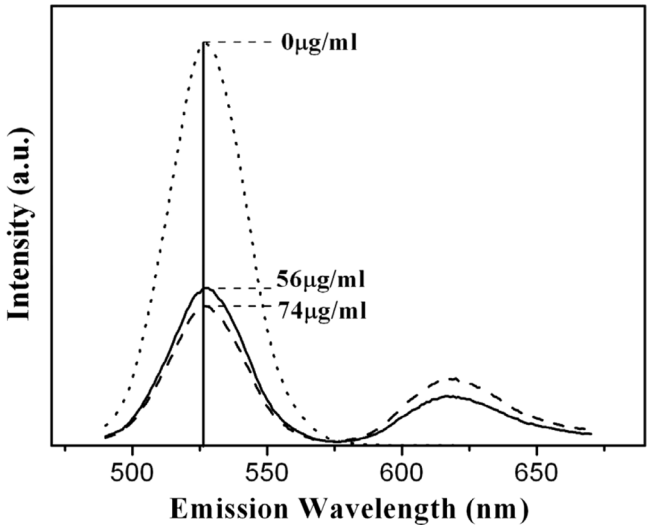

FIGURE 2 Fluorescence emission spectra from solutions of QDs-IgG(Alexa Fluor) with different concentration of IgG(Alexa Fluor). 0 μg/ml, 56 μg/ml and 74 μg/ml of IgG(Alex Fluor) were used.

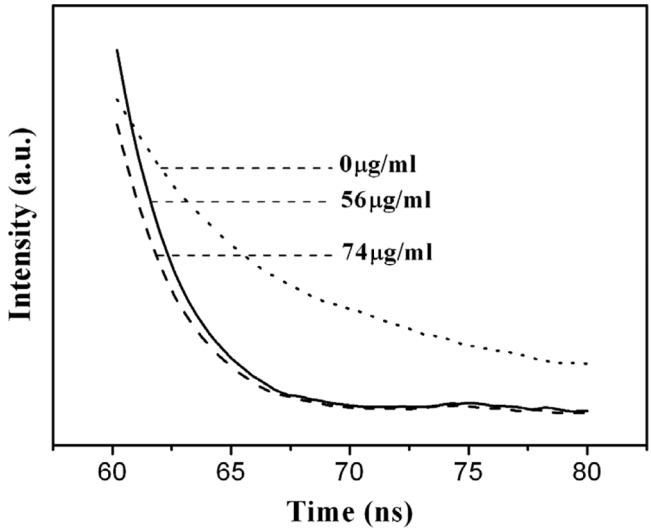

FIGURE 3 Plot of the 340 nm QDs-IgG(Alexa Fluor) photoemission intensity versus time immediately after a short pulse excitation signal. Data are shown for various QDs-IgG(Alexa Fluor) conjugate configurations where the concentration of IgG(Alexa Fluor) was increased from 0 to 74 μg/ml.

FRET parameters obtained from samples with concentration of 56 μg/ml and 74 μg/ml are listed in Table 1. The FRET efficiencies obtained from lifetime data coincide with the values obtained from fluorescence emission spectra. This shows that QDs-IgG(Alexa Fluor) conjugates form FRET system. Figure 4 shows the fluorescence emission spectra from QDs-IgG(Alexa Fluor) with various concentration of IgG(Alexa Fluor). The emission intensity from Alexa Fluor increases with increase of Alexa Fluor concentration, while emission intensity

TABLE 1 FRET Parameters Obtained from Samples of QDs-IgG(Alexa Fluor)

	Sample 1 (56 μg/ml Alexa Fluor)	Sample 2 (74 μg/ml Alexa Fluor)
R_0	39.24	39.24
I_D	8229	8229
I_{DA}	3221	2858
E	0.609	0.653
r_{DA}	36.461 Å	35.327 Å
K_{ET}	3.7922e + 008 (1/s)	4.5836e + 008 (1/s)

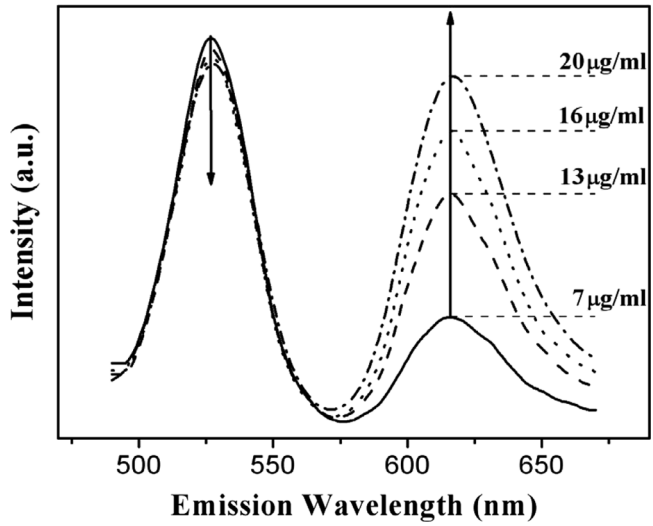

FIGURE 4 Fluorescence emission spectra from QDs-IgG(Alexa Fluor) with various concentration of IgG(Alexa Fluor).

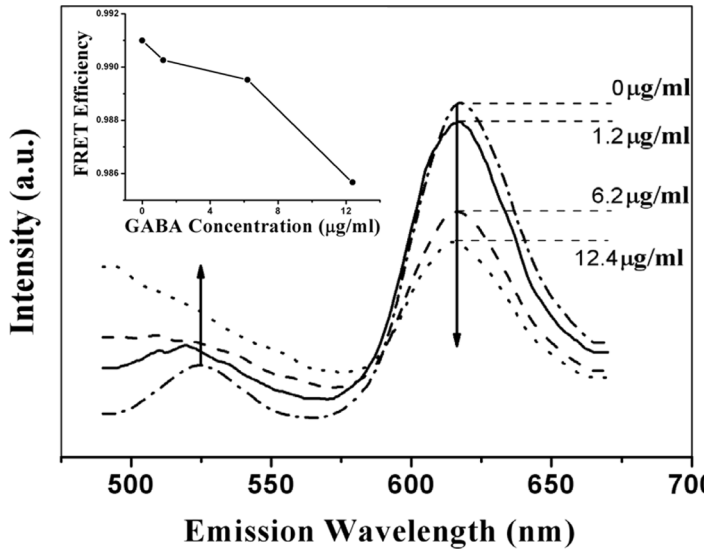

FIGURE 5 The fluorescence emission spectra from solution of QDs-IgG (Alexa Fluor)-rabbit anti-GABA-GABA.

from QDs decreases. This phenomenon might be due to increase of Alexa concentration and energy transfer from QDs to Alexa Fluor by FRET.

Figure 5 shows the fluorescence emission spectra obtained from solution of QDs-IgG(Alexa Fluor)-rabbit anti-GABA-GABA. Here the concentration of GABA was varied from 1.2 μg/ml to12.4 μg/ml, and the effect of GABA concentration on FRET efficiency was investigated. The inset figure shows that FRET efficiency depends on GABA concentration. As the GABA concentration increases, the fluorescence intensity from QDs increases while that from Alexa Fluor decreases. This results in the decrease of FRET efficiency. This phenomenon could be explained by the donor-to-acceptor distance change due to conformational change of IgG(Alexa Fluor). As anti-GABA-GABA bind to QDs-IgG(Alexa Fluor), the distance between QDs and Alexa Fluor increases and FRET efficiency is decreased.

CONCLUSIONS

Quantum dots have been used for the detection of GABA by using FRET phenomena between QDs and organic fluorescent dyes. FRET process was confirmed by measuring FRET efficiency from fluorescence spectra and lifetime of QDs- IgG(Alexa Fluor) conjugates. The dependence of FRET efficiency on GABA concentration shows that QDs combined with fluorescent dyes can be used for the detection of neurotransmitters.

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