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Development of Fluorescence Based DNA Biosensor Utilizing Quantum Dot for Early Detection of *Ganoderma Boninense*

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Abstract. We developed an optical DNA biosensor based on fluorescence resonance energy transfer (FRET) utilizing quantum dot (QD) as sensor. The modified QD was conjugated with single-stranded DNA probe (ssDNA). The target DNA was sandwiched with conjugated QD-ssDNA and reporter probe labeled with Cy5. Hybridization of the sandwich hybrid allowing detection of related sequences of *Ganoderma boninense* gene by constructed the FRET signals which the binding between those DNAs bring them in close distance for fluorescence emission. The developed biosensor capable to detect different concentration of target DNA as low as 1 nM in 10 minutes hybridization. TEM results show the particle size of QD varies in range between 5 to 8 nm. This approach provides simple, rapid and sensitive method for early detection of specific DNA.

Keywords: DNA biosensor, Quantum dot, Fluorescence resonance energy transfer, *Ganoderma boninense.*

1. Introduction

Oil palm became one of the important sources for vegetable oil in Asia mostly for Malaysia and Indonesia. This valuable source is facing serious infection called Basal Stem Rot (BSR) infected by a single species*, Ganoderma boninense* [1]. If not detected in early stage, the plants will end to death whereas no amount of chemicals can save the plants once the symptoms appear. BSR can cause appearance of fruiting bodies at the base of the stem, unopened spears, yellowing crown, deep cracks at the stem's base [2].

Recently, the studies of nanoparticles as sensor for diagnosis and detection of disease become greatest interest. The currently used nanoparticle known as quantum dot (QD)is able to overcome the limitationproperties of the organic dyes. QD is a semiconductor (CdSe, CdS, CdTe)that has diameter range between 2 to 10 nm, resistant to photobleaching, easily used, size-dependent and highly sensitive for detection of biomolecules (DNA, protein, peptide) that act as luminescent probes in biological system [3].

In analysis of biological application, QD involves in reaction that applyFRET based system where QD act as energy donors [4].FRET can be applied in optical field since QD has broad adsorption so QD can be excited far from its excitation range of the acceptor molecule and the absorption spectrum of acceptor can be paired with narrow emission of QD. When the donor and the acceptor are in close distance, the excitation energy ofthe donor is transferred to the acceptor which shows the bindingof the two molecules. In this system, specific DNA target can be detected when applyingFRET as detection mode.

In this report, we applied a simple andpractical detection method to detect our specific DNA sequence from pathogen *Ganoderma boninense.* In our approach, surface of QD is modified to bind with functional thiol (-S) groups in the presence of DNA probe and Cy5-labelled reporter probe which both probes complementary with our target DNA. The changes in fluorescence can be observed after hybridization of the specified DNA and the changes can be a marker for early detection of the pathogen.

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2. Experimental

2.1. Materials and Reagents

Lumidot CdSe/ZnS quantum dot (CdSe/ZnS QD) nanoparticle solution in toluene (core-shell type, 640 nm),toluene, 3-mercaptopropionic acid (MPA, 99%) were purchased from Sigma-Aldrich. Acetone and ethanol (EtOH) were purchased from HmBG. Methanol (MeOH) was purchased from J.T.Baker. All the chemicals were used directly without further dilution and purification. Activating reagents, 1-ethyl-3-[3-dimethylaminopropyl] carbodiimide hydrochloride (EDC, ≥ 98%) and *N*-hydroxysulfosuccinimide (NHS, ≥ 98.5%) were purchased from Fluka. Potassium hydroxide (KOH) was purchased from Systerm. 1x phosphate buffer saline (1 x PBS) (137 mM NaCl, 10 mM phosphate, 2.7 mM KCl) (pH 7.4), sodium bicarbonate buffer ($NaHCO_3$) (50 mM, pH 9.0), phosphate buffer (20 mM, pH 6.0) and hybridization buffer solution (100 mM Tris–HCl, 10 mM $(NH_4)_2SO_4$, 3 mM of $MgCl_2$) (pH 8.0) were self prepared using deionised water (Sartorius Basic Meter PB-10). All oligonucleotides for *Ganoderma boninense* were purchased from 1st BASE Laboratories Sdn Bhd, Selangor, Malaysia. The base sequences are as follows:

- Amine modified single stranded DNA (ssDNA) (20-mer):
  5'-/NH_2C_{12}/TGG GTT GTA GCT GGC CTT CC- 3'
- Complementary target DNA (35-mer):

5'-GCT AGT CAA GGT AAC GGA AGG CCA GCT ACA ACC CA-3'

- Non-complementary DNA (35-mer):
  5'-GTA AGG TGC TTG AAT TCG TTA GGC TTG GTT TCG AT-3'
- Reporter probe (15-mer):
  5'-GTT ACC TTG ACT AGC/Cy5/-3'

All oligonucleotides (100 µM) were diluted using TE buffer (10 mM Tris-HCl, 1 mM EDTA) (pH 8.0) and stored in frozen condition.

2.2. Preparation of Water Soluble CdSe/ZnS QD

Water soluble CdSe/ZnS QD was prepared by using ligand exchange reaction method. 500 µL of QD in toluene was reacted with 100 µL of MPA. The mixture was covered with aluminium foil and stored in the fridge for overnight. After this incubation time, 1 ml of 1.0 M KOH was transferred into the solution and the upper layer was discarded. Then, 1 ml of toluene was added which resulting emulsion discarded. The excess of MPA was removed by 3 repeated cycles of precipitation of the particles with 1 ml of acetone. Precipitate obtained was then dissolved with 1 ml of 1 x PBS and stored as stock solution in fridge for further experiment.

2.3. Activation of COOH Functional Group on CdSe/ZnS QD with EDC-NHS

The mixtureof 200 µL 1:1 (v/v) EtOH/H_2O, 30 mg of EDC and 15 mg of NHS were transferred into 200 µL of phosphate buffer. The mixture then added with 200 µL of 1:1 (v/v) CdSe/ZnS QD/H_2O. The solution was sonicated in a soniclean bath for 1 minbefore allowed to stand at room temperature for 1h. The solution was then centrifuged at 4000 rpm for 20 min and the clear supernatant was removed. The pellet was carefully washed with water (2 x 200 µL). Then, 175 µL of 1:1 (v/v) MeOH/H_2O was added to pellet followed by 25 µL of C_6 amine modified ssDNA (100 µM) and 100 µL of $NaHCO_3$ buffer. The solution was sonicated for 2 min to break up the pellet and stored at 4 °C for overnight. After this reaction time,the sample was centrifuged at 4000 rpm for 20 min and the clear supernatant was carefully separated from the pellet. The pellet was washed with 200 µL of 1:1 (v/v) MeOH/H_2O before 0.5 mL of deionized water was added and sonicated for 5 min to obtain a clear stock solution. It was stored in the dark at 4 °C until further usage.

2.4. Hybridization

The total volume of the hybridization solution was 1500 µL which contain calculated amount of CdSe/ZnS QD-ssDNA conjugate, reporter probe, target DNAand1x PBS buffer. The solutionmixed into Eppendorf tube and shake thoroughly in order to make it homogenous. The hybridization time reaction was carried out for 2 hoursat room temperature before fluorescence emission were taken.

2.5. Fluorescence Emission

Sample was transferred to a quartz cuvette with a micropipette. The fluorescence emissions for all samples were carried out by spectrofluorophotometer using spectrum measurement mode. A fixed excitation wavelength at 488 nm with scan rate of 2 nm/s, bandwidth of 4 nm and slidwidth of 10 nm were used to obtain emission spectra (500-800 nm range) of all samples.

2.6. Transmission Electron Microscopy (TEM)

The samples particle size was measured using a HITACHI H-7100 TEM instrument. Samples of water soluble CdSe/ZnS QD and CdSe/ZnS QD-ssDNA were directly dropped to the copper grid separately for drying and spreading process. Then, the samples were measured with the magnification of 200,000x at scale of 100 nm.

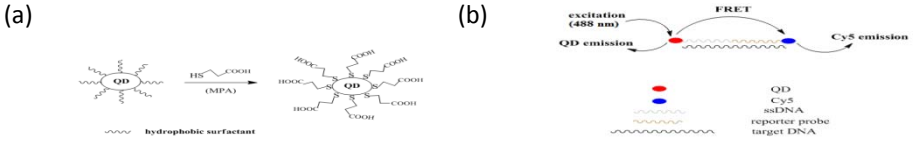
3. Result and Discussion

3.1. Water Soluble CdSe/ZnS QD

Schematic 1 shows the original CdSe/ZnS QD was modified with bifunctional hydrophilic ligand, MPA. In this reaction, the hydrophobic ligandsaround surface of commercializeCdSe/ZnS QDwere replaced by MPA which contain -S functional groups that attached at the surface of QD leading the carboxylic (COOH) groups to be extended away from the QD.CdSe/ZnS QD was left to react with MPA for overnight in order to make sure all the hydrophobic ligands were totally exchanged with COOH functional groups. Normally, nanoparticle QD was modified for bioconjugation and to improve the optical properties. Since the organic ligands do not compatible in aqueous condition, so the introduction of COOH groups enhances the compatibility of QD to react with DNA in aqua medium and increase its solubility in the water.The water soluble CdSe/ZnS QD was stable up to 1 week.

3.2. FRET of Hybrid System

Conjugation of CdSe/ZnSQD and ssDNA allow hybridization of specific target DNA. The changes of the fluorescence signalsfor CdSe/ZnS QD-ssDNA and hybrid system contained target DNAs (complementary and non-complementary DNA) were studiedfor the developed system. As shown in Figure 1 (a), CdSe/ZnS QD-ssDNA and CdSe/ZnS QD-dsDNA show emission peak at 640 nm which are according to emission of QD. No FRET is observed for CdSe/ZnS QD-ssDNA but FRET is observed for CdSe/ZnS QD-dsDNA at 680 nmconfirming hybridization.To confirm the FRET signal is due to specific hybridization, experiment for non-complementary DNA was carried out where there is no FRET so that Cy5 and QD are not in a position where FRET can occur.Hybridization solution contains reporter probe labelled with Cy5 and CdSe/ZnS QD-ssDNA. Upon hybridization with target DNA, the reporter probe and CdSe/ZnS QD-ssDNA will bind with the target forming sandwich hybrid. Hybridization occurs in this system since the sequences of the ssDNA and reporter probe completely match with the sequence of target DNA.The resulting interaction of the hybrid brings the acceptor, Cy5 and donor, QD into close distance. The energy obtained from excitation of QD at 488 nm is transferred to the acceptor and causing fluorescence emission from acceptor by means of FRET illumination [5]. Therefore, the detection of fluorescence emission from acceptor, Cy5 indicates the presence of target DNA and hybridization process occurred in the solution.It is clear that FRET can provide such important information whether the target DNA hybridized or notin the system fordetection of specific sequences of target DNA.



Schematic 1: (a)Modification of hydrophobic surface by MPA (b) FRET between donor, QD and acceptor, Cy5

Fig. 1 (b) and (c) presented thetime- and temperature-dependent for hybrid system. As can be seen, hybridization about to complete at 10 minutes hybridizationas FRET efficiency starts to slow down as time increased. The highest FRET intensity also obtained when hybridization performed at 25 °C. The intensity of

FRET decreases gradually as the temperature increases becausehigh temperature will increase the molecular motion which results in more molecular collisions and subsequent loss of energy so, QD will lose its PL properties [6]. These results suggest that our developed system contains DNAs that are not significantly hindered and readily to hybridized at normal conditions.

The sensitivity of developed DNA biosensor also was studied by allowing CdSe/ZnS QD-ssDNA to hybridize with different concentration of target DNA. As illustrated in Figure 1 (d), the corresponding FRET intensities were gradually decreaseddue to increase in negative charge density of the formation of a DNA duplex which increases the repulsive electrostatic forces between QD and DNA resulting in larger distances and lower efficiency. The designed DNA biosensor can produce FRET to detect *Ganoderma boninense* as lowas at 1 nM with limit of detection of $1.12x10^{-12}$M.

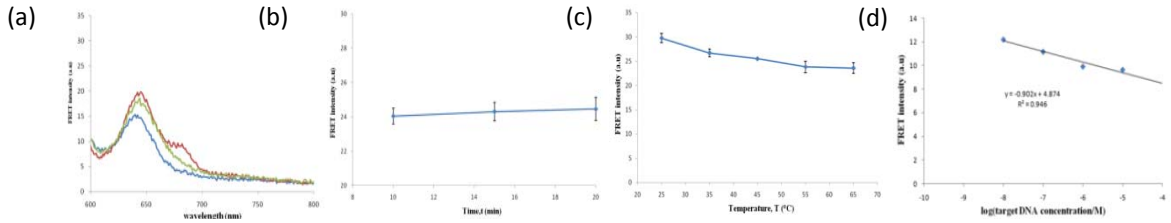


Fig. 1: (a) Fluorescence spectra of (i) CdSe/ZnS QD-ssDNA, (ii) CdSe/ZnS QD-dsDNA (complementary) and (iii) CdSe/ZnS QD-dsDNA (non-complementary) (b) Effect of hybridization time and (c) Effect of hybridization temperature and (d) Different concentration of target DNA

3.3. Measurement of Particle size of QD

Fig. 2 (a) and (b) shows TEM images in 100 nm scale. It can be viewed that the size of the particles were in the range of 2 to 10 nm. These results also proved that the modification and conjugation of QD did not affect its particle size. As can been seen in Figure 2(a), single particle of QD is observed while in Figure 2(b) agglomeration occur possibly due to ssDNA.

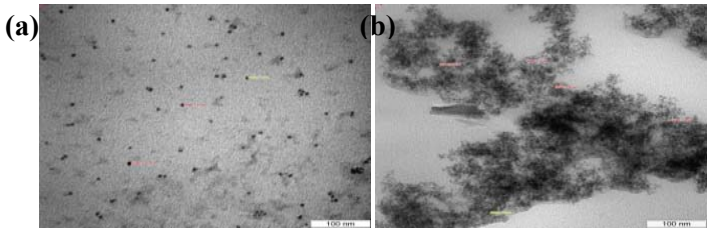


Fig. 2: TEM particle size of (a) water soluble CdSe/ZnS QD and (b) CdSe/ZnS QD-ssDNA

3. Conclusion

The DNA biosensor based on fluorescence was successfully developed utilizing FRET. The modification of QD allowed hybridization process to take place successfully in this system via ligand exchange reaction method for conjugation with DNA probe. The changes of the FRET before and after hybridization confirmed that only hybridized system gave out FRET signal and give indication of close distance between donor QD and acceptor, Cy5. We have shown the sensitivity of the detection system can be applied for the low concentration of target DNA. Our developed DNA biosensor system is suitable for rapid, simple and sensitive detection of specific sequences of DNA.

4. Acknowledgement

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6.References

[1] G. Panchal and P.D. Bridge. Following basal stem rot in young oil palm plantings. *Mycopathologia*. 2005, **159** (1): 123-127.

[2] F. Breton, Y. Hasan, L.Z. Hariadi and H. De Franqueville.Characterization of parameters for the development of an early screening test for basal stem rot tolerance in oil palm progenies. *Journal of Oil Palm Research*. 2006, (special issue): 24-36.

[3] A.J.Sutherland, Quantum Dots as Luminescence Probes in Biological Systems.*Solid State and Materials Science*. 2002, **6**:365-370.

[4] W.R. Algar, and U.J. Krull. Quantum Dots as Donors in Fluorescence Resonance Energy Transfer for the Bioanalysis of Nucleic Acids, Proteins and Other Biological Molecules. *Analytical and Bioanalytical Chemistry*. 2008, **391**:1609-1618.

[5] C-Y. Zhang, H-C. Yeh, H-C.M.T. Kuroki and T-H. Wang. Single-quantum-dot-based DNA Nanosensor. *Nature Materials*. 2005,**4**:826-831.

[6] G.G. Guilbault. *Practical Fluorescence*. New York: Taylor & Francis Publisher. 1990,pp. 28.