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Boronate-based fluorescent carbon dot for rapid and selectively bacterial sensing by luminescence off/on system

Cheong A Choi[a], Zihnil Adha Islamy Mazrad[b], Gibaek Lee[a], Insik In[b,c], Kang Dae Lee[d,*], Sung Young Park[a,b,**]

[a] Department of Chemical and Biological Engineering, Korea National University of Transportation, Chungju 380-702, Republic of Korea
[b] Department of IT Convergence, Korea National University of Transportation, Chungju 380-702, Republic of Korea
[c] Department of Polymer Science and Engineering, Korea National University of Transportation, Chungju 380-702, Republic of Korea
[d] Department of Otolaryngology–Head and Neck Surgery, Kosin University College of Medicine, Busan 49267, Republic of Korea

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ABSTRACT

Boronic acid, which can bind chemo-selectively and reversibly to diols, could be used for the early detection of bacteria through its affinity-binding reaction with diol groups on the bacterial cell wall. Herein, we describe the use of a diol-modified fluorescent probe (DYE) conjugated to a nanosensor consisting of phenylboronic acid-functionalized fluorescent carbon dot (FCD) to allow quenching via the Förster resonance energy transfer (FRET) process. Phenylboronic acid is well-known for its preferential affinity for diol-containing molecules through cyclic ester bond formation. Therefore, in the presence of glucose-containing bacteria, the DYE in the cyclic ester form will be released from the FCD and replaced by the bacterial cell forming a new cyclic boronate ester bond with the nanoparticle, inducing recovery of the fluorescence. Quantitatively, the system's detection performance at various bacterial concentrations (10^1–10^7 CFU/mL) reached ~100% after 60 min, indicating that the high binding affinity of the diol moeity on the peptidoglycan (sugar)-rich bacteria was enough to displace the DYE from the boronic acid-functionalized FCD platform. Our facile and tunable fluorescence switch-on system was tested for its ability to detect bacteria in water from a contaminated river. Incredibly, the system was most successful in detecting bacteria in the contaminated river water, thus proving it to be a less expensive and more robust affinity biosensor for the detection of contaminating pathogens in various chemoselective ligand-based environments.

1. Introduction

Microorganisms, especially bacteria, have become fundamental research targets for many microbiologists in terms of acquiring information about their affects and contaminating processes, as well as evaluating the effectiveness of antimicrobial agents [1–3]. One of the traditional methods for detecting pathogenic bacteria is the use of affinity biosensors through their interaction with immobilized biological components, such as antibodies, DNA, and receptor proteins on the transducer surface and target molecules [4,5]. Despite their high sensitivity, selectivity, and low detection limit of bacteria, biological-sensing elements have some limitations, including low stability, high cost, and laborious techniques. Chemoselective ligands have been regarded as alternatives to biological receptors, owing to their greater resistance to chemical biodegradation, better determination of the kinetic parameters of affinity interactions, and cheaper and faster analytical requirements [6–8].

The bacterial cell surface comprises polysaccharide components such as lipopolysaccharide, teichoic acid, and teichuronic acid, which consist of diols groups that might be either associated with other surface components or totally dissociated from the microbial cell [9–11]. Through these surface polysaccharides, bacteria can recognize and complex with other molecules by strong chemical bonding [12,13]. Boronic acid has been proven to be able to form complexes with compounds containing vicinal diol groups (boronate ester bond) under basic conditions through simple mixing, which have been extensively used for carbohydrate sensors and transporters [14]. Furthermore, the boronate ester bond with 1,2- or 1,3-diols is reversible, and therefore has been widely

* Corresponding author.
** Cooresponding author at: Department of Chemical and Biological Engineering, Korea National University of Transportation, Chungju, 380-702, Republic of Korea.
E-mail addresses: kdlee59@gmail.com (K.D. Lee), parkchem@ut.ac.kr (S.Y. Park).

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applied to functionalize biosensors for the recognition of saccharides and glycosylated biomolecules on bacterial surfaces though surface plasmon resonance, quartz crystal microbalance, and electrochemical methods [15–17]. However, there are no detection methods using fluorescent carbon dot (FCD) in a fluorescence quenching switch-on system activated through chemoselectivity of diol-containing compounds on the bacterium cell wall.

The staining of bacteria with fluorescent dyes (e.g., propidium iodide, ethidium bromide, and SYTO BC) in flow cytometry has been successfully investigated in the routine assessment of bacterial counts [18,19]. However, these techniques require expensive equipment. In our previous work, a new class of fluorescent materials, FCD, proved useful in life science applications, particularly for bacterial detection using ionic complex and hydrophobic interaction between cationic FCD and anionic phosphatase groups on the cell surface [11,12]. The tiny size, excellent photoluminescent properties, and high biocompatibility of the FCD (obtained by a simple condensation reaction as well as carbonization and dehydration processes) give them great potential for application in various fields of biological research. In addition, in order to acquire accurate results in bacterial detection, quantitative analysis is extremely important, but was limited in our previous research. Therefore, in this present study, our FCD system was further improved to allow for a more accurate determination of the number or concentration of bacteria captured.

With these aims in mind, we developed a diol-modified fluorescent probe (DYE) in this study, to quench the carbonized Pluronic-grafted poly(dimethylaminoethyl methacrylate)–quaternized boronic acid by the Förster resonance energy transfer (FRET) mechanism. Upon the addition of *Escherichia coli* (*E. coli*) and *Staphylococcus aureus (S. aureus*) to the DYE-conjugated FCD (D-FCD) solution, the diol groups of polysaccharides on the bacterial cell surface form new cyclic boronate ester bonds with the FCDs and replace the DYE molecules. Consequently, the DYE molecules are released into solution and remain in the supernatant after centrifugation, while the bacteria-bound FCDs are precipitated as the cell pellet. Quenching of the FCDs on the bacterial cell surface restores their higher fluorescence intensity in a manner similar to the capillary electrophoresis–laser-induced fluorescence phenomenon [20]. Besides exploring the fluorescence changes, the released DYE also allows for quantitative analysis through measurement of its UV–vis spectroscopy absorbance. This study intended to test the usefulness of cyclic voltammetry (CV) in monitoring the initial bacterial binding through detection of the electron transfer between the bacterial surface and the electrode surface. The electrode surface coverage by bacteria was also estimated from cyclic voltammograms to validate the potential of CV as the quantitative detection method of bacterial attachment. This tunable platform may be suitable as a confirmatory test, as it displayed high sensitivity and chemical specificity for bacteria detection, in addition to being relatively simple and inexpensive.

2. Materials and methods

2.1. Materials and characterization

Pluronic F-127, 2-(dimethylamino)ethyl methacrylate (DMA), 4-chlorophenylboronic acid, anhydrous toluene, hexane, ethanol, diethyl ether, 5-(2-aminoethylamino)-naphthalene-1-sulfonic acid, Trizma base, potassium ferricyanide ($K_3[Fe(CN)_6]$), potassium chloride (KCl), Trizma HCl, glyceraldehyde, dialysis membrane (Mw = 500–1000), and concentrated sulfuric acid (H_2SO_4) were purchased from Sigma-Aldrich (Seoul, Korea). The DYE molecules were synthesized as described in a previous report [21–23].

Proton NMR spectra were recorded using a Bruker AVANCE III 400 MHz spectrometer with dimethyl sulfoxide (d-DMSO) as the solvent. Absorption spectra were acquired on an Optizen 2120UV spectrophotometer (Mecasys, Yuseong-gu, South Korea). Fluorescence properties were analyzed by means of an L550B luminescence spectrometer from Perkin-Elmer (Waltham, MA, USA). Field-emission scanning electron microscopy (FE-SEM) micrographs were obtained using a JEOL JSM-6700 F SEM (Tokyo, Japan). X-ray photoelectron spectroscopy (XPS) measurements were acquired using an ESCALAB apparatus (Omicron Vacuumphysik GmbH, Taunustein, Germany) and a PHI Quantera-II system (Ulvac-PHI, Chigasaki, Japan). Analysis of the system's bacteria detection performance was conducted under an LSM 510 confocal laser-scanning microscope (CLSM; Carl Zeiss, Oberkochen, Germany) using 405-, 488-, and 543-nm emission filters at 20× magnification. Fluorescence lifetimes were measured using a NanoLED laser light source (Horiba Jobin Yvon NanoLog spectrophotometer; Horiba Korea Ltd, Seoul, Korea) at 375-nm excitation wavelength, and the data were fitted by a multi-exponential decay model. The samples for the fluorescence lifetime measurements were prepared by dissolving the FCD system in an aqueous solution at very low concentrations (1.0 mg/mL). Screen-printed carbon electrodes (SPCEs) were purchased from EGTechnology (Seoul, Korea) and used for the CV analyses using the μStat 4000 P Multi Potentiostat (DropSens, Asturias, Spain).

2.2. Synthesis of carbonized pluronic-grafted poly(dimethylaminoethyl methacrylate)–quaternized boronic acid (FCD)

The method used for the synthesis of Pluronic-grafted poly(dimethylaminoethyl methacrylate)–quaternized boronic acid (B-PgP) was as described in a previous report [13,26]. To fabricate the FCDs, 1 g of B-PgP was dissolved in 5 mL of deionized Millipore water at 25 °C. Then, 10 mL of 36 N H_2SO_4 was added, and the mixture was stirred for 1 min. The final reaction mixture was then added to 185 mL of double-deionized water (DDW) and prepared for dialysis in water using a membrane (MWCO: 1000). Finally, the product was freeze-dried and collected.

2.3. Preparation of the diol-modified fluorescent probe-conjugated FCD

The B-PgP-functionalized FCD (100 mg) and DYE (0.005 mg/mL) were dissolved in 10 mL of Tris-buffered saline (TBS) at pH 12.0. The solution was allowed to react for 24 h at room temperature to obtain D-FCDs. The reaction solution was then dialyzed (MWCO: 500–1000) for 24 h and freeze-dried.

2.4. Bacteria detection technique

To detect bacteria *via* the boronate ester bonding behavior, we used *S. aureus* (gram-positive, strain ATCC 25,323) and *E. coli* (gram-negative, strain ATCC 25,922) as the test pathogens, cultured in 50 mL of MRS and LB media, respectively, at 37 °C for 24 h. After that, bacterial cells were harvested by centrifugation, and the pellet was resuspended in PBS 7.4 buffer. Then, various concentrations of each bacterial cell solution (10^7, 10^5, 10^3, or 10^1 colony-forming units (CFU/mL) were incubated with 1 mg/mL of D-FCDs. At different interval times, the bacteria-bound nanoparticles were pelleted by centrifugation and washed 3 times. The cell pellet labeled with D-FCD was resuspended in PBS and examined on an L550B luminescence spectrometer (Excitation wavelength: 380 nm, Emission wavelength: 400 nm), as well as under the LSM 510 CLSM to verify the performance of the D-FCDs for detection of bacteria [11,13]. At the same time, the supernatant from the centrifugation was inves-