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Metal free bioimaging reagent for intracellular citrate in prostate cancer cells using aryl boronate derivative

Kanagaraj Rajalakshmi [a,1], Yun-Sik Nam [b,1], Muthusamy Selvaraj [a], Yeonhee Lee [b], Kang-Bong Lee [a,*]

[a] Green City Technology Institute, Korea Institute of Science & Technology, Hwarang-ro 14-gil 5 SeongBuk-gu, Seoul 02792, Republic of Korea
[b] Advanced Analysis Center, Korea Institute of Science & Technology, Hwarang-ro 14-gil 5 SeongBuk-gu, Seoul 02792, Republic of Korea

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ABSTRACT

A boronate group integrated on a triphenylimidazoleoxadiazolephenyl (TPIOP) moiety exhibited unusual sensing and detection abilities towards citrate. TPIOP boronate exhibited a bathochromic shift in its fluorescence emission upon interaction with citrate. Quantification of trace levels of citrate ions in the human body may aid in the diagnosis of prostate cancer and kidney malfunctions. Herein, a novel, metal-free sensing strategy for citrate was developed based on fluorimetric detection with TPIOP boronate. The fluorescence of TPIOP boronate increased linearly and was gradually red-shifted upon addition of citrate. The enhanced emission intensity was attributed to aggregation induced emission. The vacant 'p' orbital of boron in TPIOP boronate acts as a Lewis acid, and accepts electrons from citrate, which is a Lewis base. Upon binding, the hybridization geometry of boron changes from sp^2 trigonal planar to sp^3 tetrahedral. The TPIOP boronate-citrate aggregates increase the polarity of TPIOP-boronate, which caused the red shift in the fluorescence. Citrate could be detected visually using a UV lamp, as the probe changed from blue to green upon interaction with the analyte. The developed probe was applied for the visualization of endogenous citrate in PC3 live cells.

1. Introduction

Citrate is one of the most targeted anion due to its vital role in the Krebs cycle, as it is a significant species in cell metabolism and important for energy transfer and biomolecule synthesis [1]. Citrate levels in biological fluids have been employed to diagnose various such as kidney dysfunction, nephrolithiasis and glycogen storage issues [2–4]. In prostate cancer, citrate levels can be as low as 2–20 mM, while those in healthy males are 50–200 mM [1,5–7]. Therefore, monitoring citrate concentrations in biological fluids may facilitate prostate cancer screening during early stage. Various assay for citrate determination in living specimens have been developed [8–12]. Fluorescent sensors have been widely used to monitor ionic compounds and biomolecules due to their excellent sensitivity and selectivity, on-site visualization, ease of operation, and low-cost imaging equipment [13–16]. Fluorescent chemosensors based on disulfonated aluminum complexes were recently reported as sensing ensemble for the detection of citric acid [12]. Zhu et al. reported a chemogenic sensor based on a dinuclear zinc complex, which formed via a zinc-citrate coordination linkage [17].

Generally, fluorescent citrate sensors are indicator displacement assays (IDA), which consist of two steps [12,17,18]. The fluorescent probe forms complexes with metal ions, and citrate ions coordinate with the metal center of the complexes in the second step. The citrate ions displace the ligand from the complex, which leads to recovered fluorescence. Since metal ions have a vacant orbital available for citrate coordination, IDA-type chemosensors often bear Al^{3+}, Cu^{2+} and Zn^{2+} complexes as binding motifs [12,17,18]. Although IDA-type chemosensors have been successfully utilized for the detection of citrate ions, they have some disadvantages: (i) IDA-type probes can only sense citrate, if the binding affinity of citrate towards the metal is higher than that of metal and ligand in the probe: (ii) metal-probe complexes can induce side effects in biological systems: (iii) and IDA-type sensors typically exhibit low sensitivity and slow responses, due to indirect direction of citrate. Thus, metal-free chemosensors may be superior, due to the direct and single step detection of citrate with rapid and high sensitivity. Such probes will be incredibly useful if they are viable for biological applications. Therefore, the development of metal-free molecular probes for the detection of

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* Corresponding author.
E-mail address: leekb@kist.re.kr (K.-B. Lee).
[1] These authors contributed equally to this work.

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citrate anions under physiological conditions is desired. The vacant orbital and positive charge on the metal atom will play an important role IDA-based strategies. We introduced a boronate moiety to replace the metal-complexes due to its (boronate) Lewis acid-like behavior. The long standing history of Lewis acidic boronate species prompted us to utilize boron as a positively charged center to facilitate direct citrate sensing. In boronate species, boron has an empty ‘p’ orbital with sp^2 hybridization and a trigonal planar geometry [19–23]. Boron is known to act as a receptor for anions such as fluoride (F^-), hydroxide (OH^-) and cyanide (CN^-). In this case, the citrate can act as a Lewis base and donate electrons to the vacant ‘p’ orbital of boron (Lewis acid), resulting in a transition from sp^2-trigonal planar to sp^3-tetrahedral (Scheme S1).

Herein, we describe a novel Lewis acidic triphenylimidazoleoxadiazolephenyl (TPIOP) boronate sensor, which was used as a probe for citrate detection in prostate cancer (PC3) cells. The TPIOP boronate probe is comprised of phenyl rings at the 4- and 5-positions and a phenyl amidoxime moiety at the 2-position of the imidazole ring, which contains a 4-carboxyphenylboronic acid pinacol ester. The long π–conjugation and heteroatoms enhanced the electron delocalization, and created a positive charge on boron. TPIOP boronate exhibits a switch on fluorescence response towards citrate and its emission shifts from blue to green upon binding. To the best of our knowledge, this is the first citrate sensor based on a metal free-TPIOP boronate probe. The enhanced emission intensity of the probe was attributed to aggregation induced emission (AIE) [24–28]. TPIOP boronate exhibited a fast response about 15 min, high sensitivity with a limit of detection (LOD) of 10 nM, and high selective color change from blue to green with citrate in the physiological conditions. Thus, it was utilized for citrate tracking in PC3 cells.

2. Material and methods

2.1. Chemicals and reagents

1,1′-Carbonyldiimidazole (CDI), hydroxylamine hydrochloride ($HONH_2{\cdot}HCl$), Hepes buffer solution, fetal bovine serum (FBS), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), Dulbecco’s phosphate buffered saline (DBS) Roswell Park Memorial Institute (RPMI) medium 1640, penicillin, streptomycin and other cell culture materials were obtained from Sigma-Aldrich (St. Louis, MO, USA). PC3 cells (human prostate cancer cells) were purchased from Korean Cell Line Bank (Seoul, Republic of Korea). 4-Carboxyphenylboronic acid pinacol ester was purchased from Alfa Aesar (Ward Hill, MA, US). N′-hydroxy-4-(4,5-diphenyl-1H-imidazol-2-yl)benzamidine was prepared from its cyanide precursor, which was purchased from TCI (Chuo-ku, Tokyo, Japan). Dimethyl sulfoxide (DMSO) and other common solvents were purchased from Daejung Chemicals (Kyunggi-do, Republic of Korea). Carboxylic acids and interfering ions were obtained from Sigma-Aldrich (St. Louis, MO, USA) and AccuStandard (New Haven, CT, USA), respectively. All reagents were used as received without further purification.

2.2. Instrumentation

UV–vis absorption spectra were measured in the range of 300–800 nm, using polystyrene cells with 1 mm path length on an S-3100 spectrophotometer (Sinco, Seoul, Republic of Korea). Fluorescence spectra were recorded on an LS-45 luminescence spectrometer (Perkin Elmer, Waltham, MA, US). ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker Avance 400 MHz spectrometer. Mass spectra were obtained using an HR-MS-Synapt G2 in positive mode. The pH of the solutions was determined by using an HI 2210 pH meter (Hanna instruments, Woonsocket, RI, USA). Scanning Electron Microscopy (SEM) images were obtained using JSM-7610F (Jeol, Peabody, MA, USA). The samples (TPIOP and TPIOP with citrate) were prepared by spotting on a quartz plate and then dried at room temperature prior to measurements. Particle size distributions were measured using a Zetasizer apparatus (Beckman Coulter, Brea, CA, USA). A SpectraMax M2 microplate reader (Molecular devices, Sunnyvale, CA, US) was used in the cytotoxicity tests. Fluorescence images were acquired on a confocal laser scanning microscope (LSM 700, Carl Zeiss, Germany) equipped with a 63x oil immersion objective lens, and a diode laser light source.

2.3. Synthesis of TPIOP borate

The synthetic route for TPIOP boronate is shown in Scheme S2. A solution of 4-carboxyphenylbonronic acid pinacol ester **(1)** (1.00 mM, 1.00 equiv.) in dry DMF (5 mL) was added to carbonyldiimidazole (CDI) (1.20 mM, 1.20 equiv.) and the reaction mixture was stirred for 1 h at ambient temperature. Next, N’-hydroxy-4-(4,5-diphenyl-1H-imidazol-2-yl)benzamidine **(2)** (1.20 mM, 1.20 equiv.) was dissolved in anhydrous DMF with dropwise addition, and the reaction mixture was heated at 110 °C for 18 h. The reaction mixture was cooled to room temperature, and poured into ice-cold water. The reaction mixture was extracted with methylene chloride (MC) and the combined organic phases were washed with brine, and dried over anhydrous Na_2SO_4. The resulting mixture was filtered, and concentrated in vacuo. The obtained residue was purified by silica gel column chromatography to obtain pure TPIOP (IUPAC Name: 5-(4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)-3-(4-(4,5-diphenyl-1H-imidazol-2-yl)phenyl)-1,2,4-oxadiazole) **(3)** boronate as a brown solid in an excellent yield of 87%.

^{1}H NMR (400 MHz, DMSO-d_6) δ 12.94 (brs, 1H), 8.30 (d, J = 8.4 Hz, 2H), 8.20 (dd, J = 6.8, 8.1 Hz, 4H), 7.93 (d, J = 8.1 Hz, 2H), 7.58–7.52 (m, 4H), 7.47–7.39 (m, 6H), 1.32 (s, 12H) (Fig. S1). ^{13}C NMR (100 MHz, DMSO-d_6) δ 175.7, 168.5, 145.0, 135.8, 133.6, 129.1, 129.0, 128.7, 128.0, 127.7, 126.2, 126.1, 125.8, 84.7, 25.2 (Fig. S2). The found mass value was 567.46 (Fig. S3A).

2.4. Spectroscopy measurements

A solution of TPIOP boronate was prepared in 10 mM Hepes buffer at pH 7.4 with 2 vol% of DMSO. The final concentrations of the probe solutions for UV–vis and fluorescence measurements were 20 and 2 μM TPIOP boronate, respectively in 10 mM Hepes buffer at pH 7.4 with 2 vol% of DMSO. The other interfering carboxylic acid solutions were prepared in double distilled water, and the final concentrations of the carboxylic acid solutions, which were added to the probe solution, were 0.5 mM in water.

2.5. Cell culture, cytotoxicity test, and confocal microscopy imaging

PC3 cells (human prostate cancer) were cultured in RPMI 1640 with 10% FBS, 100 μg/mL penicillin, and streptomycin. Cells were maintained in an incubator at 37 °C under 5% CO_2. The cells were cultured in 96-well plates in culture media for the cytotoxicity tests. After overnight culture, the cells were incubated with different concentrations of TPIOP boronate (0–100 μM) for 24 h. To determine the viability of the cells, 0.5 mg/mL of MTT media was added and the cells were incubated for about 4 h. The produced formazan crystals were dissolved in 0.1 mL DMSO and were read with a SpectraMax M2 microwell plate reader. The cytotoxicity of TPIOP boronate was calculated using Eq. (1):

$$\mathrm{Cellviability}(\%) = \mathrm{Absorbanceofsample}/\mathrm{Absorbanceofcontrol} \times 100 \quad (1)$$