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Synthesis and Characterization of a Cationic BODIPY-Conjugated Polymer as a Fluorescent Probe for Bacterial Sensing †

Carolina P. Urquiza [1], María E. Pérez [1,2], Sofía C. Santamarina [1,2], María E. Milanesio [1,2] and Edgardo N. Durantini [1,2,*]

[1] Instituto para el Desarrollo Agroindustrial y de la Salud (IDAS), Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Ruta Nacional 36 Km 601, Río Cuarto X5804BYA, Córdoba, Argentina; email1@email.com (C.P.U.); email2@email.com (M.E.P.); email3@email.com (S.C.S.); email4@email.com (M.E.M.)

[2] Departamento de Química, Facultad de Ciencias Exactas, Físico-Químicas y Naturales, Universidad Nacional de Río Cuarto, Ruta Nacional 36 Km 601, Río Cuarto X5804BYA, Córdoba, Argentina

* Correspondence: edurantini@exa.unrc.edu.ar; Tel.: +54-935-846-76157

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Abstract

The development of fluorophores capable of detecting pathogens represents a promising strategy for the diagnosis and control of resistant infections. In this work, 8-pentafluorophenyl-1,3,5,7-tetramethyl pyrromethene fluoroborate (BDP) was synthesized from pentafluorobenzaldehyde and 2,4-dimethylpyrrole in 47% yield. Conjugation of BDP to polyethylenimine (PEI) was carried out by stirring at room temperature for 72 h, resulting in complete conversion of BDP into the corresponding conjugate (BDP-PEI). The UV-visible absorption spectra of these compounds showed a main absorption band at 505 nm with fluorescence emission at 515 nm ($\Phi_F = 0.18$). Furthermore, BDP-PEI exhibited strong green fluorescence emission in *Staphylococcus aureus* cells, indicating its potential application as a fluorophore for pathogen sensing.

Keywords: polyethyleneimine; BODIPY; fluorophore; microorganism

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1. Introduction

The incidence of infections caused by resistant pathogens has been steadily increasing. In this context, the accurate detection of microorganisms is essential to optimize antimicrobial therapy. This approach enables a rapid response to infections, reduces the indiscriminate use of antimicrobials, and allows for efficient treatment monitoring [1].

Among the numerous detection methods, the use of fluorescent probes has emerged as a promising strategy for the rapid identification of bacteria, as it enables the direct, sensitive, and real-time visualization of microbial cells [2]. To achieve this goal, an ideal fluorophore must combine high photostability and quantum yield, efficient interaction with bacterial structures, biocompatibility, low toxicity, and structural versatility for chemical adaptation.

In this context, boron-dipyrromethene derivatives (4,4-difluoro-4-boro-3a,4a-diaza-*s*-indacene, BODIPY) has been proposed as fluorophores [3]. These compounds can be

conjugated to positively charged precursor polymers. In this regard, polyethylenimine (PEI) is a polycationic aliphatic polymer due to the presence of amine groups, which can enhance interactions with bacterial envelopes [4].

In this work, the synthesis of a hybrid fluorophore based on the conjugation of BODIPY with PEI (BDP–PEI), along with its spectroscopic characterization and its interaction with Gram-positive bacteria (*Staphylococcus aureus*) using fluorescence microscopy. The results demonstrate the potential of this system as a fluorescent marker for pathogen detection, providing a novel strategy to address the challenge of resistant infections. Therefore, in this work we report the synthesis of a hybrid fluorophore based on the conjugation of BODIPY with PEI (BDP–PEI), together with its spectroscopic characterization and its interaction with *S. aureus* cells. The results highlight the potential of this system as a fluorescent marker for pathogen detection, offering a novel strategy to address the challenge of resistant infections.

2. Materials and Methods

2.1. Chemical Reagents and Equipment

All reagents were obtained from Sigma-Aldrich (Milwaukee, WI, USA) and used without further purification. Thin-layer chromatography (TLC) plates coated with silica gel (250 μm) were purchased from Analtech (Newark, DE, USA), while silica gel 60 (particle size 0.040–0.063 mm, 230–400 mesh) was supplied by Merck (Darmstadt, Germany). Proton nuclear magnetic resonance spectra were acquired on a Bruker Avance DPX400 FT-NMR spectrometer operating at 400 MHz (Bruker BioSpin, Rheinstetten, Germany). High-resolution mass spectrometry (HRMS) analyses were carried out on a Bruker micrOTOF-QII instrument (Bruker Daltonics, Billerica, MA, USA) equipped with an electrospray ionization (ESI) source. Absorption spectra were recorded on a Shimadzu UV-2401PC spectrophotometer (Shimadzu Corporation, Tokyo, Japan), and fluorescence measurements were performed with a Spex FluoroMax spectrofluorometer (Horiba Jobin Yvon Inc., Edison, NJ, USA). Microscopic imaging was conducted using an inverted fluorescence microscope (BIM500FL, Bioimager, ON, Canada) fitted with a 100× objective (Carl Zeiss CP ACRHOMAT, NA = 1.25).

2.2. Synthesis of 1,3,5,7-Tetramethyl-8-[2,3,4,5,6-Petafluorophenyl]-4,4-difluoro-4-bora-3a,4a-diaza-s-Indacene (BDP)

A solution of 2,3,4,5,6-pentafluorobenzaldehyde (593 mg, 3.02 mmol) and 2,4-dimethylpyrrole (0.62 mL, 6.0 mmol) in dichloromethane (DCM, 150 mL) was prepared under an argon atmosphere. Trifluoroacetic acid (TFA, 0.05 mL) was added, and the reaction mixture was stirred at room temperature for 22 h. Subsequently, a solution of 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ, 680 mg, 3.0 mmol) in DCM (5 mL) was added dropwise, and the mixture was stirred for an additional 4 h under an oxygen atmosphere. Triethylamine (TEA, 9 mL, 65 mmol) and $BF_3{\cdot}OEt_2$ (9 mL, 73 mmol) were then introduced, and the reaction was maintained at room temperature for 3 h. The organic phase was washed with water (3 × 50 mL), and the solvent was removed under reduced pressure. The crude product was purified by flash column chromatography on silica gel using cyclohexane/DCM (3:1) as eluent, affording BDP in 47% yield. The obtained compound was characterized by spectroscopic methods, and the data were consistent with those previously reported [5].

2.3. Synthesis of BDP-PEI

A solution of BDP (30 mg, 0.072 mmol) and PEI (185 μL, M_W = 800 g/mol, 172 mg, 0.216 mmol) in 5 mL *N*,*N*-dimethylformamide (DMF) was stirring for 72 h at room

temperature in argon atmosphere. The progress of the reaction was followed by TLC (silica gel, DCM). Under these conditions, the conjugates remained retained at the origin. The solvent was removed under reduced pressure and the conjugates were washed three times (5 mL each) with hexanes. The products were dried under reduced pressure to obtain 178 mg of BDP-PEI.

2.4. Spectroscopic Studies

Spectroscopic measurements were conducted at room temperature in DMF, utilizing a quartz cuvette with a 1 cm path length. The steady-state fluorescence emission spectra were performed by exciting the solutions at 483 nm. The fluorescence quantum yield (Φ_F) of the BDP-PEI was determined by comparison of the area under the emission spectrum with that of BDP, which was used as a reference ($\Phi_F = 0.98$) [6].

2.5. Microscopic Images of Individual S. aureus Cells

Fluorescence microscopy studies were conducted following the described methodology [7]. *S. aureus* (ATCC 43300) were cultivated aerobically on tryptic soy (TS) agar overnight at 37 °C. A suspension of *S. aureus* (1 mL) was incubated in a chamber comprised of a polymeric cylinder affixed to a coverslip for 30 min at 37 °C. Non-adherent bacterial cells were then eliminated from the chamber through washing with PBS. After that, 600 μL of PBS was added to the glass surface of the chamber and bacteria were treated with 1.0 μM BDP-PEI for 10 min in the dark. Fluorescence images were obtained with a CMOS camera using the blue fluorescence channel (EX BP 480–550, DM 570, BA 590).

3. Results and Discussion

3.1. Synthesis of BDP-PEI

The synthetic procedure of the BDP started by mixing pentafluorobenzaldehyde and 2,4-dimethylpyrrole in DCM, catalyzed by the addition of TFA. The mixture was stirred overnight followed by the addition of DDQ as the oxidant. After 4 h, TEA together with $BF_3{\cdot}OEt_2$ was added to close the dipyrromethene ring, obtaining the BDP in 47% yield.

The conjugation of BDP to PEI was carried out in DMF by stirring at room temperature for 72 h. At the end of the reaction, TLC analysis showed complete consumption of the starting BDP, with the resulting conjugate (BDP-PEI) remaining at the origin of the chromatographic plate. The solvent product was evaporates and the resulting solid was washed several times with hexanes to obtain 178 mg of BDP-PEI.

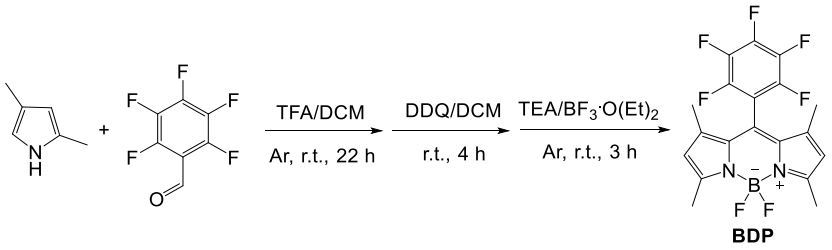

Figure 1. Synthesis of BDP.

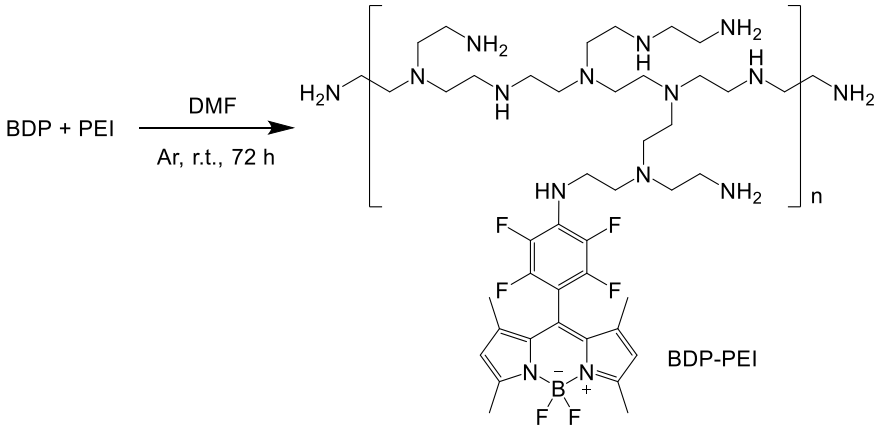

Figure 2. Synthesis of BDP-PEI conjugate.

3.2. Spectroscopic Properties

The UV−visible absorption spectra of BDP and its respective conjugate BDP-PEI in DMF are shown in Figure 3A. In both cases, a sharp absorption of the Soret band was obtained at about 505 nm, indicating that these compounds are mainly dissolved as monomers in DMF. This absorbance was attributed to the 0−0 vibrational band of the $S_0 \rightarrow S_1$ transition [6].

The fluorescence emission spectra of both BODIPY derivatives displayed a band at 515 nm, corresponding to the 0–0 vibrational band of the $S_1 \rightarrow S_0$ electronic transition. From these data, a Φ_F of 0.18 was determined for BDP-PEI. The reduced fluorescence intensity of BDP in the conjugate is attributed to its interaction with the polymer structure. Nevertheless, this value remains sufficiently high to support the application of BDP-PEI as a fluorophore [6].

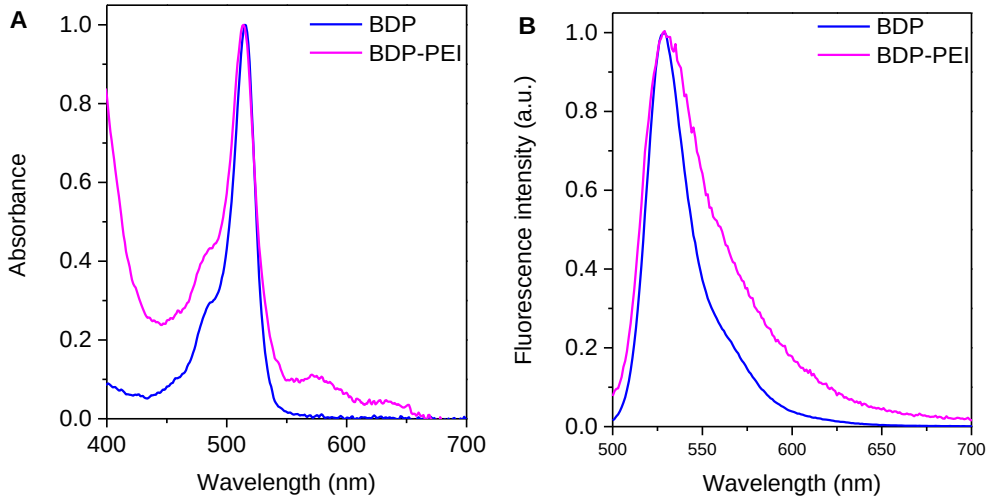

Figure 3. (**A**) Absorption and (**B**) fluorescence emission spectra of BDP and BDP-PEI (λ_{exc} = 483 nm) in DMF.

3.3. Fluorescence Visualization of S. aureus Using BDP-PEI

To investigate the cellular imaging of *S. aureus*, fluorescence microscopy was employed to assess the uptake of BDP-PEI by bacterial cells [7]. In these experiments, bacterial suspensions were incubated in a sealed chamber, allowing the cells to adhere to the glass surface through their pili. Non-adherent planktonic cells were subsequently removed by washing, leaving only surface-attached bacteria. For uptake studies, BDP-PEI (1.0 μM in PBS) was introduced into the chamber, and the results are presented in Figure

4. Bright-field microscopy revealed individual cells anchored to the glass surface (Figure 4A), whereas fluorescence images demonstrated the association of BDP-PEI with *S. aureus*, evidenced by green emission from the bacterial cells (Figure 4B). The fluorescence detected in the blue channel, attributable to BDP-PEI, confirmed its internalization or strong binding to the bacteria. Comparable findings have been described for other BODIPY derivatives interacting with microbial cells [8]. The pronounced emission of BDP-PEI in this biological context highlights its potential as a fluorophore for diagnostic cellular imaging.

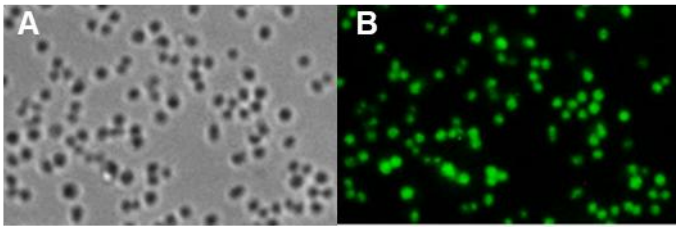

Figure 4. Microscopy images of *S. aureus* cells attached to a glass surface treated with 1.0 μM BDP-PEI for 10 min in the dark; cells under (**A**) bright field and (**B**) blue fluorescence channel.

3. Conclusions

In this work, a cationic BOD-PEI conjugate was successfully synthesized and characterized as a potential fluorescent probe for bacterial detection. The conjugate retained the characteristic absorption band at 505 nm and exhibited fluorescence emission at 515 nm, with a quantum yield of 0.18, confirming its suitability as a fluorophore. Fluorescence microscopy studies demonstrated the ability of BDP-PEI to associate with *S. aureus* cells, producing strong green emission that enabled clear visualization of individual bacteria. These findings indicate that BDP-PEI combines favorable spectroscopic properties with effective microbial labeling, highlighting its potential application as a diagnostic tool for pathogen sensing and monitoring.

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Conflicts of Interest: The authors declare no conflicts of interest.

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