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Article

Synthesis of amphiphilic poly(fluorene) derivatives for selective imaging of *Staphylococcus aureus*

Ping He [a,b], Fengting Lv [a,b], Libing Liu [a,b,*], Shu Wang [a,b,*]

[a] *Key Laboratory of Organic Solids, Institute of Chemistry, Chinese Academy of Sciences, Beijing 100190, China*
[b] *College of Chemistry, University of Chinese Academy of Sciences, Beijing 100049, China*

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ABSTRACT

Three amphiphilic poly(fluorene-*co*-phenylene) derivatives with different side chains (PFP-1, PFP-2, PFP-3) were designed and synthesized for exploring their detection and imaging of pathogens. Upon incubation with six kinds of different pathogens, it was found the three polymers could selectively interact with *Staphylococcus aureus* (*S. aureus*). Their selective imaging towards *S. aureus* were thus realized. The selective imaging towards *S. aureus* was also confirmed even under the blend of microbes. PFP-3 shows stronger fluorescence imaging signal than PFP-1 and PFP-2. Zeta potential and isothermal titration microcalorimetry (ITC) tests demonstrated that both electrostatic interactions and hydrophobic interactions played important roles in the binding between PFPs and pathogens. Thus, amphiphilic PFP-3 exhibits great potential for specific imaging of *S. aureus* in a simple and rapid manner.

1. Introduction

As a public health and security issue, pathogen infection plays a fatal role in food and water poisoning, which can cause severe diseases and even death [1–5]. *Staphylococcus aureus* (*S. aureus*) is one of the most common opportunistic pathogens of human beings, especially susceptible to infection in hospital [6–9]. To reduce the morbidity and mortality rates of pathogen infections, efficient detection of pathogens is of great importance. Present methods for pathogenic bacteria detection mainly include microorganism culture, polymerase chain reaction and other signal assay integrated targets identification [10]. However, these existing methods are either high-cost or time-consuming. Therefore, the exploration of simple and convenient methods in pathogen detection is still required. Conjugated polymers (CPs) have been drawing lots of attention because of their good optical and electrical properties, such as large extinction coefficient, signal amplification capacity, good photostability and biocompatibility [11–13]. Moreover, water solubility of CPs could be realized by introducing hydrophilic groups into the side chain, such as oligo(ethylene glycol) (OEG), quaternary ammonium, and carboxylate groups. These aforementioned superiorities endow CPs with extensive application in chemical and biochemical sensing, pathogens detection, drug screening and delivery, and photo-therapy [14–17].

In this work, we designed and synthesized three amphiphilic poly(fluorene) derivatives (PFP-1, PFP-2, PFP-3) which were constituted of both hydrophilic side chains and hydrophobic backbones. In view of the differences of the superficial composition among different kinds of pathogens [18–20], we explored the interactions between PFPs (PFP-1, PFP-2, PFP-3) with pathogens and discovered the specific recognition of *S. aureus* by PFPs. Furthermore, we also studied the possible interaction mechanism between PFPs and pathogens by zeta potential and ITC measurement.

2. Materials and methods

2.1. Materials and measurements

All the organic solvents were purchased from Beijing Chemical Works and used as received except for ethyl ester which was redistilled. Other reagents and chemicals were purchased from Aldrich Chemical Company, Acros, Alfa-Aesar or Sinopharm Chemical Reagent Co., Ltd. and used as received. Phosphate buffer saline (PBS) was purchased from Hyclone. HaCaT cell line was obtained from the cell culture center of Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences (Beijing, China). Fetal bovine serum (FBS) was purchased from Sijiqing Biological Engineering Materials (Hangzhou, China). Eagle's minimum essential medium (EMEM) was purchased from WISENT INC (Beijing, China). *Bacillus subtilis* (*B. subtilis*), *Candida albicans* (*C. albicans*), *Saccharomyces cerevisiae* (*S. cerevisiae*) and *Pseudomonas aeruginosa* (*P. aeruginosa*)

* Corresponding authors.
E-mail addresses: liulibing@iccas.ac.cn (L. Liu), wangshu@iccas.ac.cn (S. Wang).

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were purchased from China General Microbiological Culture Collection Center. *Staphylococcus aureus* (*S. aureus*) was purchased from Chinese Academy of Sciences Institute of Microbiology. Ampr *Escherichia coli* (*E. coli*) was purchased from the Beijing Bio-Med Technology Development Co., Ltd. (China).

^{1}H NMR and ^{13}C NMR spectra were collected by Bruker Avance 400 MHz or Bruker Avance 300 MHz spectrometer. UV–Vis spectra were measured on a JASCO V-550 spectrophotometer and the fluorescence spectra were recorded on a Hitachi F-4500 fluorometer equipped with a Xenon lamp as excitation source. The fluorescent images were taken on inverted fluorescence microscope (Olympus 1X71, Japan) with a 100 W mercury lamp as light source or laser scanning confocal microscope (Olympus, FV1200-IX61). The zeta potentials and hydrodynamic diameter were measured on the Zeta-Potential meter (Nano ZS 90, UK). The TEM images were taken on a Hitachi HT7700 (Japan) transmission electron microscope. The enthalpy changes were measured on the ITC (MicroCal iTC200, USA).

2.2. Preparation of PFPs

2.2.1. PFP-1

To a solution of monomer 2 (64 mg, 0.09 mmol) and monomer 1 (60 mg, 0.09 mmol) in *N*,*N*-dimethylformamide (DMF, 4 mL), 2 mL of 2.0 mol/L aqueous K_2CO_3 was added. Then a certain amount of [1,1′-bis(diphenylphosphino)ferrocene]dichloropalladium(II) (Pd(dppf)Cl_2) was added under nitrogen atmosphere [1], and the resulted mixture was stirred at 90 °C for 48 h. After cooling down to room temperature, the mixture was extracted with 75 mL of chloroform for three times after 30 mL of distilled water added. After dried over anhydrous magnesium sulfate, the solution was concentrated under vacuum and then the residue was dissolved in as little chloroform as possible. The solution was dispersed in approximately 20-folds diethyl ether and the mixture was centrifuged for 5 min in 8000 r/min. The sediment was dried under vacuum at 45 °C and then the target polymer was obtained as a brownish black solid (25 mg, 20%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.93–7.36 (m, 20H), 7.10 (s, 2H), 4.74 (s, 1H), 4.01 (s, 3H), 3.54–3.35 (m, 11*H*), 3.32 (s, 9H), 3.08 (s, 5H), 2.92 (d, 4H), 2.46 (s, 4H), 1.76 (s, 9H), 1.44 (s, 27H).

2.2.2. PFP-2-Br

To a solution of monomer 3 (112 mg, 0.15 mmol) and monomer 1 (101 mg, 0.15 mmol) in 3 mL of DMF and 3 mL of toluene, 2 mol/L aqueous K_2CO_3 (3 mL) was added. The following procedures were same as that of PFP-1 synthesis. Finally, the sediment was dried under vacuum at 45 °C and then the target polymer was obtained as a brownish black solid (29 mg, 14%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.73 (dd, 8H), 7.12 (s, 2H), 4.64 (s, 1H), 3.95 (dd, 7H), 3.08 (s, 4H), 1.99 (s, 9H), 1.74 (s, 5H), 1.38 (d, 31H), 1.14 (s, 6H), 0.81 (s, 4H).

2.2.3. PFP-2

To a solution of PFP-2 (10 mg) in tetrahydrofuran (THF), trimethylamine in methanol (3.2 mol/L, 5 mL) was added. The solution was stirred at 40 °C for 24 h and then the excess trimethylamine and solvent were removed by the rotary evaporators [2]. The residue was dissolved in methanol and dialyzed through a dialysis bag with molecular weight cut-off of 3500 Da for two days to give a brownish black solid. ^{1}H NMR (400 MHz, DMSO) δ 7.75 (t, 8H), 7.15 (s, 2H), 6.74 (s, 1H), 4.22 (s, 1H), 4.04 (s, 3H), 3.84 (d, 2H), 3.38 (q, 2H), 3.32 (d, 6H), 3.25 (d, 2H), 3.17 (d, 1H), 3.13–3.04 (m, 1H), 2.90 (s, 4H), 2.50 (s, 20H), 1.91 (s, 3H), 1.68 (s, 4H), 1.36 (s, 31H), 1.09 (t, 10H).

2.2.4. PFP-3-Br

To a solution of monomer 3 (57 mg, 0.08 mmol), monomer 2 (54 mg, 0.08 mmol) and monomer 1 (100 mg, 0.08 mmol) in DMF (4 mL) and toluene (2 mL), 3 mL of 2 mol/L aqueous K_2CO_3 was added. The following procedures were same as that of PFP-2-Br synthesis. The target polymer was obtained as a brownish black solid (24 mg, 11%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.87–7.44 (m, 9H), 7.11 (s, 2H), 4.68 (d, J = 26.1 Hz, 2H), 4.08–3.80 (m, 5H), 3.58–3.35 (br, 4H), 3.31 (s, 5H), 3.04 (t, J = 21.4 Hz, 6H), 2.46 (s, 2H), 1.99 (s, 5H), 1.75 (s, 5H), 1.44 (s, 28H), 1.14 (s, 3H), 0.80 (s, 3H).

2.2.5. PFP-3

To a solution of PFP-3 (9 mg) in THF, trimethylamine in methanol (3.2 mol/L, 3 mL) was added. The following procedures were same as that of PFP-2 synthesis. ^{1}H NMR (400 MHz, DMSO) δ 7.78 (dd, 14H), 7.16 (s, 2H), 6.72 (s, 2H), 4.03 (s, 3H), 3.84 (d, 2H), 3.60 (t, 1H), 3.41–3.33(m, 16H), 3.16 (s, 5H), 3.09 (s, 1H), 2.89 (s, 6H), 1.91 (s, 3H), 1.72 (d, 5H), 1.36 (s, 38H), 1.09 (t, 8H), 0.70 (s, 2H).

2.3. Fluorescence imaging assay

The liquid spawn (10 μL) of *S. aureus* was added into 10 mL of liquid NB (1% tryptone, 0.3% beef extract, 0.5% NaCl) culture medium and cultivated in the orbital shaker at 37 °C overnight. The bacterium was collected by centrifugation at 7100 r/min for 2 min and washed with PBS (1×) twice. The suspension of *S. aureus* was diluted to OD_{600} = 1.0. 100 μL of above *S. aureus* solution was then further diluted into 500 μL with PBS and incubated without or with PFP-3 under 1, 5 and 10 μmol/L at 37 °C for 15 and 30 min. Then the systems were washed by PBS and resuspended in 10 μL PBS, of which 3 μL was added on a clean glass slide and immobilized by a coverslip. The fluorescent images were taken under the inverted fluorescence microscopy for PFP channel with the exposure time of 100 ms. Magnification of the object was 100×. The type of light filter was D380/30 nm exciter and D460/50 nm emitter. Amp^r *E. coli* shared the same experiment conditions and procedures as *S. aureus* except for the culture medium which was LB (1% tryptone, 0.5% yeast extract, 1% NaCl) and *C. albicans* also shared most of the processes besides the OD_{600} = 2.0 and the YPD (1% yeast extract, 2% tryptone, 2% glucose) culture medium [3].

Likewise, *P. aeruginosa* (Gram-negative) and *C. albicans* (Fungi) shared the same operations with *E. coli* and *S. cerevisiae* respectively. *B. subtilis* (Gram-positive) was the same as *S. aureus* aside from its BPY (0.5% beef extract, 1% tryptone, 0.5% yeast extract, 0.5% glucose, 0.5% NaCl) culture medium.

As for the blend of *S. aureus* (100 μL, OD_{600} = 1.0), *E. coli* (100 μL, OD_{600} = 1.0) and *C. albicans* (100 μL, OD_{600} = 2.0), the mixture was incubated with PFPs (10 μmol/L at 37 °C for 30 min and then observed under the laser scanning confocal microscope (LSCM) with a 405 nm laser as light source.

2.4. Zeta potential measurements

The Gram-positive bacteria *S. aureus* and *B. subtilis*, Gram-negative bacteria *E. coli* and *P. aeruginosa* were incubated without or with PFPs at 37 °C for 30 min, while fungus *C. albicans and S. ceresiae* were incubated without or with PFPs at 30 °C for 30 min ([PFPs] = 10 μmol/L in RUs). After the incubation, the systems were washed with PBS for once to remove the unbound and weakly interacted PFPs by centrifugation at 7100 r/min for 2 min. Then the

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