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Fluorescent probes for Cu^{2+}, Hg^{2+} and amino acids in aqueous solutions based on two mechanisms

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ABSTRACT

The new polythiophene-based conjugated polymers Poly{3-[(4-{[2-(dimethylamino) ethyl]amino}cyclohexylidene)methyl]thiophene} (**P1**), Poly{3-[(4-{[3-(dimethylamino)propyl]amino} cyclohexylidene)methyl]thiophene} (**P2**) and Poly{3-{[4-(4-methylpiperazin-1-yl)cyclohexylidene] methyl}thiophene} (**P3**) have been synthesized by $FeCl_3$ oxidative polymerization. Among the common metal ions tested, **P1**, **P2** and **P3** exhibited high selectivity and sensitivity toward Hg^{2+} in methanol aqueous solutions at pH 3 with a turn-off mode in which the detection limit of **P3** for Hg^{2+} reached a level of 10^{-8} M. Additionally, the fluorescence of **P1** in Tris–HCl buffer solution could be dramatically quenched in the presence of Cu^{2+} with a detection limit of 10^{-9} M. These sensing processes for Hg^{2+} and Cu^{2+} have been shown to be mediated by electrostatic effects and complexation. In addition, the **P1**–Cu^{2+} system was also employed as a turn-on fluorescence probe for the label-free detection of amino acids.

1. Introduction

Pollution caused by heavy metal ions has posed severe risks to the environment and human health. For example, mercury and its derivatives are widespread global pollutants and have caused grave consequences [1–4]. Copper (Cu^{2+}) is an essential trace metal that is important for human life but can be toxic to organisms such as algae, fungi, bacteria, or viruses [5–7]. Therefore, the design and synthesis of chemosensors for highly noxious heavy metal ions is currently of great importance. Recently, sensitive chemosensors for heavy metal ions based on amplified fluorescence quenching of conjugated polymers have been developed [8–13]. Fluorescent chemosensors typically consist of two parts: a metal-discerning unit to selectively recognize the ion, and a fluorosphore to translate the metal binding event into a useful signal response. Generally, they have been based on three interactions: (1) electrostatic, (2) coordination and (3) conformational changes caused by aggregation.

To achieve improved analyte recognition, molecular probes which exhibited good sensing properties for the detection of multiple species or stimuli were designed using different mechanisms. Recently, dual sensing fluorescent probes were reported for the ratiometric detection of different metal ions [14,15] or metal ions along with proteins and sugars [16,17]. Bai et al. [18] prepared an amphiphilic conjugated polymer as an aggregation based multifunctional sensing platform for Ag^{2+} and Hg^{2+}. Then Jayakannan et al. [19] reported a triple-action carboxylic di-stilbene fluorescent polymer chemosensor for Cu^{2+} and amino acids. Despite this there has been no report until now of a multifunctional polymer probe for detecting heavy metal ions based on an entirely different mechanism.

To address this, we designed three probes **P1**, **P2** and **P3**, based on polythiophenes, which are known for their high quantum yield and significant photochemical stability [20–23]. To enhance the fluorescence intensity, binding ability and selectivity toward a special ion, amino groups were introduced as a side chain of the polymers. The synthesized polymers displayed excellent selectivity and sensitivity toward Hg^{2+} in CH_3OH aqueous solution at pH 3 and Cu^{2+} in Tris–HCl buffer solution respectively, with a turn-off mode. This result conforms to our anticipation that these bi-dentate amine containing polymers can undergo complexation in a neutral environment and have electrostatic interactions in acidic conditions with different metal ions because the diamino groups were uncharged and positively charged respectively. Furthermore the non-fluorescent **P1**–Cu^{2+} complex can be used as an "off-on" probe

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to differentiate naturally occurring amino acids, which is of great importance as amino acids play important roles as metabolic intermediates in the human body.

2. Experimental

2.1. Materials

All metal salts such as $CuCl_2{\cdot}2H_2O$, $NiCl_2{\cdot}6H_2O$, $BaCl_2{\cdot}3H_2O$, $AlCl_3{\cdot}6H_2O$, $AgNO_3$, $CdCl_2{\cdot}2.5H_2O$, $PbCl_2$, $CoCl_2{\cdot}6H_2O$, $SrCl_3{\cdot}6H_2O$, $CrCl_3{\cdot}6H_2O$, $ZnCl_2$, $HgCl_2$, $MnCl_2{\cdot}4H_2O$ and LiCl were analytical grade and used without further purification. All other organic reagents were purchased and used as received.

2.2. Measurements

UV–vis spectra were recorded on a Shimadzu 3100 spectrometer. Fluorescence measurements were carried out using an Edinburgh Instruments Ltd-FLS920 fluorescence spectrometer. ^1H NMR and ^{13}C NMR spectra were recorded on a Bruker AV III 400 MHz NMR spectrometer. Infrared spectra were recorded on a Bruker Vertex 70 FT-IR spectrometer using KBr pellets. Gel permeation chromatography (GPC) measurements were performed with a Waters 2410 refractive-index detector at 35 °C and THF was used as the eluent at a flow rate of 1.0 ml min^{-1}. GPC data were calibrated with polystyrene standards. Mass spectra were obtained using a Perkin Elmer Clarus 500 mass spectrometer.

2.3. Sample preparation

All tests described in this paper were carried out at room temperature (25 °C) with distilled water. In the experiments involving titration with various metal ions, the polymers were dissolved in MeOH/H_2O (1/1, v/v) or THF/H_2O (1/9, v/v) to afford the test solutions (5×10^{-5} M). Stock solutions (1×10^{-5} M) of the metal salts in water were prepared.

2.4. Synthesis of 3-[(4-oxocyclohexylidene)methyl]thiophene

A solution of 3-(1, 4-dioxaspiro[4.5]dec-8-ylidenemethyl) thiophene (3.72 g, 16 mmol) and concentrated hydrochloric acid (12 ml) in 25 ml methanol was stirred for 2 h. The solvent was removed, and pure water (20 ml) was added. The mixture was extracted with $CHCl_3$ (3× 20 ml). The organic phase was washed with water several times and dried over Na_2SO_4, the crude product was purified by thin layer chromatography (TLC) on silica gel (n-hexyl acetate:ethyl acetate = 6:1) to give the product (2.11 g, 68.7%) as a faint yellow oil. Ms (ESI): $m/z = 192.2$ $[M]^+$. FTIR (KBr, cm^{-1}): 1658 (C=O), 1454 (CH=C). ^1H NMR ($CDCl_3$, 400 MHz, ppm): $\delta = 7.29$–7.31 (d, 1H, CH, $J = 8$ Hz), 7.11 (s, 1H, CH), 7.05–7.07 (d, 1H, CH=CH, $J = 8$ Hz), 6.41 (s, 1H, CH=C), 2.81–2.84 (t, 2H, CH_2), 2.66–2.69 (t, 2H, CH_2), 2.46–2.52 (m, 4H, CH_2). ^{13}C NMR ($CDCl_3$, ppm, δ): 211.3, 138.3, 136.6, 128.4, 125.2, 122.3, 119.8, 41.0, 40.1, 34.1, 27.2.

2.5. Synthesis of N,N-dimethyl-N′-(4-thiophen-3-ylmethylene-cyclohexyl)-ethane-1,2-diamine (*1*)

A mixture of 3-[(4-oxocyclohexylidene)methyl]thiophene (0.5 g, 2.60 mmol) in 10 ml methylene chloride and 5 ml acetic acid was stirred at room temperature. Then a solution of N, N-Dimethyl-1, 2-ethylenediamine (0.5 g, 5.67 mmol) in methylene chloride (10 ml) was added dropwise over 30 min and the reaction mixture was stirred for 1 h. After adding sodium cyanoborohydride (0.16 g, 2.55 mmol), the reaction mixture was kept in an ice bath for 1 h and subsequently for 3 h at room temperature. The final reaction mixture was diluted with water and extracted with $CHCl_3$ (3× 50 ml), the organic layer was washed with water (3× 50 ml) and dried over anhydrous Na_2SO_4. After filtration and evaporation, the crude product was isolated by preparative TLC on silica gel to yield compound **2** (0.4 g, 61.5%) as a yellowish oil. Ms (ESI): $m/z = 265.2$ $[M+H]^+$. FTIR (KBr, cm^{-1}): 1459 (CH=C), 1276 (C—N). ^1H NMR ($CDCl_3$, 400 MHz, ppm): $\delta = 7.24$–7.26 (d, 1H, CH, $J = 8$ Hz), 7.02 (s, 1H, CH), 6.99–7.01 (d, 1H, CH=CH, $J = 8$ Hz), 6.16 (s, 1H, CH=C), 2.915–2.953 (m, 1H, CH_2), 2.75–2.79 (m, 2H, CH_2), 2.68–2.73 (m, 1H, CH), 2.45–2.48 (m, 2H, CH_2), 2.34–2.40 (m, 2H, CH_2), 2.33 (s, 1H, NH), 2.24 (s, 6H, CH_3), 1.98–2.00 (m, 1H, CH_2), 1.26–1.39 (m, 4H, CH_2). ^{13}C NMR ($CDCl_3$, ppm, δ): 141.6, 138.9, 128.7, 124.8, 121.6, 115.4, 59.2, 56.6, 45.8, 45.4, 44.5, 35.0, 34.3, 33.5, 27.3.

2.6. Synthesis of poly{3-[(4-{[2-(dimethylamino)ethyl]amino}cyclohexylidene)methyl]thiophene} (*P1*)

Anhydrous ferric chloride (1.2 g, 4.44 mmol) was suspended in 20 ml dry chloroform and stirred for 30 min under nitrogen and then compound **1** (0.29 g, 1.09 mmol) was added drop-wise. The mixture was stirred at 0 °C for 24 h. Treatment of the reaction mixture with methanol (10 ml) resulted in the precipitation of the polymer which was carefully de-doped by repeatedly treating a chloroform solution of the polymer with 25% ammonium hydroxide at ambient temperature. The crude product was obtained as a red solid after evaporation of the chloroform. The solid polymer was then purified by Soxhlet extraction using n-hexane/tetrahydrofuran (10:1) to remove impurities and oligomers. Finally **P1** (0.15 g, 50.9%) was obtained as a red solid after evaporation of the Soxhlet extraction solvents. FTIR (KBr, cm^{-1}): 1456 (CH=C), 1278 (C—N). ^1H NMR ($CDCl_3$, 400 MHz, ppm): $\delta = 6.99$ (s, 1H, CH), 6.17 (s, 1H, CH). GPC: Mn = 6587 g/mol, PDI = 1.37.

2.7. Synthesis of N,N-dimethyl-N′-[4-(thiophen-3-ylmethylidene)cyclohexyl]propane-1,3-diamine (*2*)

A mixture of 3-[(4-oxocyclohexylidene)methyl]thiophene (0.3 g, 1.56 mmol) in 10 ml methylene chloride and 5 ml acetic acid was stirred at room temperature. Then a solution of N, N-dimethyl-1,3-propylene diamine (0.5 g, 6.75 mmol) in methylene chloride (10 ml) was added drop-wise over 30 min, and the reaction mixture was stirred for 1 h. After the addition of sodium cyanoborohydride (0.1 g, 1.56 mmol) the reaction mixture was kept in an ice bath for 1 h and subsequently for 3 h at room temperature. The final reaction mixture was diluted with water and extracted with $CHCl_3$ (3× 50 ml), the organic layer was washed with water (3× 50 ml) and dried over anhydrous Na_2SO_4. After filtration and evaporation, the crude product was isolated by preparative TLC on silica gel to yield monomer **2** (0.24 g, 56.0%) as a yellowish oil. Ms (ESI): $m/z = 279.2$ $[M+H]^+$. FTIR (KBr, cm^{-1}): 1460 (CH=C), 1181 (C—N). ^1H NMR ($CDCl_3$, 400 MHz, ppm): $\delta = 7.21$–7.23 (d, 1H, $J = 8$ Hz), 6.99 (s, 1H, CH), 6.96–6.98 (d, 1H, CH=CH, $J = 8$ Hz), 6.13 (s, 1H, CH=C), 2.87–2.91 (m, 1H, CH_2), 2.72–2.75 (m, 2H, CH_2), 2.69–2.70 (m, 1H, CH), 2.37 (s, 1H, NH), 2.31–2.34 (m, 2H, CH_2), 2.21 (s, 6H, CH_3), 2.156 (m, 1H, CH_2), 1.93–2.05 (m, 2H, CH_2), 1.66–1.73 (m, 2H, CH_2), 1.22–1.36 (m, 4H, CH_2). ^{13}C NMR ($CDCl_3$, ppm, δ): 141.2, 138.9, 128.7, 124.7, 121.7, 117.1, 58.2, 56.3, 45.8, 45.7, 45.4, 34.9, 34.0, 33.2, 31.9, 28.3.