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The development of a conjugated polyelectrolytes derivative based fluorescence switch and its application in penicillamine detection

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ABSTRACT

A sensitive and simple method for detecting penicillamine (PA) has been developed based on fluorescence quenching and recovery. The fluorescence probe was designed based on poly 2,5-[3-(1,1-dimethyl-piperidine methylene) thiophene] chloride(PDPMT-Cl), a water-soluble cationic polythiophene derivative. The fluorescence of PDPMT-Cl was quenched by Au^{3+} due to the spontaneous formation of Au^{3+}–PDPMT-Cl assembling dyads. The addition of PA created stable Au^{3+}–PA complex leading the release of PDPMT-Cl and fluorescence recovery. The fluorescence intensity of PDPMT-Cl fluorescence "off–on" switch system was linear to PA concentration varying from 0.01 to 20 μmol/L ($\gamma = 0.9981$) with a detection limit of 3.26×10^{-9} mol/L.

1. Introduction

Penicillamine (2-amino-3-methyl-3-sulfanyl-butanoic acid, PA) is derived from hydrolytic degradation of penicillin. The structure is described as follows (Fig. 1), PA is a sulfur-containing amino acid which belongs to the aminothiol family and it strongly chelates with a majority of heavy metal ions [1]. PA exists in either D- or L-enantiomeric form with different biological and toxicological properties. D type is clinically useful and it is used in treating hepatolenticular degeneration (Wilson's disease), rheumatoid arthritis, primary biliary cirrhosis, scleroderma, fibrotic lung diseases, cystinuria, heavy element poisoning, and progressive systemic sclerosis [2,3]. However, there are about 50% of patients experiencing one or more adverse effects by D-PA, such as anorexia, loss of taste, oral ulceration, skin rashes, hematological effects, glomerulonephritis and nephrotic syndrome [4,5], which makes it important to monitor D-PA in urine and plasma.

Various analytical methods have been reported for D-PA determination in both pharmaceutical preparations and biological samples. These methods include high performance liquid chromatography [6], fluorimetry [7], spectrophotometry [8], chemiluminescence [9], capillary electrophoresis [10], and electrochemistry [11]. Recently, fluorescent sensor [12–14] has drawn increased attention for D-PA detection. However, most of the sensors were based on a fluorescence turn-on probe [15–17] or a fluorescence quenching probe [18–20]. The construction of a fluorescent chemo-sensor with "off–on" response based on water-soluble polythiophene derivatives is very novel.

As optical transducers, water-soluble conjugated polymers (CPs) provide a useful platform for constructing high sensitive chemo-sensors and bio-sensors [21–28]. Thanks to delocalized electronic structures in CPs, it is applicable to rapidly transfer excitation through an isolated conjugated polymer chain [29]. CPs offer many sensing advantages [30–34] as fluorescence sensors comparing to quantum dots [35,36] and small molecule dyes. A sensitive and simple method for detecting penicillamine (PA) has been developed based on fluorescence quenching and recovery. The fluorescence probe was designed based on poly 2,5-[3-(1,1-dimethyl-piperidine methylene) thiophene] chloride(PDPMT-Cl), a water-soluble cationic polythiophene derivative. The fluorescence of PDPMT-Cl was quenched by Au^{3+} due to the spontaneous formation of Au^{3+}–PDPMT-Cl assembling dyads. The addition of PA created stable Au^{3+}–PA complex leading the release of PDPMT-Cl and fluorescence recovery.

2. Experiments

2.1. Reagents and apparatus

All chemicals have a purity of analytical grade or greater. PDPMT-Cl was synthesized according to previous report [37]. PA was purchased from Sigma-Aldrich. Stock standard solutions of PA, PDPMT-Cl and Au ions (4.0×10^{-4} mol/L) were individually prepared in double distilled water and intermediate solutions (4.0×10^{-5} mol/L) were prepared weekly from the stock standard solutions by appropriate double distilled water dilution. Tris–HCl buffer solutions with different pHs were prepared by mixing 0.1 mol/L Tris and 0.1 mol L^{-1} HCl in proportion. Infrared spectra (IR) were recorded in potassium bromide pellets

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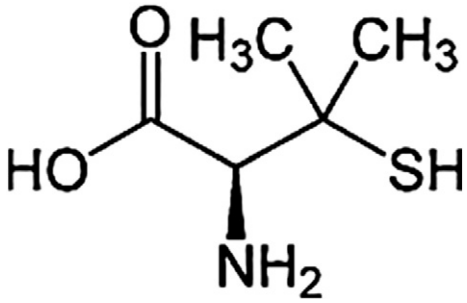

Fig. 1. Structure of PA.

on a Perkin Elmer 1750. ^{1}H NMR and ^{13}C NMR were measured by Bruker AV III400. Fluorescence measurements were measured by RF-5301 fluorescence spectrophotometer. A pHS-3C meter (Shanghai Scientific Instruments Company, China) was used to measure the pH of the solution.

2.2. Synthesis of PDPMT-Cl

PDPMT-Cl was synthesized according to literature [32]. 3-methylthiophene was used as starting material through bromination, Witting–Horner reaction, and methylation to obtain the monomer. The polymer was synthesized by oxidative polymerization in chloroform using $FeCl_3$ as oxidant. The structure is described as follows (Fig. 2).

2.3. Fluorescence measurement

In a 10 mL colorimeter tube, 1.0 mL Au^{3+} solution at 4.0×10^{-5} mol/L was added to the mixture of 1.0 mL PDPMT-Cl at 4.0×10^{-5} mol/L and 1.0 mL Tris–HCl buffer solution at pH 7.5. Upon adding PA into PDPMT-Cl, the fluorescence of PDPMT-Cl was recovered and recorded at emission 562 nm (excitation 455 nm) with 10 nm slit width.

2.4. Preparation of PA and human samples

Twenty PA tablets (125 mg/tablet) were powdered and the equivalent of one tablet (125 mg as PA) was weighed and extracted with 100 mL HCl at 0.10 mol/L. The extraction solution was sonicated for 15 min with vortex mixing at 5 min intervals before filtering through an ordinary filter paper with 0.10 mol/L HCl for three times. The filtrate and washings were diluted to a 250 mL calibrated flask. The sample solutions were diluted with water to obtain solutions at expected concentrations within the calibration range before assays.

Human blood samples were obtained from a rheumatoid arthritis patient treated with PA at oral dose of 1.2 g/day. The samples were immediately centrifuged at 6000 r/min for 15 min upon collection. The obtained plasma was aliquoted to 5 mL and stored at −20 °C until analysis. For protein deposit purpose, 0.50 mL plasma sample was diluted with 0.50 mL 0.60 mol/L trichloroacetic acid solution before a 1 min vigorous shake, then the sample was left at 0 °C for 1 h. After

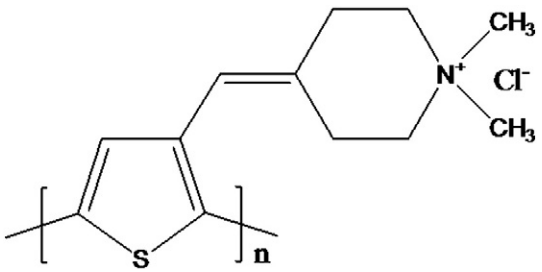

Fig. 2. Structure of PDPMT-Cl.

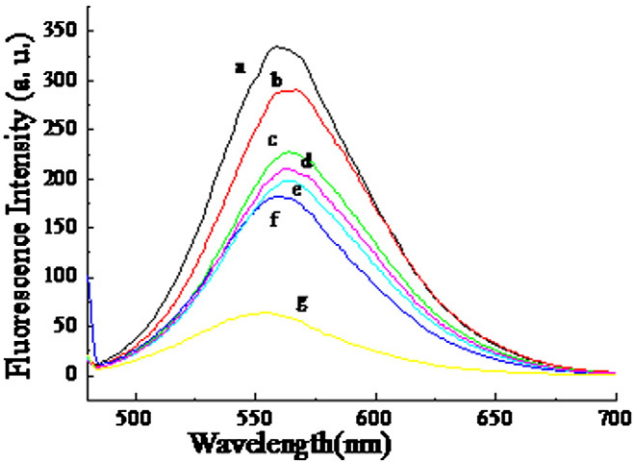

Fig. 3. Fluorescence emission spectra of PDPMT-Cl upon addition of different metal ions. [PDPMT-Cl] = 4.0×10^{-6} mol/L; Tris–HCl buffer solution pH = 7.5. a: PDPMT-Cl; b: Ru^{3+}; c: Cd^{2+}; d: Cu^{2+}; e:Rh^{3+}; f:Pd^{2+}; g:Au^{3+}.

being centrifuged at 10,000 r/min for 20 min, the supernatant was transferred into another 1.5 mL vial and kept at 4 °C.

3. Results and discussion

3.1. Fluorescence response of PDPMT-Cl to metal ions

Fluorescent experiments were carried out with different ions (Ru^{3+}, Cd^{2+}, Cu^{2+}, Rh^{3+}, $Pd2^{+}$, Au^{3+}). Fig. 3 shows the fluorescence response toward different ions. As can be seen from Fig. 3, fluorescence was quenched effectively by Au^{3+} for 5 min. The fluorescence was quenched for 6 h with the existence of Au^{3+}. Therefore, Au^{3+} was used as quenching ion.

3.2. Au^{3+} caused fluorescence intensity changes of PDPMT-Cl

The fluorescence reduction efficiency increased quickly in the first few minutes of reaction and the quenching system was able to maintain stable for at least 6 h. Upon adding Au^{3+}, the fluorescence intensity of PDPMT-Cl significantly decreased, which indicates the strong interaction between PDPMT-Cl and Au^{3+}. Fluorescence response of PDPMT-Cl in the presence of Au^{3+} in various concentrations was investigated. The fluorescence intensity decreased upon the addition of Au^{3+}. The relative fluorescence intensity (initial fluorescence intensity/the

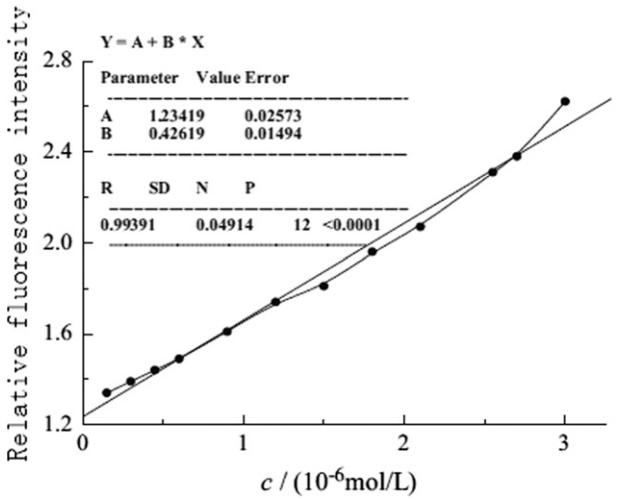

Fig. 4. Stern–Volmer plots of fluorescence quenching.