Talanta 77 (2008) 319–324

Contents lists available at ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

Sensitive and selective detection of aspartic acid and glutamic acid based on polythiophene–gold nanoparticles composite

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ARTICLE INFO

Article history:
Received 22 February 2008
Received in revised form 16 June 2008
Accepted 19 June 2008
Available online 5 July 2008

Keywords:
Polythiophene derivative
Gold nanoparticles
Aspartic acid and glutamic acid
Fluorometry

ABSTRACT

In recent years, gold nanoparticles and water-soluble fluorescent conjugated polymers are promising materials in terms of their potential applications in a variety of fields, ranging from monitoring DNA hybridization to demonstrate the interaction between proteins, or detecting diseased cell, metal ions and small biomolecular. In order to exploit some new properties of the both, many attempts have been devoted to achieve nanoparticle–polymer composite via incorporating metal nanoparticle into polymer or vice versa, however, only few of them are put into practical application. In the present paper, we utilize the "superquenching" property of AuNPs to polythiophene derivatives for detecting aspartic acid (Asp) and glutamic acid (Glu) in pure water, and discuss the factors accounting for fluorescence quenching and recovery via modulating pH. Thus an exceptionally simple, rapid and sensitive method for detecting Asp and Glu is established with a limit of detection (LOD) is 32 nM for Asp and 57 nM for Glu, the linear range of determination for Asp is 7.5×10^{-8} M to 6×10^{-6} M and 9.0×10^{-8} M to 5×10^{-6} M for Glu. The system is applied to real sample detection and the results are satisfying. Otherwise the composite is very sensitive to pH change of solution, we expect it will be possible to use as pH sensor with wide range in the future.

1. Introduction

For decade years, the application of gold nanoparticles (AuNPs) and water-soluble fluorescent conjugated polymers (conjugated polyelectrolytes, CPEs) has gained great attention due to their unique performance for use as highly sensitive chemical and biosensors [1–10]. For AuNPs, several features make them exceptional appealing to many researchers. First, AuNPs possess high extinction coefficient in the visible region, the extraordinary optical features make AuNPs an ideal color reporting group for signaling molecular recognition events and render them to function as efficient quenchers for most fluorophores [11–15]; secondarily, by modification with thiol ligands, such as antibody, DNA/RNA, AuNPs could specifically recognize target biomolecular [16,17]; and thirdly, the good conductivity of AuNPs also makes them be widely used for electrochemical assay [18–20]. For example, Kim et al. used the AuNPs–RNA–dye conjugates to detect a complementary single-stranded DNA target through the fluorescence changes from the conjugates [21]; by taking advantage of different agglomeration states of AuNPs can result in distinctive color changes. Chen et al. [22] exploited AuNPs-based competitive colorimetric assay for detection of protein–protein interactions. On the other hand, CPEs, characterized by a backbone with a π-delocalized electronic structure and pendant groups bearing ionic functionalities, also have been one of the best candidates as components in high performance and rapid response chemical and biological sensor applications [23–25]. The utility of these CPEs in biosensors comes from their high absorption coefficients (excellent "light harvesters") together with high fluorescent quantum yields, furthermore, when appropriately modified, they can detect, transduce, and possibly amplify chemical, biological and physical changes into measurable optical or electrical signals [26]. In order to exploit the best use of the both, several groups have focused their interests on incorporating metal nanoparticles into conducting polymers or vice verse to obtain integrated functional multicomponent devices [27–30]. However, these efforts are often restricted to study the structure of the composite, only few cases aim at practical application [31–33].

In this regard, we had for the first time utilized water-soluble fluorescent polythiophene derivative and AuNPs composite for detecting aspartic acid (Asp) and glutamic acid (Glu). Polythiophene derivatives have received widespread attention in chemical and biological sensing field, one hand, they are easy modification and preparation, thiophene polymer can be obtained through oxidation of the corresponding monomers by $FeCl_3$ or other coupling reagents [34]; on the other hand, the interactions between the side-chain (ligands) and different analytes (targets) can readily be

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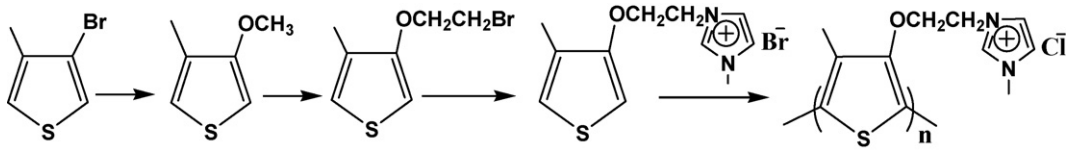
doi:10.1016/j.talanta.2008.06.040



Scheme 1. The synthesis route of polythiophene derivate, (i) CH_3OH, CH_3ONa, CuBr, NMP; (ii) toluene, $NaHSO_4$, $BrCH_2CH_2OH$; (iii) CH_3CN, 1-methylimidazole; and (iv) $FeCl_3$, $CHCl_3$, Bu_4NCl/ion exchange.

detected from the changes in both side-chain and backbone conformations without any tagging process [35]. Glu and Asp both play critical roles in maintaining natural functions of body, high-level Glu content in plasma will lead to acute ischemic stroke [36], and Glu is precursor for γ-amino butyric acid, which is the main inhibitory neurotransmitter in the central nervous system [37]; while Asp can facilitate tricarboxylic acid cycle, potassium salt of Asp is used to cure heart, liver disease and diabetes [38,39]. The general approach for determination of free amino acids is by liquid, gas chromatography or electrochemical strategies [40–43], but these chromatography methods have some drawbacks, such as the lengthy cleanup and derivatization steps, electrochemical methods also need complex treatment of electrodes.

2. Experimental

2.1. Materials and methods

Poly(1*H*-imidazolium-1-methyl-3-{2-[(4-methyl-3-thienyl)-oxy]ethyl})chloride (PT) was synthesized according to the previously published literatures [44,25] (Scheme 1). $HAuCl_4{\cdot}3H_2O$ was purchased from Beijing Chemical Company. Trisodium citrate dehydrate was from Sinopharm Company, China. 20 free amino acids were obtained from Shanghai Sangon Biological Engineering Technology & Services Co., Ltd. Sample Asp and Glu were from Wuhan Hezhong Fine Chemical Factory (90 ± 2% according to the label). Tris(hydroxymethyl)aminomethane (Tris) was purchased from Sigma. Unless other specified, the rest reagents were of analytical grade and used without further purification. The water used was purified through a Water Pro Plus system (Labconco Co., USA).

2.2. Preparation of gold nanoparticles (AuNPs)

All glassware used in the following preparations was thoroughly cleaned in a bath of fresh aqua regia (HNO_3:HCl = 3:1), rinsed in pure water prior to use. AuNPs were prepared by one-step citrate reduction of $HAuCl_4$ at ambient temperature following Grabar's method [45]. In short, 100 mL aqueous solution consisting of 1 mM $HAuCl_4$ was brought to a vigorous boil with stirring in a round-bottom flask fitted with a reflux condenser; 38.8 mM trisodium citrate (10 mL) was then added rapidly to the solution. The solution was heated under reflux for another 15 min, during which time its color changed from pale yellow to deep red. The solution was cooled to room temperature while being stirred continuously. After filtration and centrifugalization, the resulted solution was stored at 0–4 °C for further use. The sizes of the nanoparticles were verified by TEM (JEF2010, Japan) analysis with an average size of 13.8 ± 1.5 nm (about 60 particle samples) (Fig. 1). The particle concentration was 13.0 nM according to Beer's law using an extinction coefficient of $2.08 \times 10^8\ M^{-1}\ cm^{-1}$ at 517 nm.

2.3. Standard and sample solutions

The stock solutions of each amino acid (0.2 mM) were prepared in pure water and used by appropriate dilution of the stock solution. Sample solutions, which were equivalent to about 20 mg of Asp and Glu according to the label content of the tablet supplier, were transferred to 100-mL volumetric flasks, sonicated for 15 min and stored for analysis.

2.4. Instrumentation

UV–vis absorption was taken on a TU-1900 Spectrophotometer (Beijing Purkinje General Instrument Co., Ltd). Fluorescence measurements were acquired in 3-mL quartz at room temperature using a LS-55 Luminescence Spectrometer (PerkinElmer, UK). The pH of solution was measured using Sartorius PB-10 pH Meter (Sartorius, Germany). TEM measurements were made on a transmission electron microscope operating at 200 kV. The sample for TEM was prepared by placing a drop of the colloidal solution on the carbon-coated micro-grid and drying at room temperature.

3. Results and discussion

In pure water, PT exhibited a strong emission with maximum wavelength at 514 nm (Fig. 3a), which had a perfect spectral overlap with the absorption of AuNPs, as a result, the fluorescence of PT could be quenched dramatically by AuNPs even at very low concentration via a highly efficient energy transfer between them (Fig. 3b). Linear Stern–Volmer plots thus provided a traditional and convenient means to determine K_{SV} value, the K_{SV} was related to PT efficiency via the relationship:

$$\frac{F^0}{F} = 1 + K_{SV}\ [\text{quencher}] \quad (1)$$

where F^0 and F were PT fluorescent intensity in the absence and presence of quencher, respectively. A plot of F^0/F vs quencher concentration showed the highly efficient "superquenching" originated from the PT fluorophore–quencher pair, here the K_{SV} was $1.29 \times 10^{10}\ M^{-1}$ with the concentration ranging from 0 pM to 26 pM.

Because of the adsorption of citrate during the preparation, AuNPs were typically negatively charged, did not quench the

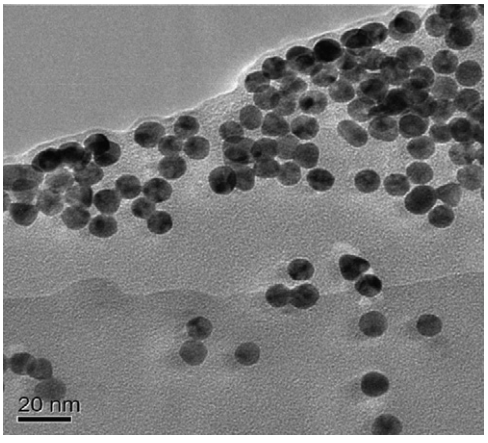


Fig. 1. TEM image and corresponding particle size of AuNPs.