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A facile, sensitive and selective fluorescent probe for heparin based on aggregation-induced emission

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ABSTRACT

A facile, rapidly responsive fluorescence turn-on probe for heparin with high selectivity and sensitivity was reported in this paper. The probe could aggregate on the negatively charged heparin template through electrostatic interactions and then display intense fluorescence due to its aggregation-induced emission (AIE) characteristics. Under optimal condition, the probe showed high selectivity to heparin over chondroitin sulfate(ChS), hyaluronic acid (HA), dextran (DeX) and other substances, with a linear range of 0.2–14 μg/mL, and a detection limit of 57.6 ng/mL. In diluted serum, it also showed good performance.

1. Introduction

Heparin is a highly sulfated glycosaminoglycan with high density of negative charges [1,2]. It is known as an anticoagulant in surgery and treatment of thrombotic diseases due to its quick anticoagulant effect by accelerating the inactivating of thrombin and several other coagulation factors [3]. At high concentrations, however, heparin could induce hemorrhages, thrombocytopenia and hyperkalemia [4,5]. Therefore, it is important to monitor heparin levels in medical applications.

Traditional methods for heparin detection include activated clotting time (ACT), activated partial thromboplastin time (APTT) and anti-factor Xa activity [6–9]. However, these methods are indirect, costly and lack of specificity [10,11]. In order to monitor heparin more effectively, in recent years, many new methods have been developed, such as fluorimetry [12–21], colorimetry [22–26], capillary electrophoresis [27–30], and electrochemical methods [31–34]. Among these methods, fluorescent chemosensors have been demonstrated useful because of their high sensitivity and low cost. However, they still have some drawbacks, such as long time lag [14], short wavelength emission [14], analyte-induced fluorescence quenching [12,14,20], requiring more rigorous testing media [18,21], restricted applicability in real samples [12,13,15,19], and a narrow dynamic response window [12,16]. For example, Anslyn et al. reported a heparin receptor with phenylboronic acids and ammonium groups showed remarkable selectivity and affinity for heparin, but the receptor undergo fluorescence quenching upon the binding of heparin [14]. In contrast to fluorescence quenching, Liu et al. developed a multicolor biosensor for heparin detection based on polymer/heparin complex formation to induce fluorescence resonance energy transfer between the fluorene fragments and the 2,1,3-benzothiadiazole units in the cationic polyfluorene derivative, but its application in complex biological media was not described [15]. Therefore, rapidly responsive fluorescent turn-on sensors that could be applied to quantify heparin in complex biological media with high selectivity and sensitivity are still imperatively demanded.

In 2001 Tang, Zhu and co-workers first reported the phenomenon of aggregation-induced emission (AIE), molecules of which are weakly fluorescent in solution but exhibit intense fluorescence after aggregation [35]. Since then, many AIE active dyes have been described by various research groups, and they have already been successfully used further as fluorescent sensors for metal ions, temperature, pH, and biosensing systems [36–41]. Our group has recently reported a series of salicylaldehyde azine derivatives with AIE characteristics [42]. Because of their long emission wavelength and large stokes shift, they have been applied in the detections of human serum albumin (HSA), bovine serum albumin (BSA) and protamine, as well as applied as optical materials [43–45]. To extend the potential applications of the AIE active salicylaldehyde azine derivatives for biomolecule detection, we developed a new ammonium group functionalized salicylaldehyde azine derivative N,N'-Bis[4-[[3-(trimethylammonio)ethyl] oxy]salicylidene]ethylenediamine bromide (BTASE) with AIE

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characteristics for heparin detection in diluted horse serum through charge–charge interaction in this paper.

2. Experimental

2.1. Apparatus

Fluorescence spectra measurements were recorded on a JASCO FP-6500 spectrofluorimeter (Tokyo, Japan) equipped with a xenon discharge lamp, 1 cm quartz cells. All pH tests were made with a Model pHS-3C pH meter (Shanghai, China). NMR spectra were measured using a JOEL JNM-ECA300 spectrometer operated at 300 MHz. Elemental analyses were carried on a FLASH EA1112 elemental analyzer. All of the measurements were operated at room temperature of 298 K.

2.2. Reagents

All reagents and solvents used in this paper were of analytical grade without further purification. Heparin sodium salt of 150 U/mg and hyaluronic acid were purchased from Beijing Kehaijunzhou Biotechnology Development Center (Beijing, China). Chondroitin sulfate and dextran were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). Human serum albumin was purchased from Beijing Biodee Biotechnology Co. Ltd. (Beijing, China). Other reagents were purchased from Alfa Aesar Co. (Tianjin, China). Deionized water was used throughout the experiment. Tris–HCl solutions were prepared by adding HCl/NaOH to reach proper pH. The 1 mM stock solution of the dye reagent was prepared by dissolving the compound in dimethyl sulfoxide.

2.3. Analytical procedure

For heparin detection, to a 5.0 mL flask with 3.88 mL buffer (10 mM Tris–HCl at pH 7.0) containing different amount of heparin, 0.12 mL of the BTASE stock solution was added to each flask by a pippet to obtain solutions of 30 μM BTASE. After well mixed, the solution was allowed to stand at room temperature for 2 min. Then 2.0 mL of the solution was transferred to a 1 cm cell for fluorescence measurement by excitation/emission at 391/530 nm.

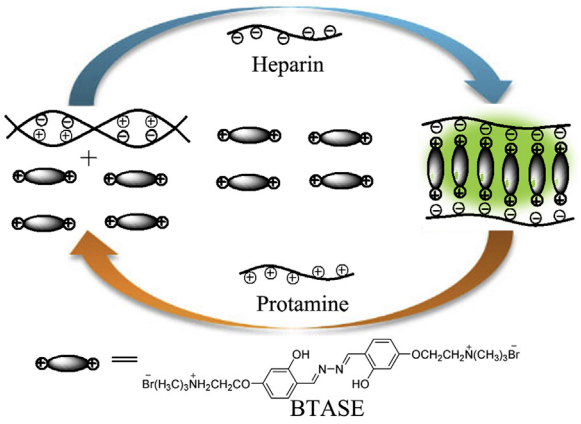
Heparin detection in horse serum was carried out as follows. Horse serum was diluted in buffer (10 mM Tris–HCl at pH 7.0) to produce a 1% serum sample. Then 0.12 mL of the BTASE stock solution was added to a 5.0 mL flask with 3.88 mL serum sample containing different amount of heparin. After well mixed, the solution was allowed to stand at room temperature for 2 min. Then 2.0 mL of the solution was transferred to a 1 cm cell for fluorescence measurement by excitation/emission at 391/530 nm.

3. Results and discussion

3.1. The fluorescence turn-on detection of heparin

As shown in Scheme 1, positively charged tertiary amines was introduced into BTASE to improve its water solubility. BTASE displayed very weak fluorescence in aqueous solution. Upon addition of heparin, BTASE could aggregate on the negatively charged heparin template due to electrostatic interactions between the positively charged tertiary amines in BTASE and the negatively charged sulfate and carboxylate groups in heparin, and thus display intense AIE fluorescence.

In 10 mM Tris–HCl at pH 7.0, 30 μM BTASE displayed very weak fluorescence at 530 nm, while the fluorescence increased in the presence of heparin. Nearly 40-fold fluorescence enhancement



Scheme 1. The design principal of the fluorescence turn-on detection of heparin based on AIE characteristics of BTASE.

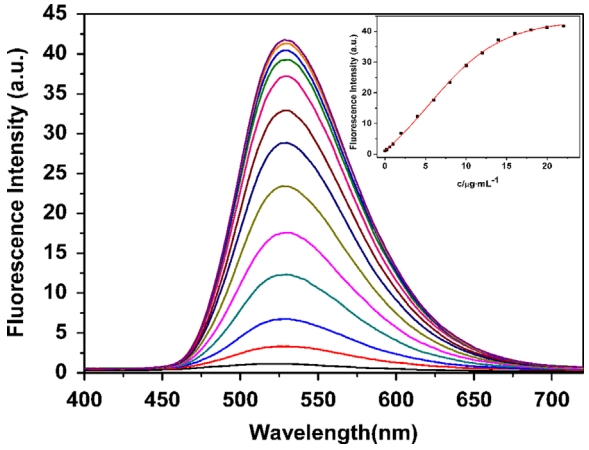


Fig. 1. Fluorescence spectra of BTASE (30 μM in 10 mM Tris–HCl buffer solution, pH=7.0) in the presence of different amounts of heparin (from 0 to 22 μg/mL), λ_{ex}=391 nm.

was observed when the concentration of heparin reached 22 μg/mL (Fig. 1), which should be attributed to the aggregation of BTASE on the negatively charged heparin template through electrostatic interactions. The formation of aggregates of BTASE-heparin aggregates was directly observed using TEM (transmission electron microscope): particle size of micrometre sizes were detected in the solution of BTASE containing 16 μg/mL heparin; while no particle was observed in the solution of BTASE without heparin (Fig. S1).

3.2. Optimization of the analytical condition

3.2.1. Effect of BTASE concentration

In 10 mM Tris–HCl at pH 7.0 containing 16 μg/mL heparin and 30–40 μM BTASE, a maximum fluorescence intensity could be obtained (Fig. 2). The excessive BTASE (> 40 μM) were well dispersed in solution and displayed almost no fluorescence, but could absorb the excitation light and induce inner filter effects, which led to fluorescence decrease of BTASE and heparin aggregates [46]. Thus, 30 μM BTASE was used for the following tests.

3.2.2. Effect of pH

The effect of pH on fluorescence intensity is shown in Fig. 3. In 10 mM Tris–HCl with 30 μM BTASE and 16 μg/mL heparin, the