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Highly Selective and Sensitive Heparin Probing from Supramolecular Assembly of Pyrene Derivatives

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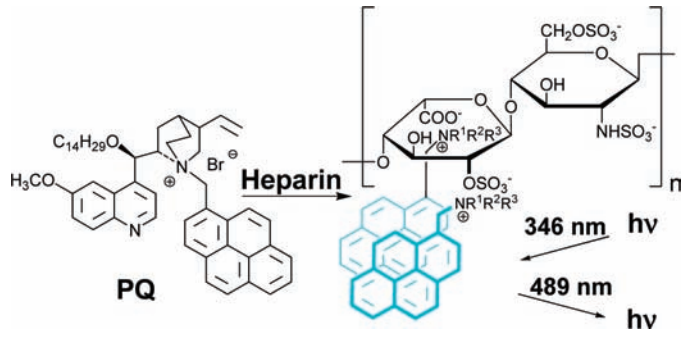
ABSTRACT



A new quinine derivative PQ, bearing pyrenyl as a fluorophore, was prepared to recognize heparin in aqueous solution. PQ exhibits good selectivity and sensitivity for heparin over other biological molecules. Upon binding with heparin, PQ shows a typical excimer emission peaked at 489 nm along with a weak monomer emission at 376 nm. Moreover, PQ also shows good performance to detect the heparin in serum.

Heparin has been clinically used as a major anticoagulant during cardiopulmonary surgery and in emergency deep venous thrombosis (DVT) conditions. However, heparin overdose can induce some adverse effects, such as hemorrhages and heparin-induced thrombocytopenia.[1,2] Therefore, an assay to quantify the concentration of heparin in serum is of crucial importance. Up to now, some assays have been developed to monitor the concentration of heparin, including the activated clotting time (ACT), the activated partial thromboplastin time, potentiometric assays, anti-Xa, and protamine complexation.[3] However, these methods are indirect and are not sufficiently reliable, accurate, or amenable to clinical settings.[4] Thus, many researchers have attempted to develop new methods for the detection of heparin. Of these attempts, fluorescent chemosensors have received considerable attention because of their high sensitivity and simplicity.[5,6] Indeed, some fluorescent chemosen-

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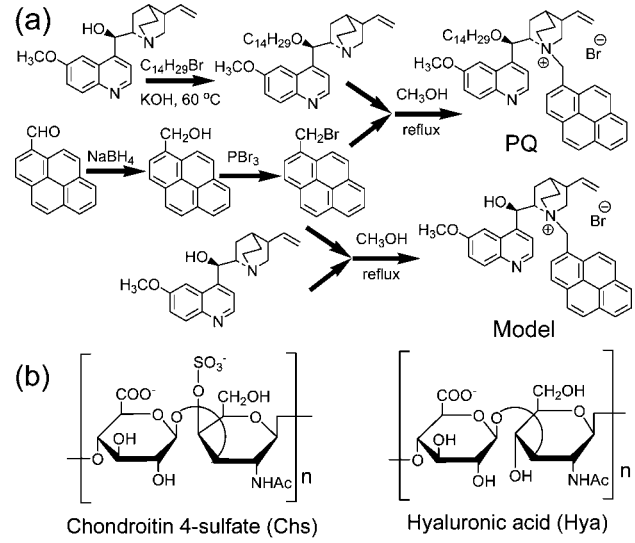
sors have already been designed and utilized for heparin assays,[7] though some of them have limitations including poor selectivity and short emission wavelength. Therefore, there is still an urgent demand for the development of ratiometric sensors with good selectivity, high sensitivity, and long emission wavelength.[8,9]

In the present work, quinine, a widely available natural product carrying quinuclidine, was chosen as the basic unit for heparin recognition. We first modified its molecular structure by covalently attaching a long hydrophobic chain to the hydroxy group to increase the hydrophobic interaction. On the other hand, the pyrenyl group was introduced to the molecule to form a positively charged fluorescent unit for efficient electrostatic interaction with the negatively charged heparin.[10] As a result, we can reasonably envision that this fluorescent sensor (PQ) will benefit from both the electrostatic interaction and the hydrophobic effect to recognize heparin in aqueous solution. PQ is expected to show monomer fluorescence emission in aqueous solution. However, in the presence of heparin, ammonium groups in PQ would interact with the negative sulfate and the carboxylate groups on the same side of the pyranosyluronic acid unit in heparin, resulting in excimer emission of pyrene. Thus, sensitive responses of fluorescence should be observed due to the supramolecular assembly of pyrene derivatives in the recognition process of heparin. The synthetic pathway of fluorescent sensor (PQ) and its model compound without the long alkyl chain is summarized in Scheme 1a. The detailed synthetic procedures are described in the Supporting Information.

Scheme 1. (a) Synthetic Pathway of Sensor (PQ) and the Model Compound and (b) Structures of the Polymeric Anionic Analysts



From the absorption spectra of PQ in a solution of C_2H_5OH/H_2O (v/v = 1:3), it could be found that PQ displays an absorption band with a peak at 346 nm, which is corresponding to the pyrenyl band (Figure 1). Upon binding

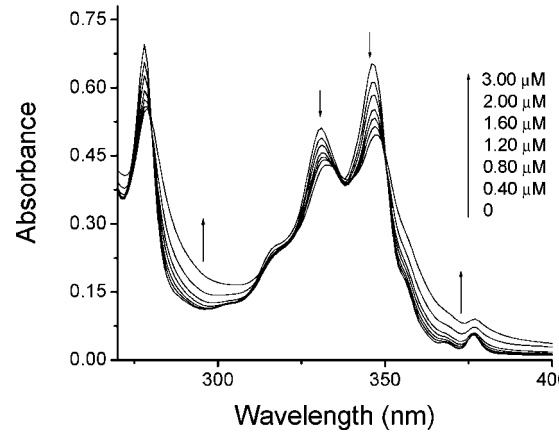


Figure 1. Changes in the absorption spectra of PQ (10.0 μM) upon addition of heparin in 10.0 mM HEPES buffer (pH 7.4).

of PQ to heparin in the HEPES buffer solution, a significant red shift was observed, as shown in Figure 1. The band broadening and red shift in the UV spectra of PQ upon the addition of heparin are attributed to the favorable intermolecular $\pi-\pi$ stacking of the two pyrene residues in the ground state.[11] This provides evidence for the formation of an intermolecular excimer of PQ when bound to heparin.

The formation of a pyrene excimer is also supported by a fluorescence titration experiment. A characteristic emission

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band of pyrene with a peak at 376 nm was observed, which is corresponding to the monomer pyrene (Figure S10, Supporting Information). To confirm the monomer emission, the solvent effect on fluorescence spectra was investigated (Figure S11, Supporting Information). The results indicate that the fluorescence enhancement is not induced by the solvent or the environmental effect. Upon addition of heparin, a broad and structureless emission band centered at 489 nm was observed, and the fluorescence emission intensity at 376 nm decreased concomitantly. The excimer signal of PQ solution was enhanced by 12-fold in the presence of 1.60 μM heparin, as shown in Figure 2a. However, the model

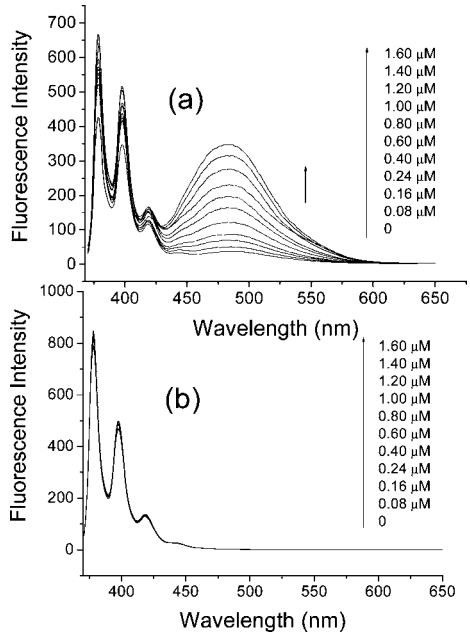

Figure 2. Changes in (a) fluorescence spectra of PQ (10.0 μM) and (b) the fluorescence spectra of the model compound (10.0 μM) upon gradual addition of heparin to HEPES buffer solution (10.0 mM, pH 7.4). Excitation wavelength, 346 nm.

compound without alkyl ether has no fluorescence response to heparin in the same conditions (Figure 2b), indicating that the long-chain alkyl ether plays an important role via hydrophobic interaction and leads to the assembly of PQ in aqueous solution. The significant fluorescence enhancement of PQ at the longer wavelength (489 nm) was attributed to the formation of an excimer by pyrene, and the proposed mechanism was demonstrated in Figure 3.

To further investigate the interaction between PQ and heparin in aqueous solution, the time-resolved fluorescence decay of PQ in the presence of heparin was determined (Figure S12, Supporting Information). The fluorescent lifetime was 14.2 ns monitored at 376 nm before the addition of heparin. Meanwhile, the fluorescence decay of PQ could not be detected at 489 nm, suggesting that excimer was not formed before adding heparin. However, the fluorescence intensity of PQ at 489 nm increased with the addition of heparin, and the fluorescent lifetime monitored at 489 nm was 35.6 ns, which could be attributed to

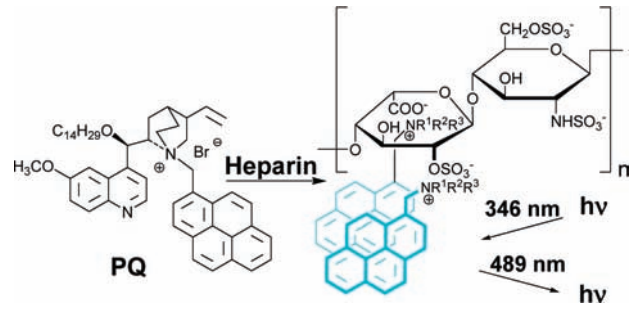

Figure 3. Schematic illustration of the proposed fluorescence "turn-on" sensing mechanism of PQ for heparin.

the formation of an intermolecular pyrene excimer on the same side of the disaccharide units.

The high sensitivity of the sensor for heparin can be used to create a calibration curve for the quantitative measurement of heparin. Therefore, heparin was added at different concentrations, ranging from 0.08 to 1.6 μM, and the fluorescence intensity of PQ (10.0 μM) at 489 nm was recorded to generate a calibration curve. A good linearity was found between the fluorescence intensity of the sensor and the heparin concentrations at these clinically relevant concentrations (Figure 4a). Heparin is usually administered at doses of 2.0−8.0 U mL^{-1}

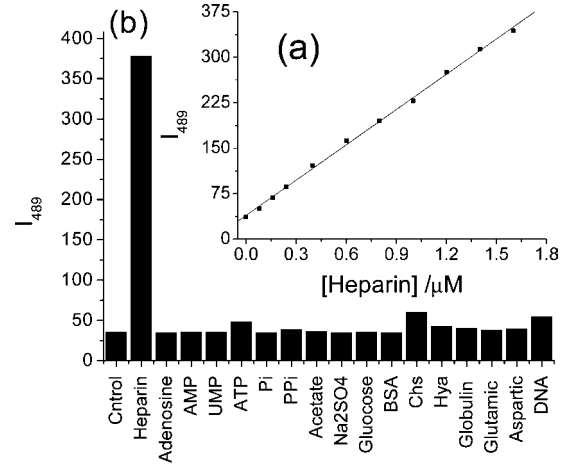

Figure 4. (a) Variation of the fluorescence intensity of PQ (10.0 μM in 10.0 mM HEPES buffer solution, pH 7.4) vs the concentrations of heparin, $I_{489} = 39.12 + 194.07 \times$ [heparin], $R = 0.9992$. (b) The selectivity of PQ (10.0 μM) to some important biological molecules in 10.0 mM HEPES buffer solution (pH 7.4). Adenosine, AMP, ATP, Pi, PPi, UMP, glucose, sodium sulfate, glutamic acid, aspartic acid, BSA, globulin, heparin, Hya, Chs, and DNA were added at 1.60 μM in 10.0 μM sensor solutions, respectively. The control is 10.0 μM fluorescent sensor solution in 10.0 mM HEPES buffer (pH 7.4).

(0.8−3.2 μM) during cardiopulmonary surgery and emergency deep venous thrombosis (DVT) conditions to prevent excessive clotting.[6] Meanwhile, heparin is used at doses of 0.2−2.0 U mL^{-1} (0.08−0.8 μM) in postoperative and long-term antico-

agulant care of DVT. Therefore, the concentrations of heparin used clinically are within the range of the calibration curve used here.

To use PQ to directly measure the heparin concentration in clinical samples, the selectivity of PQ for heparin must be considered. Thus, a series of biomolecules were tested, including adenosine, adenosine monophosphate (AMP), adenosine triphosphates (ATP), phosphate (Pi), pyrophosphate (PPi), uridine monophosphate (UMP), DNA, glucose, sodium sulfate, bovine serum albumin (BSA), and globulin. In addition, molecules with molecular structures similar to heparin, including hyaluronic acid (Hya) and chondroitin sulfate (Chs) (Scheme 1b), and typical amino acid containing two carboxylates (glutamic acid and aspartic acid), were also investigated. The selectivity shown in Figure 4b is in the order heparin $\gg$ chondroitin sulfate > hyaluronic acid, suggesting that the conformation of the sugar dimer and electrostatic interactions play a dominant role in binding. These results indicate that PQ shows highly selective fluorescence "turn-on" sensing for heparin in aqueous solution. The high selectivity might be due to the structural compatibility between PQ and heparin. The sulfate and carboxylate groups attached on the same side of the pyranosyluronic acid unit can "catch" two PQ molecules (pyrenyl ammonium), resulting in excimer emission, which differentiates heparin from Chs, Hya, and other similar biological entities.

To explore the practical application of PQ in a "turn-on" assay for heparin, bovine serum was used in this experiment. The fluorescence intensity of PQ at 489 nm increases with increasing concentrations of heparin in the presence of diluted bovine serum, as shown in Figure 5. It could be even detected with the naked eye. The strong fluorescence and large Stokes shift of PQ in heparin can overcome the interference of autofluorescence from blood, as shown in Figure S13 (Supporting Information).

In summary, we have successfully developed a highly sensitive and selective fluorescent sensor, which exhibits fluorescence "turn on" sensing for heparin in aqueous solution. This is due to the favorable intermolecular $\pi-\pi$ stacking of pyrenes by the supramolecular assembly. The fluorescent sensor shows excellent selectivity for heparin with

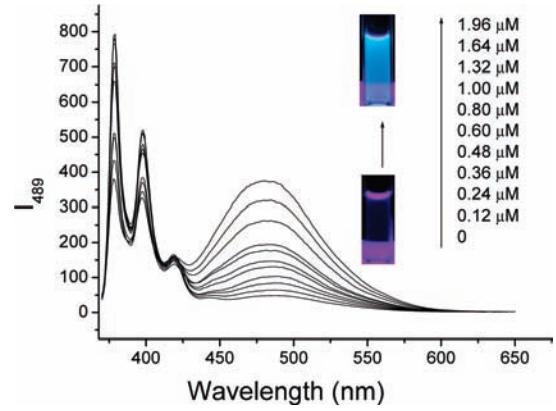

Figure 5. Responses of PQ (10.0 μM) to heparin with diluted bovine serum in 10.0 mM HEPES buffer solution (pH 7.4).

30 nM sensitivity at pH 7.4 in 10.0 mM HEPES buffer solution. Moreover, a good linear relationship was found between the fluorescence intensity of the sensor and the heparin concentrations at clinically relevant concentrations (0.08−3.20 μM). The fluorescent sensor was used to detect heparin in serum with high sensitivity. This work not only provides a simple and practical approach for the detection of heparin but also opens a new perspective to rationally design a sensor for other molecules by supramolecular self-assembly.

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Supporting Information Available: Synthetic procedures, characterization of new compounds and additional spectroscopic data. This material is available free of charge via the Internet at http://pubs.acs.org.

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