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A polyadenosine–coralyne complex as a novel fluorescent probe for the sensitive and selective detection of heparin in plasma

Szu-Ying Hung [a], Wei-Lung Tseng [a,b,c,*]

[a] Department of Chemistry, National Sun Yat-sen University, Kaohsiung 804, Taiwan
[b] School of Pharmacy, College of Pharmacy, Kaohsiung Medical University, Kaohsiung, Taiwan
[c] Center for Nanoscience and Nanotechnology, National Sun Yat-sen University, Kaohsiung, Taiwan

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ABSTRACT

This study presents the development of a simple, label-free, sensitive, and selective detection system for heparin based on the use of a complex of 20-repeat adenosine (A_{20}) and coralyne. Coralyne emits relatively weak fluorescence in an aqueous solution. In the presence of A_{20}, coralyne molecules complexed with A_{20} through A_2-coralyne-A_2 coordination. An increase in the fluorescence of coralyne was observed because coralyne remained separate from water in the hydrophobic environment of the folded A_{20}. The presence of heparin and the formation of the coralyne–heparin complex caused coralyne to be removed from the A_{20}–corlayne complex. Because heparin promoted coralyne dimerization, the fluorescence of coralyne decreased as a function of the concentration of added heparin. This detection method is effective because the electrostatic attraction between heparin and coralyne is substantially stronger than the coordination between A_{20} and coralyne in a 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) buffer at pH 7.0. Under optimal conditions (5 μM coralyne, 1 μM poly A_{20}, and 10 mM HEPES), this probe exhibited high selectivity (> 90-fold) toward heparin over hyaluronic acid and chondroitin sulfate. The probe's detection limit for heparin was determined to be 4 nM (75 ng/mL) at a signal-to-noise ratio of 3. This study validates the practicality of using the A_{20}–corlayne complex to determine the concentration of heparin in plasma.

1. Introduction

Heparin, which consists primarily of trisulfated disaccharide repeating units, is the most highly charged polysaccharide in biological systems. Because of its crucial roles in regulating cell growth and differentiation, immune defense, and blood coagulation, heparin has been used clinically as an anticoagulant drug for over 80 years. Heparin is an effective drug in treating and preventing venous thromboembolism and blood clotting because it is capable of accelerating the inactivation rate of coagulation factors such as fibrin-bound thrombin. The recommended therapeutic ranges of heparin levels are 2–8 U/mL (17–67 μM) during cardiovascular surgery and 0.2–1.2 U/mL (1.7–10 μM) during postoperative and long-term care. The accurate quantification of heparin is essential during anticoagulant therapy and surgery because a high heparin dose leads to adverse effects such as hemorrhaging and thrombocytopenia (Girolami and Girolami, 2006; Warkentin et al., 1995).

Because of its clinical importance, numerous methods have been devised for quantifying heparin levels in the blood. Activated clotting time (ACT) assays, such as the activated partial thromboplastin time and chromogenic antifactor Xa assay, are traditional clinical procedures for quantifying heparin levels (Murray et al., 1997; Raymond et al., 2003). However, the ACT method is influenced by many variables such as hypothermia, hemodilution-induced dilution of clotting factors, and interpatient differences in antithrombin levels (Despotis et al., 1997; Machin and Devine, 2005). The Hepcon HMS assay system, which is based on the titration of heparin with protamine and clot formation in the end point, enables more accurate monitoring of heparin concentrations; however, this method involves indirect sensing, and is time consuming and expensive (Ramamurthy et al., 1998). Researchers have developed various methods for identifying and quantifying heparin, such as anion exchange chromatography (Ander et al., 2001), capillary electrophoresis (Volpi et al., 2012), potentiometric methods (Crespo et al., 2012), and ion mobility mass spectrometry (Seo et al., 2012). Although all of these reported methods have demonstrated high sensitivity to heparin, they are costly, time consuming, and nonportable.

In response to these shortcomings, massive sensors have been devised for the simple and rapid sensing of heparin, using

* Corresponding author at: Department of Chemistry, National Sun Yat-sen University, 70, Lien-hai Road, Kaohsiung 804, Taiwan. Tel.: +886 7 5252000; fax: +886 7 3684046.
E-mail address: tsengwl@mail.nsysu.edu.tw (W.-L. Tseng).

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fluorophores (Dai et al., 2011; Wang et al., 2008; Wang and Chang, 2008), chromphores (Zhong and Anslyn, 2002), cationic polymers (Pu and Liu, 2009; Zhan et al., 2010), boronic acid-containing copolymers (Sun et al., 2007), polyethyleneimine-modified quantum dots (Yan and Wang, 2011), graphene oxide (Fu et al., 2012), positive-charged gold nanoparticles (Cao and Li, 2011), and 4-mercaptopyridine-modified silver nanoparticles (Wang et al., 2013). In general, the mechanism for these sensors relies on electrostatic binding between a cationic sensor and anionic heparin, the ability of the boronic acid group to chelate to sugar diol units, or both. Nevertheless, most of these sensors cannot distinguish heparin from its analogues, such as hyaluronic acid (HA) and chondroitin sulfate (ChS). We recently proposed a molecular beacon (MB) strategy for the fluorescence turn-on detection of heparin in plasma (Kuo and Tseng, 2013), Because coralyne can drive adenosine (A)—A mismatches to form A_2-coralyne-A_2 coordination (Lin and Tseng, 2011, 2012), the designed MB containing 16-mer A bases formed a hairpin structure in the presence of coralyne. When a hairpin-shaped MB encounters heparin, the electrostatic force between coralyne and heparin splits the stem and switches on the MB fluorescence. Although this MB sensor provides high sensitivity and selectivity for heparin, chemically modifying the MB with fluorophores and quenchers is relatively complex and expensive.

Herein, this study presents a convenient, label-free, selective, and sensitive assay for standard (unfractionated) heparin through the competitive binding between heparin and 20 repeat A (A_{20}) to coralyne. We demonstrated that the presence of A_{20} can efficiently enhance the fluorescence of coralyne and the selectivity of coralyne toward heparin. Scheme 1 illustrates the mechanism for a fluorescence turn-off assay of heaprin using the A_{20}–coralyne complex. Coralyne drives the A–A mismatches to form a stable A_2-coralyne-A_2 complex in the hydrophobic environment of A_{20}, causing an enhancement in the fluorescence of coralyne at a neutral pH. The presence of heparin promotes the dimerization of coralyne through electrostatic attraction, thereby removing coralyne from the formed A_{20}–coralyne complex. Because of dimer-promoted fluorescence quenching of coralyne, the fluorescence of the A_{20}–coralyne complex was switched off in the presence of heparin. To demonstrate the practicality of this probe, we applied it to quantifying heparin in plasma.

2. Experimental section

2.1. Chemicals

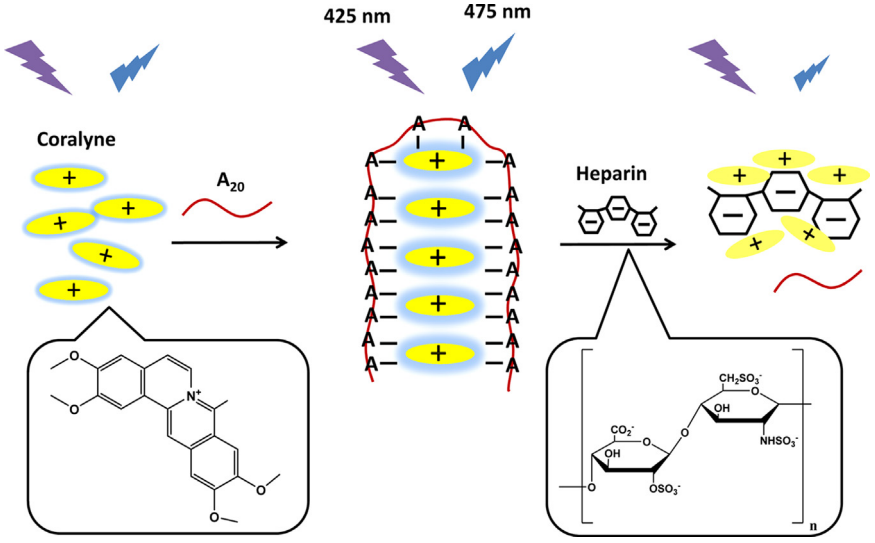
4-(2-Hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), sodium chloride (NaCl), protamine sulfate (from salmon), coralyne sulfoacetate, heparin (sodium salt; MW 18,000) from porcine intestinal mucosa, ChS (sodium salt) from bovine trachea from, and HA (sodium salt) from bovine vitreous humor were purchased from Sigma-Aldrich (St. Louis, MO, USA). All DNA samples were obtained from Neogene Biomedicals Corporation (Taipei, Taiwan). Milli-Q ultrapure water (Milli-Pore, Hamburg, Germany) was used in all of the experiments.

2.2. Apparatus

The absorption and fluorescence spectra of coralyne were recorded using JASCO V-670 spectrophotometer (JASCO, Tokyo, Japan) and a Hitachi F-7000 fluorometer (Hitachi, Tokyo, Japan). Fluorescence polarization was recorded using a Hitachi F-7000 fluorometer equipped with an autopolarization measurement. Circular dichroism (CD) was performed on a JASCO model J-815 CD spectropolarimeter (JASCO, Tokyo, Japan).

2.3. Sample preparation

All DNA samples and polysaccharides were prepared in a solution containing 10 mM HEPES (pH 3.0–10.0) and 0–160 mM NaCl. Polyadenosine (0–8 μM, 100 μL) was incubated with coralyne (20 μM, 100 μL) at ambient temperature for 30 min. Polysaccharides (0–2 μM, 200 μL), including heparin, Chs, and HA, were added to an equal volume of the resulting solutions (200 μL). After 0–20 min incubation at ambient temperature, the mixed solutions were transferred separately into a 4 mL quartz cuvette. Their fluorescence sepctra were collected using a Hitachi F-7000 fluorometer at an excitation wavelength of 425 nm. For sensing protamine, heparin (50 μL, 0–10 μM) was incubated with protamine (50 μL, 0–70 μM) at ambient temperature for 10 min. The mixture (100 μL) was added to a solution (400 μL) containing 5 μM coralyne, 1 μM A_{20}, 10 mM HEPES (pH 7.0). After 5 min, the fluorescence spectra of the resulting solutions were recorded using a Hitachi F-7000 fluorometer at the same excitation wavelength.



Scheme 1. Turn-off fluorescence detection of heparin based on competitive binding between heparin and the A_{20}–coralyne complex to coralyne.