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Turn-on fluorescence sensor for the detection of heparin based on rhodamine B-modified polyethyleneimine–graphene oxide complex

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ABSTRACT

Rhodamine B modified Polyethyleneimine (PEI) (called RB–PEI) with strong fluorescence intensity was synthesized, which contained high positively charged amine groups. The fluorescence intensity of RB–PEI can be quenched by graphene oxide (GO). The high efficiency of the quenching was caused by the non-covalent binding of RB–PEI to the GO surface by electrostatic and π–π interactions, forming a RB–PEI/GO complex. However, adding heparin caused intense fluorescence to be recovered, and this was caused by the anionic heparin removing the RB–PEI from the GO surface, forming a fluorescent RB–PEI/heparin complex. Under optimal conditions, the change in the fluorescence intensity when heparin was added was proportional to the heparin concentration over the range 0.09–0.9 U mL^{-1}. The detection limit for heparin was 0.00132 U mL^{-1}, which was lower than that has been achieved using other methods. This approach offers a new quantitative method for determining heparin.

1. Introduction

Heparin is produced and stored in mast cells in animal tissues, and it has the highest negative charge density of any known biological molecule (Scheme 1a) (Cao et al., 2014). As we know, heparin has many medical applications. For example, it is widely used as an anti-thrombotic (Liu et al., 2014a, 2014b, 2014c), inhibits the growth and replication of the human immunodeficiency virus, and can suppress tumor growth and metastasis by inhibiting angiogenesis (Falanga and Marchetti, 2007). The therapeutic dose of heparin during cardiovascular surgery and to treat deep venous thrombosis in an emergency is 2–8 U mL^{-1}, and the therapeutic dose for postoperative and long-term care is 0.2–1.2 U mL^{-1} (Hung and Tseng, 2014). An overdose of heparin can cause many side effects, the most frequent of which are hemorrhage, heparin-induced thrombocytopenia, and osteoporosis (Kaminski et al., 2008). Therefore, it is important to be able to detect heparin in therapeutic drugs and in blood. At present, the most commonly used methods for determining heparin include colorimetric (Zhan et al., 2010; Fu et al., 2012), absorption (Klajnert et al., 2009; Liu et al., 2013), and electrochemical immunoassays (Dey and Raj, 2012; Zhao et al., 2013). These methods offer reasonable sensitivities and specificities, but some are inaccurate, complicated, time-consuming, or have poor reproducibilities. Therefore, a simple, rapid and sensitive method to detect heparin is highly desirable.

Fluorescence methods are the most promising alternatives for detecting heparin because they offer advantages over other techniques, including their ease and speed of use and their sensitivities (Cao et al., 2014; Szelke et al., 2010; Hung and Tseng, 2014; Chen et al., 2011; Liu et al., 2014a, 2014b, 2014c; Cheng et al., 2013; Shao and Wang, 2013; Wu et al., 2013). A large number of fluorescence probes have been developed for the sensitive detection of heparin, but, to the best of our knowledge, few devices for detecting heparin using fluorescence turn-on sensors have been developed (Cao et al., 2014; Cheng et al., 2013; Shao and Wang, 2013; Wu et al., 2013). Fluorescence turn-on sensors have more advantages than do fluorescence quenching sensors, giving less chance of false positive signals and giving better sensitivities and selectivities, as has been demonstrated in numerous studies (Rurack et al., 2000). Further developing an environmentally friendly turn-on probe for detecting heparin is, therefore, still a challenge.

Carbon materials are more environmentally and biologically friendly than are inorganic materials because carbon is one of the most common elements (Chung et al., 2012). Graphene oxide (GO) has unique features, such as being easily dispersed in water, not being cytotoxic, having a surface that is easily modified, and having low manufacturing costs, effective electron transfer properties that make it a powerful fluorescence quencher, that make it a useful new type of nanomaterial for use in biosensors (Chung et al., 2012; Liang et al., 2013; Othmana et al., 2013; Li et al., 2013;

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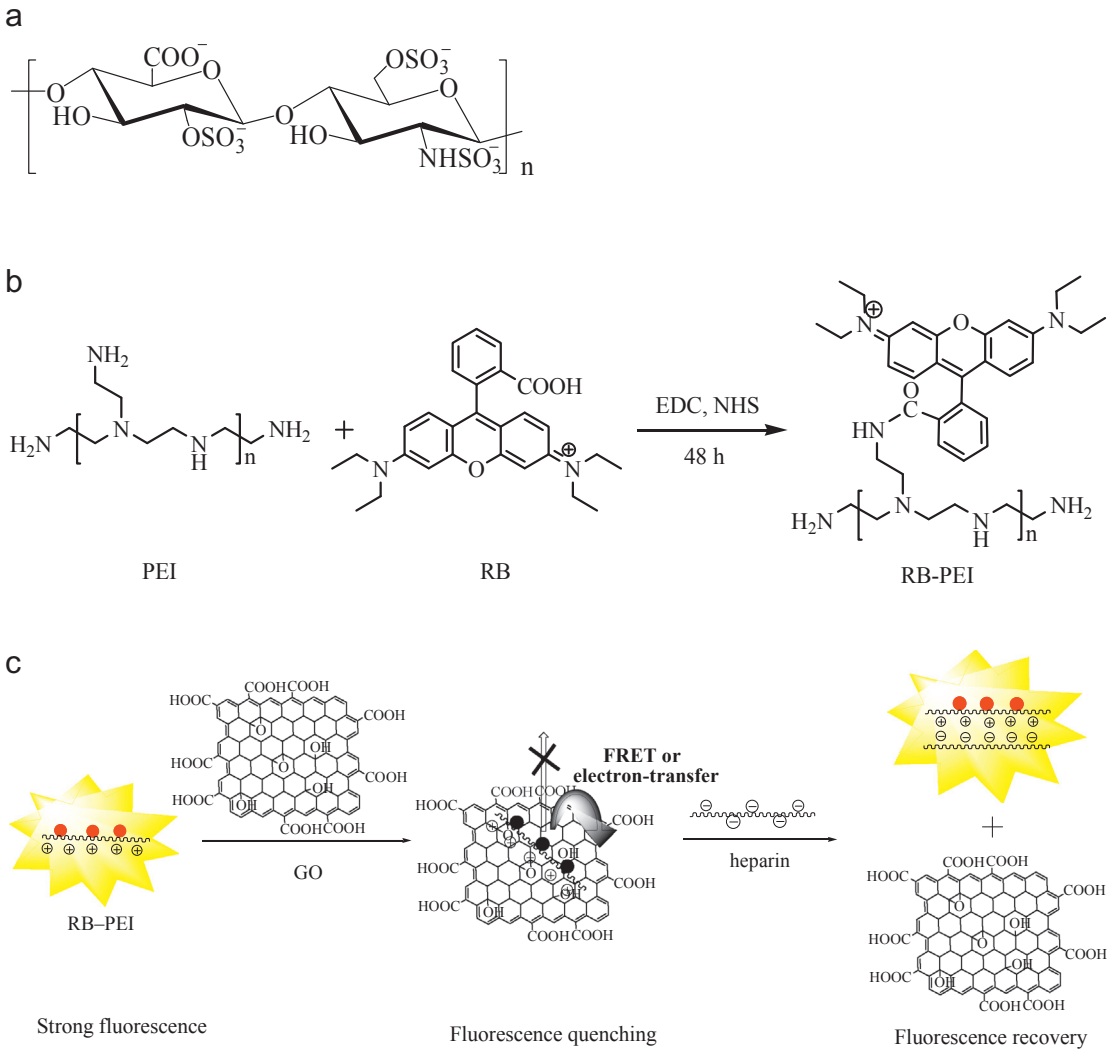

Scheme 1. (a) The structure of the major repeating disaccharide unit in heparin, (b) reaction scheme for the synthesis of rhodamine B-modified polyethyleneimine (RB–PEI), and (c) illustration of the design rationale for the detection of heparin using a fluorescence sensor based on RB–PEI and graphene oxide (GO). The RB–PEI structure shown is only one of the possible structures, RB being able to form amide bonds with any of the amine groups in PEI.

Zhu et al., 2013). However, to the best of our knowledge, this type of GO-complex fluorescence sensor has not been developed for detecting heparin. Polyethyleneimine (PEI) is a cationic polymer that has the potential to have a high positive charge density (Harpe et al., 2000), which makes it a good candidate for strongly interacting with negatively charged GO or heparin. Rhodamine B (RB) is a fluorescent dye. RB can be used to modify PEI, giving RB–PEI, to improve the positive charge density of RB. It would be expected that RB–PEI, which will have a high positive charge density, will have a strong binding affinity for GO or heparin compared with the binding affinity of RB for GO or heparin.

Here, we describe a simple and efficient indicator displacement assay (IDA)-based fluorescence method for detecting heparin. RB–PEI is used as an indicator in this method, and the sensitive detection of heparin is achieved from competition between the GO and heparin to bind with the indicator (shown in Scheme 1). Rather weak fluorescence of RB–PEI adsorbed, by electrostatic and π–π attraction, onto the GO surface (RB–PEI/GO) was expected in an aqueous solution. However, in the presence of heparin, cationic RB–PEI will form a stable inter-polyelectrolyte complex with anionic heparin through strong electrostatic interactions, leading the RB–PEI to desorb from the GO surface and recover the fluorescence signal. The RB–PEI/GO complex could, therefore, be used to detect heparin through the fluorescence turn-on process.

2. Experimental

2.1. Materials and chemicals

PEI was obtained from Wuhan Qianglong New Chemical Materials Co., Ltd. Heparin sodium, hyaluronic acid salt (HA, 95%), chondroitin sulfate (CS), vitamin PP (VPP), inosine, adenosine-5′-triphosphate disodium salt (ATP), *N*-hydroxysuccinimide (NHS), DL–Homocysteine (HCT), glycine and natural graphite powder were purchased from Sinopharm Chemical Reagent Co., Ltd. In this paper, U mL^{-1} is used as the unit of heparin concentration because of the complexity of heparin and the uncertainty in its molecular weight (Wang and Chang, 2008). *N*-(3-Dimethylaminopropyl)-*N*′-ethylcarbodiimide (EDC) was purchased from Shanghai Medpep Co., Ltd. RB was purchased from the Tianjin Institute of Fine Chemicals retrocession. All of the reagents were of analytical grade and were used as received without any further purification. All solutions were prepared in deionized water under ambient conditions.