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Highly sensitive and surface-renewable electrochemical quartz crystal microbalance assays of heparin and chondroitin sulfate based on their effects on the electrodeposition of neutral red

Rongmin Yu, Ming Ma, Lingling Wang, Qingji Xie*, Zhijun Cao, Xueqin Jiang, Shouzhuo Yao

Key Laboratory of Chemical Biology and Traditional Chinese Medicine Research (Ministry of Education of China), College of Chemistry and Chemical Engineering, Hunan Normal University, Changsha 410081, PR China

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ABSTRACT

The electrochemical quartz crystal microbalance (EQCM) technique was used to investigate the electrochemistry of neutral red (NR) in phosphate buffer solution (PBS) and the effects of coexisting heparin (Hep) or chondroitin sulfate (CS) for the first time. The pH dependence of the electrochemistry of NR was examined, and a V-shaped frequency response (versus time) was observed during the cyclic voltammetric experiment of NR in a nearly neutral medium (pH ca. 6.10–7.00), being due to the electrodeposition and stripping of the poorly soluble reduced product of NR (NR_{Red}) at these pH values. The effects of potential scan rate, the concentration of NR, and several supporting electrolytes were examined at pH 6.80. The V-shaped response to the redox switching of NR was weakened by the introduction of Hep or CS, being due to the increased inhibition of the NR_{Red} electrodeposition probably via the electrostatic interaction of the NR and especially the NR_{Red} with Hep or CS. The height of the V-shaped response decreases with the increase of Hep or CS concentration, with limits of detection down to 3 nmol L^{-1} for Hep and 2 nmol L^{-1} for CS, respectively. The novel and surface-regenerable EQCM assay protocol based on the electrochemically switchable deposition of a dye is highly recommended for wide biosensing applications.

1. Introduction

Heparin (Hep, Scheme 1), consisting of repetitive sulfated ($-OSO_3^-$, $-NHSO_3^-$) and carboxylated ($-COO^-$) linear polysaccharide with repeating uronic/glucuronic acid and glucosamine residues, is a kind of highly negatively charged polysaccharide (Comper, 1981). Hep has been widely used to prolong or inhibit blood coagulation for patients in medical treatments. Hence, a simple and accurate monitoring of Hep is important, and several chemical methods for Hep detection were developed, for example, spectrophotometry (Band and Lukton, 1982; Jiao and Liu, 1998; Němcová et al., 1999; Qiu et al., 2005; Zhang et al., 2002), surface plasmon resonance analysis (Gaus and Hall, 1998; Liu et al., 2001), capillary chromatography (Zhou et al., 1998), electrochemical (Langmaier et al., 2006; Lewinski et al., 1997; Sun et al., 2006a,b; Xiao et al., 2001) and quartz crystal microbalance (QCM) (Cao et al., 2007; Cheng et al., 2001), etc.

Chondroitin sulfate (CS, Scheme 1) is a homopolymeric glycosaminoglycan having disaccharide repeating units formed by a hexuronic acid and a hexosamine residue, which also carries many negative charges. CS plays an important role in physiological activities, e.g. anticoagulation, lowering of blood lipids as well as antiarthritic, antitumoral, and antiatherosclerotic natures, thus it is widely used in treating neuralgia, angina pectoris, arthritis, ulcers, and hyperlipemia (Lu et al., 2006; Wang, 1995). Until now, the analytical methods of CS include spectrophotometry (Gao et al., 2000), high-performance lipid chromatography (HPLC) (Koshiishi et al., 1998; Du and Eddington, 2002; Choi et al., 2003; Volpi, 2000), electrophoresis (Okamato et al., 2004; Karousou et al., 2004; Volpi, 1999), turbidometry (Ji et al., 2001), and complexometry (Desaire and Leary, 2000).

The electrochemical quartz crystal microbalance (EQCM) as a very convenient and powerful tool can respond to small changes of mass (nanogram scale) and the viscoelasticity of a foreign film on the electrode during electrochemical perturbations. The mass effect from loading or removal of a rigid, thin, uniformly distributed film on the EQCM electrode can be expressed by the well-known Sauerbrey equation (Sauerbrey, 1959; Buttry, 1991) that depicts a linear relationship between the frequency shift and the electrode-mass change. The EQCM is also sensitive to the density and viscosity of the local solution near the EQCM electrode surface, and the net Newtonian–liquid–loading effect for a PQC with one side exposed

* Corresponding author. Tel.: +86 731 8865515; fax: +86 731 8865515.
E-mail address: xieqj@hunnu.edu.cn (Q. Xie).

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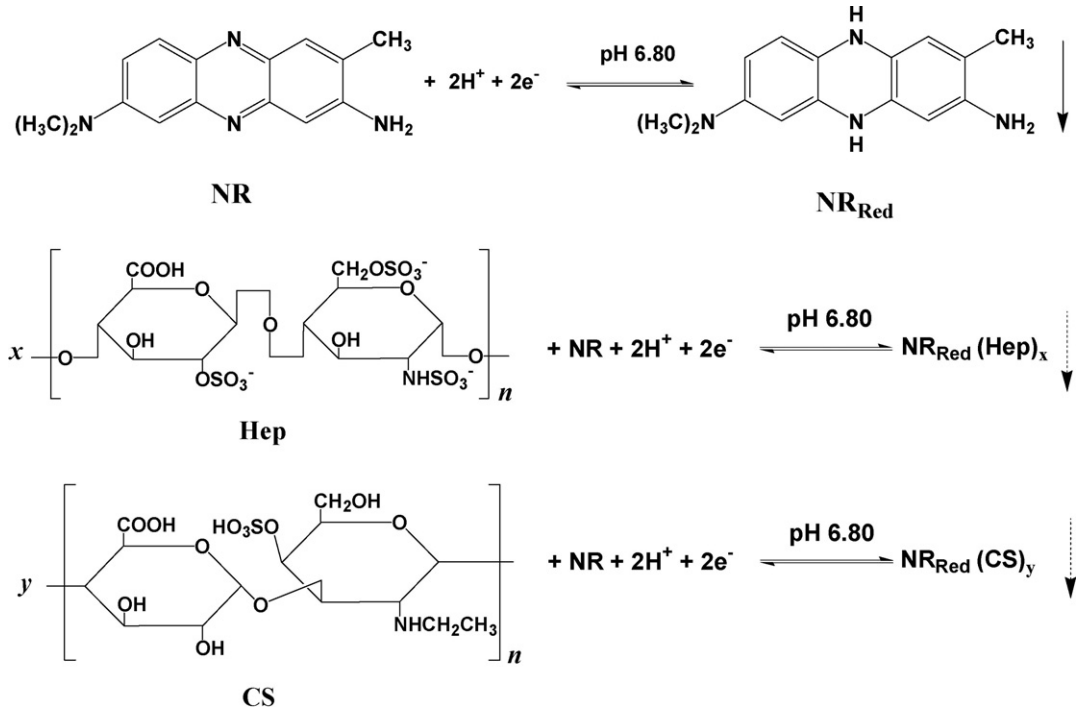

Scheme 1. Reductive electrodeposition of NR and effects of Hep and CS. The dashed arrows here indicate that the adducts possess higher solubility than the NR_{Red}, and the protonation and deprotonation of nitrogen atoms are not considered here.

to the liquid solution can be characterized by the equations proposed by Kanazawa and Gordon as well as Martin et al. (Buttry, 1991; Kanazawa and Gordon, 1985; Martin et al., 1991; Xie et al., 1999; Zhou et al., 2007). Also, the EQCM-based biosensor has been widely used for analyses of protein (Kim et al., 2008), nucleic acid (Wang et al., 1999), and enzyme (Su et al., 2008), and for immunoassay (Shi et al., 2006), etc.

It is interesting and important to establish surface-regenerable biosensing and electroanalytical methods for the sensitive quantification of biomolecules. Very recently, we described a biosensing mode with a dynamically renewed surface for the sensitive detection of Hep based on its interaction with the charge-transfer complex generated during *o*-tolidine electrooxidation (Cao et al., 2007). In this work, a novel EQCM biosensing protocol featured by a dynamically renewed surface of the detection electrode is proposed for the highly sensitive assay of Hep or CS, on the basis of the effect of the target analyte on the electrodeposition/stripping of the reduced product of neutral red (NR_{Red}) that is poorly soluble in a pH 6.80 phosphate buffer solution (PBS).

2. Experimental

2.1. Instrumentation and reagents

All electrochemical experiments were conducted on a CHI660C electrochemical workstation (CH Instruments Inc., USA) controlled by the CHI660C software. A research QCM (Maxtek Inc., USA) controlled by the Maxtek software was used to record the resonant frequency (f_0) and the motional resistance (R_1) data of the piezoelectric quartz crystal (PQC). AT-cut 9 MHz PQCs (12.5 mm in crystal diameter) with 6.0 mm diameter Au electrodes in key-hole configuration were used. The Au electrode on one side of the PQC was exposed to the solution and served as the working electrode, while that on the other side faced air. The reference electrode was a KCl-saturated calomel electrode (SCE), and all potentials reported in this work are reported versus it. The counter electrode was a carbon rod.

Neutral red (NR) was purchased from the Third Reagent Factory of Shanghai (Shanghai, China) and its stock solution (4 mmol L^{-1}) was stored in the dark due to the optical sensibility of the dye. Hep sodium salt and Hep sodium injection were purchased from Shanpu Chemical Company (Shanghai, China) and Tianjin Pharmaceutical Co., Ltd. (Tianjin, China), respectively. All calculations for Hep are in terms of its molecular weight of 12000 Da (Malsch et al., 1994). Shark CS (molecular weight: 10,000–30,000, and the average molecular weight of 20,000 is used for the calculation of its molar concentration) and CS ophthalmic solution were purchased from Jiehui Biological Technology Co., Ltd. (Changsha, China) and Shandong Haishan Pharmaceutical Co., Ltd. (China), respectively. The PBS (pH 6.80, except otherwise specified) contained 0.20 mol L^{-1} NaH_2PO_4–Na_2HPO_4 + 50 mmol L^{-1} Na_2SO_4. All other reagents were of analytical grade or better quality. All solutions were prepared fresh prior to use, and Milli-Q ultra-pure water (Millipore, 18 MΩ cm) was used throughout. The experiments were conducted at room temperature around 20 °C.

2.2. Procedures

The surface of the gold electrode was treated with one drop of the mixture of H_2O_2 and H_2SO_4 (v/v 1:3) for 15 s, followed by rinsing with copious Milli-Q ultra-pure water and dried with a stream of pure nitrogen. The treatment was repeated thrice. The treated electrode was then scanned between 0 and 1.5 V at 30 mV s^{-1} in 0.20 mol L^{-1} $HClO_4$ solution for sufficient cycles to obtain reproducible cyclic voltammograms. The electrode was washed again with copious Milli-Q ultra-pure water and dried with a stream of pure nitrogen. The electrode was then subject to potential cycling (between −0.100 and 0.500 V vs SCE at 30 mV s^{-1}) in an aqueous solution of 2.00 mmol L^{-1} $K_4Fe(CN)_6$ + 0.10 mol L^{-1} Na_2SO_4. The peak-to-peak separation was generally between 60 and 80 mV, indicating that a clean electrode surface had been obtained (Chen et al., 2007). In the EQCM experiments, the electrodes were inserted into the electrochemical cell, and the simultaneous responses of