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Tailoring chemically converted graphenes using a water-soluble pyrene derivative with a zwitterionic arm for sensitive electrochemiluminescence-based analyses

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ABSTRACT

We report a method to tailor chemically converted graphenes (CCGs) using a water-soluble pyrene derivative (**1**) with a zwitterionic arm, and the feasibility of the tailored CCGs to sensitive electrochemiluminescence (ECL)-based analyses. The compound **1** serves the dual purpose of improving the dispersion of the CCGs in aqueous solutions and further tailoring the catalytic activity of the CCGs with dendrimer-encapsulated catalytic nanoparticles. As a model system, we conjugated dendrimer-encapsulated Pt nanoparticles to the **1**-functionalized CCGs on indium tin oxide (ITO) electrodes. The resulting ITOs exhibited significantly increased ECL emission of the luminol/H_2O_2 ECL system; *i.e.* two orders-of-magnitude enhancement in the ECL compared to that obtained from bare ITOs, which allowed a *ca.* 154 times more sensitive ECL-based analysis of cholesterol using the modified ITOs compared with the use of bare ITOs.

1. Introduction

Among several effective techniques developed for preparing graphene sheets, the chemical reduction of exfoliated graphene oxides (GOs) allows large-scale synthesis of graphenes at relatively low cost (Allen et al., 2010), which is beneficial in a number of advanced analytical applications such as ECL sensors, field-effect sensors, paper-based analytical devices, and electrochemical sensors/biosensors (Cate et al., 2015; Kimmel et al., 2012; Stine et al., 2013; Su and Lv, 2014). Nevertheless, the practical applications of the graphene sheets, usually denominated as chemically converted graphenes (CCGs), are often limited by two major challenges: how to improve the dispersibility of the CCGs in solutions for practical processability and how to tailor the properties of the CCGs further depending on applications (Su et al., 2009). Several effective methods have been developed to circumvent the obstacles via chemical functionalization of CCGs. They can be classified into two general approaches: the non-covalent functionalization of CCGs through the physisorption of aromatic organic molecules onto basal planes of CCGs, and the covalent functionalization of CCGs with reactive organic species through covalent bond formation onto CCGs (Georgakilas et al., 2012; Kim et al., 2012). Of these two methods, the non-covalent functionalization of CCGs with pyrene derivatives is particularly attractive since it can provide versatile chemical functionalities onto CCGs without degrading the intrinsic properties of the graphenes. For example, the non-covalent functionalization of graphene with 1-pyrenecarboxylic acid was demonstrated to achieve stable aqueous dispersions of graphenes for multifunctional applications of the graphenes (An et al., 2010).

Electrochemiluminescence (ECL) is a unique type of chemiluminescence in which electrogenerated species are involved to form excited states emitting light in the vicinity of electrode surfaces (Forster et al., 2009; Richter, 2004). The ECL provides the beneficial features of chemiluminescence even with better temporal and spatial controllability due to the intrinsic light-generation process of ECL on electrode surfaces. The ECL technique has been thus developed as a powerful tool in many analytical applications such as immunoassays, DNA analysis, and molecular diagnosis of environmentally or clinically relevant compounds (Dennany et al., 2004; Miao, 2008). Recently, the ECL technique has also been used with graphene-related nanomaterials because graphene sheets present many excellent properties including high surface-to-volume ratio ($\sim$2,600 $m^2\,g^{-1}$) and high electron transfer rate (up to 200,000 $cm^2\,V^{-1}\,s^{-1}$) for efficient ECL signal transduction (Allen et al., 2010; Schedin et al., 2007). However, the translation of the extraordinary properties of graphenes into the

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practical ECL applications has some major challenges. Specifically, graphene sheets are only dispersible in a limited number of solvents, such as *N*-methyl pyrrolidone, *N*,*N*-dimethylacetamide, and γ-butyrolactone, with high surface tension (Hernandez et al., 2008), and are often re-aggregated via π-π stacking interactions even in these limited solvents, which makes handling and processing of graphene sheets challenging (Mali et al., 2015). The reactivity of pristine graphenes is also relatively low compared to other carbon allotropes such as carbon nanotubes and fullerenes (Mali et al., 2015), which often requires the use of highly catalytic species for catalyzed electrochemical reactions leading to sensitive ECL-based assays (Gu et al., 2015; Huang et al., 2016; Jiang et al., 2014; Li et al., 2015; Zhao et al., 2015). For example, the use of hemin as a biomimetic catalyst was reported to facilitate the electrocatalytic reduction of oxygen on graphene sheets, resulting in ultrasensitive ECL-based immunoassays (Deng et al., 2013). Recently, our group also reported the covalent decoration of graphenes with amine-terminated dendrimers encapsulating catalytic nanoparticles, which can be utilized for enhanced stable ECL of $Ru(bpy)_3^{2+}$/tripropylamine (Kim et al., 2012; Kim and Kim, 2014).

In this context, we report the functionalization of CCGs using a water-soluble pyrene derivative **1** (3-((pyren-1-yl)methyl)imidazolium-1-propionate), and the feasibility of the functionalized CCGs to sensitive ECL-based analyses. The compound **1** consisted of a pyrene appended with a 3-(imidazolium)propionate zwitterionic arm (Scheme 1a), and served the dual purpose of improving the dispersion of the CCGs in aqueous solutions and further tailoring the catalytic activity of the CCGs with dendrimer-encapsulated catalytic nanoparticles (DENs). The aromatic pyrene moiety of **1** enables the compound **1** to be anchored onto the hydrophobic surface of the CCG sheets. The stability of the aqueous dispersion of the resulting CCGs was greatly enhanced due to the hydrophilic zwitterionic arm in **1** anchored onto the CCGs, which thus facilitates the processability for integration of the CCGs onto the ITO substrates to develop ECL signal-transduction platforms. In addition, the carboxylic group of the zwitterionic arm in **1** allows the facile secondary functionalization of the CCGs on ITOs with catalytic DENs for sensitive ECL-based analyses. Specifically, we synthesized the pyrene derivative **1** with a 3-(imidazolium) propionate zwitterionic arm, and prepared stable aqueous dispersions of **1**-functionalized CCGs. Based on this dispersion, we functionalized the ITO surfaces by spin-coating of the **1**-functionalized CCGs onto ITOs and the subsequent covalent conjugation of catalytic Pt nanoparticles (diameter 1.8 ± 0.2 nm) encapsulated inside amine-terminated polyamidoamine dendrimers (Pt DENs) to the **1**-functionalized CCGs on ITOs (Scheme 1b). The resulting ITOs, which we denote as Pt DEN-**1**-CCG/ITOs, exhibited highly enhanced catalytic activity for the electrochemical redox reactions of the luminol/H_2O_2 ECL system, leading to significantly improved ECL emission. The Pt DEN-**1**-CCG/ITOs provided two orders-of-magnitude enhancement in the ECL of luminol/H_2O_2 compared to ECL obtained from bare ITOs, which enabled the sensitive ECL-based analysis of cholesterol.

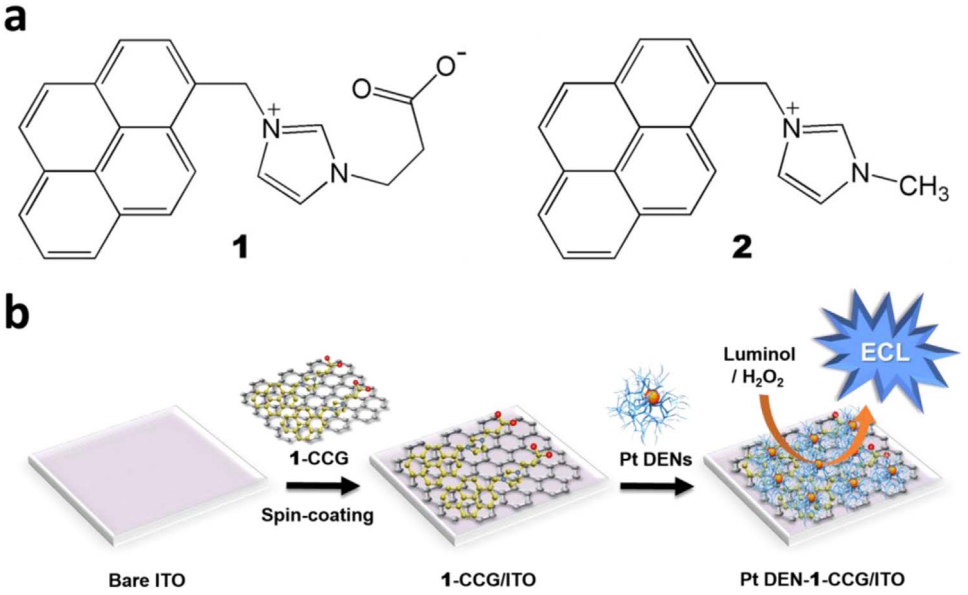
2. Experimental

2.1. Preparation of functionalized CCG dispersions

Functionalized CCGs were prepared by the hydrazine-based chemical reduction of GOs in the presence of the synthesized pyrene derivative **1** or **2** (Supplementary Information for details on the synthesis of **1** and **2**). The GOs were synthesized according to a modified Hummer's method as we reported previously (Supplementary Information for details) (Kim et al., 2012). The synthesized GOs were then chemically reduced by adding 0.4 μL of hydrazine (35 wt%) and 2.8 μL of NH_4OH (28 wt%) to 4 mL of the GO solution (*ca.* 0.05 mg/mL) in the presence of the synthesized pyrene derivative **1** (2 mM) or **2** (10 mM). The chemical reduction was carried out at 80 °C for 90 min. The resulting functionalized CCGs were collected, purified by centrifugation, and washed with deionized (DI) water several times. The functionalized CCGs solutions were additionally purified by extensive dialysis up to 10 days to remove any free pyrene derivative **1** or **2**. As a control experiment, the CCGs were also prepared using the same method as that used for the functionalized CCGs, but in the absence of **1** or **2**.

2.2. Modification of ITO electrodes with *1*-functionalized CCGs and Pt DENs

ITO electrodes were modified by spin-coating of **1**-functionalized CCGs on the surface of ITOs and subsequent conjugation of Pt DENs onto the **1**-functionalized CCGs on ITOs. The resulting ITOs, *i.e.* Pt DEN-**1**-CCG/ITOs, were used as signal transduction platforms



Scheme 1. (a) Structure formulas of pyrene derivatives (**1** and **2**). (b) Schematic illustration of preparation process of Pt DEN-**1**-CCG/ITO.