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Highly sensitive electrochemiluminescence immunosensor based on ABEI/H_2O_2 system with PFO dots as enhancer for detection of kidney injury molecule-1

Huiyun Yang, Haijun Wang, Chengyi Xiong, Yaqin Chai*, Ruo Yuan*

Key Laboratory of Luminescent and Real-Time Analytical Chemistry (Southwest University), Ministry of Education, College of Chemistry and Chemical Engineering, Southwest University, Chongqing 400715, PR China

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ABSTRACT

In this work, poly[9,9-dioctylfluorenyl-2,7-diyl] (PFO) dots is discovered to display an appealing dual enhancement effect for the electrochemiluminescence (ECL) system of N-(aminobutyl)-N-(ethylisoluminol)/hydrogen peroxide (ABEI/H_2O_2), which not only enhances the ECL intensity of ABEI but also catalyzes decomposition of H_2O_2 to further amplify the ECL signal of ABEI. Owing to the electronegative property of PFO dots, electropositive ABEI-PEI as ECL reagent could be adsorbed on their surface and thus form a novel luminescence emitter (ABEI-PEI-PFO dots) with high ECL efficiency based on electrostatic attraction. Meanwhile, the water solubility and stability of this emitter are improved in virtue of the amine-rich property of ECL reagent (ABEI-PEI), which could increase the luminous efficiency of ECL reaction in aqueous solution. To increase the electron transfer efficiency, Pt nanoparticles (PtNPs) supported on reduced graphene oxide nanosheets (RGOs) via a onepot synthetic strategy are chosen as immobilizing platform for the ECL emitter (ABEI-PEI-PFO dots). Herein, the obtained dual-amplifed ABEI-PEI-PFO dots-RGOs/PtNPs complex is served as an ideal nanocarrier to capture detection antibody (Ab_2). According to sandwiched immunoreaction, a highly sensitive ECL immunosensor is constructed for the detection of kidney injury molecule-1 (KIM-1) with a linearity from 50 fg mL^{-1} to 1 ng mL^{-1} and a detection limit of 16.7 fg mL^{-1}. The developed ECL emitter combining dual amplified property for signal enhancement purpose would provide new thought and potential for sensitive bioanalysis and clinical application.

1. Introduction

Classical methods of diagnosing kidney injury commonly include measurement of creatinine, serum urea nitrogen and estimated glomerular filtration rate (Mohammadi et al., 2016; Work and Schwartz, 2008). But when these indicators increase, the kidney injury is likely to be serious or has caused irreversible damage (Rosner, 2009). Many studies show that kidney injury molecule-1 (KIM-1), an early protein biomarker of kidney injury, could reflect damage and recovery process of nephropathy with speediness and specificity, suggesting an indispensable significance for searching sensitive strategy in improvement of timely accurate detection of KIM-1 (Vaidya et al., 2006; Yin and Wang, 2016). In numerous analytical methods, Electrochemiluminescence (ECL) assay with inherent advantages, such as high sensitivity, wide dynamic range and outstanding controllability, has attracted continuous attention in fundamental research, which possesses superiority to meet the requirement for accurate detection of KIM-1 (Hu and Xu, 2010; Ke et al., 2018). Up to now, using ECL strategy to detect KIM-1 has been rarely reported. Among various ECL reaction system, N-(aminobutyl)-N-(ethylisoluminol) (ABEI)/hydrogen peroxide (H_2O_2) is regarded as one kind of classical ECL reaction system (Yang et al., 2002). Besides, the luminophore ABEI is easy to be labeled with other reagent for new ECL reagent formation or immobilized via the amino groups, leading little reduction in its luminescence activity (Xie et al., 2016).

In the aim of constructing ECL biosensor to precisely determine protein biomarker at ultralow levels, the amplification of detection signal is a key point to improve the detection sensitivity of biosensor (Cao et al., 2018; Wang et al., 2017; Zhou et al., 2017). For ECL system of ABEI/H_2O_2, endeavor has been made to enhance its ECL signal. On the one hand, several reagents have been investigated to enhance the ECL intensity of ABEI (Jiang et al., 2016; Wang et al., 2018). In our previous work, based on the enhancement effect of polyethylenimine (PEI) to ECL intensity of ABEI, a complex of ABEI derivative (ABEI-PEI)

* Corresponding authors.
E-mail addresses: yqchai@swu.edu.cn (Y. Chai), yuanruo@swu.edu.cn (R. Yuan).

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with self-enhanced property was prepared to be a new ECL reagent (Yang et al., 2017). Meanwhile, the properties of electropositivity and high hydrophilicity were successfully introduced into the obtained ECL reagent by virtue of abundant amino of PEI. On the other hand, catalysts like peroxidase or mimic peroxidases could distinctly catalyze H_2O_2 to form reactive oxygen species (ROSs) like hydroxyl radical ($OH^{\bullet}$) and superoxide radical ($O_2^{\bullet -}$), which could oxidize ABEI to obtain the amplified ECL signal (Liu et al., 2017; Jiang et al., 2017). Although the above strategies were utilized for the purpose of amplifying ECL signal, making efforts to improve the ECL signal amplification and achieve preferable luminous efficiency was still necessary. Therefore, it is meaningful and challenging to explore novel materials that directly enhance ECL intensity of ABEI and catalyze the decomposition of H_2O_2 for further signal amplification of ABEI at the same time.

Poly(9,9-dioctylfluorenyl-2,7-diyl) (PFO) dots is one kind of typical conjugated polymer dots synthesized by conjugated polymer (Wu and Chiu, 2013). Owing to the nontoxic features and simple synthesis procedures, PFO dots have aroused interest in applification of chemical sensing (Chen et al., 2017; Wu et al., 2015). Besides, their electronegative surface could resort to electrostatic approaches for the further modification purposes (Clafton et al., 2010). In this report, we explored an appealing phenomenon that PFO dots had an obvious enhancement effect for the ECL intensity of ABEI. Meanwhile, PFO dots could react like mimic peroxidases to catalyze H_2O_2 for further amplifying the ECL signal of ABEI. This dual amplified effect incorporated by PFO dots could achieve preferable luminous efficiency in the aim of obtaining a strong ECL signal, which was serviceable to improve the detection sensitivity of biosensor in aspect of trace analysis. However, owing to the high hydrophobicity of conjugated polymer, the prepared conjugated polymer dots were short of water solubility and stability, which might astrict the employment of PFO dots in the ECL biosenser field (Feng et al., 2013). Thus, achieving higher water solubility of PFO dots is meaningful for further expanding its potential application.

Herein, ABEI-PEI-PFO dots as a novel ECL emitter was prepared for the construction of ECL immunosensor to sensitively detect KIM-1. The obtained ABEI-PEI-PFO dots with high ECL efficiency was ascribed to the dual amplified property of PFO dots. Meanwhile, the water solubility of the ECL emitter was increased in virtue of the amine-rich property of ECL reagent, which was beneficial for the stability of ECL reaction in aqueous solution. Subsequently, taking advantage of excellent electroconductibility of metal nanoparticles and graphene nanomaterials for increasing the electron transfer efficiency, Pt nanoparticles (PtNPs) supported on reduced graphene oxide nanosheets (RGOs) via a onepot synthetic strategy were utilized as the loading platform of ABEI-PEI-PFO dots. Lastly, with bovine serum albumin (BSA) blocking the nonspecific binding sites, the obtained composites (ABEI-PEI-PFO dots-RGOs/PtNPs) with high-efficiency luminescence were applied as ideal nanocarrier of detection antibody (Ab_2) to form ABEI-PEI-PFO dots-RGOs/PtNPs@Ab_2-BSA complex (Ab_2 bioconjugates). In addition, the capture antibody (Ab_1) was loaded on Au nanoparticles (AuNPs). And through sandwiched immunoreaction among Ab_1, KIM-1 and Ab_2 bioconjugates, a high-sensitive ECL immunosensor was constructed for KIM-1 detection. The preparation of the ECL emitter, the construction of immunosensor, and the signal enhanced mechanism were demonstrated in Scheme 1. In conclusion, the introduction of conjugated polymer dots for dual amplified purpose would broaden the applification of ABEI/H_2O_2 system in various areas. Besides, the immunosensor for KIM-1 detection offers new means for possible early diagnosis of kidney injury related disease.

2. Experimental section

2.1. Preparation of PFO dots and RGOs/PtNPs

The detailed prepared process of PFO dots and RGOs/PtNPs were shown in Scheme 1. Firstly, PFO polymer was dissolved in tetrahydrofuran (THF) to form homogeneous solution (2 mg mL^{-1}). Then, 200 μL of the mixture solution was injected into 5 mL of ultrapure water under the vigorous sonication condition for 10 min. After removing THF by partial vacuum evaporation and followed by filtration through a 0.2 μm membrane filter, the resultant solution of PFO dots was obtained.

RGOs/PtNPs was synthesized according to the previous literature with a little modification (Li et al., 2014). Firstly, the aqueous solution of the GOs suspension was ultrasonicated for 40 min. Then, 5.0 mL of the GOs suspension (0.5 mg mL^{-1}), 3 mL of H_2PtCl_6 (10 mM), and 0.5 mL of NaOH (0.1 M) solutions were mixed together under stirring. After homogeneous mixing, 0.5 mL of freshly prepared ascorbic acid (AA, 0.1 M) solution was slowly added to the mixed solution under stirring. The mixture was heated in a water-bath at 60 ℃ for 30 min without any agitation. The final products were collected by centrifugation at 8000 rpm for 10 min, thoroughly washed with ultrapure water.

2.2. Preparation of ABEI-PEI-PFO dots-RGOs/Pt NPs@Ab_2-BSA complex

First, 3 mL PEI (0.005%) and 1 mL ABEI (0.005 M) were mixed together. And then, 50 μL GA (0.1%) was added into above solution and kept stirring for 3 h at room temperature (RT) to sufficiently cross-link PEI and ABEI. Next, based on the electrostatic attraction between ABEI-PEI and PFO dots, 2 mL of the prepared PFO dots solution was added into above ABEI-PEI solution to form ABEI-PEI-PFO dots complex. After that, 500 μL as-prepared RGOs/PtNPs was added to form ABEI-PEI-PFO dots-RGOs/PtNPs nanoplatform, due to the specific interaction between amino group of ABEI-PEI-PFO dots and RGOs/PtNPs hybrid. As-prepared ABEI-PEI-PFO dots-RGOs/PtNPs was centrifuged at 7000 rpm for 10 min to discard excess reagents, and the sediment was dispersed in 2 mL double distilled water. After that, based on specific interaction between amino group and noble metal (Pt), 200 μL anti-KIM solution (Ab_2) was mixed into the obtained composite and reacted at 4 ℃ with stirring overnight. To block the remaining active binding sites, 1 mL BSA (1%) was added into the obtained ABEI-PEI-PFO dots-RGOs/PtNPs@Ab_2 complex with stirring for 50 min. After centrifugation at 6000 rpm for 8 min to discard excess reagents, the bioconjugates of ABEI-PEI-PFO dots-RGOs/PtNPs@Ab_2-BSA (Ab_2 bioconjugates) was obtained.

2.3. Fabrication process of the modified electrodes

Previous to modification, the GCE (4 mm in diameter) was polished with 0.3 μm and later with 0.05 μm alumina slurry, and then thoroughly cleaned with ethanol and double distilled water with 5 min ultrasonic processing. Next, the GCE immersed in $HAuCl_4$ solution (1%) was electrochemically deposited (deposition potential: −0.2 V; deposition time: 30 s) to obtain a thin layer of Au nanoparticles (AuNPs). After drying, the electrode surface was incubated with 16 μL capture KIM antibody (Ab_1) at 4 ℃ for 12 h. Then to block the nonspecific binding sites, 16 μL blocking agent BSA (1%) was dropped onto the electrode surface for 50 min at RT. Subsequently, the modified electrode was incubated with 16 μL KIM antigen (Ag) and kept at 4 ℃ for 50 min with concentrations linearly increasing. Ultimately, 16 μL Ab_2 bioconjugate was modified on the obtained electrode through the immune binding between antigen and antibody. In procedure of the electrode fabrication, every step of the resultant electrode was softly washed by double distilled water to remove the excess and unreacted reagents.

3. Results and discussion

3.1. Characterization of the nano-composite

SEM was used for characterization of the prepared RGOs/PtNPs,