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Spectroscopic detection of thrombin with peptides self-assembled on gold nanoparticles hybridized graphene oxide

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ABSTRACT

Thrombin is an important kind of proteases that can cleavage specific peptide bonds and plays a crucial role in numerous diseases, which leads to large amounts of efforts on its detection. In this study, a novel peptide self-assembled biosensor was developed based on gold nanoparticles functionalized graphene oxide (GO) for specific detection of thrombin. Through sulfhydryl design, peptides containing thrombin cleavage sites could self-assemble with the nanocomposites of the graphene material and gold particles, which brought the biosensor unique optoelectronic properties and high biological sensitivity to transduce bio-molecular interactions into optical signals. Spectroscopic measurements were carried out in the presence of thrombin to observe an increase of the absorption peak at ~528 nm and a decrease of the absorbance at wavelength larger than 600 nm. The biosensor showed a linear dose-dependence and stable response to thrombin. And with a 528–680 nm absorbance ratio as the sensitive detection parameter, the self-assembled biosensor exhibited high sensitivity and specificity for the thrombin detection. Thus, it revealed convenient and sensitive detection abilities for protease of thrombin, indicating a promising prospect for other related proteases, such as matrix metalloproteinases to cancer related diseases.

1. Introduction

Proteases are an important class of enzymes that catalyze the hydrolytic cleavage of peptide bonds in target proteins with specificity and break up proteins into smaller fragments in the metabolic processes [1,2]. They serve significant physiological roles and also participate in a variety of disease pathologies, exhibiting great research value [3,4]. As one typical kind of proteases, thrombin acts as the main effector enzyme in blood coagulation cascade [5,6]. It can cleave twelve known substrates under the assistance of cofactors with both pro- and anti-coagulant functions, and plays an crucial role in thrombosis, vascular damage, inflammation, and tissue injury repairs [7]. Moreover, it has been verified that thrombin was closely related to plenty of bad diseases such as atherosclerosis, and even regulates dormant tumor phenotype [8]. Thus, it is of great significance to develop highly sensitive detection approaches for thrombin in fields of clinic diagnosis and treatment [9].

Numerous efforts have been made on thrombin detection, and among them, biosensors were popular for their high sensitivities that come from their biometric elements [10,11]. Classified by the biological elements, there were three main kinds of biosensors for thrombin detection: One was that composed of proteins like fibrinogen, which took the advantage of fundamental functions of thrombin, but need much time for detection and were not suitable for conservation [12]. The other two were biosensors composed of nucleic acid aptamers and peptides, respectively [13,14]. On one hand, aptamers and peptides both had advantages such as controllable structure, ease of synthesis, long-term preservation, higher affinity, and faster response. On the other hand, peptides constituted of more than twenty different amino acids exhibit more diverse interaction formats and conformations than nucleic acid aptamers, which can result in higher selectivity and binding capacity, as well as better ease-of-assembly characteristics [9]. All these imply that a biosensor with peptides as biometric elements will be an ideal choice for thrombin detection.

For small molecules like peptides and nanoparticles, graphene oxide (GO) was an ideal building block because of its large specific

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surface area and rich binding sites [15,16]. Besides, GO functionalized by nanoparticles can exhibit unique physical and chemical properties such as ultrasensitive features and excellent catalytic properties [17,18]. On the basis of the tailored optoelectronic properties of the graphene material and superior biological nature of biomolecules, the combination of nanoparticles functionalized graphene oxide and small biomolecules shows promising potential in construction of optical biosensors.

In this study, a novel biosensor was constructed for spectroscopic detection of thrombin by equipping composites of graphene oxide and gold nanoparticles (GNPs) with peptides containing specific cleavage sites of thrombin. The graphene material hybridized with the nanoparticles through its oxygen-containing groups without internal bridges. Then, specifically designed peptides were chosen and applied to self-assemble with the nanocomposites for the establishment of the biosensing system. Spectroscopic detection was carried out for rapid and high-throughput measurements based on the optical properties of the nanocomposites. High sensitivity and selectivity of the biosensor could be observed toward thrombin detection.

2. Materials and methods

2.1. Peptides self-assembled on gold nanoparticles hybrized graphene oxide

Composites of graphene oxide and gold nanoparticles (GO/GNPs) were synthesized as our previous work [19]. In brief, 1.225 mL of 1% chloroauric acid ($HAuCl_4$) was mixed with 3 mL well-dispersed GO solution (~0.5 mg/mL) and 150 mL deionized water. After standing in the dark for 30 min, the mixture was heated to 80 °C with continuously stirring, and then 2.1 mL of 1% trisodium citrate ($Na_3C_6H_5O_7$) was added for reduction. The heating was kept at 80 °C for 1 h, while the stir was lasted for 75 min. With further centrifugated at 3000 r.p.m. for 10 min, the nanocomposites were finally obtained. For comparison with GO/GNPs, GNPs were prepared similar to the preparation steps of the nanocomposites with the only difference that no graphene oxide was added. Moreover, for investigation on the effect of the gold particle size on GO/GNPs for thrombin detection, similar procedure with different doses of sodium citrate (4.2 mL and 1.05 mL) added in the preparation process was also performed.

Specifically designed octapeptides (Cys-Leu-Val-Pro-Arg-Gly-Ser-Cys, CLVPRGSC) and tetrapeptides (Cys-Arg-Gly-Cys, CRGC) were synthesized by solid phase peptide synthesis (SPSS) [14,20]. These two kinds of peptides were dissolved in phosphate buffer solution (PBS, pH = 7.2) for solutions at 1.2 mM, respectively. The stock solution of octapeptides was then diluted to concentrations of 0.12 mM and 0.012 mM. For self-assembly of the biosensor for thrombin detection, all those prepared solutions (150 μL) were respectively mixed with the GO/GNPs dispersions (3 mL) and then oscillated at 2800 r.p.m. for 1 min. The peptides could spontaneously link with the nanocomposites due to the sulfydryl groups on them. The obtained solutions were then used as peptides self-assembled biosensors for thrombin. The brief synthesis process was shown in Fig. 1.

2.2. Optical spectroscopy for characterization and optimization of the peptides self-assembled nanocomposites

For characterization of the nanocomposites and peptides assembled biosensors, transmission electron microscopy (TEM) images were taken by JEM-1230 (120 kV, JEOL Ltd., Japan) and spectra were monitored by Ocean Optics USB2000+ Spectrometer (Dunedin, USA) with the wavelength ranging from 400 nm to 800 nm (0.38 nm optical path) in an absorbance mode. Before usage, the spectrometer should be calibrated and adjusted with light intensity and other parameters to prevent light saturation and determine the reference spectrum. Different total light intensity would be applied in different groups of experiments, which would not affect the comparisons in the same group and final statistical results. GO dispersion, GO/GNPs solution and the biosensor assembled with 1.2 mM octapeptides obtained above were all detected and compared with each other by both approaches.

Besides, the size of the nanocomposites used in the work was detected by dynamic light scattering on a Zeta Sizer Nano Series instrument (Malvern). Moreover, for the biosensor optimization, the optimal category and concentration of peptides applied here were also investigated through absorption spectra. As mentioned above, biosensors containing octapeptides or tetrapeptides at same dose (1.2 mM) and those including octapeptides at different concentrations (0.012 mM–12 mM) were all measured. Their performance under addition of thrombin (from bovine plasma, Sigma-Aldrich Co., LLC, 40-300 NIH units/mg protein) at 27 μM were also detected with a mixing volume ratio of 21:1. Similar process was then performed for investigation of effects of particle size and solution concentration of GO/GNPs on thrombin detection.

2.3. Thrombin detection using the peptides self-assembled nanocomposites

After the optimization measurements, GO/GNPs/OPs were chosen for further detection. Dose-dependence and specificity experiments were both performed through absorption detection. 3 mL GO/GNPs was mixed with 150 μL octapeptides at 1.2 mM and oscillated at 2800 r.p.m. for 1 min. Then, 150 μL thrombin solutions at different concentrations (1 fM–10 pM) were added respectively into the mixed solutions and oscillated at 2800 r.p.m. for another 1 min and incubated for 10 min before dose-dependence measurements, with PBS applied as control. Subsequently, those mixtures were immediately placed into the quartz cuvette for absorption spectroscopic measurement.

To evaluate the specificity of the biosensor, human serum albumin (HSA) and insulin (porcine) were chosen for the comparison with thrombin detection. HSA was a serum albumin protein composed of several hundreds of amino acids like thrombin, which was the major component of blood. While insulin was an important protein hormone with only 51 amino acids, which regulated the sugar level in the blood. All of them were essential proteins in the blood. And these three proteins were dissolved in PBS in this study at 27 μM for the specific comparison, respectively. Same operating steps were repeated for detection of HSA and insulin as that for thrombin, and PBS instead of proteins was detected as the control trail. All of the reagents except peptides were obtained from Sigma-Aldrich Co., LLC.

3. Results

3.1. Optical spectroscopy for characterization of the nanocomposites

With abundant oxygen functional groups on its graphene-like flexible nanosheets, graphene oxide could act as the growth substrate for gold nanoparticles [21]. In the presence of sodium citrate, GNPs could be reduced from chloroauric acid, and nucleate and grow on the GO nanosheets due to electrostatic interactions between particles and the oxygen groups of the graphene material. As shown in the TEM image of Fig. 2A, nanoparticles could be seen to disperse on the graphene sheets. Besides, compared the nanocomposites with graphene oxide through absorption spectra