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A novel peptide/Fe_3O_4@SiO_2-Au nanocomposite-based fluorescence biosensor for the highly selective and sensitive detection of prostate-specific antigen

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ABSTRACT

Highly selective and sensitive detection methods are very important for the early diagnosis of prostate-specific antigen (PSA). Here, we present a novel peptide/Fe_3O_4@SiO_2-Au nanocomposite-based fluorescence biosensor for highly selective and sensitive detection of PSA. The biosensor was made by self-organizing 5-FAM labeled peptides onto the surface of magnetic Fe_3O_4@SiO_2-Au nanocomposites (MNCPs), resulting in efficient quenching of the FAM fluorescence. The PSA specifically recognized and cleaved the 5-FAM-labeled peptides, leading to the fluorescence recovery. This is the first report of the MNCPs by in situ growth of Au nanoparticles (AuNPs) on the SiO_2 encapsulated single Fe_3O_4 nanocubes. The MNCPs feature robust salt stability, and allow for effective fluorescence quenching and easy magnetic separation, which greatly decrease the background fluorescence. The peptide/MNCPs-based fluorescence biosensor measure a wide range of concentrations of PSA, from 1.0×10^{-12} to 1.0×10^{-9} g/mL, with a limit of detection (LOD) of 3.0×10^{-13} g/mL in both standard solutions and serum samples, demonstrating the great potential of this biosensor platform for use in clinical and biological assays.

1. Introduction

Prostate cancer is a fairly common malignancy with mortality rates ranked second out of various cancers in men [1–6]. Early and precise diagnosis of tumor biomarkers will be of great help for the cancer therapy [7–10]. However, the levels of tumor biomarkers are often remarkably low in biological samples obtained from patients during early disease progression [11,12]. Currently, prostate-specific antigen (PSA) is widely used in the early diagnosis of prostate cancer [13–15]. There is the great possibility for people with more than 4 ng/mL of PSA in serum suffering from prostate cancer [16–18]. Therefore, it is imperative to explore a highly selective and sensitive determination method for detection and quantification of PSA. The conventional immunoassay methods, such as surface plasmon resonance [19], electrogenerated chemiluminescence (ECL) [20,21], electrochemical immunoassay [22], and fluorescence [23] are developed for monitoring serum PSA. Among these techniques, fluorescence immunoassay has attracted much attention due to its merits, including facile process and high sensitivity [24]. However, the existing fluorescence immunoassays are largely based on antibodies as recognition elements, which generally involve several weaknesses including high cost, high time consumption, and easy denaturalization and deactivation [25].

Recently, oligopeptides with specific sequences have emerged as a compelling alternative to antibodies for protein assays. Oligopeptides have many advantages over antibodies, such as cost-effectiveness, stability, reliability, resistance to harsh environments, and facile chemical synthesis [26,27]. Since PSA has shown molecular recognition and enzymatic activity for an oligopeptide (HSSKLQ) [28], several peptide-based methods have been proposed for the PSA detection with high specificity, including ECL, electrochemical, and fluorescence assays [29–31]. Among these approaches, peptide-based fluorescence methods have aroused some interest due to its rapidity, simplicity, and wide linear range. For instance, Choi et al. constructed a peptide-based fluorescence biosensor using gold nanoparticles (AuNPs) as the quencher for measuring the enzyme activity of PSA, in which AuNPs must be centrifuged to facilitate detection. Generally, AuNPs are laborious to separate and possess poor salt stability [32,33], tending to readily agglomerate and impair the performance of AuNPs-based biosensors. Thus, the development of functional AuNPs-based nanocomposites is necessary to achieve highly selective and sensitive detection of PSA.

To date, various materials including metal-organic frameworks

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(MOFs) [34,35], silicon nanowires (SiNWs) [36], PbS [37], Ag [38], Pd [39], SiO_2 [40,41], and Fe_3O_4 [42,43] have been explored in the construction of functional AuNPs-based nanocomposites. Among these, SiO_2 is a promising material to improve the salt stability of AuNPs, due to its good hydrophilicity, biocompatibility, and mature surface functionalization [44–46]. Fe_3O_4 can be used simultaneously to simply and rapidly separate interfering molecules [47–49]. Therefore, magnetic Fe_3O_4@SiO_2-Au nanocomposites (MNCPs) might be an ideal platform for monitoring the enzyme activity of PSA with high specificity. The main objective of this work is to develop a peptide/MNCPs-based fluorescence biosensor for the highly selective and sensitive detection of PSA. A specific oligopeptide (CHSSKLQK) labeled by 5-FAM (FAM-peptides) was designed as a fluorescence emitting and molecular recognition element, and MNCPs were used as the fluorescence quenchers and magnetic splitters. The MNCPs significantly exhibit excellent stability at high salt concentrations (0.1 M), and allow for the effective quenching to FAM-peptides and facile magnetic separation from solution, which greatly decrease the background fluorescence. In the presence of PSA, the FAM-peptides are specifically recognized and cleaved by PSA, releasing the fluorescence emitting species from the MNCPs surface and leading to the recovery of fluorescence. Thus, highly selective and sensitive detection of PSA, both in standard solutions and serum samples, was achieved using this peptide/MNCPs-based fluorescence biosensor.

2. Experimental section

2.1. Chemicals

FAM-peptides were synthesized by Shanghai Apeptide Co., Ltd. (China). Myoglobin (Mb) were obtained from Abcam Inc. (Cambridge, United Kingdom). PSA from human semen, thrombin (Thb), lysozyme (LYZ), glucose oxidase (GOD) , β-lactoglobulin (β-LAG), bovine serum albumin (BSA), chloroauric acid ($HAuCl_4$), polyoxyethylene (5) nonylphenylether (IgepalCO-520), 3-aminopropyltriethoxysilane (APTES), and sodium borohydride ($NaBH_4$, 99%) were all purchased from Sigma-Aldrich. Iron chloride ($FeCl_3{\cdot}6H_20$, 99%) , sodium oleate ($C_{17}H_{33}CO_2Na$, 96%), 1-octadecene ($C_{18}H_{36}$, 90%), aqueous ammonia (25–28%), and tetraethyl orthosilicate (TEOS, 98%) were purchased from Sinopharm Chemical Reagent Ltd. A solution of 10 mM phosphate-buffered saline (PBS) was made up using 10 mM Na_2HPO_4, 10 mM NaH_2PO_4, and 10 mM NaCl (pH 7.4). A Millipore filtration system was used to provide the DI water in the experiments.

2.2. Instrumentation

Transmission electron micrograph (TEM) experiments were performed on a JEM-2100 transmission electron microscope (JEOL, Japan). A Kratos Analytical Axis Ultra photoelectron spectrometer (Kratos Analytical ltd, UK) was used for the X-ray photoelectron spectroscopy (XPS) measurements. Energy-dispersive X-ray analysis (EDX) was conducted with an environmental scanning electron microscopy (FEI Quanta 200). The fluorescence spectra were obtained from 500 to 600 nm with an excitation at 486 nm using a LS 55 Fluorescence Spectrometer. All fluorescence spectra were measured at least in triplicate.

2.3. Synthesis of hydrophobic Fe_3O_4 nanocubes

The $C_{54}H_{99}FeO_6$ was first synthesized according to the method reported by Park et al. [50]. Briefly, $C_{18}H_{33}NaO_2$ (36.5 g) and $FeCl_3{\cdot}6H_2O$ (10.8 g) were added into a mixture of hexane (140 mL), DI water (60 mL), and ethanol (80 mL). The mixture was reacted to 60 °C and refluxed for 4 h. After cooling to 25 °C, the resulting waxy $C_{54}H_{99}FeO_6$ was washed with DI water. To synthesize Fe_3O_4 nanocubes [51], the $C_{18}H_{33}NaO_2$ (0.64 g) and $C_{54}H_{99}FeO_6$ (1.8 g) were added to a three-necked flask (50 mL) with a solvent of 1-octadecene (10 g). The mixture was kept at 200 °C for 1 h and then at 320 °C for 40 min. The reaction solution needs to be rapidly cooled to 25 °C, and the resulting Fe_3O_4 nanocubes were washed with hexane and ethanol. Afterward, the product was vacuum-dried at 60 °C for 12 h.

2.4. Synthesis of NH_2-modified Fe_3O_4@SiO_2 nanoparticles

A reversal microemulsion technique was used to synthesize the Fe_3O_4@SiO_2 nanoparticles [52]. Fe_3O_4 (0.0019 g) and Igepal-CO 520 (0.52 g) were mixed in cyclohexane (11 mL). Afterward, $NH_3{\cdot}H_2O$ (0.2 mL, 28–30%) and TEOS (0.28 mL) were added to the solution with continuous stirring. The resulting Fe_3O_4@SiO_2 nanoparticles were centrifuged, washed with ethanol and hexane, and finally redispersed in ethanol. Finally, APTES was dropped into an ethanol solution of Fe_3O_4@SiO_2 nanoparticles to react for 24 h. The NH_2-modified Fe_3O_4@SiO_2 nanoparticles were centrifuged, and washed with ethanol and DI water.

2.5. Preparation of MNCPs

These MNCPs were synthesized on the basis of a reported procedure [53]. The NH_2-modified Fe_3O_4@SiO_2 nanoparticles (0.008 g) were redispersed in DI water (20 mL), and mixed with 1 wt% $HAuCl_4$ aqueous solution (0.5 mL). After stirring at 25 °C for 1 h, 50 mM $NaBH_4$ solution (10 mL) was dropped into the reaction mixture, and kept the stirring for another 1 h. The products were centrifuged, washed with copious DI water, and finally dried at 60 °C for 8 h.

2.6. PSA detection

Scheme 1 illustrates the schematic of peptide/MNCPs-based fluorescence biosensor for the PSA detection. FAM-peptides were self-organized on the surface of MNCPs via Au-S bonds, leading to the efficient quenching of FAM fluorescence by the AuNPs. In the presence of the target, PSA specifically cleaved the FAM-peptides and released the fluorescence emitting species from the MNCPs surface, which led to the fluorescence recovery.

2.6.1. Fluorescence quenching by MNCPs

In fluorescence quenching experiments, different volumes of MNCPs (200 μg/mL) were mixed with 20 μL of FAM-peptides (10 μM) in 10 mM PBS and the volumes were adjusted to 200 μL with 10 mM PBS for further fluorescence detection. The mixture was kept with oscillation at 25 °C for 0.5 h before testing. The final concentrations of MNCPs in the cuvettes were 0, 10, 20, 30, 40, 50, 60, 80, 100, and 150 μg/mL.

2.6.2. Fluorescence recovery by PSA

In fluorescence-recovery experiments, a variable amount of PSA (1.0×10^{-9} g/mL) and 20 μL of FAM-peptides (10 μM) in 10 mM PBS were thoroughly mixed and kept at 37 °C for 1 h. An aliquot of 30 μL MNCPs (200 μg/mL) in 10 mM PBS was introduced and the volumes were adjusted to 200 μL with 10 mM PBS. Finally, the mixture was kept at 25 °C with oscillation for 0.5 h before testing.

3. Results

3.1. Characterization of materials

TEM, XPS, and EDX were first performed to determine the features of the materials and confirm the synthesis of MNCPs. Monodisperse Fe_3O_4 nanocubes of ca. 12 nm were successfully synthesized by thermal decomposition of the iron-oleate [51] (Fig. 1A). As shown in Fig. 1B, the uniform sphere-shaped Fe_3O_4@SiO_2 nanoparticles of ca. 38 nm were readily prepared by encapsulating single Fe_3O_4 nanocubes with the SiO_2 shells of ca. 13 nm using a reversal microemulsion technique