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The simultaneous detection of free and total prostate antigen in serum samples with high sensitivity and specificity by using the dual-channel surface plasmon resonance

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ABSTRACT

Free/total prostate antigen (f/t-PSA) ratio in serum as a promising parameter has been used to improve the differentiation of benign and malignant prostate disease. In order to obtain the accurate and reliable f/t-PSA ratio, the simultaneous detection of f-PSA and t-PSA with high sensitivity and specificity is required. In this work, the dual-channel surface plasmon resonance (SPR) has been employed to meet the requirement. In one channel, t-PSA was directly measured with a linear range from 1.0 to 20.0 ng/mL. In another channel, due to the low concentration of f-PSA in serum, the asynchronous competitive inhibition immunoassay with f-PSA@Au nanoparticles (AuNPs) was developed. As expected, the detection sensitivity of f-PSA was greatly enhanced, and a linear correlation with wider linear range from 0.010 to 0.40 ng/mL was also achieved. On the other hand, a simple method was explored for significantly reducing the non-specific adsorption of co-existing proteins. On basis of this, the f/t-PSA ratios in serum samples from prostate cancer (PCa) or benign prostatic hyperplasia (BPH) patients were measured. And it was found that there was significant difference between the distributions of f/t-PSA ratio in BPH patients ($16.44 \pm 1.77\%$) and those in PCa patients ($24.53 \pm 4.97\%$). This present work provides an effective method for distinguishing PCa from BPH, which lays a potential foundation for the early diagnosis of PCa.

1. Introduction

Statistics from World Health Organization (WHO) show that prostate cancer (PCa) has been the most common disease and the second primary cause of cancer mortality in many developed countries (Fernández-Sánchez et al., 2005). Moreover, once the disease metastasizes to other organisms in the body, no a curative therapy can be available. Therefore, it is very important to identify tumors before they have spread outside the prostate (Balk et al., 2003). Currently, prostate-specific antigen (PSA) has been identified as the most reliable tumor marker for the early diagnosis and prognosis of PCa (Etzioni et al., 2003; Fernández-Sánchez et al., 2004; Yu et al., 2004).

PSA is a 34-kDa serine protease enzyme produced by prostate gland. It appears in different forms, mainly free PSA (f-PSA) and a complex with protease inhibitor alpha 1-antichymotrypsin (PSA–ACT, MW 90 kDa) (Lilja et al., 1991). The total PSA (t-PSA) refers to the sum of free and complexed PSA forms (Uludag and Tothill, 2012). Because f-PSA is typically less than 1.0 ng/mL in serum (Liu et al., 2008), most analytical methods display a limited sensitivity for the detection of f-PSA. In general, a t-PSA concentration above 10.0 ng/mL means the high possibility of PCa, and that less than 4.0 ng/mL indicates the low possibility. However, as a biochemical phenomenon, there is a diagnostic grey zone for patients with t-PSA levels between 4.0 and 10.0 ng/mL, which cannot be directly solved by a more sensitive assay (Escamilla-Gómez et al., 2009). So far, the ratio of f-PSA to t-PSA (f/t-PSA) has been considered as a promising parameter for distinguishing PCa from BPH, and improving the diagnostic specificity of PCa (Ballentine, 2012; Escamilla-Gómez et al., 2009; Fernández-Sánchez et al., 2004; Pannek et al., 1998).

In order to obtain the f/t-PSA ratio, the simultaneous detection of f-PSA and t-PSA is in crucial need. Many analytical techniques (e.g. fluorescence, electrochemistry and piezoelectric) have been employed to design immunosensor for the simultaneous detection of t-PSA and f-PSA (Escamilla-Gómez et al., 2009; Grubisha et al., 2003; Jung et al., 2000). However, there are great challenges to

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achieve the accurate and credible f/t-PSA ratio in human serum because of the following elements: (a) the level of f-PSA at the early stage of cancer is particularly too low to be detected, the detection sensitivity is required to improve; (b) the non-specific adsorption of other coexisting proteins in human serum is difficult to be eliminated (Ayela et al., 2007; Trevino et al., 2009; Uludag and Tothill, 2012); (c) most of the reported immunosensors for the PSA detection are generally expensive, require skilled personnel and are associated with time-consuming sample processing (Hoa et al., 2007; Wang et al., 2007; Yuan et al., 2009). In view of these problems above, it is significant to explore an optimal and simple method to enhance the detection sensitivity and specificity for f-PSA and t-PSA, and further to achieve the accurate f/t-PSA ratio, which would be useful for the early diagnosis of PSA in the urological clinic and general practice environments.

Surface plasmon resonance (SPR), as one of the most common optical techniques, has the attractive features including the high sensitivity, label-free and real-time measurement, relatively simple procedure, and low sample consumption (Hanken et al., 1998; Phillips and Cheng 2007; Xia et al., 2010). In recent years, the surface plasmon resonance technique has been widely employed for the analysis of PSA. The PSA detection limit of 0.1 ng/mL was achieved by using standard SPR (Jung et al., 2009). Moreover, the PSA detection limits at fM or aM level were achieved by LSPR (Lee et al., 2011; Truong et al., 2012; Wang et al., 2009). However, in these reports, the preparation of plasmonic Au nanodisks arrays (Lee et al., 2011), or other complex nanostructures (Truong et al., 2012; Jung et al., 2009) are time-consuming and troublesome. Therefore, taking advantage of the excellent properties of the label-free SPR technique, more attempts should be made to design a simple and effective strategy for the analysis of PSA.

The multi-channel SPR device has some advantages of saving analytical time and cost, and also offsetting the error and signal interference. Therefore, in this work, the simultaneous detection of f-PSA and t-PSA was carried out by using the dual-channel SPR device. The concentration of t-PSA can be directly detected by SPR because of its large molecular weight and high concentration in serum. However, for the f-PSA detection, the direct SPR method is limited due to the limitation of the technique itself and the low concentration of f-PSA in serum (lower than 1 ng/mL). Therefore, in order to achieve a higher detection sensitivity of f-PSA, the asynchronous competitive inhibition assay with AuNPs-antigen (f-PSA@AuNPs) was developed. Comparing to the sandwich f-PSA assay (anti-f-PSA/f-PSA/anti-f-PSA@AuNPs) and the traditional competitive inhibition assay, our method provides the higher sensitivity and a wider linear correlation, which is significant for the detection of low level f-PSA in human serum samples. In addition, the detection of f-PSA and t-PSA in serum samples is often affected by the non-specific adsorption of other coexisting proteins in serum samples. Herein, a simple approach for significantly reducing the interference from the co-existing proteins in serum was explored. Therefore, comparing with those reported literatures (Lee et al., 2011; Jung et al., 2009; Truong et al., 2012; Wang et al., 2009), this present strategy is not only effective for achieving the high sensitivity and specificity for the detection of f-PSA and t-PSA in human serum, but also the accurate and credible f/t-PSA ratios can be obtained. Moreover, it was found that there was significant difference between the distributions of f/t-PSA ratio in BPH patients and those in PCa patients, which is significant for clinical application of the early PCa diagnosis.

2. Experimental

2.1. Regents and instruments

Sodium citrate and $HAuCl_4 \cdot 4H_2O$ were obtained from Shanghai Fine Chemicals Factory (China). Monoclonal t-PSA and f-PSA antibody (anti-t-PSA and anti-f-PSA), f-PSA and t-PSA (prepared from human seminal fluid) were purchased from Linchao Biotechnology Limited Company (Shanghai, China). PSA–ACT complex (pI: 5.5–6.2) was obtained from BiosPacific, Inc. The human serum samples were obtained from Xiangya Hospital (Changsha, China) with ethical approval. Mercaptoundecanoic acid (MUA), N-hydroxysuccinimide (NHS), ethanolamine hydrochloride (EA) and 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDC) were acquired from Sigma Aldrich (Shanghai, China). Other reagents are all of analytical purity and used as received. Deionized water was treated with a water purification system (Simplicity 185, Millipore Corp., Billerica, MA). Phosphate buffered saline (PBS, 0.01 mol/L, pH = 5.6) was used throughout.

The SPR measurements were conducted with a BI-SPR 3000 system (Biosensing Instrument Inc., USA) equipped with a dual-channel flow cell. The solutions were preloaded into 200 μL sample loop on a six-port valve and then delivered to the flow cell with a flow rate of 20 μL/min. The instrument is capable of cutting off the dispersed front and tail ends of injected sample plugs prior to introducing samples into the SPR sensing areas. All the experiments were carried out at 25 °C.

2.2. Procedures

2.2.1. Fabrication of f-PSA@AuNPs and anti-f-PSA@AuNPs

Au nanoparticles (AuNPs, 20 nm in diameter) were synthesized by referring the literature (Storhoff et al., 1998). The obtained AuNPs colloidal suspension was then adjusted to the pH value of 9.0. In order to obtain the f-PSA@AuNPs conjugates or anti-f-PSA@AuNPs, f-PSA or anti-f-PSA was mixed with the treated AuNPs colloidal suspension, incubating for 2 hours. Afterwards, the mixture solution was centrifuged at 9000 rpm for 10 min at 4 °C, and the supernatant was pipetted out to remove the excess f-PSA or anti-f-PSA. The precipitated f-PSA@AuNPs or anti-f-PSA@AuNPs conjugates were re-dispersed in PBS (0.344 μg/mL) and stored in refrigerator (4 °C) for later use.

2.2.2. Preparation of human serum sample

The blood samples of 5 PCa patients, 6 BPH patients diagnosed by the aspiration biopsy, and 2 normal middle-aged men were obtained from Xiangya Hospital (Hunan, China). These obtained samples were centrifugated at 2000 rpm for 15 min to get the blood serum. And then, the separated serum samples were treated with 100 kDa ultra-filtration membranes to filter some coexisting proteins with larger molecular weight in serum, reducing the interference from the nonspecific adsorption of co-existing proteins on the practical detection of f-PSA and t-PSA. At last, the ultra-filtered serum samples were stored at −80 °C for later use. Before the detection of f-PSA in serum sample, the real serum sample was diluted ten times using phosphate buffer solution (pH = 5.6). And for the detection of t-PSA, the serum sample was diluted two times.

2.2.3. Preparation of SPR sensor for dual-channel detection

Gold film was firstly divided into two independent zones by scraping the Au disk. Then the whole film was annealed with hydrogen flame to remove contaminants attached on the surface. A self-assembled monolayer was formed on the gold surface through submerging the Au film into 4 mmol/L MUA solution for 12 h. Afterwards, the film surface was activated by dipping into a