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Gold nanoparticle labeling with tyramide signal amplification for highly sensitive detection of alpha fetoprotein in human serum by ICP-MS

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ABSTRACT

In this work, we developed an immunoassay based on tyramide signal amplification (TSA) and gold nanoparticles (Au NPs) labeling for highly sensitive detection of alpha fetoprotein (AFP) by inductively coupled plasma mass spectrometry (ICP-MS). AFP was captured by anti-AFP1 coating on the 96-well plate and labeled by anti-AFP2-horseradish peroxidase (HRP), in which the HRP can catalyze the deposition of biotinylated tyramine on the nearby protein. Then the streptavidin (SA)-Au NPs was labeled on the deposited biotinylated tyramine as the intensive signal probe for ICP-MS measurement. Under the optimal experimental conditions, the limit of detection of the developed method for AFP was 1.85 pg/mL and the linear range was 0.005–2 ng/mL. The relative standard deviation for seven replicate detections of 0.01 ng/mL AFP was 5.2%. The proposed method was successfully applied to the detection of AFP in human serum with good recoveries. This strategy is highly sensitive and easy to operate, and can be extended to the sensitive detection of other biomolecules in human serum.

1. Introduction

Cancer is one of the most threatening killers of human beings, and a growing number of people are suffering from cancer worldwide [1]. Currently, accurate diagnosis of cancer at its early stage is regarded as the key to reduce mortality and decrease the therapy costs [2]. However, the concentration of the cancer biomarkers (DNA [3], RNA [4], protein [5], circulating tumor cells (CTCs) [6]) is extremely low and biological matrix is very complex, which make accurate highly sensitive detection of trace amounts of biomarkers still a challenge. Alpha fetoprotein (AFP) mainly generates from the liver, yolk sac and fetal human gastrointestinal tract [7]. High concentration of AFP found in adult serum is a symptom of disease, which can be taken as a clinical indicator of primary liver cancer [8]. Thus, developing simple, sensitive and reliable methods for the quantification of AFP in human serum is of great importance.

Currently, a lot of techniques have been developed for the detection of AFP, such as enzyme-linked immunosorbent assay (ELISA) [9], electrochemical luminescence (ECL) [10], electrochemical method [11,12], molecular mass spectrometry (MS) [13], inductively coupled plasma mass spectrometry (ICP-MS) [14], quartz crystal microbalance (QCM) [15] and surface plasmon resonance (SPR) [16]. Among the techniques mentioned above, ICP-MS immunoassay with elemental tags (metal chelate, metal-containing water-soluble polymer, and metal-containing nanoparticles (NPs)) has emerged and grown up as a revolutionary technique for the detection of biomolecules (protein [17,18] and DNA [19]), cells [20–22], and bacteria [23] due to its high sensitivity, wide linear range, good tolerance to sample matrix and multi-elements detection ability. Some ICP-MS based immunoassays with lanthanide chelates, such as Eu^{3+}, Sm^{3+}, Pr^{3+} chelates have been reported for the detection of AFP with LOD in the range of 0.2–8.2 ng/mL [24–26]. Metal-containing NPs have also been explored as the elemental tags for ICP-MS based immunoassay for the detection of AFP due to their high specific surface area, good biological compatibility, easy modification by biomolecules and large number of metal atoms in one NP. Compared with metal chelates, metal-containing NPs have a signal amplification effect in the methods based on element labeling and ICP-MS detection. Hu et al. [27] reported a sensitive ICP-MS based immunoassay for the detection of AFP with Au NPs labeling, in which the single-particle mode detection instead of traditional integral mode was employed. The concentration of Au NPs tagged immunocomplex could be quantified by the frequency of transient signals, and the limit of quantification was 16 pg/mL for AFP. Baranov et al. [28] proposed a new ICP-MS based immunoassay with Au nanocluster labeling for AFP detection with a LOD of 0.22 ng/mL obtained. Ko et al. [29] synthesized metal-doped inorganic NPs as elemental tag for the detection of

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AFP by a sandwich-type ICP-MS immunoassay, and a LOD of 0.37 ng/mL was reported. Zhang et al. [14] combined magnetic immunoassay with ICP-MS detection and developed a new method for simultaneous quantification of AFP and CEA in human serum with Au NPs and Ag NPs as elemental tags, respectively, and the LODs were 86 pg/mL and 54 pg/mL for AFP and CEA, respectively. Liu et al. [30] used upconversion nanoparticles (UCNPs) for the first time as elemental tags for the ICP-MS detection of AFP in human serum, and the LOD was reported to be 0.22 ng/mL. These studies indicated that ICP-MS with suitable elemental tags has great potential in sensitive detection of biomarkers such as AFP.

To further improve the sensitivity of ICP-MS based immunoassay, many signal amplification strategies have been involved. Liu et al. [17] proposed an ICP-MS based method with the signal amplification of catalytic silver deposition on immunogold tag for the determination of CEA, and the LOD was 0.03 ng/mL. Garcia-Cortes et al. [31] reported a method based on the signal amplification of Au catalytic deposition on Mn-doped ZnS QDs followed by specific ICP-MS detection of Au, which allowed the detection of prostate-specific antigen (PSA) at the low attog/mL level. Li et al. [32] developed an ICP-MS based triple-amplification system by combination of nicking-displacement, rolling circle amplification (RCA) and bio-bar-code probes for the detection of DNA target, and the LOD of 3.2×10^{-17} mol/L was obtained. He et al. [33] reported an ICP-MS based method for sensitive and specific quantification of DNA in human serum via a dual amplification of RCA and Au NPs labeling, and the LOD of 1.0×10^{-16} mol/L DNA was obtained. Zhang et al. [34] developed a highly selective and sensitive ICP-MS based method by combining Au NPs labeling with hybridization chain reaction (HCR) as the dual amplification strategy for the detection of CTCs, the LOD of 15 cells was reported. Tyramide signal amplification (TSA) is a horseradish peroxidase (HRP) mediated signal amplification method in which HRP reacts with hydrogen peroxide and the phenolic part of tyramine to produce a radical-containing quinone-like structure on the C2 group. This “activated” tyramine then covalently binds to tyrosine residues of nearby protein molecules [35]. Based on this, HRP as an immunoassay label can catalyze the deposition of a fluorophore-tyramine conjugating on the enzyme site and nearby proteins, resulting in localized enhancement of fluorescent signal. Due to its good amplification effect and simple operations, TSA has been widely used in ELISA [36], flow cytometry [37], immunohistochemistry [38], and in situ hybridization [39] for the detection of protein [40], cell [41], DNA [42] and virus [43,44].

In order to develop a simple and sensitive method for the highly sensitive detection of biomarkers by ICP-MS based immunoassay, a dual amplification strategy by combining TSA with Au NPs labeling was developed. To the best of our knowledge, this is the first report on the ICP-MS immunoassay with TSA for protein quantification. With AFP as a model protein, a sandwich immunoreaction was conducted firstly to capture target AFP on the 96-well plate, and the captured AFP was then labeled with anti-AFP2-HRP. HRP can trigger the deposition of biotin-tyramine on the nearby proteins in the presence of H_2O_2, thereafter streptavidin (SA)-Au NPs was added to couple with the biotin on the tyramine. Thus, AFP can be easily detected by ICP-MS detection of ^{197}Au with dual amplification effect. Various factors affecting the immunoassay were investigated, and the analytical performance of the proposed immunoassay was evaluated. The proposed method was applied to the detection of AFP in human serum for validation.

2. Experimental section

2.1. Instruments

An Agilent 7500a ICP-MS (Agilent, Tokyo, Japan) with a Babington nebulizer was used for the detection of ^{197}Au. The instrumental parameters for ICP-MS are listed in Table S1. An XS105 Dual Range microbalance (Mettler Toledo Instruments Co., Ltd., Shanghai, China) and BS110S electronic balance (Beijing Sartorius Instrument Systems, Inc., Beijing, China) were used for weighing the reagents. The pH values were adjusted with a Mettler Toledo 320-S pH meter (Mettler Toledo Instruments Co., Ltd., Shanghai, China).

2.2. Reagents

Anti-AFP1-coating was purchased from Linc-Bio Science Co. LTD (Shanghai, China). Anti-AFP2-HRP was purchased from Abcam (Cambridge, England). Gold chloride tetrahydrate ($HAuCl_4{\cdot}4H_2O$) was obtained from Shanghai Chemical Reagent Co. (Shanghai, China). Citric acid, formic acid and acetic acid were purchased from Sinopharm Chemistry Reagent Co. Ltd (Shanghai, China). Bovine serum albumin (BSA) and nitric acid were obtained from Aladdin Reagent Inc. (Shanghai, China). Biotin-tyramine was obtained from Makewonderbio Co. LTD (Beijing, China). The phosphate buffer saline (PBS, pH 7.4) consists of 137 mmol/L NaCl, 2.7 mmol/L KCl, 1.5 mmol/L KH_2PO_4, and 8 mmol/L K_2HPO_4. PBST consists of 10 mmol/L PBS and 0.1% Tween 20. All reagents used were analytical reagent grade unless stated otherwise. Ultrapure water (18.25 MΩ cm, Milli-Q Element, Millipore, Billerica, MA) was used throughout this work.

2.3. Preparation of Au NPs and SA-Au NPs

15 nm and 30 nm citrate-stabilized Au NPs were prepared according to the procedures reported in our previous work [22]. All glass containers and magneton were soaked in aqua regia (HNO_3:HCl = 1:3, v/v) for 3 h, then washed thoroughly with ultrapure water. $HAuCl_4$ solution (0.01% (w/v), 50 mL) was heated to boiling with vigorous magnetic stirring, and then 1% (w/v) trisodium citrate (2.5 and 1.0 mL for preparation of 15 nm and 30 nm Au NPs, respectively) was added quickly. The solution was boiled for another 10 min with stirring until the color of the solution changed from pale yellow to deep red. After the solution was cooled to room temperature with continuous stirring, 15 nm and 30 nm Au NPs were obtained. 3 nm citrate-stabilized Au NPs was prepared by reducing $HAuCl_4{\cdot}4H_2O$ with $NaBH_4$ [45]. 0.2 mL of 1% (w/v) $HAuCl_4$ solution was added into 18 mL ultrapure water with vigorous magnetic stirring before the addition of 0.4 mL of 1% (w/v) trisodium citrate. After stirring for 10 min, 0.2 mL of 0.075% $NaBH_4$ (w/v) in 1% (w/v) trisodium citrate was added. The solution kept continuous stirring for 5 min until the color of the solution changed from pale yellow to pink. Then 3 nm Au NPs was obtained. The prepared Au NPs were stored in a brown glass bottle at 4 °C.

For the preparation of SA-Au NPs conjugate, 200 μL Au NPs (58 mg/L as Au for 3 nm, 15 nm and 30 nm Au NPs, adjusted to pH 8 by 100 mmol/L K_2CO_3) were mixed with 10 μg SA overnight under 4 °C. Then the SA-Au NPs conjugate was blocked by 40 μL of 5% (w/v) BSA for 1 h, followed by centrifugation to remove the unreacted SA. Finally, the SA-Au NPs conjugate was stored in 200 μL PBS for further use.

2.4. Immunoassay procedure

The principle of the proposed ICP-MS based immunoassay was shown in Fig. 1. Firstly, the 96-well plate was washed three times with PBS. Then, 5 μg/mL anti-AFP1-coating in carbonate buffer was immobilized on the 96-well plate by adsorption at 4 °C overnight. After washing three times with PBST, 100 μL of PBS with 1% (w/v) milk powder was added into each well and incubated for 1 h to saturate the uncoated active sites. Then, the sample solution containing AFP was added to react with the anti-AFP1-coating on the 96-well plate for 1 h. Afterward, the plate was rinsed three times, and 100 μL of 100 ng/mL of anti-AFP2-HRP in PBS was added into each well and incubated for 1 h at room temperature. The excess antibody was removed by