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A sensitive and selective resonance Rayleigh scattering method for quick detection of avidin using affinity labeling Au nanoparticles

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ABSTRACT

Avidin is a glycoprotein with antinutritional property, which should be limited in daily food. We developed an affinity biosensor system based on resonance Rayleigh scattering (RRS) and using affinity biotin labeling Au nanoparticles (AuNPs). This method was selective and sensitive for quick avidin detection due to the avidin–biotin affinitive interaction. Under optimal conditions, RRS intensity of biotin–AuNPs increase linearly with an increasing concentration of avidin from 5 to 160 ng/mL. The lower limit of detection was 0.59 ng/mL. This rapid and selective avidin detection method was used in synthetic samples and egg products with recoveries of between 102.97 and 107.92%, thereby demonstrating the feasible and practical application of this assay.

1. Introduction

The detection and quantification of proteins plays an essential role in fundamental research [1]. Establishing a rapid, sensitive and specific technology for protein analysis was of great importance. Resonance Rayleigh scattering (RRS) is a phenomenon of elastic light scattering. When the excitation wavelength is near the absorption band of a scattering particle, the RRS intensity is considerably higher than the conventional light scattering intensity [2]. Therefore, RRS spectra and scattering intensities can be obtained using conventional fluorescence spectrophotometer [3]. RRS techniques gradually gained attention due to its convenient performance, simple apparatus, and high sensitivity [4,5]. To date, the RRS technique has been extensively applied in the analysis of biomacromolecules such as nucleic acids, proteins, and polysaccharides [5,6]. Recently, its use spread to the analysis of inorganic ions [7], surfactants [8], and drugs [9]. Many probes have been used with the RRS technique, such as Au nanoparticles (AuNPs), bromocresol green, graphite oxide, and rhodamine [10,11]. AuNPs were an important nanomaterials and have been widely applied as probes owing to its unique physical and chemical characteristics, such as good stability and biocompatibility [12–16]. The RRS spectrum and localized surface plasmon resonance absorption of AuNPs is sensitive to their aggregation state. So it was a good scattering probe to detect proteins.

Avidin is a glycoprotein with antinutritional property. It had a good thermal stability. And cooking times were not sufficient to adequately heat all cold spot areas [17]. Overdosing on avidin will lead to biotin-malnutrition because of interaction between avidin and biotin. Thus, the concentration of avidin, an antinutritional factor in food products, should be limited [18]. However, avidin plays an important role in the treatment of cancer. It plays an important role in the positioning and imaging of cancer cells [19]. With further research, avidin became widely used in genetic engineering because of its antimicrobial activity and insecticidal properties [20], which may cause avidin residue. Hence, the selective and rapid determination of avidin is necessary and of great significance.

Until now, a variety of methods have been investigated for the determination of avidin. The earliest method of avidin detection was a microbiological method, which required long culture times and was easily affected by the environment [21]. The efforts for avidin analysis also focused on fluorescence labeling methods [22–25]. However, these methods were vulnerable to background interference. Other methods based on immunology, such as the enzyme-labeled assay [26] had a low detection limit but were time consuming. Biosensors were also used in the study of avidin such as biotinylated gold electrode sensor [27]. This method had a low detection limit and a relatively wide detection range. But it needed tedious electrode preparation and modification, involved complex operation and equipment [28]. The existing methods for avidin detection were age-old. And in recent years, there were little new method for sensitive and rapid avidin detection.

We described a bioaffinity nanoprobe system for avidin detection using RRS technology in this work. Avidin and biotin were broadly used in biochemistry, immunochemistry, and immunoassays because they form a highly specfic and stable complex [29]. The affinity constant of the avidin–biotin complex is approximately 10^{-5} M^{-1}, which making it one of the strongest known non-covalent bonds [30]. To make

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the detection more effective and specific, biotin were used to modify AuNPs in our work.

Thus biotinylated AuNPs were used as specific scattering probes in this study. As the result of the specific interaction between biotin and avidin, RRS intensity of biotin–AuNPs was enhanced gradually. The signaling principle of the affinity biosensor was depicted. The linear relation between biotin–AuNPs and avidin based on RRS technique was established. Under optimal conditions, the limit of detection (LOD) can reach to 0.59 ng/mL with the avidin ranging from 5 to 160 ng/mL. Compared with other methods, this study was simple without a tedious pretreatment [22,25,31,32] and avidin can be selectively detected with lower LOD and wilder linear variation. The method was also applied to the detection of avidin content in food samples to prove its feasibility and practicability.

2. Experimental

2.1. Reagents And Chemicals

All chemicals used were of analytical grade. Avidin was purchased from Sigma-Aldrich (USA). $HAuCl_4$ was purchased from the Shanghai Chemical Reagent Company (China). NaOH, salts (Na^+, K^+), glucose, lactose, sodium citrate, Na_2HPO_4, NaH_2PO_4, K_2CO_3, and vitamin C were acquired from the Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Biotin, glycine, cysteine, glutathione, aspartic acid, L-serine, tyrosine, lysozyme, and BSA were purchased from the Biosharp Company (China). PEG20000 was acquired from Beijing Solarbio Science and Technology Company (China). HCl was acquired from the Xinyang Chemical Reagent Factory (China). Conalbumin and mucin were purified in our lab. Doubly distilled water (DDW) was used throughout.

Avidin was prepared in a 1 mg/mL stock solution and diluted to 1 μg/mL as working solution. Biotin was prepared in 0.2 mg/mL. All the solution was stored at 4 °C. The 0.1 mg/mL $HAuCl_4$ and 10 mg/mL sodium citrate solutions were prepared with DDW. Phosphate buffered solutions (PBS) with different pH values were prepared by mixing 0.02 mol/L Na_2HPO_4 and 0.02 mol/L NaH_2PO_4, according to certain proportions.

Egg products (egg white powder and whole egg powder) were purchased from the Kangde Biological Products Co. Ltd. (China).

2.2. Preparation of the AuNP solution

The glass apparatus used for preparing AuNPs was immersed in aqua regia (HNO_3: HCl = 1:3, v/v) for 48 h, washed by DDW several times, and then dried before use.

AuNPs were prepared according to previously published methods [33]. In a 250 mL beaker, 100 mL of 0.1 mg/mL $HAuCl_4$ was heated at 95 °C until boiling. Next, 5 mL of 10 mg/mL sodium citrate solution with the same temperature was quickly added to the beaker drop by drop and the solution was stirred at 120 rpm on a magnetic heater stirrer. AuNPs formed in 2–3 min [34]. The solution was boiled with stirring for 10 min. The color of the solution turned from blue-black to bright red. The cooled solution was stored at 4 °C, sealed and in the dark. The concentration of the above AuNP solution was 47.8 μg/mL.

2.3. Preparation of real sample

We selected egg white powder and whole egg powder as real samples to detect the avidin concentration because they are frequently used as raw materials in food processing. The egg products were pretreated by the separation process. The powder samples (178.5 mg) were added to 10 mL DDW. These mixtures were centrifuged at 5000 rpm for 15 min at 4 °C to deplete high-abundance proteins, ovalbumin and mucoprotein, using a 3-30N bench type cryogenic centrifuge (sigma, Germany). The supernatant was collected and diluted as food samples for future detection.

2.4. Conjugation of AuNPs with biotin

To prepare the biotin–AuNP nanoprobe, 1 mL of 0.2 mg/mL biotin was added to 10 mL of 47.8 μg/mL AuNPs with the appropriate pH with magnetic stirring. After stirring for 10 min, 170 μL of 3% PEG-20000 was added as a stabilizer. Next, the mixture was stirred for 15 min and stored at 4 °C [35]. The conjugation process was optimized by altering the PEG20000 concentration.

We also optimized pH value for the conjugation. The combination of AuNPs and biotin can be controlled by adjusting the pH in relation to the electrostatic interaction. The pH values of the AuNPs were adjusted using 0.1 mol/L K_2CO_3 or 0.1 mol/L HCl (PHS-3C pH meter from Shanghai Precision & Scientific Instrument Co. Ltd, China). In a 10 mL tube, 0.5 mL of 47.8 μg/mL AuNPs with different pH values and 0.05 mL of 0.2 mg/mL biotin were added. After 5 min, 50 μL of 10% KCl was added. The solution was diluted with DDW to 5 mL and mixed thoroughly. The RRS intensity of the mixed solution was detected after 30 min.

2.5. TEM detection of AuNPs and binding products

A JEM-2100 transmission electron microscope (TEM) (JEOL Ltd, Japan) was used to observe the formation of AuNPs and to measure their diameters. AuNPs and the binding products were diluted to suitable concentrations and ultrasonically dispersed for 10 min. One to two drops were placed on a copper grid and the copper network was placed on the rod sample for TEM detection after water volatilization. The acceleration voltage was 200 kV.

2.6. Procedures of RRS detection of avidin

The principle of AuNP detection of avidin is shown in Scheme 1. In this process, AuNPs were labeled with biotin and used to target avidin through the specific conjugation between biotin and avidin. The RRS intensity of biotin–AuNPs was very weak. However, it can be greatly enhanced after the adding of avidin. What's more, the color of the blank was red (Scheme 1A). After adding avidin, the color of the AuNPs turned to blue (Scheme 1B). Based on this principle, we can detect avidin using biotin–AuNPs.

In a 10 mL tube, 0.5 mL of biotin–AuNP solution, various amounts of avidin (5 to 160 ng/mL) or real samples, and 2.5 mL PBS were added. The mixture was diluted to 5 mL with DDW, and mixed thoroughly. The concentration of avidin in the food sample solutions was diluted until it reached the appropriate the linear range of the calibration curve established in this study.

An RF-5301 Spectrofluorometer (Shimadzu, Japan) was used to record RRS spectra and to measure the RRS intensity. The absorption spectra were obtained using a Nanodrop 2000c spectrometer (Thermo, USA). The RRS spectra of the solution were recorded by means of synchronous scanning at $\Delta\lambda = 0$ ($\lambda_{em} = \lambda_{ex}$) through the wavelengths ranged from 225 to 800 nm. The excitation and emission slit width were 5.0 nm. The RRS intensity of the binding product and the blank were measured at the maximum wavelength. The change in RRS intensity was obtained by subtraction of the RRS intensity of the binding product from that of the blank.

3. Results and discussion

3.1. The characterization of AuNPs and the binding system

The TEM images of the obtained AuNPs and the binding system are shown in Fig. 1A, B, C. Fig. 1A shows that the obtained AuNPs were almost spherical in morphology, and were homogeneously distributed in the solution. The relatively mean diameter of AuNPs was 11 nm. The results were similar with the literature values [12]. From the Fig. 1B, we can see that AuNPs still dispersed well even after conjugation