Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy 162 (2016) 75–80

Contents lists available at ScienceDirect

Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy

journal homepage: www.elsevier.com/locate/saa

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

Qi Wang, Xi Huang, Xuan Fu, Huan Deng, Meihu Ma, Zhaoxia Cai *

National Research and Development Center for Egg Processing, College of Food Science and Technology, Huazhong Agricultural University, Wuhan, Hubei 430070, PR China

ARTICLE INFO

Article history:
Received 13 July 2015
Received in revised form 24 February 2016
Accepted 29 February 2016
Available online 9 March 2016

Keywords:
Avidin
Resonance Rayleigh scattering
Au nanoparticle
Biotin
Egg products

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

* Corresponding author.
E-mail address: caizhaoxia@mail.hzau.edu.cn (Z. Cai).

http://dx.doi.org/10.1016/j.saa.2016.02.047
1386-1425/

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.

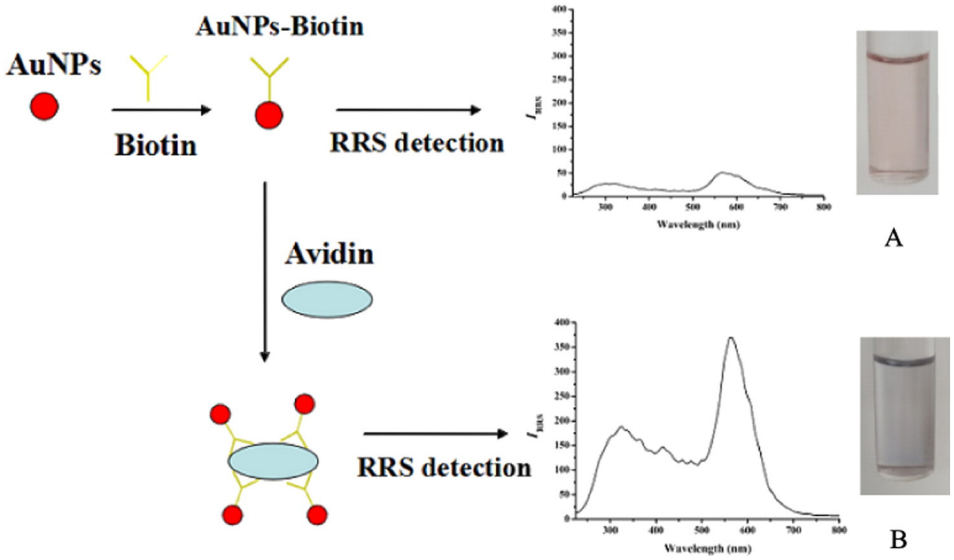
In a 10 mL tube, 0.5 mL of biotin–AuNP solution, various amounts of avidin (5 to 160 ng/mL) or real samples, and 2.5 mL PBS were added. The mixture was diluted to 5 mL with DDW, and mixed thoroughly. The concentration of avidin in the food sample solutions was diluted until it reached the appropriate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



Scheme 1. Principle of the AuNP RRS assay to detect avidin. Inset: Photography of the biotin–AuNPs without (A) and with (B) avidin under natural light.

with biotin without affecting AuNPs distribution properties. However, biotin-AuNPs aggregated with short interparticle distance after addition of avidin to the solution due to the strong and affinity interaction between avidin and biotin (Fig. 1C).

The RRS spectra of AuNPs and the avidin-biotin-AuNPs system are shown in Fig. 1D. The RRS intensities of avidin (Fig. 1D a), biotin-AuNPs (Fig. 1D b), and AuNPs (Fig. 1D c) were weak. The maximum scattering wavelength of AuNPs was 565 nm. We can see that after conjugation with biotin, the RRS intensity of the AuNPs decreases (Fig. 1D c to b). This may be the result of the PEG20000 acting as the dispersant in the conjugation system [36]. And it was identical with the conclusions in the TEM images. The RRS intensity of the conjugated AuNPs at 565 nm were greatly enhanced with the addition of avidin (Fig. 1D b to d). This indicated that biotin-AuNPs reacted with avidin and formed avidin-biotin-AuNP complex, which was mainly responsible for the enhancement of RRS intensity.

3.2. The possible mechanism of the RRS enhancement

Biotin-AuNPs were used as specific scattering probes to capture avidin in this work. Fig. 2 showed the comparison of adsorption and RRS spectra of avidin-biotin-AuNP binding products. The absorption spectrum indicated that avidin-biotin-AuNPs had a wide range of absorption, with the absorption peak at 525 nm (Fig. 2b). The RRS of biotin-AuNPs increased when avidin added (Fig. 2a). This resulted from the reaction and aggregation of the biotin-AuNPs with avidin to form a nanostructure complexation. In addition, the scattered band at 325–450 nm and the scattered peak of 565 nm were located at the absorption band at 300–550 nm. Thus, RRS happened and the scattering intensity of the binding product was much higher than conventional light scattering intensity. RRS is an unique elastic light scattering technique. When the RRS spectrum of the avidin-biotin-AuNPs binding products

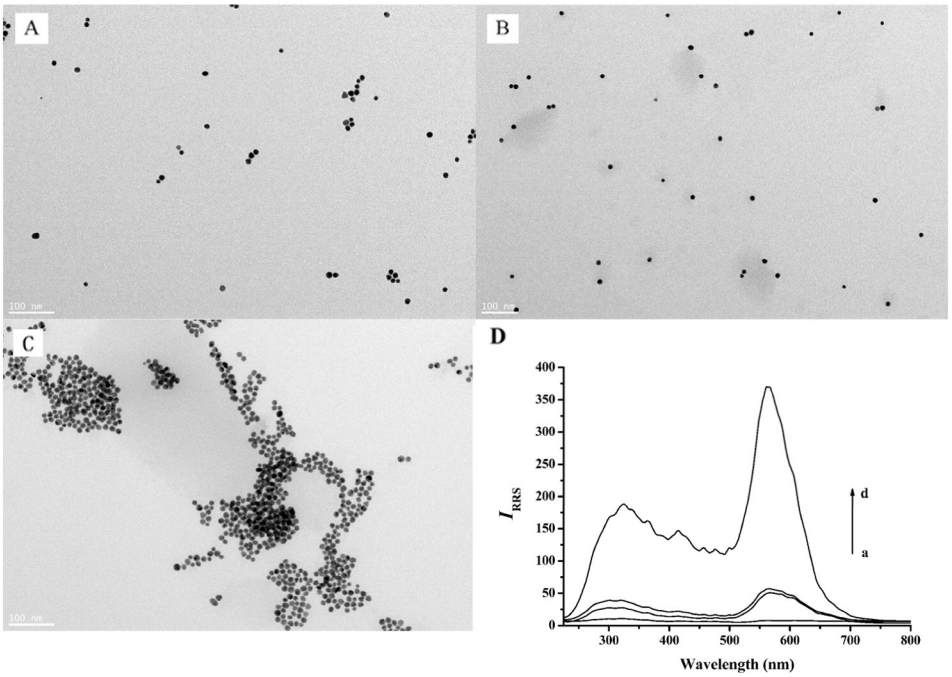


Fig. 1. TEM images of AuNPs (A), biotin–AuNPs (B), and avidin–biotin–AuNPs (C). RRS spectra of the system (D): avidin (a), AuNPs–biotin (b), AuNPs (c), and avidin–biotin–AuNPs (d).

were located at or near its absorption band, the scattering resonated with the light absorption to produce the RRS. The intensity of RRS was several orders of magnitude higher than ordinary Rayleigh scattering and did not abide to Rayleigh's scattering law [34]. Meanwhile, the TEM images in Fig. 1A and B showed that AuNPs distributed well and the interparticle distance was wider compared with avidin-biotin-AuNPs. So, we speculate the main reason of the change of RRS spectra and TEM images was due to the interaction between avidin and biotin-AuNPs.

Several mechanisms for the interaction between the biotin-AuNPs and avidin are possible, including electrostatic attraction, hydrophobic interaction, and surface bound complexation equilibrium attraction [37]. In aqueous solution, AuNPs were negatively charged supramolecular compounds with amount of citrate anion adsorbed to its surface and a strongly hydrophilic characteristic. Whereas, avidin was positively charged and strongly hydrophilic above its isoelectric point (pH 10.5). Therefore, biotin-AuNPs bind avidin through the "bridge" of citrate anions surrounding the surface of the AuNPs. The NH_3^+ moiety of amino acid residues in a peptide chain within the avidin molecule bind to the –COO of the citrate ion by electrostatic force [38]. Thus, the hydrophobicity of the binding products increased with a liquid-solid interface form between the complex and water. This produced surface-enhanced scattering and enhanced the scattering intensity. What's more, the interaction force between avidin and biotin is one of the strongest known non-covalent bonds. It was about ten thousand times than the interaction force between antigen and antibody. Avidin can binding to the ketone of imidazole ring. All of this leading to result that avidin can binding to biotin stability and specificity, not affecting by other factors such as the concentration of reagent and the pH. This combination is irreversible [30].

3.3. Optimum conditions for the reaction between AuNPs and biotin

3.3.1. Effect of PEG20000 concentration on the binding system

PEG20000 acted as the dispersing agent in this system. Appropriate concentration of PEG20000 could improve the anti-jamming ability of the system [36]. So the effect of the PEG20000 concentration on the binding system was considered (Supplemental Data Fig. S1). Increasing concentrations of PEG20000 resulted in decreasing and then stabilizing RRS intensities of both the binding product and the blank. This may be due to the aggregated AuNPs dispersal with the increasing concentration of PEG20000 in the conjugation system. However, when the PEG20000 concentration was too high, it could prevent the aggregation of AuNPs and the change in RRS intensity was small, which likely indicates that

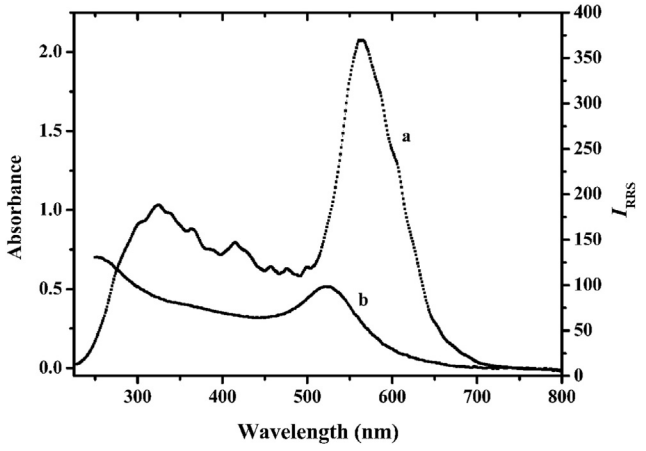


Fig. 2. RRS spectra (a) and absorption spectra (b) of the avidin–biotin–AuNP binding system.

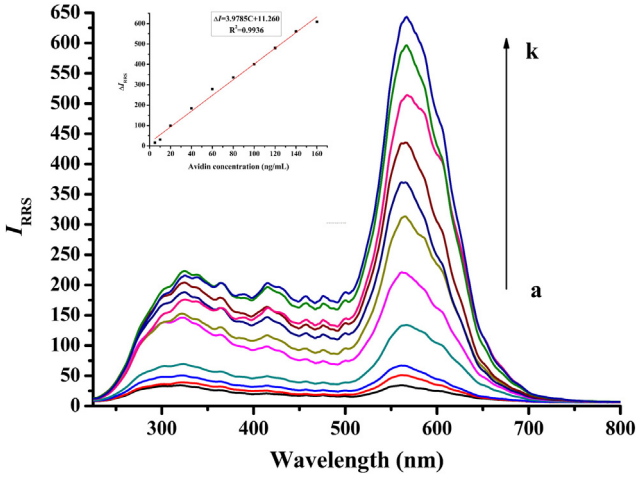


Fig. 3. RRS spectra of biotin–AuNPs with different concentrations of avidin. Concentration of avidin from (a) to (k) (ng/mL): 0, 5, 10, 20, 40, 60, 80, 100, 120, 140, and 160. Inset: Linear relationship between the change in RRS intensity of avidin–biotin–AuNPs and the avidin concentration. Avidin concentrations were 5 ng/mL, 10 ng/mL, 20 ng/mL, 40 ng/mL, 60 ng/mL, 80 ng/mL, 100 ng/mL, 120 ng/mL, 140 ng/mL, and 160 ng/mL. Biotin–AuNP concentration: 4.78 μg/mL; pH 7.5.

PEG20000 acted as a dispersing agent [36]. However, the low concentration of PEG20000 can stabilize the system. Therefore, 0.054 μg/mL PEG20000 concentration was selected for this study.

3.3.2. Effect of pH on the conjugation between biotin and AuNPs

pH played an important role on the conjugation between biotin and AuNPs. So we optimized the pH on the conjugation between biotin and AuNPs. AuNPs aggregating in the KCl solution would lead to RRS intensity increasing. When biotin conjugated with the AuNPs, it coated on the surface of AuNPs and prevented the aggregation of AuNPs in salt solution [39]. Thus, the RRS intensity of biotin-AuNPs was low. The results are shown in Supplemental Data Fig. S2. The RRS intensities of the AuNPs-biotin system decreased with increasing pH. The RRS intensity of biotin-AuNPs was high because biotin couldn't binding on AuNPs under acidic conditions. The intensity was decreased and the system stabilized because the biotin coating prevented AuNPs aggregation in the KCl solution in the neutral and alkaline range. The pH of AuNPs solution we made using sodium citrate reduction method was 7.5.

Table 1
Effect of coexisting substances on the binding system.

Coexisting Substances	Concentration (10^{-7} mol/L)	Change in RRS intensity (%)
Na^+	5000	−3.85
K^+	1000	−3.75
Glucose	1000	+3.13
Lactose	1000	+4.91
Glycine	1	−2.67
Cysteine	10	−4.49
Glutathione	100	−3.49
Aspartic acid	10	−3.90
L-serine	1	−2.67
Tyrosine	0.1	+2.33
Lysozyme	1	−4.49
BSA	0.1	−2.20
Conalbumin	0.1	−2.60
Mucin	0.001	−2.90
Vitamin C	10	−3.83

Table 2
Results for avidin determination in synthetic samples.

Synthetic sample	Main interferences (10^{-8} mol/L)	Change in RRS intensity (%)
1	K^+ 200, Glycine 2, Glucose 20, Cysteine 2, Mucin 0.002	−9.23
2	Na^+ 200, L-serine 2, Lactose 20, BSA 0.02, Glutathione 2	−9.89
3	K^+ 200, Tyrosine 2, Lactose 20, Conalbumin 0.002, Vitamin C 0.2	−6.94
4	Na^+ 200, Aspartic acid 2, Glucose 20, Cysteine 2, BSA 0.002	−7.58
5	K^+ 200, Aspartic acid 2, Lactose 20, Vitamin C 0.2, Lysozyme 0.02	−9.23

3.4. Optimum conditions for avidin detection

3.4.1. Effect of pH on the conjugation between avidin and biotin-AuNPs

Electrostatic interaction contributed to the combination of avidin and biotin. So the effect of pH on the RRS intensity of the system was investigated (Supplemental Data Fig. S3). The RRS intensity of AuNPs almost did not change with the increase of pH. However, the RRS intensity of avidin-biotin-AuNPs system changed significantly with increasing pH, showing the trend of first rise then falling. The maximum change in RRS intensity appeared at pH 7.5. When the pH was lower or higher than 7.5, the change of RRS intensity would decline because avidin has a low biological activity to biotin-AuNPs and boitin-AuNPs cannot aggregate. Therefore, pH 7.5 PBS buffer solution was chosen for this study.

3.4.2. Effect of detection temperature on the conjugation system

The influence of the temperature in the detection was investigated (Supplemental Data Fig. S4). With the increasing temperature, the maximum change in RRS intensity occured at 25 °C. When the temperature was too high, the change in RRS intensity declined because the AuNPs were slightly aggregated at a high temperature making the blank value high. When the temperature was too low, the change in RRS intensity also declined because avidin showed lower biological activity towards the biotin-AuNPs and could not occupy all the binding sites of biotin-AuNPs. However, on the whole, the influence of temperature on the detection system was little. The detection temperature chosen for further study was 25 °C.

3.4.3. Effect of the mixing sequence for each reagents on binding system

Mixing of the various reagents in a different sequence had a influence on the RRS intensity. This is in accordance with previous reports [34]. The results are shown in Supplemental Data Table S1. To obtain high sensitivity, the addition, in order, of biotin–AuNPs, PBS buffer solution, and avidin to the working solution was selected. In this mixing sequence, the highest change in RRS intensity was obtained.

3.4.4. Stability of the RRS intensity of the binding system

Under the optimum experimental conditions, biotin–AuNPs and avidin formed the binding complex and reacted completely in 10 min. Moreover, the RRS intensity remained stable for 120 min. The results are shown in Supplemental data Fig. S5. This indicated that the binding complex had good stability. After adding all reagents and mixing well, the RRS intensity could be measured within 120 min. After 120 min, the intensity increased slowly because of the aggregation of the binding product. The detection system we established in this study had a good stability. So it was significance for practical application.

3.5. The detection of avidin using conjugated AuNPs

3.5.1. Calibration curve

According to the above procedures, the calibration curve for avidin determination was constructed under the optimal conditions. The RRS spectra of biotin–AuNPs with different concentrations of avidin are presented in Fig. 3. With the increased concentration of avidin (from 5 to 160 ng/mL), the RRS intensity of the avidin–biotin–AuNP complex was enhanced proportionately. Under the optimal condition, the calibration curve was constructed according to the relationship between the avidin concentration (C) and their corresponding RRS intensity change (ΔI). The inset of Fig. 3 illustrates the linear increase in the RRS intensity with the increasing concentration of avidin. The linear ranges for avidin were from 5 to 160 ng/mL and the standard regression equation was $\Delta I = 3.9785C + 2.0808$ (C: ng/mL), with the correlation coefficient $R^2 = 0.9936$. The LOD was defined by the equation $LOD = 3S_0/K$, where S_0 was the standard deviation of blank measurements ($n = 11$) and K was the slope of the calibration graph. Here, S_0 was 0.7876 and the LOD was 0.59 ng/mL.

3.5.2. Analytical performance

The influence of coexisting substances such as Na^+, K^+, glucose, lactose, glycine, cysteine, glutathione, aspartic acid, L-serine, tyrosine, lysozyme, BSA, vitamin C, conalbumin, and mucin were investigated. The experimental results are listed in Table 1. Many of the coexisting substances, such as ions, amino acids, vitamins, and proteins, had little influence on the determination of avidin within the permission of 5.0% relative error. Therefore, this method was resistant to disturbance.

According to the interference of foreign substances, Table 2 displays the analysis of five synthetic samples. In real egg products, salts, amino acids, proteins, saccharides, vitamins, and others were present. Therefore, in each synthetic samples, we selected salts, amino acids, proteins, saccharides, and vitamins to simulate the real sample and to assess the anti-interference of our method. Table 2 showed that a certain concentration of the synthetic samples had little influence on the detection system. The strong interaction between biotin and avidin might contribute to the results. Thus, the detection of avidin had a strong anti-interference ability. The proposed method proved to be sensitive, simple and reliable.

To further prove the feasibility and practicability of this method, recovery and RSD values of avidin detection in the synthesized samples using the RRS method were investigated. The avidin concentration in the synthetic samples was set at 20 ng/mL. The concentration of proteins, amino acids, and ions added to the solution are shown in Table 3. Avidin in the synthetic samples was precisely determined by this method. The results were reliable with good recovery and RSD values.

To examine the applicability of this method, we applied this method in real products. We chose egg powder to detect because avidin had a relatively high concentration in egg white. Two egg powder samples were

Table 3
Recovery and RSD values of avidin detection in the synthesized samples using the RRS method.

Samples	Concentration of avidin (ng/mL)	Coexisting substances (10^{-8} mol/L)	Determined avidin (ng/mL, $n = 5$)	Recovery (%, $n = 5$)	RSD (%, $n = 5$)
1	20.00	Lysozyme 2.5; Conalbumin 0.25; BSA 0.25; Mucin 0.0025	21.49	107.47	7.02
2	20.00	Glycine 2; Cysteine 2; Aspartic acid 2; L-serine 2; Tyrosine 0.2	21.80	109.01	5.13
3	20.00	K_2HPO_4 12,505; NaH_2PO_4 12,505; NaCl 2500; KCl 2500	19.43	97.14	5.70

Table 4
Comparison of the linear ranges and LODs of several methods for determination of avidin.

Method	Linear range (μg/mL)	LOD (μg/mL)	Reference
Radioimmunoassay	0.1–0.2	1×10^{-3}	[32]
Fluorophore marker titration method	NM[a]	0.13	[22]
Magnetoelastic bioaffinity sensor	0.2–0.75	0.2	[31]
Biocytin fluorescence polarization assay	0.065–6.83	NM[a]	[25]
This study	0.005–0.16	5.9×10^{-4}	

Note: NM[a] indicates no mention in the paper.

pretreated to remove the high-abundance proteins by the separating procedures mentioned in Section 2.3. According to the experimental procedure, the concentration of avidin in food samples was determined. The content of avidin in egg white powder and whole egg powder were 0.56 and 0.65 μg in per mg egg powder. The avidin content we detected was lower compared with the standard value of avidin concentration in eggs, 50 mg avidin per liter egg white [18]. This may be due to the avidin loss or avidin removal during the process. Thus, the method established in this study was suitable for the determination of avidin concentration in egg products.

The data of the linear ranges and LOD obtained by various methods of avidin detection were collected and presented in Table 4 to compare the different methods. We can see from the table that radioactive labeling had a relatively low LOD value and a strong specificity [32]. However, radioactive isotope can harm the operators and pollute the environment. The LOD value of the titration method was high, thus requiring an improvement in the sensitivity of this method. Detection of avidin using a magnetoelastic bioaffinity sensor had a relatively wide linear range [31]. However, the cost was higher and the detecting process was more complex compared with other methods. The method proposed in this study had a relatively lower LOD and higher sensitivity without a long analysis time, complex operations and use of an expensive apparatus.

4. Conclusions

The RRS method based on specific interactions made the avidin detection method using biotin–AuNPs more sensitive, rapid and selective. In the established RRS system, avidin could interact with biotin–AuNPs, thus resulting in the RRS intensity increase from conjugated AuNPs. Under optimum conditions, the linear calibration equation was obtained with the detection limit 0.59 ng/mL. The RRS method established in this work was applied to quantitative determination of the avidin in synthetic samples and egg products with stable recovery and RSD values. This study highlights the sensitive and selective detection of proteins using RRS technology and specific molecular interactions.

Acknowledgements

This research was supported by the National Natural Science Foundation of China (No. 31371810) and Fundamental Research Funds for the Central Universities (2662015PY080).

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.saa.2016.02.047.

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