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Gold nanoparticles as a label-free probe for the detection of amyloidogenic protein

Hai-Jie Zhang[a], Hu-Zhi Zheng[a,*], Yi-Juan Long[a], Geng-Fu Xiao[b], Ling-Yan Zhang[a], Qin-Long Wang[a], Mei Gao[a], Wen-Jun Bai[a]

[a] College of Chemistry and Chemical Engineering, Southwest University, Chongqing 400715, PR China
[b] Wuhan Institute of Virology, Chinese Academy of Science, Wuhan 430071, PR China

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ABSTRACT

Because amyloidogenic proteins, such as prion protein, β-amyloid peptide and α-synuclein, are associated with a variety of diseases, methods for their detection are important. Recombinant prion protein (rPrP) can selectively induce aggregation of dihydrolipoic acid capped gold nanoparticles (DHLA-AuNPs), which reduces the absorbance of the DHLA-AuNPs and changes their color from red to blue. These changes were used for label-free qualitative and quantitative detection of amyloidogenic protein. The addition of NaCl improved the detection sensitivity considerably, and the detection limit was as low as 33 pmol/L.

1. Introduction

Proteins can adopt misfolded conformations under certain conditions, and this can lead to β-strand mediated insoluble fibril formation [1]. There are 20 different known syndromes associated with the formation of amyloid fibrils [2]. Abnormal deposition of amyloid β peptide (Aβ) is related to Alzheimer's disease [3], and abnormal expression and fibril formation of α-synuclein to Parkinson's disease [4]. The transformation of normal prion protein (PrP^C) to the pathological conformer (PrP^{Sc}) and the following aggregation of PrP^{Sc} may cause Creutzfeldt–Jakob disease, kuru disease, and other prion diseases [5]. Studies have shown that Aβ is an important biomarker of Alzheimer's disease, and abnormal concentrations of Aβ can be used for early diagnosis of the disease [6]. Parkinson's disease is characterized by Lewy bodies, and α-synuclein has been identified as the main component of Lewy bodies in sporadic Parkinson's disease [7]. While the abnormal prion protein act as a marker for transmissible spongiform encephalopathies infections is considered to be the causative agent [8,9]. Amyloid-related disease biomarkers have been detected by a number of methods, including immunoprecipitation and mass spectroscopy [10], enzyme-linked immunosorbent assay [11,12], nanomechanical resonator arrays and secondary mass labeling [13], surface enhancement Raman spectroscopy [14], R-hemolysin pores [15], capillary electrophoresis [8,16], fluorescence spectroscopy [17,18], and fluorescence correlation spectroscopy [19]. Some of these methods, such as immunoassays, are time and labor intensive, while others, such as fluorescence detection, require complicated labeling procedures. Xiao et al. [20] utilized aptamer modified quantum dots and magnetic microparticles in a sandwich structure with prion protein for sensitive and quantitative detection of PrP^{Sc} and PrP^C. While the use of distinct rPrP binding aptamers improves the sensitivity and specificity in prion disease diagnosis, the modification process and detection method are complicated. Therefore, the development of a simple and sensitive detection method is essential.

Gold nanoparticles (AuNPs) have excellent optical, electrical and magnetic properties. They can be assembled in various forms and have a wide range of applications in analytical chemistry, catalysis, and biology [21–23]. In addition, they are highly biocompatible, and their surface chemistry is versatile. Their distance-dependent surface plasmon resonance (SPR) absorption provides a sensitive detection strategy, and a classic example of this is the application of AuNPs to DNA hybridization detection [24]. And thiol-modified AuNPs have been used as probes for metal ion detection [25,26].

Recently, our group reported that prion protein might induce aggregation of quantum dots [27]. Based on this phenomenon, we developed a simple, fast, highly selective, and label-free detection

* Corresponding author at: College of Chemistry and Chemical Engineering, Southwest University, Chongqing 400715, PR China. Tel.: +86 23 68252360; fax: +86 23 68254000.
E-mail address: zhenghz@swu.edu.cn (H.-Z. Zheng).

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method for prion protein. However, the sensitivity of this method needs to be improved.

Here, we report prion protein induced aggregation of dihydrogen lipoic acid (DHLA) modified AuNPs. Based on this phenomenon, using prion protein as a model of amyloidogenic protein and DHLA-AuNPs as a probe, we established a new method for visual qualitative and spectrophotometry quantitative detection of amyloidogenic proteins. Addition of NaCl was investigated for improving the detection sensitivity.

2. Experimental

2.1. Reagents

Chloroauric acid ($HAuCl_4$), cytochrome c and chymotrypsin were purchased from Aladdin Reagent Co., Ltd. (Shanghai, China). Sodium citrate was purchased from Beijing Chemical Works (Beijing, China). Lipoic acid was purchased from Shanghai Bangcheng Chemical Industry Co., Ltd. (Shanghai, China). Sodium borohydride was purchased from Chengdu Kelong Chemical Reagent Factory (Chengdu, China). Tetramethylammonium hydroxide solution (1 mol/L), human serum albumin (HSA), hemoglobin (Hb), horseradish peroxidase (HRP) and glucose oxidase (GOx) were purchased from Sigma–Aldrich (St. Louis, MO). All regents used for purification of rPrP were purchased from Genview (Tianjin, China). L-glycine (Gly), L-tyrosine (Tyr), L-methionine (Met), L-phenylalanine (Phe), L-alanine (Ala), L-histidine (His), L-arginine (Arg), L-lysine (Lys), L-glutamic acid (Glu), L-aspartate (Asp), L-leucine (Leu), L-isoleucine (Ile), L-valine (Val) and bovine serum were purchased from Beijing Dingguo Changsheng Biotechnology Co., Ltd. (Beijing, China). Bovine serum albumin (BSA) was purchased from Shanghai BioLife Science & Technology Co., Ltd. (Shanghai, China). Pepsin was purchased from Shanghai Institute of Biological Products (Shanghai, China). Cellulase was purchased from Dongfeng Biochemistry Technology Co., Ltd. (Shanghai Institute of Biological Science, China). Thrombin was purchased from Heilongjiang Dilong Pharmaceutical Co., Ltd. (Suihua, China). All of the solutions were prepared with Milli-Q ultrapure water.

2.2. Measurements

The dynamic light scattering signal of DHLA-AuNPs was recorded by a Zetasizer Nano ZS90 apparatus (Malvern Instruments Ltd, Malvern, UK). The morphology and dispersivity of the DHLA-AuNPs were measured by a Tecnai G^2 F20 S-TWIN field emission transmission electron microscope (TEM) (FEI, Hillsboro, OR).

2.3. Preparation and modification of AuNPs

AuNPs (13 nm) were synthesized according to the procedures described by Frens [28]. The concentration of the resulting AuNPs (16 nmol/L) was determined according to its molar absorptivity and absorption recorded at 520 nm [29] by a UV-2450 spectrophotometer (Shimadzu, Kyoto, Japan). Because of strong covalent interaction between the sulfhydryl group and gold, we modified the AuNPs with DHLA [30]. Briefly, 50 mL of AuNPs was mixed with a toluene solution of DHLA by gentle stirring for 30 min under N_2. Then, tetramethylammonium hydroxide was added into the mixture, and the solution was stirred overnight. The organic phase was discarded and the aqueous phase was allowed to stand for 48 h at room temperature. Finally, DHLA-AuNPs were harvested by centrifugation at 10,000 rpm using a LRH-250-Z Z383K high speed bench centrifuge (HERMLE Labortechnik GmbH, Wehingen, Germany) for 1 h, and then redispered in ultrapure water. After modification, only a slight shift in the SPR band from 520 nm to 523 nm was observed (Fig. S1).

2.4. Purification of rPrP

The plasmid of recombinant human prion protein (23–231) was expressed in *Escherichia coli BL21 (DE3)* [31] and purified using a nickel ion-charged Sepharose column as described previously [32]. The protein concentration was determined with a Bio-Rad (Hercules, CA) protein assay kit.

2.5. Detection of rPrP

A solution of DHLA-AuNPs (5.0 nmol/L) was prepared in 0.40 mL of phosphate buffered saline (PBS, pH 7.40, 0.10 mol/L) at room temperature. Then a certain volume of rPrP solution was added to the mixture, and ultrapure water was added to bring the total volume to 1.0 mL. After incubating for 30 min at room temperature, the mixture was centrifuged at 3000 rpm for 10 min, and then the absorption spectra of the supernatant were record by a UV–vis spectrophotometer.

2.6. Addition of NaCl to improve the detection sensitivity

A solution of DHLA-AuNPs (5.0 nmol/L) and NaCl (50 mmol/L) was prepared in 0.40 mL of PBS (pH 7.40, 0.10 mol/L). Then a certain volume of rPrP solution was added to the mixture, and ultrapure water was added to bring the total volume to 1.0 mL. After incubating for 30 min at room temperature, the mixture was centrifuged at 3000 rpm for 10 min and absorption of the supernatant was recorded.

2.7. Determination of rPrP in real samples

Concentrations of rPrP in bovine serum and lysate of *E. coli.* expressed rPrP were determined by the standard addition method. Before determination, BSA in bovine serum was removed by a centrifugal filter device (molecular weight cutoff (MWCO) is 50,000, Millipore, Carrigtwohill, Ireland).

3. Results and discussion

3.1. Aggregation of DHLA-AuNPs induced by rPrP

There was no notable change in the size or dispersion of the AuNPs after modification with DHLA (Fig. S2). After addition of 50 nmol/L rPrP to the DHLA-AuNPs, the solution rapidly changed color from red to blue and the SPR band near 523 nm decreased in intensity (Fig. S3). These changes could be used for qualitative and quantitative detection of amyloidogenic protein. TEM images indicated that the DHLA-AuNPs aggregated after addition of rPrP, and the aggregation was increased when the rPrP concentration increased (Fig. 1). Dynamic light scattering results also verified the hydrodynamic diameter of the DHLA-AuNPs changed considerably when the rPrP concentration increased (Fig. S4).

The effect of coexisting metal cations, amino acids, and proteins that are commonly found in biological samples on the protein detection was evaluated by adding these compounds and cations to the sample solution. Amino acid concentrations in biological samples usually less than 0.50 mmol/L, while proteins such as Hb and thrombin are typically present in blood samples at low micromolar levels. In the present study, 0.75 mmol/L solutions of positively charged (His, Arg, and Lys) and negatively charged (Gly, Tyr, Met, Phe, Ala, Glu, Asp, Leu, Ile, and Val) amino acids were added to the PBS to 15 μmol/L and did not interfere with the protein detection. When 0.1 mmol/L solutions of metal ions (Ca^{2+}, Ag^{+}, Cd^{2+}, K^{+}, Pb^{2+}, Ni^{2+}, Zn^{2+}, Cu^{2+}, Co^{3+}, Al^{3+}, Cr^{3+}, Hg^{2+}, and Mg^{2+}) and different concentration of proteins (BSA, HRP, pepsin, GOx, Hb, thrombin, cellulose, HSA, cytochrome c, and chymotrypsin) were added to

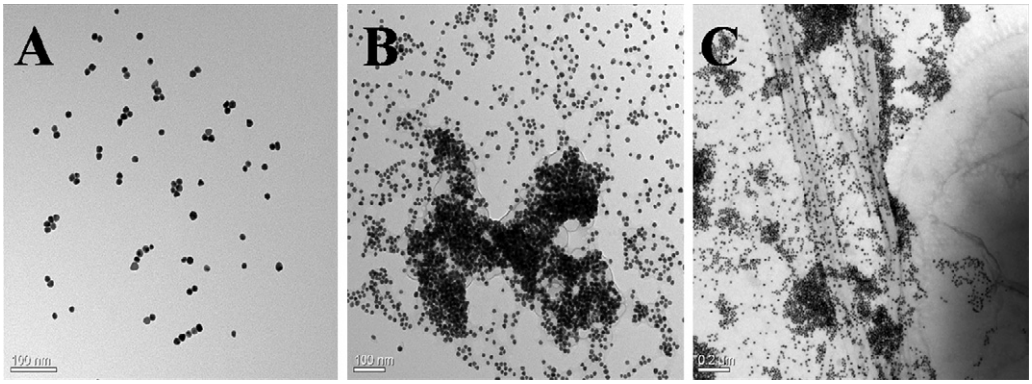


Fig. 1. TEM images of DHLA-AuNPs mixed with rPrP. (A) DHLA-AuNPs and 20 nmol/L rPrP. (B) DHLA-AuNPs and 100 nmol/L rPrP. (C) Precipitation of DHLA-AuNPs and 200 nmol/L rPrP negatively stained with phosphotungstic acid.

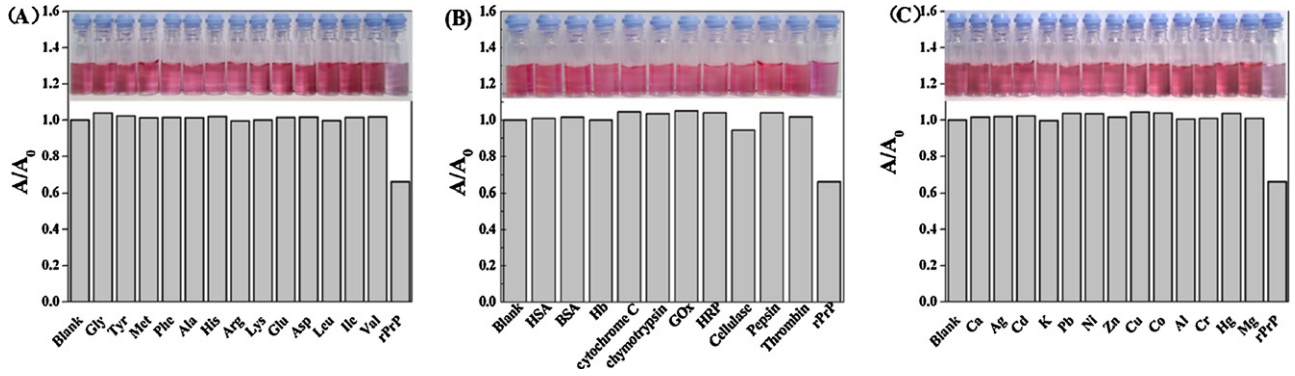


Fig. 2. Selectivity of amyloidogenic protein detection (rPrP, 15 nmol/L; AuNPs 5.0 nmol/L) with the following interference: (A) amino acids (15 μmol/L), (B) proteins (BSA 1.5 μmol/L; HRP 3 μmol/L; GOx 0.75 μmol/L; HSA 0.6 μmol/L; cytochrome c, Hb and chymotrypsin 0.3 μmol/L; pepsin and thrombin 0.15 μmol/L; cellulase 0.12 μmol/L) and (C) metal ions (0.75 μmol/L). A_0 is the absorption of the DHLA-AuNPs solution. A is the absorption of the DHLA-AuNPs in the presence of amino acids, proteins, metal ions, or rPrP.

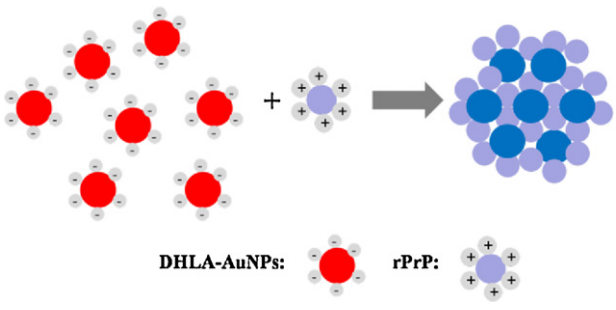
the DHLA-AuNPs solution, no aggregation or absorption change was observed (Fig. 2). Although the concentrations of BSA and GOx used in the present study are a little lower than physiological concentrations, diluting the biological sample and ultra-filter could reduce their influence. The molecular weight of BSA and GOx are 67 kDa and 160 kDa, respectively. They can be removed by a centrifugal filter device with a MWCO of 50,000. These results confirm the DHLA-AuNPs have high selectivity for the detection of amyloidogenic protein, and this probe could be used with biological samples. By visual analysis, 50 nmol/L of rPrP could be detected using DHLA-AuNPs as a probe.

The isoelectric point (pI) of rPrP is about 10.0. Under the present experimental conditions (pH 7.40), rPrP is positively charged and the DHLA-AuNPs are negatively charged, and electrostatic interaction occurs. The large surface area of the DHLA-AuNPs provides a suitable binding site for rPrP, which increases local protein concentrations and promotes oligomer formation on the AuNPs surfaces [33,34]. Oligomer formation could then lead to aggregation of rPrP. The electrostatic interaction between rPrP and free DHLA-AuNPs then results in further absorption of the AuNPs onto the surfaces of the rPrP aggregates. This process continues and finally forms large aggregates (Scheme 1). When the aggregates become large enough, they precipitate very rapidly. The precipitated aggregates contain many DHLA-AuNPs, which considerably reduces the distance between the AuNPs. This means that rPrP induce aggregation of the DHLA-AuNPs turns the solution from red to blue.

The electrostatic interaction between rPrP and DHLA-AuNPs was beneficial for the aggregation of DHLA-AuNPs, but it would not be the only contributing factor to DHLA-AuNPs aggregation. Under the same conditions, positive charged interferences, such as 15 μmol/L Arg (pI 10.76), Lys (pI 9.60) and 40 nmol/L cytochrome c (pI 9.8), chymotrypsin (pI 8.1), did not induce DHLA-AuNPs precipitation. This demonstrates that the aggregation is not only caused by the electrostatic interaction. The TEM image showed that both aggregation of DHLA-AuNPs and fibril formation of rPrP occurred (Fig. 1C). Because the DHLA-AuNPs promoted the fibril formation, and fibril formation accelerated precipitation of the DHLA-AuNPs, this provides a very sensitive and selective method for amyloidogenic protein detection.

3.2. Quantitative detection of rPrP

After mixing the DHLA-AuNPs with rPrP, the AuNPs precipitated. As a result, the SPR band of the DHLA-AuNPs near 523 nm decreased in intensity. As the rPrP concentration increased, the SPR absorption decreased linearly.



Scheme 1. Interaction between DHLA-AuNPs and rPrP.

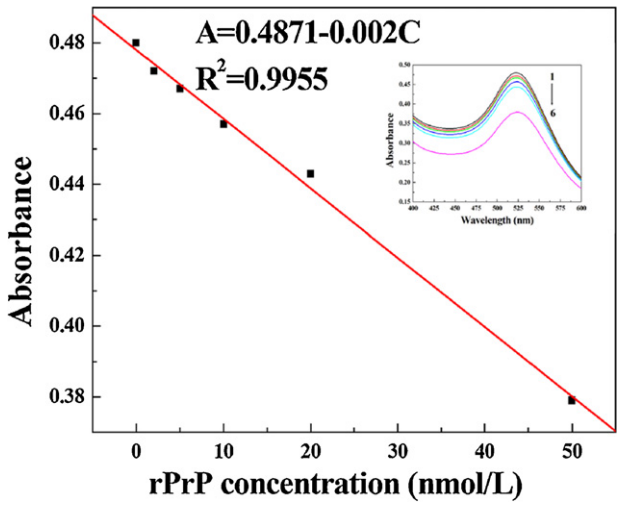


Fig. 3. Linear plot of the SPR band intensity at 523 nm for the DHLA-AuNPs solution (5.0 nmol/L) against the rPrP concentration in PBS. Concentration of rPrP: (1) 0, (2) 2.0, (3) 5.0, (4) 10, (5) 20, and (6) 50 nmol/L.

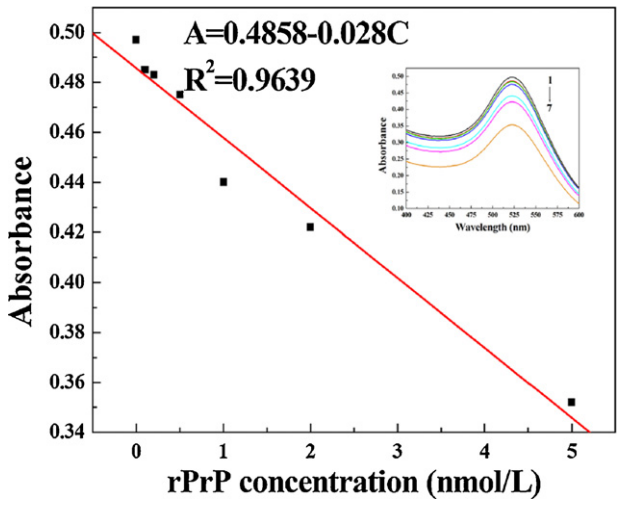


Fig. 4. Linear plot of the SPR band intensity at 523 nm of the DHLA-AuNPs solution (5.0 nmol/L) against the rPrP concentration at pH 7.40 PBS (50 mmol/L) in the presence of 50 mmol/L NaCl. The insert shows the corresponding spectrogram. Concentrations of rPrP: (1) 0, (2) 0.10, (3) 0.20, (4) 0.50, (5) 1.0, (6) 2.0, and (7) 5.0 nmol/L.

The effect of pH on rPrP detection was investigated (Fig. S5A). The highest detection sensitivity was obtained at pH 4.00, but the method had poor repeatability because the stability of the DHLA-AuNPs solution is strongly dependent on the pH. At pH ≤ 3, precipitation occured and the solution turned blue immediately. Therefore, we chose pH 7.40 as the optimum pH for high repeatability. The concentration of DHLA-AuNPs and the incubation time were also optimized at 5.0 nmol/L (Fig. S5B) and 30 min (Fig. S5C), respectively.

Under the optimum conditions, the absorbance at 523 nm exhibited a linear relationship with the concentration of rPrP from 2.0 to 50 nmol/L. The linear regression equation was $A = 0.4871 - 0.002\,C$ ($R^2 = 0.9955$) (Fig. 3), where A is the absorbance at 523 nm and C is the concentration of rPrP. The detection limit was 0.7 nmol/L (3σ), and the relative standard deviation was 1% ($n = 11$) for 15 nmol/L rPrP. It should be noted that traditional tests for prion protein, such as enzyme-linked immunosorbent assay, required expensive and sophisticated equipment or a high level of training. Protein misfolding cyclic amplification [35] technology, which is time consuming (days to months), is not amenable to large-scale screening purposes and is limited by accuracy. By comparison, with our spectroscopic method using AuNPs as a label-free probe, prion protein can be detected colorimetrically in minutes and quantitatively at 2.0–50 nmol/L concentrations in half an hour.

3.3. Addition of NaCl to improve the detection sensitivity

In the presence of 10 mmol/L NaCl, the citrate capped AuNPs changed color rapidly from red to blue because of aggregation. By contrast, the DHLA-AuNPs remained stable with the high concentration of NaCl, and no absorption change was observed even in the presence of 100 mmol/L NaCl. Interestingly, after addition of NaCl, the detection sensitivity for rPrP improved dramatically (Fig. S6). Amyloidogenic protein fibril formation mainly depends on the hydrophobic and electrostatic interactions of protein [36–38]. The addition of NaCl will decrease the repulsive electrostatic interaction between the protein molecules and increase their hydrophobicity, which will allow the protein to fold easily. This acceleration in fibril formation will promote aggregation of the DHLA-AuNPs. Therefore, the detection sensitivity was improved dramatically by the addition of NaCl.

The experiments with addition of amino acids, proteins, and metal ions were repeated to evaluate the effect of interferences in the presence of 50 mmol/L NaCl in pH 7.40 PBS with the DHLA-AuNPs. No aggregation or absorption changes were observed (Fig. S7). These results show that only rPrP can decrease the absorption. As the rPrP concentration increased from 0.10 nmol/L to 5.0 nmol/L in the solution containing DHLA-AuNPs and NaCl, the intensity of the absorption peak at 523 nm decreased linearly (Fig. 4). The detection limit was approximately 33 pmol/L (3σ). The linear equation was $A = 0.4858 - 0.028\,C$ ($R^2 = 0.9639$), where A is the SPR absorbance intensity of the supernatant of the DHLA-AuNPs solution after addition of various concentration of rPrP, and C is the concentration of rPrP. Transmission studies indicate that prions may also be present in blood, potentially allowing for ante mortem diagnosis, but the sensitivity of currently available analytical methods is insufficient for the detection of the extremely low prion concentrations that can be expected in body fluids [39]. In addition, it is possible that some infected patients may never develop clinical symptoms but will remain asymptomatic carriers who can potentially transmit the disease to other individuals [40,41]. The increased sensitivity of the label-free AuNPs probe with NaCl may be suitable for to detection of the low concentrations of prion protein in some biological samples.

3.4. Interference from coexisting substances

The AuNPs-based label-free method was highly selective for amyloidogenic protein detection. In biological samples, such as human serum, there are many coexisting metal ions, amino acids, and proteins that may interfere with the detection of the amyloidogenic protein. A number of coexisting substances (Table S1) were studied for potential interferences. With these substances, a relative error of less than ±5% was considered to be acceptable. Most of the amino acids, proteins and metal ions tested did not affect the determination. For example, in human serum, most amino acids are present at concentrations lower than 0.50 mmol/L, and after dilution of the serum, there were no interferences.

3.5. Detection of rPrP in a solution of E. coli lysate

Purified rPrP (0.5–10 nmol/L) was spiked into 10_fold_diluted *E. coli* lysate. The standard addition method was used to detect rPrP concentration. A calibration curve (Fig. 5) was constructed for the measurement of an unknown concentration of rPrP in the presence of 50 mmol/L NaCl. There was a linear relationship

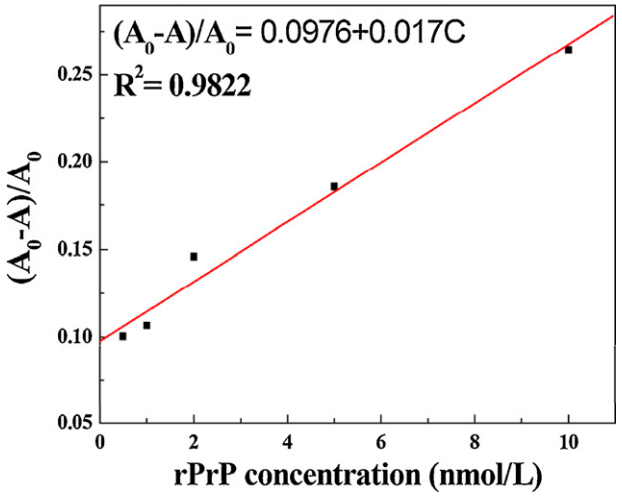


Fig. 5. Detection of rPrP spiked in a solution of lysate from *Escherichia coli* in pH 7.40 PBS. *A* is the SPR absorption of the supernatant of the DHLA-AuNPs solution after adding lysate from *E. coli* spiked with standard solutions of purified rPrP and A_0 is the SPR absorption of DHLA-AuNPs solution in the presence of lysate without purified rPrP. The concentration of DHLA-AuNPs was 5.0 nmol/L.

$((A_0 - A)/A_0 = 0.0976 + 0.017\,C,\ R^2 = 0.9822)$ between the SPR band intensity and rPrP concentration. The concentration of rPrP in the dilute solution of lysate from *E. coli* expressing rPrP was determined to be 5.7 nmol/L, and that in the undiluted lysate was 2.8 μmol/L. The prion protein was obtained by expression in *E. coli*, and after incubation for 12 h the *E. coli* was centrifuged. The prion protein was purified through a nickel ion-charged Sepharose column, and its concentration before purification was unknown. This method could be used to determine the concentration before purification. The good linear relationship between the UV–vis absorption and rPrP concentration confirmed that the probe could be applied to biological samples.

3.6. Detection of rPrP in bovine serum

Purified rPrP (0–20 nmol/L) was spiked into 10_fold_diluted bovine serum (Fig. 6). Using the calibration curve $((A_0 - A)/A_0 = 0.0003 + 0.0178\,C,\ R^2 = 0.9715)$ suitable for the measurement of an unknown concentration of rPrP in the presence of 50 mmol/L NaCl, the concentration of rPrP in the bovine serum sample was calculated at 8.5 nmol/L. The concentration of prion protein in serum is less than 50 ng/mL [42,43], some infected animals may never develop clinical symptoms but will remain asymptomatic carriers who can potentially transmit the disease to other individuals. The ability to accurately detect rPrP in the presymptomatic stages would potentially help to reduce the risk that many more animals will be infected by this fatal and terrible disease.

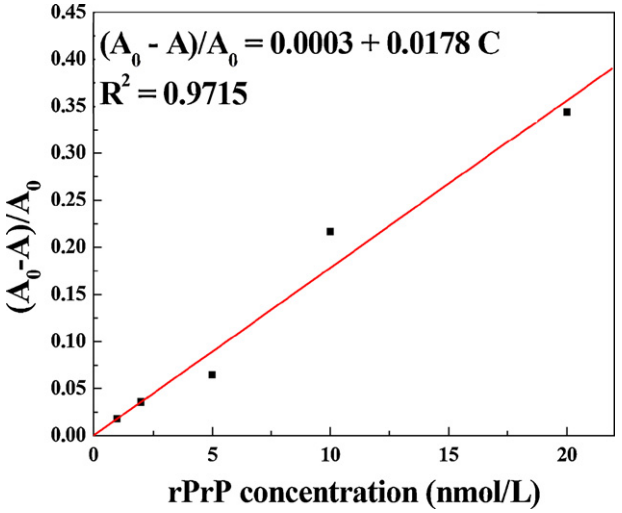


Fig. 6. Detection of rPrP spiked in bovine serum. *A* is the SPR band intensity for the supernatant of the DHLA-AuNPs solution after addition of bovine serum spiked with standard solutions of purified rPrP, and A_0 is the SPR band intensity of the DHLA-AuNPs solution in the presence of bovine serum without purified rPrP. The concentration of DHLA-AuNPs was 5.0 nmol/L in pH 7.40 PBS.

4. Conclusion

Amyloidogenic proteins, such as prion protein, β-amyloid peptide and α-synuclein, are associated with many diseases. A label-free, highly selective, and sensitive method for amyloidogenic protein detection was established using DHLA-AuNPs probes. Addition of NaCl to the probe solution notably improved the detection sensitivity, and gave a detection limit of as low as 33 pmol/L. Accurate detection of rPrP in the presymptomatic stages could reduce the infection risk to other biologicals. This method could be adapted to the detection of related diseases.

Acknowledgments

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Appendix A. Supplementary data

The following material is available free of charge via the Internet at http://www.sciencedirect.com/: Normalized UV–vis absorbance spectra of the prepared AuNPs before (Black) and after (Red) DHLA modification (Fig. S1); TEM images of the prepared AuNPs dispersion before (A) and after (B) DHLA modification (Fig. S2); UV–vis absorbance spectra of DHLA-AuNPs before (Black) and after (Red) addition of 50 nmol/L rPrP (50 mmol/L PBS, pH 7.40, DHLA-AuNPs concentration 5.0 nmol/L) (Fig. S3); (A) Hydrodynamic diameter (Size) and polydispersity index (PDI) of DHLA-AuNPs in the presence of different concentrations of rPrP (0, 2.0, 20, 30, 40, 50 and 100 nmol/L), hydrodynamic diameter of DHLA-AuNPs in the absence (B) and presence (C) of 100 nmol/L rPrP in 50 mmol/L pH 7.40 PBS, DHLA-AuNPs concentration: 5.0 nmol/L (Fig. S4); Optimization of the detection conditions. (A): pH (DHLA-AuNPs 5.0 nmol/L, rPrP concentration 15 nmol/L), (B): DHLA-AuNPs concentration (50 mmol/L PBS, pH 7.40, rPrP concentration 15 nmol/L), (C): reaction time (A and A0 are the absorption of DHLA-AuNPs in the presence and absence of rPrP respectively; DHLA-AuNPs 5.0 nmol/L; rPrP 15 nmol/L, 50 mmol/L PBS, pH 7.40) (Fig. S5); Sensitization effect of NaCl on the UV–vis absorbance spectra at 523 nm, Black: 5.0 nmol/L DHLA-AuNPs solution, Red: 5.0 nmol/L DHLA-AuNPs solution containing 2.0 nmol/L rPrP, (NaCl concentration: 0, 10, 20, 50 and 100 mmol/L, 50 mmol/L PBS, pH 7.40) (Fig. S6); Selectivity of the analysis of rPrP in the presence of 50 mmol/L NaCl, (A): amino acids (His, Lys, Arg, Gly, Val, Glu, Ala, Met, Phe, Tyr, and Asp 1.5 μmol/L; Leu and Ile 0.75 μmol/L), (B): proteins (cellulose, pepsin, thrombin, HSA, GOx, and BSA 20 nmol/L; cytochrome c 16 nmol/L, chymotrypsin 10 nmol/L; HRP 6.6 nmol/L; Hb 3.4 nmol/L), (C): metal ions (Cd^{2+}, Mg^{2+}, Al^{3+}, Co^{3+}, K^{+}, Cr^{3+}, and Cu^{2+} 1 μmol/L; Pb^{2+}, Zn^{2+}, Ni^{2+}, Ag^{+}, and Hg^{2+} 0.5 μmol/L, Ca^{2+} 0.3 μmol/L). A0 is the absorption of the DHLA-AuNPs solution. A is the absorption of DHLA-AuNPs in the presence of amino acids, proteins, metal ions or rPrP. rPrP concentration: 2.0 nmol/L, DHLA-AuNPs concentration: 5.0 nmol/L, 50 mmol/L phosphate buffer,

pH 7.40 (Fig. S7); and Effect of coexisting substances on the determination of rPrP (2.0 nmol/L) using the DHLA-AuNPs (5.0 nmol/L) probe containing 50 mmol/L NaCl (Table S1).

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.talanta.2011.12.052.

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