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ORIGINAL PAPER

An Immunonanogold Resonance Scattering-Quenching Probe for Rapid and Sensitive Assay of Microalbumin

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Abstract A novel and sensitive immunonanogold resonance scattering (RS) spectral probe was obtained for rapid detection of microalbumin (Malb), using 10 nm gold nanaoparticle to label goat anti-human Malb. It was based on that the gold-labeled anti-Malb took place nonspecific aggregation and exhibited a strong RS peak at 577 nm in pH 5.2 $C_6H_8O_7$–Na_2HPO_4 buffer solution containing polyethylene glycol (PEG), and the immunocomplex formed after specific reaction of gold-labeled anti-Malb with Malb, which led to a decrease in the intensity of RS peak at 577 nm considerably. The decreased RS intensity at 577 nm (ΔI_{577nm}) was linear to the concentration of Malb in the range of 4–128 ng/mL, with a detection limit of 3.2 ng/mL. The proposed method was applied to detect Malb in healthy human urine samples with satisfactory results.

Keywords Microalbumin · Nanogold-labeled · Resonance scattering quenching

Introduction

Microalbuminuria (MAU) is defined as urinary albumin excretion between 20–200 mg/L at a short time or 30–300 mg/24 h [1]. Recent studies have shown MAU is an early sign of incipient renal disease and a marker of its progression. Furthermore, MAU is a key indicator of the need for intensified treatment in diabetes mellitus and hypertension, and is one of the best predictors of cardiovascular disease in nondiabetic individuals [2–4]. Thus, the quantitative measurement of MAU was helpful to early diagnosis and the improving prognosis of diabetes. Additionally, it has an important diagnostic value for hypertensive renal disease, eclampsia, and renal injury induced by toxicants [5–8]. Some methods have been used to assay Malb, such as enzyme-linked immunosorbent assay (ELISA; [9]), radioimmunoassay (RIA; [10]), immunoturbidimetry (IT; [11]), high performance liquid chromatography (HPLC; [12–13]), magnetic immunoassay (MIA; [14]), chemiluminescene immunoassay (CLIA; [15]), fluorescence immunoassay (FIA; [16]), time-resolved fluoroimmunoassay (TRF; [17]), chip electrophoresis (CE; [18]). The above-mentioned methods have some shortages including multi-step operations, long time, using harmful regents, poor sensitivity, expensive regents and instruments. Thus, it is significant to set up a new method for Malb, with rapidity, sensitivity, economic and nontoxic reagent.

Resonance scattering spectral (RSS) method, with high sensitivity, rapidity and simplicity, has been applied to analysis of trace inorganic ions and organic compounds such as nucleic acid [19], carbohydrates [20], and proteins [21] with satisfactory results. In recent years, gold nanoparticles attract great interests because of easy preparation, unique chemical and physical properties such as RS effects and good biocompatibility [22]. After three labels including radioisotope, enzyme and fluorescent, the colloidal gold has become the fourth label, and widely applied to bioassay, immunoblotting and protein staining [23]. Our research group has developed a high sensitive, selective and simple

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RSS method to determine some antigens, combined with nanogold-labeled immunoreaction and RS effect of nanogold [24–26]. Up to date, there is no report about study of coupling RS quenching effect with nanogold-labeled immunoreaction for rapid and sensitive assay of Malb. In this paper, a new immunoresonance scattering-quenching probe was proposed for quantitative determination of Malb in human urine, with simple operation, good selectivity and high sensitivity.

Experimental

Instruments and reagents

A Model of Cary Eclipse fluorescence spectrophotometer (Varian Company, Australia), with excited slit and emission slit of 5 nm, PMT detector voltage of 400 V, was used to record the RS spectra and the RS intensity at 577 nm. A TU-1901 double beams UV-visible spectrophotometer (Beijing Purkinje General Instrument Limited Co., China) was used to record the absorption spectra. A 79-1 magnetic heat stirrer (Zhongda Instrumental Plant, Jiangsu, China) was used to mix well. A SK8200LH ultrasonic reactor (Kedao Ultrasonic Instrument Limited Company, Shanghai, China) was used to reduce incubation time. A Model of H-600 transmission electron microscope (TEM) (Electronic Stock Limited Company, Japan) was used to characterize gold particles and gold-labeled anti-Malb particles and to measure their diameters. A HH-S constant temperature water bath (Dadi Automatic Instrument Plant, Jiangsu, China) was used to control the temperature. A model of NaNo-ZS90 particle sizer and Zeta potentiometer analyzer (England) were used to record the particle size distribution and the average particle size.

Human Malb (240 mg/L) standard reagent and goat anti-human Malb (anti-Malb) serum were purchased from Jiemen Biological Reagent Limited Company, Shanghai, China (Batch number: 06040452). A 2.40 mg/L Malb working solution was obtained by diluting the stock solution (240 mg/L) with water. A working goat anti-human Malb serum solution was prepared as follows, put 1.0 mL the goat anti-human Malb serum into 10 mL volumetric flask, diluted to the marker with water. A $HAuCl_4$ solution (National Pharmaceutical Group Chemical Reagents Company, China) and a sodium citrate solution (Shanghai Chemical Reagent Plant, Shanghai, China) were used to prepare mean diameter 10 nm gold nanoparticles. Na_2HPO_4-NaH_2PO_4, Tri-HCl, citric acid–Na_2HPO_4 and citric acid–$Na_3C_6H_5O_7$ buffer solutions were prepared. The 100 g/L KCl and 0.1 mol/L K_2CO_3 stock solutions were prepared with water. 100 mg/L of PEG-4000, PEG-6000, PEG-10000, PEG-20000 were also used. All reagents used were analytical grade and the water was doubly distilled.

Preparation and characterization of colloidal gold

Gold nanoparticles in size of 10 nm were prepared by citrate reduction of $HAuCl_4$ according to the procedure described in Frens [27] with a slight modification. All glassware used in the following procedures were thoroughly cleaned with freshly prepared HNO_3–HCl (HNO_3:HCl=1:3, by volume), washed with double distilled water, and dried prior to use. In brief, a 100 mL of 0.01% $HAuCl_4$ solution was heated to boiling. After 2 min, 2.75 mL of 1% sodium citrate solution was added with vigorous stirring. The color immediately changed from pale yellow to purple then to red in end after 2~3 min, which indicated that the gold nanoparticles formed. After 7 min, the heating was stopped. The solutions were stirred continuously until it cooled to room temperature. Finally, the cooled solution was diluted with water to 100 mL and stored in the refrigerator at 4 °C.

Two procedures were used to measure the size of nanogold. TEM was accuracy, spectrophotometry was simple. We used transmission electron microscope to observe the size and uniformity of the gold particles, the diameter of gold particles was 10 nm. The gold nanoparticles had the maximum absorption wavelength at 518 nm. According to the report described in Ref. [28], the equation between gold nanoparticles size X (nm) and the maximum absorption wavelength Y (nm) was $Y=0.4271X+514.56$. Based on this equation, we could estimate the diameter of gold particles was about 8 nm, when the maximum absorption wavelength of the gold was controlled at about 518 nm.

Optimal condition for conjugation nanogold and the antibody

Optimization of pH

Gold nanoparticles were hydrophobic colloid with negative charge. The anti-Malb was absorbed on the colloidal gold particle surfaces, mainly by van der Waals force and hydrophobic interaction [29]. When the pH was proper that equal to or a little more than the isoelectric point of the antibody, the surface of antibody had no electrostatic charge with strong hydrophilic interaction that could accelerate the conjugation. In this experiment, a RS method was used to test the influence of different pH on colloidal gold labeling. We adjusted pH from 4.0 to 9.0 in 1.0 mL colloidal gold solution by adding 0.1 mol/L K_2CO_3 or 0.1 mol/L HCl to each calibration test tube and then dropped 20.0 μL anti-Malb working solution into each test tube. After 5 min, we

added 0.10 mL 100 g/L KCl. Two hours later, we diluted the solution with water to 3.0 mL. Then we took the solution to a quart cell, and placed into the Cary Eclipse fluorescence spectrophotometer. The scanning speed of 1,200 nm/min was chosen and PMT detector voltage of 400 V was used to record the RS spectra and the RS intensity by means of synchronous scanning excited wavelength λ_{ex} and emission wavelength $\lambda_{em}(\lambda_{ex}-\lambda_{em}=\Delta\lambda=0)$. The conjugation could be determined by comparing the maximum RS intensity at 545 or 577 nm (See in Fig. 1a). At the pH<6.0, the addition of the anti-Malb did not stabilize colloidal gold, the intensity enhanced since gold nanoparticles aggregated accompanying a color change from red to blue as dropping of KCl. When the pH>6.0, the system was in red, the intensity was least and stable. It could be interpreted that coating of gold particles by anti-Malb prevented aggregation of gold colloidal by the KCl. A pH 6.5 of colloidal gold was considered to be suitable for the experiment.

Optimization of antibody amounts

We added anti-Malb solution (0–35 μL) to each 1.0 mL colloidal gold solution with adjusting pH 6.5. After 5 min, we added 0.10 mL 100 g/L KCl solution to each tube and mixed well. After 2 h, we diluted the solution with water to 3.0 mL. Then we measured the intensity as the process of optimization of pH mentioned above. From Fig. 1b, we concluded the minimal anti-Malb that stabilized 1.0 mL colloidal gold was approximately at 20.0 μL. In the experiment, a 20.0 μL anti-Malb was chosen for labeling 1.0 mL colloidal gold.

Preparation of gold labeled anti-malb

We adjusted 80 mL colloidal gold solution to pH 6.5 under the magnetic stirring by adding 1.6 mL 0.1 mol/L K_2CO_3 solutions. Then we added 1.6 mL anti-Malb working serum slowly to the above colloidal gold solution, controlling the dropping time within 8 min. After stirring 10 min, we added 1.42 mL 3% PEG-20000 (the end concentration was 0.05%) as stabilizer. The mixture was stirred for 15 min and stored at 4 °C.

Procedure

We added 0.40 mL of pH 5.2 citric acid–Na_2HPO_4 buffer solution, 0.60 mL 57.96 mg/L gold labeled anti-Malb, 0.50 mL 100 mg/L PEG-4000 solution and a certain amount of Malb solution successively to 5-mL graduated tubes, mixed well. Then we diluted the mixture to 2.0 mL with water. Subsequently, we placed these tubes to ultrasonic reactor for incubation 20 min at room temperature. Took the reacted solution to a quart cell, recorded the RS spectra and the RS intensity at 577 nm (I) by means of synchronous scanning excited wavelength λ_{ex} and emission wavelength $\lambda_{em}(\lambda_{ex}-\lambda_{em}=\Delta\lambda=0)$. The blank I_b without Malb was recorded. A $\Delta I=I_b-I$ value was obtained.

Results and discussion

Principle

The colloidal gold was stable in solution by virtue of a balance electrostatic among the particles. However, addition of strong electrolyte, the balance was broken resulting an aggregation accompanying color change from red to blue. In this experiment, the gold labeled anti-Malb was stabile because the colloidal gold coated by anti-Malb, and a certain concentration of KCl could not make them aggregation. The RS intensity for gold labeled anti-Malb was very weak. However, the intensity enhanced since gold-labeled anti-Malb nanoparticles were unspecific aggregation in pH 5.2–25 mg/L PEG-4000 solutions. Gold-labeled anti-Malb and Malb had complementarity and compatibility in structure, they took place immune reaction and formed immunogold complex, which had compact structure and deposited in the end, leading to decease unspecific aggregations, which cause a decrease in the RS

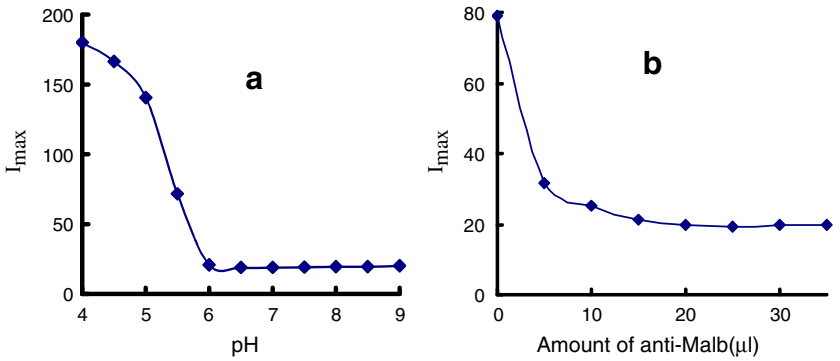

Fig. 1 Effect of conjugation pH and antibody amounts. **a** 19.32 mg/L colloidal gold-20 μL anti-Malb; **b** 19.32 mg/L colloidal gold—pH 6.5

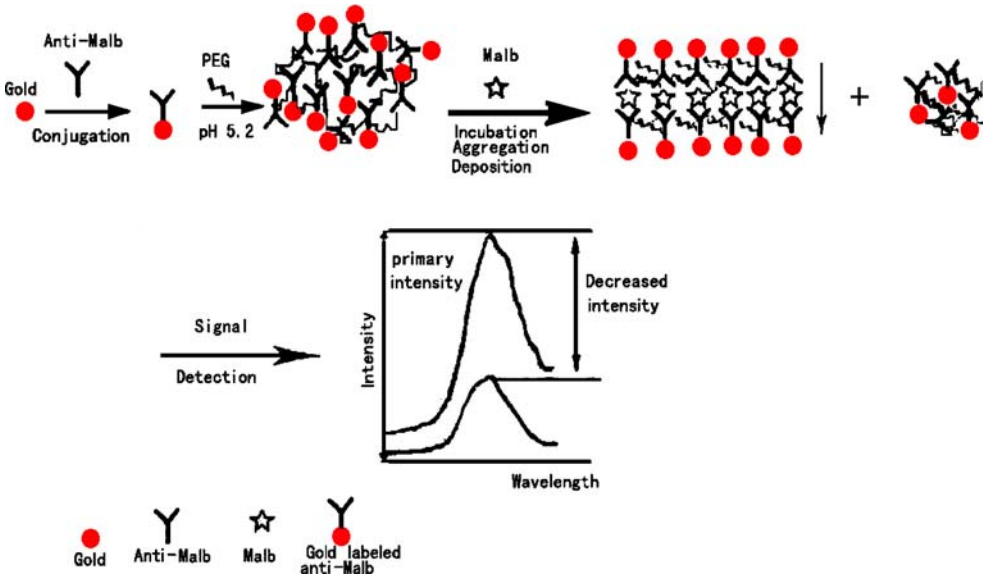

Fig. 2 The principal of gold-labeled anti-Malb and Malb immunoreaction

intensity, illustrated in Fig. 2. The decreased RS intensity was proportional to the addition of Malb concentration in a certain range. Thus, we set up a rapid and sensitive assay for Malb, coupling the RS quenching effect with nanogold-labeled immunoreaction.

Transmission Electron Microscopy (TEM)

Nanogold particles and immunonanogold particles were monodispersed in water solution (Fig. 3a), as well as diameter distribution was narrow. In pH5.2 buffer solution and in presence of 25 mg/L PEG-4000, the immunonanogold particles aggregated. TEM observations indicated that the aggregation led to cross linking of the immunonanogold clusters (Fig. 3b). The big size immunocomplex formed and deposited after immunoreaction took place (Fig. 3c), which led to a decrease in the remnant immunonanogold clusters.

Laser scattering images

We obtained the size distribution of the clusters of the gold-labeled anti-Malb before and after immunoreaction in laser scattering images (see in Fig. 4). As shown in Fig. 4a, the gold-labeled anti-Malb took place unspecific aggregations in pH 5.2–25 mg/L PEG-4000 solutions, the particle size was about 491 nm. After immunoreaction, we obtained the particle size was around 300 nm by adding 0.024 mg/L Malb (seen in Fig. 4b). We concluded that the immunoreaction resulted in a decrease the unspecific aggregations of immunogold in solution.

Absorption spectra

Figure 5 showed the absorption spectra for nanogold and the conjugates. The gold nanoparticles exhibited a characteristic surface plasmon resonance band at 518 nm (A in Fig. 5a). Upon the binding of antibody, the absorption band shifted around 4 nm to longer wavelengths compared to the nanogold particles, shown in B in Fig. 5a. It seemed that this behavior was due to surface modification, as well as the electrolyte in the solution that could affect the plasmon resonance band due to changes in screening effects and the dielectric constant of the medium [30]. However, the absorption band of gold-labeled anti-Malb shifted to long

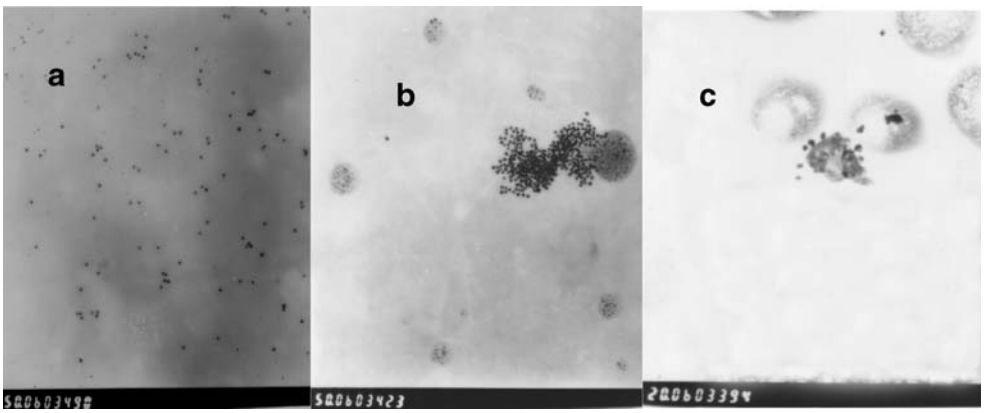

Fig. 3 TEM images. **a** 57.96 mg/L colloidal gold; **b** pH 5.2–17.39 mg/L gold-labeled anti-Malb-25 mg/L PEG-4000; **c** *B*+0.024 mg/L Malb

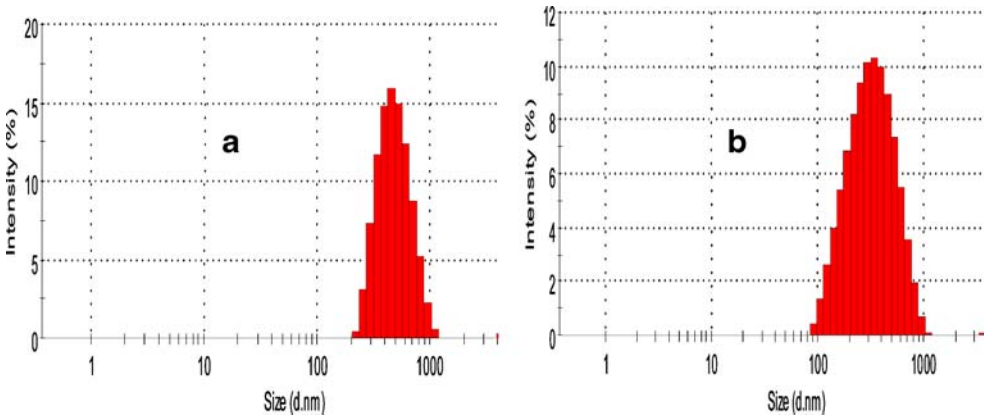

Fig. 4 Particle size distribution from laser light scattering image. **a** pH 5.2–17.39 mg/L gold labeled anti-Malb-25 mg/L PEG-4000; **b** *A*+0.024 mg/L Malb

wavelength about 532 nm in pH 5.2–25 mg/L PEG-4000 solution (see in Fig. 5b), which indicated that there were aggregations. But the immunoreactions took place between gold labeled anti-Malb and different concentrations of Malb, the absorbance decreased and the absorption band shifted to shorter wavelength. When the concentration of Malb was 0.008 and 0.024 mg/L, the absorption band was 529 and 527 nm respectively, and the absorbance decreased. It is known that the absorption band of gold particle shift to shorter wavelength along with decrement particle size.

Fluorescence spectra

The immunoreactive of antibody depend upon the tertiary structure of antibody remaining unperturbed after formation of conjugate with nanogold [31]. The tertiary structure of the antibody could be study by fluorescence measurement, by exciting the sample at a particular wavelength and monitoring the fluorescence emission from the tryptophane or tyrosine in the antibody. Figure 4 showed the fluorescence spectra of the anti-Malb and gold-labeled anti-Malb (Fig. 6). The anti-Malb and gold-labeled anti-Malb exhibited a fluorescence peak at 345 nm in Figs 4a and d, using an excited wavelength of 280 nm. After labeling, strong fluorescence quenching was observed. The quenching possibly came from the common action of two factors including absorption and scattering effect of colloidal gold at 280 nm and conjugation function. The fluorescence intensity of the anti-Malb was 76 U (F_a). Put another blank quart cell on side of incident light, the fluorescence intensity (F_b) was 52 U, when we added colloidal gold to the blank quart cell, the fluorescence intensity (F_c) was 13 U. We inferred the absorption and scattering effect of colloidal gold at 280 nm would result in a decrease in the 39 U ($F_b - F_c$). The fluorescence intensity of gold-labeled anti-Malb (F_d) was 9 U. The quenching fluorescence intensity for the interaction between gold and the antibody was 28 U [$F_a - F_d - (F_b - F_c)$].

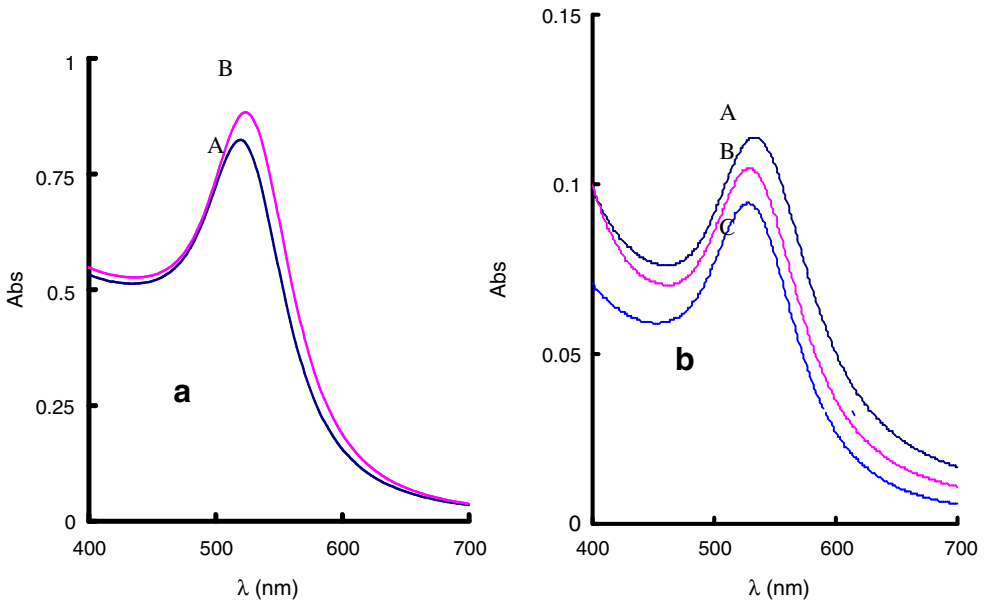

Fig. 5 Absorption spectra. **a** *A* 57.96 mg/L colloidal gold; *B* 57.96 mg/L gold labeled anti-Malb; **b** *A* pH 5.2–17.39 mg/L gold labeled anti-Malb-25 mg/L PEG-4000; *B* a+0.008 mg/L Malb; *C* *A*+0.024 mg/L Malb

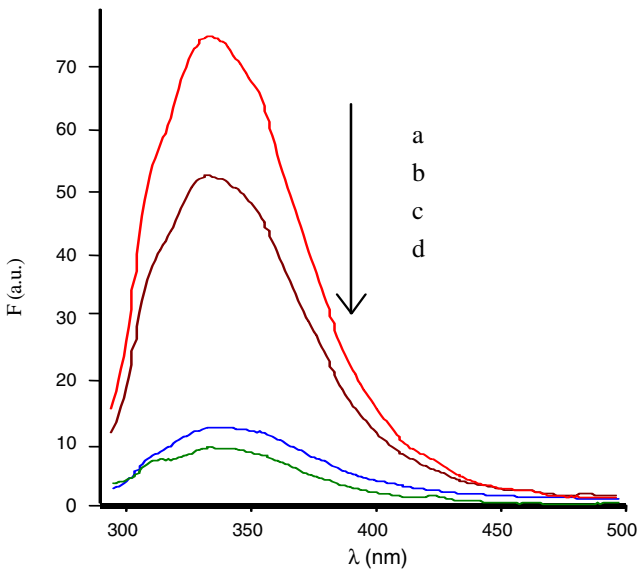

Fig. 6 Fluorescence spectra. *a* 0.04 mL anti-Malb working solution diluted to 2.0 mL; *b* a+1 cm quart cell (blank); *c* *a*+1 cm quart cell (contained 57.96 mg/L colloidal gold); *d* 2.0 mL 57.96 mg/L gold-labeled anti-Malb

Resonance Scattering Spectra (RSS)

Malb and anti-Malb are water-soluble macromolecular proteins with weak RS signal. And the gold-labeled anti-Malb also showed weak RS signals in water solution. But in pH 5.2–25 mg/L PEG-4000 solution, gold-labeled anti-Malb took place aggregation that exhibited a strongest RS peak at 577 nm (Fig. 7). After adding Malb, the immuno-complex formed and deposited, which caused a decrease in remnant of aggregation of immunonanogold and led to a decrease the RS intensity at 577 nm. This wavelength was chosen to monitor the RS intensity.

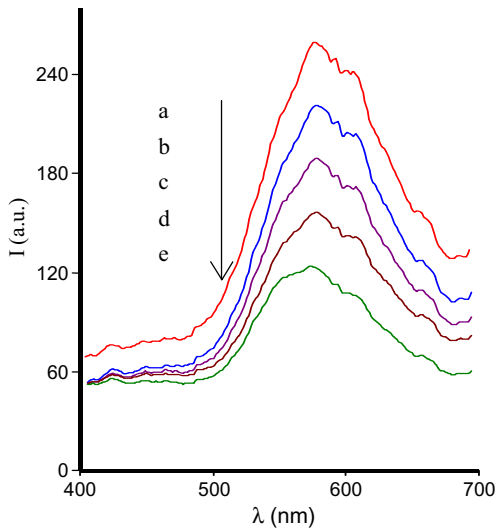

Fig. 7 Resonance scattering spectra. *a* pH 5.2–17.39 mg/L gold-labeled anti-Malb-25 mg/L PEG-4000; *b* *a*+0.008 mg/L Malb; *c* *a*+0.04 mg/L Malb; *d* *a*+0.08 mg/L Malb; *e* *a*+0.12 mg/L Malb

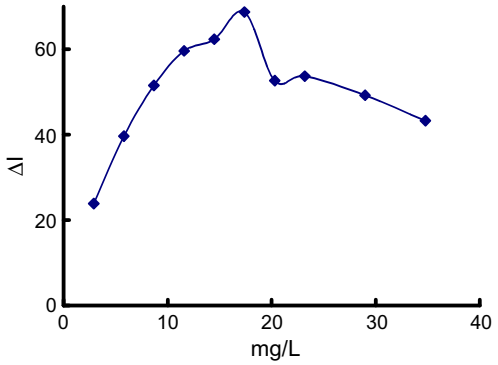

Fig. 8 Effect of labeled anti-Malb concentrations on ΔI. pH 5.2–0.024 mg/L Malb-25 mg/L PEG-4000

Effect of pH and volume of buffer solution

We tested the effect of citric acid–Na_2HPO_4 (pH 2.2–8.0), citric acid–$Na_3C_6H_5O_7$ (pH 3.0–6.6), Na_2HPO_4–NaH_2PO_4 (pH 5.0–8.0), Tri-HCl (pH 7.2–9.1) and borax–borate (pH 7.4–9.0) buffer solutions on ΔI. Results showed the optimal buffer solution was pH 5.2 citric acid–Na_2HPO_4 and its optimal volume was 0.40 mL.

Effect of gold-labeled anti-malb concentration

We studied the influence of concentration of gold-labeled anti-Malb working solution on ΔI (See in Fig. 8). In certain range, the ΔI increased along with the increase of gold-labeled anti-Malb concentration. When the concentration of gold-labeled anti-Malb was 17.39 mg/L, the system had the maximal ΔI. A 17.39 mg /L gold-labeled anti-Malb was chosen.

Effect of PEG

Low concentration of PEG could stabilize colloidal gold, but in certain pH, high concentration of PEG would lead

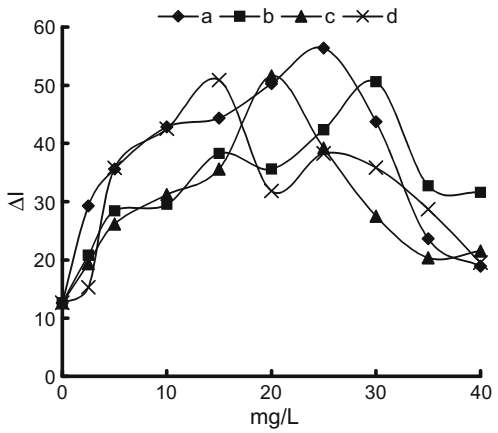

Fig. 9 Effect of different kinds and concentration of PEG on ΔI. pH 5.2–17.39 mg/L gold labeled anti-Malb-0.024 mg/L Malb; *a* PEG-4000; *b* PEG-6000; *c* PEG-10000; *d* PEG-20000

Table 1 Comparing this method and that used to assay Malb

Assays[a]	Linear range	DL	Remarks	Ref.
ET	0.015–1 mg/L	0.015 mg/L	Good sensitivity and reproducibility, but protein was absorbed by capillary tube	[1]
CL-ELISA	0.15–15 mg/L	0.089 mg/mL	High precision, high specificity	[9]
HPLC	6.1–963 mg/L	6.1 mg/L	Wide range but detected total proteins	[13]
MI	5–400 mg/L	5 mg/L	Rapid and simple but low sensitivity	[14]
FI	10–600 mg/L	4.65 mg/L	Good selectivity	[16]
TRF assay	10–320 mg/L	5.5 mg/L	Accurate	[17]
CE	5–300 mg/L	5 mg/L	Simple, robust, precision accuracy	[18]
ELISA	2–1600 ng/mL	1.2 ng/mL	Sensitive but use monoclonal antibody	[32]
IS assay	30–960 ng/mL	0.02 mg/L	Sensitive, simple, good selectivity	[33]
GIS assay	4–128 ng/mL	3.2 ng/mL	High sensitivity, good selectivity	The assay

[a] *ET* Electrophoretic technique, *CL* chemiluminescence, *MI* magnetic immunoassay, *FI* fluorescence immunoassay, *CE* chip electrophoresis, *IS* immunoresonance scattering, *GIS* gold-labeled immunoresonance scattering

aggregation of gold-labeled anti-Malb to enlarge the detection signals, and this aggregation was reversible and protein had no effect on bioactivity. We considered the influence of PEG-4000, PEG-6000, PEG-1000, PEG-20000 on ΔI (See in Fig. 9). In a certain degree, the four PEG would enhance the ΔI. When the concentration of PEG-6000 was 25 mg/L, the system had the maximal ΔI, so a 25 mg/L PEG-6000 was selected for use.

Effect of incubation time and temperature

Results showed that under the ultrasonic irradiation, the reaction relatively quick and the results kept stable after 20 min. Under the 37 °C water, the reaction relatively slow and the results kept stable after 50 min. While in 20 °C room temperature, after 60 min, the reaction still continued. So the incubation time of 20 min under the ultrasonic irradiation at room temperature was used.

Table 2 Selectivity of the assay (0.024 mg/L Malb)

Coexistent substance	Tolerance (mg/L)	Relative error (%)	Coexistent substance	Tolerance (mg/L)	Relative error (%)
L-asp	20	−1.7	Vitamin B_1	40	−2.8
L-cys	8	2.5	Vitamin B_{12}	2	−1.3
L-tyr	20	4.3	Vitamin C	8	3.4
L-lys	12	−2.2	Vitamin K_3	40	−1.6
L-arg	16	−3.1	Vitamin H	20	−4.6
L-glu	40	−4.6	Na^+ Cl^-	240	−3.2
DL-trp	7	−2.6	K^+ Cl^-	30	−2.3
DL-met	40	−3.8	Na^+ F^-	2000	−3.3
Niacin	5	2.6	Fe^{3+}	1.6	4.6
Folacin	2.7	3.9	Ca^{2+}	20	3.7
Creatine	1.8	4.8	Glucose	16	−3.9
Gly	8	−4.2	Polysucrose	3.5	−2.7
BSA	5	−3.3	Sucrose	27	−1.8
HSA	2	−1.8	Urea	13	−2.9
H-IgG	2.6	−2.5	Oxalate	12	−3.2

Linear relationship

Under the optimal condition, the ΔI for different concentration of Malb was recorded and the working curves were drawn according to the relationship between the Malb concentration C and the ΔI. The data were linearly correlated according with equation $\Delta I = 703.9C + 39.96$ with a correlation coefficient $r = 0.9983$ in the 0.004–0.128 mg/L concentration range and a 0.0032 mg/L detection limit. Compared with the methods which used to analysis of Malb in urine (See in Table 1), some needed multi-step operations, some have had poor sensitivity, others needed expensive equipment. In this paper, we coupled RS quenching effect with nanogold-labeled immunoreaction

Table 3 Results for Malb in urine sample

Sample	Found (mg/L)	Mean (mg /L)	RSD (%)	IT assay (mg /L)
1	5.32 5.67 5.03 5.26 5.41	5.39	4.3	5.96
2	8.76 8.34 8.47 8.69 8.19	8.49	2.8	7.89
3	4.21 4.56 4.27 4.28 4.15	4.29	3.6	5.26
4	11.86 11.47 11.06 11.35 11.63	11.47	2.6	12.65
5	7.63 7.86 7.32 7.54 7.65	7.60	2.6	6.80
6	17.33 18.25 17.89 17.62 17.35	17.69	2.2	17.21
7	9.52 9.31 9.57 9.83 9.65	9.58	2.0	9.03
8	8.57 8.69 8.32 8.41 8.31	8.46	1.9	9.52
9	3.54 3.67 3.58 3.21 3.33	3.41	4.5	3.71
10	6.31 6.57 6.18 6.23 6.52	6.36	2.7	5.49
11	2.16 2.14 2.35 2.17 2.29	2.22	4.2	2.76
12	10.89 10.23 10.12 10.31 10.57	10.42	3.0	10.05
13	11.37 11.86 11.59 11.67 11.83	11.66	1.7	12.36
14	5.79 5.37 5.64 5.23 5.68	5.54	4.2	4.62
15	4.72 4.35 4.26 4.57 4.69	4.52	4.5	4.06
16	7.68 7.59 7.23 7.54 7.26	7.46	2.7	7.87
17	6.37 6.54 6.59 6.21 6.43	6.43	2.3	7.01
18	15.66 15.47 15.62 15.21 15.68	15.53	1.3	15.97
19	2.46 2.52 2.31 2.51 2.36	2.43	3.8	3.01
20	7.86 7.59 7.61 7.72 7.59	7.67	1.5	8.42

to set up a new method with simple operation, good selectivity and high sensitivity, which would be widely used to assay Malb in real sample.

Effect of coexistent substances

According to the procedure, we tested the effect of coexistent substances on the determination of 0.024 mg/L Malb. When the relative error was within ±5%, the tolerated amount for coexistent substances was listed in Table 2. The result showed that the coexistent substances did not interfere with the Malb determination, which indicated that this method had good selectivity.

Analysis of samples

Twenty healthy human urine samples were centrifuged at 3,000 r/min for 10 min and the supernatants were used. The content of Malb in urine sample was determined according to the procedure (x) and IT method (y) simultaneously (See in Table 3). The regression analysis of the two assays was made, the regression equation, correlation coefficient, slope, intercept were $y=0.99x+0.26$, 0.985, 0.99, 0.26 mg/L, respectively. The data demonstrated the results of the both methods were consistent on the whole. In healthy subjects, the concentration of albumin in urine was ordinarily present in the low mg/L range [34]. The content of Malb measured by this assay was somewhat consistent with that reported previously.

Conclusion

A new immunonanogold resonance scattering assay has been proposed for determination of Malb with high sensitivity, good selectivity, based on coupling RS quenching effect with nanogold-labeled immunoreaction. This assay has been applied to detect the Malb in real sample, with satisfactory results.

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