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ORIGINAL PAPER

A Highly Sensitive Aptamer-Nanogold Catalytic Resonance Scattering Spectral Assay for Melamine

Aihui Liang · Lianping Zhou · Huimin Qin · Yi Zhang · Huixiang Ouyang · Zhiliang Jiang

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Abstract The aptamer (ssDNA) was used to label nanogold (NG) particle to fabricate an aptamer–nanogold (NGssDNA) probe for melamine. The probe was stabile in pH 6.6 Na_2HPO_4–NaH_2PO_4 buffer solutions and in the presence of high concentration of electrolyte. Upon addition of melamine, it interacted with the probe to form big NGssDNA-melamine aggregations that led to the resonance Rayleigh scattering (RRS) intensity at 566 nm increased greatly. The increased RRS intensity (ΔI) is linear to melamine concentration in the range of 1.89–81.98 μg/L, with a detection limit of 0.98 μg/L melamine. The unreacted probe in the aptamer reaction solution exhibited strong catalytic effect on the slow Cu_2O particle reaction between glucose and Fehling reagent, but the catalytic activity of NG aggregations is very weak. When melamine concentration increased, the unreacted probe decreased, the RRS peak intensity at 614 nm decreased. The decreased RS intensity is linear to melamine concentration in the range of 0.63–47.30 ng/L melamine, with a detection limit of 0.38 ng/L. The aptamer-modified nanogold catalytic RRS assay was applied to determination of melamine in milk, with high sensitivity and selectivity, simplicity and rapidity.

Keywords Melamine · Aptamer · Nanogold catalysis · Resonance Rayleigh scattering spectral assay

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Introduction

Melamine, also called as 2, 4, 6-three amino-1, 3, 5-triazine skeleton, was mainly used in the synthesis of melamine formaldehyde resins [1]. Because of melamine has as high as 66% nitrogen, it was abused in feed and food as adulterate protein-rich by unethical manufacturers [2, 3]. Melamine has low toxicity, and could form insoluble crystals in combination with cyanuric acid in the discharge process, leading to the formation of kidney stones, causing the kidney failure for infants and children, and ultimately death [2]. Thus, it was necessary greatly to explore a fast, simple, sensitive and inexpensive method to detect melamine. Currently, available techniques for detecting melamine include gas chromatography [3], liquid chromatography [4], liquid chromatography-mass spectrometry [5], colorimetric detection [6, 7], electrochemical method [8]. However, some of them were not sensitive, some were not selective, and some of them operations are complicated.

Aptamer with high affinity, low cost, good stability, easy and quick preparation in vitro and functionalized, can bind specifically to a wide range of target molecules, and has been widely used in bioanalysis [9–12]. Nanogold (NG) has unique physical and chemical properties, and has an important role in chemical and biochemical analysis [13–15]. Combined the resonance Rayleigh scattering (RRS) effect of nanogold with aptamer, several aptamer-modified nanogold RRS assays were reported to detect trace Hg^{2+} [16], urinary adenosine [17], and cysteine [18]. To enhance the sensitivity, the nanogold catalytic reaction and centrifugal separation and membrane separation techniques were utilized in RRS analysis, and several immunonanodold and aptamer-labelled nanogold catalytic RRS assays have been proposed [19–21]. However, the separation step for the

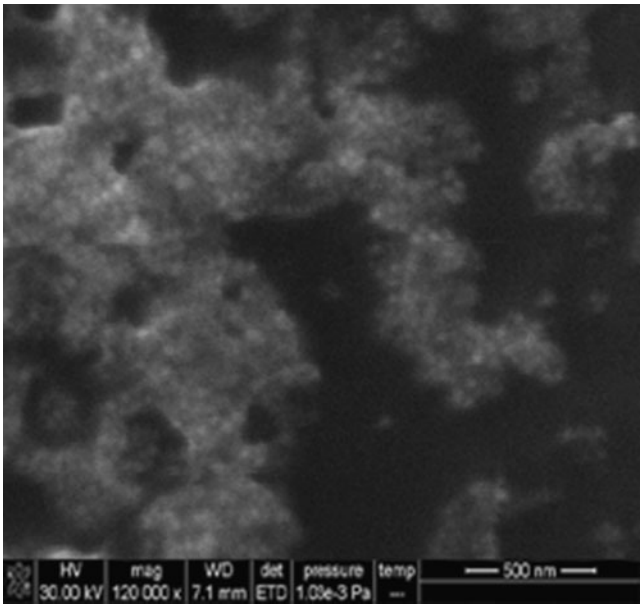

Fig. 1 SEM of NGssDNA-MA system. 11.43 μg/mL NGssDNA −50.44 μg/L MA

nanogold catalytic RRS assay was used to prolong the analytical time, and there is no report about the catalytic effect of the aptamer-modified nanogold aggregations. In this article, based on the high affinity between aptamer and nanogold, an aptamer-modified nanogold RRS probe was prepared for the direction determination of melamine. Furthermore, combining aptamer-nanogold reaction, the great difference both the NGssDNA and its aggregation catalytic activity, with the Cu_2O particle RRS effect, a highly sensitive and selective, simple and rapid aptamer-modified nanogold catalytic RRS technique was proposed to detect melamine.

Experimental

Apparatus and Reagents Cary eclipse fluorescence spectrophotometer (US Varian, Inc.), 79–1 magnetic stirrer with heating (Zhongda Instrumental Plant, Jiangsu, China), and SK8200LH ultrasonic reactor (Shanghai Kedao Ultrasonic Instrument Co., Ltd., operating frequency is 59 kHz) were used.

2.9×10^{-2} mol/L $HAuCl_4$(National Pharmaceutical Group Chemical Reagents Company, China), 1.00×10^{-5} mol/L melamine (MA) (National Pharmaceutical Group Chemical Reagents Company, China), 0.05% sodium borohydride, aptamer (ssDNA) with sequence of TTTTTTTTTTTTTTTTTTTTTTTTTTTTTT (Invitrogen Company), 1.0% trisodium citrate (Shanghai Chemical Reagent Plant, Shanghai, China), 2.0 mol/L NaCl and 10 mg/mL glucose. solution were used. Fehling solutions include a 0.28 mol/L $CuSO_4$ and 1.23 mol/L $C_4H_4O_6KNa$ containing 6.25 mol/L NaOH. A 0.20 mol/L Na_2HPO_4 and 0.20 mol/L NaH_2PO_4 solutions were used to prepare different pH phosphate buffer solution according to a certain volume ratio. Nanogolds were prepared by sodium borohydride reduction of $HAuCl_4$, according to the procedure described in the reference [22, 23]. Into a conical bottle containing 80 mL water, 1.0 mL of 2.9×10^{-2} mol/L $HAuCl_4$ and 7.0 mL of 1.0% sodium citrate solution were added under the stirring. Then 7.0 mL of 0.05% sodium borohydride solutions were dropped slowly. After 10 min, it was diluted to 100 mL with water. The concentration of nanogold was 57.13 μg/mL. The preparation steps of the aptamer-nanogold (NGssDNA) probe were followed, 2.5 mL 0.3 μmol/L ssDNA and 30.0 mL 57.13 μg/mL nanogold solution were added into a 50 mL conical flask, mixed well, and holding for 5 min at room temperature. Its concentration, calculated as Au, is 52.74 μg/mL. All reagents were of analytical grade and the water was doubly distilled.

Table 1 Relationship of gold concentration and the $\Delta I_{614\ nm}$

Form	Regress equation	Linear range (ng/mL Au)
NG	$\Delta I_{614\,nm} = 93.4C_{Au} + 2.0$	0.1–3.6
NG aggregation	$\Delta I_{614\,nm} = 12.84C_{Au} + 1.1$	0.2–3.6
NGssDNA	$\Delta I_{614\,nm} = 45.0C_{Au} + 2.1$	0.3–6.0
NGssDNA agregation [a]	$\Delta I_{614\,nm} = 7.40C_{Au} - 1.8$	0.5–6.0

[a] The NGssDNA reaction solution containing 0.50 mmol/L melamine

Preparation of Sample A 5.0 mL of 1% trichloroacetic acid solution and 1.0 mL of 0.4% lead acetate solution were added to1.0 mL of liquid milk sample in a 10 mL centrifuge tube, mixed well and ultrasonic extraction 20 min, standing for 5 min. The mixture was centrifuged at 10,000 rpm for 10 min, and the filtrate was obtained by a nylon filter in pore size of 0.15 μm. Then 1.0 mL the filtrate was transferred into another centrifuge tube and adjusted to pH 5 with 4.8 mol/L NaOH, and diluted to 10 mL with water.

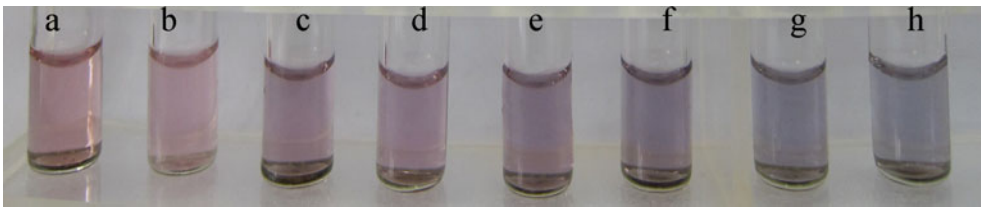

Fig. 2 The color change of NGssDNA-MA systems. The concentration of a, b, c, d, e, f, g and h was 0, 12.61, 25.22, 37.84, 50.45, 56.75 and 63.06 μg/L respectively

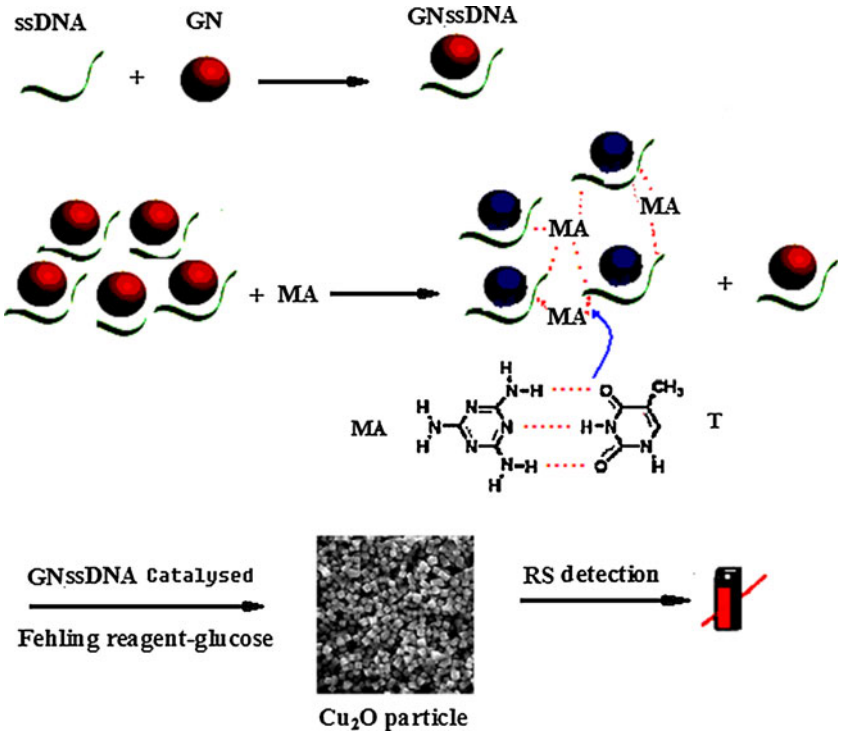

Fig. 3 Principle of NGssDNA probe catalytic RRS assay for MA

Procedure A 300 μL of 52.74 μg/mL NGssDNA, 400 μL pH 6.6 of Na_2HPO_4-NaH_2PO_4 buffer solution and a certain amount of melamine solution were added into a 5 mL marked tube in turn, and mixed well. Ten minutes later, a 35 μL 2.0 mol/L NaCl solution was added, diluted to 2.0 mL with water, and mixed well. The aptamer reaction solution was diluted 40 times for use.

A 30 μL 0.28 mol/L $CuSO_4$ solution, 0.28 mL 1.23 mol/L $KNaC_4H_4O_6$, 50.0 μL the diluted aptamer reaction solution, and 0.20 mL 10 mg/mL glucose was added successively to a 5 mL graduated test tube, diluted to 2.5 mL, mixed well, and placed the tube in a water-bath at 70 °C for 7 min. Stop the reaction by tap water cooling. A part of the solutions was transferred into a quartz cell. The settings were as follows, volt = 450 v, excited slit = emission slit = 2.5 nm, emission filter = 1%T attenuator, $\lambda_{ex} - \lambda_{em} = \Delta\lambda = 0$. The RRS intensity at 614 nm ($I_{614\ nm}$) and the blank solutions without melamine $(I_{614\ nm})_b$ were recorded. The value of $\Delta I_{614\ nm} = (I_{614\ nm})_b - I_{614\ nm}$ was calculated.

Results and Discussion

The ssDNA interact with NG to form stable NGssDNA probe through the van der Waals force and intermolecular force, in pH 6.6 Na_2HPO_4-NaH_2PO_4 buffer solutions and in the presence of 35 mmol/L NaCl solution. Upon addition of melamine, it can combined with NGssDNA to form big NGssDNA-melamine aggregations (Fig. 1), and the color changes from red to blue with the increase of concentration of melamine (Fig. 2). The

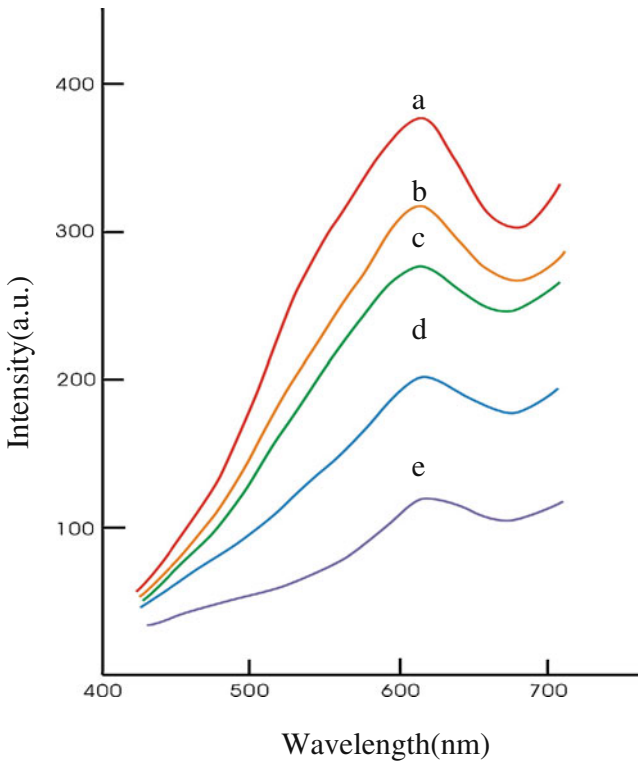

Fig. 4 RRS spectra of NGssDNA- glucose-Cu(II) particle reaction. **a**:3.35 mM $CuSO_4$-0.14 M $C_4H_4O_6KNa$-0.44 mM glucose-5 ng/mL NGssDNA-0.0 ng/L MA; **b**: a-9.46 ng/L MA; **c**: a-15.77 ng/L MA; **d**: a-31.53 ng/L MA; **e**: a-47.37 ng/L MA

Table 2 Effect of foreign substances

Foreign substance	Tolerance limit (Times)	Relative error (%)	Foreign substance	Tolerance limit (Times)	Relative error (%)
Fe^{3+}	200	5	Urea	500	5
Ca^{2+}	400	7	Glucose	500	3
Mg^{2+}	400	−7	IgG	350	−1
Cu^{2+}	300	6	IgM	100	−6
K^{+}	300	7	BSA	200	4
Zn^{2+}	600	7	HSA	350	7
NH_4^{+}	400	9	L-Tyrosine	200	−6
Hg^{2+}	100	−2	L-Valine	100	−5
L-Lysine	100	−3	L-phenylalanine	100	3

average size is 152 nm characterized by laser scattering method. When melamine concentration increased, the aggregations increased, and the RRS intensity enhanced linearly. Thus, a 1.89–81.89 μg/L melamine can be determined by the NGssDNA probe, but its sensitivity is not high, with a detection limit of 0.98 μg/L melamine. Tables 1 and 3 showed that NG exhibited strong catalytic effect on the Fehling reaction, while the nanogold aggregations exhibited weak. After NG modified by the ssDNA, its catalytic activity weaken. However, the slope of the NGssDNA catalytic system was larger than the NGssDNA aggregation system. Thus, if both the NGssDNA and its aggregation coexist in the system, the contribution of the latter may be neglected. If the unreacted NGssDNA in the aptamer reaction solution was used as nanocatalyst of Cu(II)-glucose, the cubic Cu_2O particles produced and exhibited a RRS peak at 614 nm. When melamine concentration increased, the unreacted NGssDNA decreased, and the RRS intensity decreased. The decreased RRS intensity is linear to the melamine concentration. Thus, a NGssDNA catalytic RRS assays for melamine was proposed (Fig. 3).

RRS spectra Rayleigh scattering belongs to elastic scattering, and the scattering frequency is equal to the incidence frequency. It was well known that the electron energy band (E_{SE}) occurred on the nanoparticle surface. When incidence photon energy (E_P) is closed to or equal to the surface electron energy, the photon resonated with the surface electron, and the Rayleigh scattering signal enhanced greatly. This Rayleigh scattering called as resonance Rayleigh scattering (RRS). In the pH 6.6 Na_2HPO_4-NaH_2PO_4 buffer solution and in the presence of 35 mmol/L NaCl solution, the aptamer and nanogold can form a stable aptamer–nanogold probe (NGssDNA) that exhibited a weak RRS peak at 566 nm. Upon addition of melamine, it combined with the NGssDNA to form big clusters that caused the RRS peak increased. The increased RRS intensity was linear to the melamine concentration. Thus, the probe can be utilized to assay melamine with a wavelength of 566 nm.

The reaction of glucose-Cu(II) was very slow at room temperature and indeed at 70 °C. Upon addition of nano-catalyst such as NGssDNA and NG, the Cu_2O particles produced quickly. That exhibited a RRS peak at 614 nm as in Fig. 4. The unreacted NGssDNA in the aptamer reaction solution decreased as the melamine concentration increased, and the RRS intensity at 614 nm decreased linearly. Thus, a wavelength of 614 nm was chosen for use.

Optimization of Analytical Conditions The NG aggregation reaction need at high concentration of NG, and the NG catalytic reaction take place at a level of trace NG. Thus, the diluted step was necessary to the nanocatalytic RRS assay. For the NGssDNA reaction, the effect of pH and the volume of Na_2HPO_4-NaH_2PO_4 buffer solution on the $\Delta I_{614\ nm}$ were considered. When the pH is 6.6 and the volume of the buffer solutions is 350 μL (3.5 mmol/L PO_4^{3-}), the $\Delta I_{614\ nm}$ value is the largest. So, a 350 μL pH 6.6 Na_2HPO_4-NaH_2PO_4 buffer solutions was chosen. The effect of NGssDNA concentration on $\Delta I_{614\ nm}$ was considered, the $\Delta I_{614\ nm}$ attained the maximum when the concentration is 11.43 μg/mL. Accordingly, a 11.43 μg/mL NGssDNA was chosen. The results demonstrated that $\Delta I_{614\ nm}$ attained the maximum when the NaCl concentration is 35 mmol/L. Thus, a 35 mmol/L NaCl was chosen. The effect of the aptamer reaction time was investigated. After 10 min later, the $\Delta I_{614\ nm}$ reaches its maximum, and was chosen.

Effect of the nanocatalytic reaction factors such as reagent concentration, reaction temperature and time on

Table 3 Analytical features of NGssDNA catalytic RRS assay for MA

[a] The ssDNA oncentration of 10 T, 20 T and 30 T was 3.75 nmol/L, 5.25 nmol/L and 3.75 nmol/L respectively

Principle	Base number of ssDNA[a]	Regression quation	Linear range ng/L	Correlation coefficient	Detection limit ng/L
Based on NGssDNA catalytic effect	10 T	$\Delta I_{614\,nm} = 5.1C + 10.8$	0.79–37.84	0.9979	0.52
	20 T	$\Delta I_{614\,nm} = 4.3C + 7.7$	0.79–44.14	0.9949	0.49
	30 T	$\Delta I_{614\,nm} = 5.2C + 11.1$	0.63–47.30	0.9983	0.38

Table 4 Comparing of analytical features for melamine assays reported

Methods	Linear range	Sensitivity	Comment	Reference
Colorimetric	–	400 μg/L	Complex and low sensitive.	[6]
Spectrophotometry	0.13–1261 μg/L	0.1 μg/L	Complex and sensitive.	[7]
Electrochemical	4.92–416 μg/L	1.21 μg/L	Complex and time-consuming.	[8]
Colorimetric sensor	–	2.5 mg/L	Complex and low sensitive.	[24]
Colorimetric	1–80 mg/L	0.4 mg/L	Simple and low sensitive.	[25]
AgssDNA RRS [a]	6.31–378.4 μg/L	5.09 μg/L	Simple and sensitive.	[26]
AgssDNA Cat RRS	0.02–1.06 μg/L	0.01 μg/L	Sensitive.	[26]
NGssDNA RRS	1.89–81.98 μg/L	0.98 μg/L	Simple and sensitive.	This assay
NGssDNA Cat RRS	0.00063–0.0473 μg/L	0.00038 μg/L	Highly sensitive.	This assay

[a] AgssDNA represents the aptamer modified nanosilver

the $\Delta I_{614\ nm}$ was studied separately. The results showed that a 3.35×10^{-3} mol/L $CuSO_4$, 4.4×10^{-4} mol/L glucose, 0.14 mol/L $C_4H_4O_6KNa$, and reaction temperature of 70 °C for 7 min, giving max $\Delta I_{614\ nm}$, and were chosen for use. The influence of volume of the aptamer reaction solution was examined. When the reaction solution was diluted 40 times, the $\Delta I_{614\ nm}$ increased as the volume increased within 50 μL, the $\Delta I_{614\ nm}$ attained the maximum when the filtrate amount is 50 μL. Thus, a 50 μL of the diluted reaction solution was chosen.

Effect of Foreign Substances According to the procedure, the influence of foreign substances on the determination of 31.53 ng/L melamine was tested, with a relative error of ±10%. Table 2 shows that the common metal ions, proteins and amino acids do not interfere with the determination, which indicated that this method had good selectivity.

Working Curve In the selected conditions, the RRS intensity for different melamine was recorded. The analytical features showed in Table 3, the 30 T aptamer system has low detection limit, and wide linear range. So the 30 T aptamer system was selected for use. Compared with the reported methods for melamine (Table 4) [6–8, 24–26], the NGssDNA RRS probe is more simple and sensitive, but the sensitivity was not still high. And the NGssDNA catalytic RRS assay is one of most sensitive method, with wide detection range and simplicity. Furthermore, the glucose-Cu^{2+} catalytic reaction can be stopped easily by tap water cooling.

Analytical Applications In the selected conditions, the melamine in milk sample was detected according to the procedure, and no melamine was found by the assay. In the milk sample solution, three different concentrations of melamine were added. Extracted the sample according to the sample pretreatment procedure, and 5 determinations were repeated. The analytical results showed in Table 5, with average recovery of 99.2%–100%, and relative standard deviation of 0.8%–1.7%.

Conclusions

Based on NG-modified aptamer reaction, and the catalytic active difference of nanogold and the nanogold aggregation on the Fehling reaction, and the RRS effect of Cu_2O particles, a new NGssDNA catalytic RRS method was developed for trace melamine. This method is simplicity, low-cost, sensitivity and selectivity. This principle would be utilized for detection of other target molecule.

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Table 5 Analytical results for melamine in milk sample

Sample	Content (ng/L)	Added (ng/L)	Found (ng/L)	Recovery (%)	RSD (%)
1	–	19.25	19.02, 18.97, 19.21, 19.15, 19.34	99.2	0.8
2	–	30.26	30.29, 30.19, 30.33, 29.98, 29.45	99.3	1.2
3	–	43.37	44.05, 43.31, 42.89, 42.52, 44.19	100.0	1.7

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