Talanta 112 (2013) 20–25

Contents lists available at SciVerse ScienceDirect

Talanta

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

Colorimetric detection of ractopamine and salbutamol using gold nanoparticles functionalized with melamine as a probe

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ARTICLE INFO

Article history:
Received 11 January 2013
Received in revised form
14 March 2013
Accepted 15 March 2013
Available online 25 March 2013

Keywords:
Gold nanoparticles
Ractopamine
Salbutamol
Melamine
Colorimetric

ABSTRACT

A highly selective and sensitive method is developed for colorimetric detection of ractopamine and salbutamol using gold nanoparticles (AuNPs) functionalized with melamine (MA), respectively. The presence of these β-agonists induces the aggregation of gold nanoparticles through hydrogen-bonding interaction that was accompanied by a distinct change in color and optical properties, which could be monitored by a UV–vis spectrophotometer or even naked eyes. This process caused a significant decrease in the absorbance ratio ($A_{670\ nm}/A_{520\ nm}$) of melamine–gold nanoparticles (MA–AuNPs), and the color changed from wine red to blue. The systems exhibited a wide liner range, from 1×10^{-10} M to 5×10^{-7} mol/L with a correlation coefficient of 0.995 for ractopamine, and 1×10^{-10} M to 1×10^{-5} mol/L with a correlation coefficient of 0.996 for salbutamol, with measuring the absorbance ratio ($A_{670\ nm}/A_{520\ nm}$). The detection limit of these β-agonists is as low as 1×10^{-11} mol/L. Particularly, the developed method has been applied to the analysis of real swine feed samples and has achieved satisfactory results.

1. Introduction

Recently, β-agonists received extensive attention for their illegally used as feed additives. They were original drugs for the treatment of pulmonary disease and asthma [1]. However, due to their potential roles in decreasing adipose tissue deposition and increasing protein accretion in livestock, β-agonists in particular clenbuterol, salbutamol and salbutamol were applied in animals to the production of muscle tissues [2]. Because their stable properties, the β-agonists can be easily deposited in human beings after meat consumption, and result in many serious health problems such as cardiovascular and central nervous diseases [3]. For their poisoning side-effects with people, many countries have forbidden the use of β-agonists in stockbreeding [4]. Therefore it is necessary to develop selective methods for detecting them at low concentrations with great precision and accuracy. Various analytical techniques for the determination of β-agonists have been reported, including liquid chromatography spectroscopy (LC) [5], gas chromatography spectrometry (GC) [6], electrochemical detection [7–9], and enzyme-linked immunosorbent assay (ELISA) [10]. Gupta and co-workers developed electrochemical detection for using electroactive phase in PVC based membranes, and had many achievements [11–15]. However, these methods are not very convenient for analysis of a large number of environmental samples as they generally require complex procedures and costly instruments, making it difficult for on-site and real-time determination.

Colorimetric sensors have attracted increasing considerations for their convenience of visual observation and simple operations in recent years [16–18]. Taking the advantage of their strong localized surface plasmon resonance (LSPR) absorption with extremely high extinction coefficients ($\sim3\times10^{-11}$ mol^{-1} L cm^{-1}) [19], systems based on the analyte-induced aggregation of gold nanoparticles (AuNPs) have been employed for the optical-detection applications. As the dispersed AuNPs are wine red whilst the aggregated ones are blue, this distinct change in color provide the naked-eye detection of various targets, including viruses [20], DNA [21], proteins [22], small molecules [23], metal ions [24] and cancerous cells [25]. However, the challenge to produce a colorimetric sensor is to find a suitable mediator to modify the surface of gold nanoparticles, which makes inter-particle crosslinking of AuNPs. More recently, our group has proposed a colorimetric method for the detection of clenbuterol using label-free gold nanoparticles in the presence of melamine (MA) [26]. Melamine can be connected with clenbuterol through hydrogen-bonding

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http://dx.doi.org/10.1016/j.talanta.2013.03.033

interaction [27,28] and has strong binding ability to the surface of AuNPs [29], which act as crosslinking agent between clenbuterol and AuNPs. In this paper, we improved our previous method and developed a new probe for the detection of two different β-agonists, ractopamine and salbutamol.

2. Experimental

2.1. Chemicals and materials

All reagents were of analytical grade and used without further purification. Solutions were prepared using high pure water with a resistance of 18 MΩ cm. Ractopamine, salbutamol and melamine was purchased from Sigma (USA). $HAuCl_4 \cdot 4H_2O$ was bought from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Glycine, L-glutamic acid, L-tyrosine, L-arginine, vitamin C and glucose were purchased from North Ringer Biotechnology Co., Ltd. (Beijing, China). $CaCl_2$, $MgCl_2$, and NaCl were purchased from Beijing Chemical Company (Beijing, China). The buffer was 0.01 M sodium acetate (pH 3.0–11.0, adjusted with acetic acid or sodium hydroxide). UV–vis absorption spectra were acquired on a UV-2550 spectrophotometer (Shimadzu, Japan), using 1-cm path length quartz cuvettes for measurements. IR spectra were measured with Avatar 360 FT-IR ESP (Thermo Nicolet, USA). Transmission electron microscopy (TEM) measurements were made on an H-7500 (Hitachi, Japan) at 80 kV. The pH of the solution was measured with PB-10pH meter (Sartorius, Germany).

2.2. Probe preparation

AuNPs were prepared by the reduction of $HAuCl_4$ with sodium citrate according to the method in the literature [30]. Typically, 15 mL of trisodium citrate (38.8×10^{-3} mol/L) was added rapidly into a boiling solution of 150 mL of $HAuCl_4$ (1×10^{-3} mol/L) under vigorously magnetic stirring and the mixed solution was continually boiled under stirring for another 30 min, producing a wine-red colored solution. The solution was cooled to room temperature while being stirred continuously and the citrate-stabilized suspension of gold nanoparticles were formed. The size of AuNPs was about 13 nm and the concentration was 10 nmol/L. The stock solution of AuNPs was stored in a refrigerator at 4 °C for further use.

To obtain melamine–gold nanoparticles (MA–AuNPs), 100 mL buffer and 50 mL of the prepared AuNPs were mixed to control the acidity of the aqueous solutions, then MA was added to this solution under stirring for about 15 min to make sure self-assembly of the MA onto the surface of gold nanoparticles. After this simple experiment, AuNPs functionalized with MA were prepared at room temperature.

2.3. Real swine feed samples preparation

Real swine feed samples were prepared according to previous literature [7]. Firstly, 1.0 g swine feed powder were weighed into 25.0 mL centrifuge tubes, and 3.0 mL acetone and 1.0 mol/L NaOH mixture (9:1, v-v) were spiked into the feed samples, than vibrating by supersonic for 5 min. Secondly, the mixture was centrifuged at 10,000 rpm and 5 °C for 10 min, repeated for three times and collected the clear supernatants. After that the supernatants were evaporated by nitrogen-blowing. Lastly, residues were dissolved in 1.0 mL acetate buffer (pH 5.0), then the same volume hexane were added for removing fat. After centrifuging at 10,000 rpm and 5 °C for 5 min, the remaining water phase were collected for analysis.

For the analysis of real swine feed samples, the above extracting solution was diluted 20 times with pH 5.0 acetate buffer and used to prepare a series of samples by "spiking" them with standard solutions of β-agonists.

3. Results and discussion

3.1. Mechanism

In this study, unlike the preceding method, melamine was priori modified onto the surface of Au nanoparticles by $-NH_2$ to prepare MA–AuNPs probe. The β-agonists had strong affinity to melamine through hydrogen-bonding interaction, which induced the inter-particle crosslinking of AuNPs, resulting in appreciable changes in color by naked eyes and absorption properties. Fig. 1 shows the mechanism of this platform for sensing β-agonists. Comparing with the previous research, these labeled AuNPs exhibit a more stable and convenient process. What is more, the MA–AuNPs probe can be applicable to measure other β-agonists, and the detection limit of ractopamine or salbutamol was managed down to 1×10^{-11} mol/L. In addition, to demonstrate the practicality of the present approach, it has been successfully applied to determine the concentration of β-agonists in real swine feed samples.

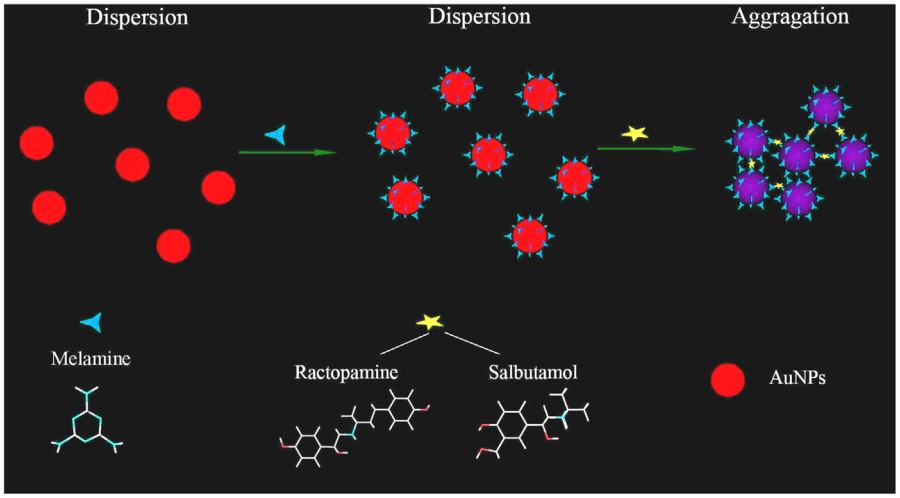

Fig. 1. Schematic illustration of the mechanism of the aggregation of MA–AuNPs induced by β-agonists.