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Fluorescent detection of clenbuterol using fluorophore functionalized gold nanoparticles based on fluorescence resonance energy transfer

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ABSTRACT

A new fluorescence method for detecting clenbuterol based on gold nanoparticles (AuNPs) and rhodamine B (RB) has been developed. It has been observed that the quenching of fluorescence of RB occurs in the present of AuNPs through the fluorescence resonance energy transfer (FRET). In the presence of clenbuterol, the FRET-based fluorescence of RB and AuNPs would be gradually recovered for the reason that clenbuterol could congregate the AuNPs and inhibit the FRET process. An assay for the determination of clenbuterol has been proposed based on the modulation in FRET efficiency between RB and AuNPs. Under the optimum conditions, the fluorescence recovery efficiency of the assay is proportional to the concentration of clenbuterol. The detection limit in swine feed was calculated to be 0.008 mg/g and the recoveries were in the range of 97%–102%. In short, the proposed method provides advantages such as rapidity, simplicity, low cost and high sensitivity, making it promising for the rapid screening of clenbuterol residues.

1. Introduction

Clenbuterol (4-amino-3,5-dichloro-α-tert-buty-laminomethyl benzyl alcohol hydrochloride) is a beta-adrenergic drug usually employed as a bronchial dilating agent for the treatment of pulmonary diseases in humans and animals (López-Erroz, Viñas, Cerdán, & Hernández-Córdoba, 2000). Due to its growth promoting effect involved in increasing lean muscle mass and reducing the fat deposition, clenbuterol is also commonly but illegally added at high doses to the feed of livestock, especially pigs and cattle, to improve the production of lean meat (Wang et al., 2013). Intake of clenbuterol may result in human food poisoning including muscle tremor, tachycardia, palpitation and dizziness (Brambilla et al., 2000). Thus it has been a threat to people's health and already been banned for adding in animal feeds. However, illegal abuse of clenbuterol never stopped, especially the incident occurring in the middle of March 2011 in China caused great concern for clenbuterol residues (Zhang, Zhao, et al., 2012).

With the great increase of public concern on food safety and a strict monitoring standard, various analytical methods for the detection of clenbuterol residue have been developed. Among them, the most commonly used methods are mainly based on chromatographic techniques due to their great sensitivity and accuracy, such as liquid chromatography with electrochemical detection (Zhang, Gan, & Zhao, 2003), ultra-high pressure liquid chromatography–tandem mass spectrometry (UHPLC–MS/MS) (Nicoli et al., 2013) and gas chromatography–mass spectrometry (GC–MS) (Corcia, Morra, Pazzi, & Vincenti, 2009; Yang et al., 2013). Besides, immunoassays especially enzyme-linked immunosorbent assay (ELISA) techniques, which were highly selective and sensitive, have been widely used to detect clenbuterol residue in animal feeds, foods and urine (Posyniak, Zmudzki, & Niedzielska, 2003; Sawaya, Lone, Husain, Dashti, & Saeed, 2000). Others such as capillary electrophoresis (Li, Du, Yu, Xu, & You, 2013) and electrochemical methods (Bo et al., 2013; Wang et al., 2013) have also been developed. However, most of the existing methods require expensive instruments, time-consuming/complicated sample pretreatment, and professional operators, which limit the application of these methods for on-site and real-time determination of clenbuterol. Therefore, it is necessary to put forward a more rapid, efficient, convenient and sensitive method for the detection of clenbuterol residue.

Nanoparticles, especially gold nanoparticles (AuNPs) have been widely used in variety of chemical and biological sensing because of their unique size-dependent optical and electronic properties (Saha, Agasti, Kim, Li, & Rotello, 2012). Due to the extremely high extinction coefficients and the strongly interparticle-distance-dependent absorption spectra, AuNPs were utilized as an ideal

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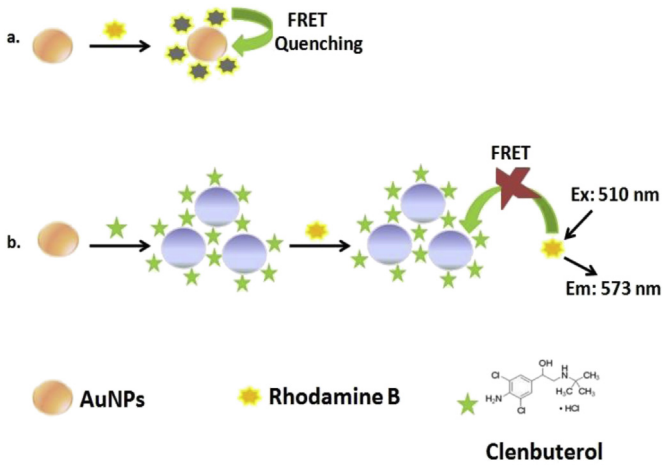
color reporter for colorimetric sensors (Zhao, Brook, & Li, 2008). In general, when AuNPs approach each other and aggregate, the color of the nanoparticles changes from red to purple or blue because of the shift of the surface plasmon band to a longer wavelength. Colorimetric assays could be convenient and attractive in many applications because they can be easily monitored with the naked eyes, without the aid of any advanced instruments. More recently, a novel strategy for the colorimetric detection of clenbuterol has been proposed using label-free AuNPs in the presence of melamine (Zhang, Zhao, et al., 2012). Melamine can be connected with clenbuterol through hydrogen-bonding interaction and has strong binding ability to the surface of AuNPs, which acts as crosslinking agent between clenbuterol and AuNPs. This method was then improved and applied for the detection of two other β-agonists, ractopamine and salbutamol (Zhou, Wang, Su, Zhao, & He, 2013).

On the other hand, the high extinction coefficient and the broad absorption spectrum overlapping well with the emission of usual fluorophore endow AuNPs with excellent fluorescence quenching ability (Wang & Guo, 2009; You et al., 2007). In fluorescence resonance energy transfer (FRET) process, AuNPs have been identified to act as excellent acceptors to replace traditional organic quenchers (Ling & Huang, 2010). AuNPs have a Stern–Volmer quenching constant (K_{SV}) that is several orders of magnitude greater than that of typical small molecule dye–quencher pairs (Huang & Chang, 2006). FRET arises from an excited-state energy interaction in which an energy donor transfers energy to an acceptor without photoemission. The efficiency of FRET is very sensitive to the distance between the donor and an acceptor. When AuNPs were used as an acceptor for FRET, the distance dependence changes from $1/R^6$ to $1/R^4$ and the calculated energy-transfer distances are as large as 70–100 nm, about 10 times longer than the typical Forster distances (R_0) (Ray, Fortner, & Darbha, 2006). Moreover, AuNPs can quench the fluorescence of dyes with different emission wavelength from the visible range to the near infrared (Guo et al., 2011). With AuNPs as superb acceptors/quenchers, the FRET-based assays for detection of ions (Huang & Chang, 2006; Shang, Jin, & Dong, 2009; Wang & Guo, 2009) and small molecules (Cai et al., 2011; Guo et al., 2011) have been reported, and their distinguished analytical performances arouse us to develop a new FRET system for clenbuterol detection. To date, the fluorescent methods for clenbuterol detection in literature are mainly focused on immunoassay-based biosensors (Song et al., 2013; Wang, Tao, & Meng, 2009), and there has been no FRET-based assay for clenbuterol.

In this study, we established a highly sensitive turn-on fluorescent method for the detection of clenbuterol in swine feed with the aid of fluorophore functionalized AuNPs. As shown in Scheme 1, the principle for AuNPs-based fluorescent determination of clenbuterol is very simple. Positively-charged rhodamine B (RB) can be readily adsorbed on the surfaces of citrate-stabilized AuNPs through electrostatic interactions, resulting in highly efficient FRET to occur between them (Guo et al., 2011; Shang et al., 2009). RB displays weak emission on the surface of AuNPs due to the highly efficient fluorescence quenching ability of AuNPs. Meanwhile, clenbuterol with aromatic anilinic NH_2 group and aliphatic NH group in its molecule may adsorb on the AuNPs through ionic and coordination bonds, respectively (Izquierdo-Lorenzo, Sanchez-Cortes, & Garcia-Ramos, 2010; Zhang, Zhao, et al., 2012). So, in the presence of clenbuterol, clenbuterol molecules can compete with RB molecules to adsorb on the surface of the AuNPs and further induce the aggregation of AuNPs, and as a result, the fluorescence of RB was restored because the ON/OFF behaviors of FRET are strongly influenced by the distance between donor and acceptor. Based on this strategy, clenbuterol detection can be realized in a simple and sensitive approach.



Scheme 1. Schematic illustration of fluorescent assay for clenbuterol which was established on the basis of its modulation on the FRET between rhodamine B and AuNPs. (a) The fluorescence of rhodamine B was quenched by AuNPs through FRET; and (b) the fluorescence of rhodamine B was restored because the presence of clenbuterol switched off FRET between rhodamine B and AuNPs.

2. Experimental

2.1. Reagents and materials

All the chemicals were of analytical grade and were used without any further purification. Doubly distilled water (DDW) was used throughout the experiments. Clenbuterol was purchased from Aladdin Reagent Company (Shanghai, China). $HAuCl_4 \cdot 4H_2O$, trisodium citrate, glucose, L-cysteine, glycine, L-lysine, tryptophane, vitamin C, $MgCl_2$, $CaCl_2$, NaCl and rhodamine B (RB) were obtained from Huishi Biochemical Reagent Company (Shanghai, China). The stock solution of RB was prepared by dissolving 24.0 mg of RB in 100 mL DDW (500 μM) and was then diluted to final 25 μM for further use.

2.2. Apparatus

All optical measurements were performed at room temperature under ambient conditions. The absorption spectra were recorded on a 2550 UV–vis spectrophotometer (Shimadzu, Tokyo, Japan). The fluorescence spectra were acquired on a RF-5301 fluorescence spectrophotometer (Shimadzu, Tokyo, Japan) at the excitation wavelength of 510 nm, with both of the exciting and emission slits set at 5 nm. Transmission electron microscopy (TEM) measurements were made on a JEM-2100F (JEOL Co., Japan) operated at an accelerating voltage of 200 kV. Zeta potential (ζ) measurement was performed on a Zeta Sizer Nano ZS particle analyzer. The centrifugation was carried out on a CR20B2 refrigerated centrifuge (Tokyo, Japan). Vortex mixing was performed on a WH-3 vortex mixer (Huxi, Shanghai, China).

2.3. Preparation and characterization of citrate-stabilized AuNPs

The colloidal solution of 13 nm citrate-stabilized AuNPs was prepared using Frens' method described in a previous report (Grabar, Freeman, Hommer, & Natan, 1995). All pieces of glassware were thoroughly immersed and cleaned with newly prepared aquaregia, completely rinsed with DDW and fully dried before use. Typically, in a 250 mL round-bottom flask equipped with a condenser, 100 mL of 1 mM $HAuCl_4$ was heated to a rolling boil with vigorous stirring. Rapid addition of 10 mL of 38.8 mM sodium