Analytica Chimica Acta xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

Analytica Chimica Acta

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

A fluorescent polymer dots positive readout fluorescent quenching lateral flow sensor for ractopamine rapid detection

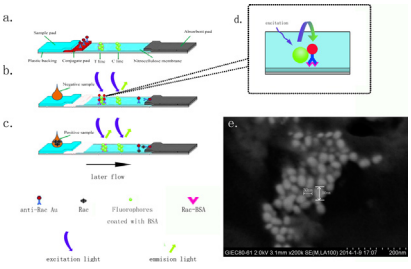
Cong Ying Shi [1], Ning Deng [1], Jia Jie Liang, Ke Nan Zhou, Qiang Qiang Fu, Yong Tang *

Department of Bioengineering, Guangdong Province Key Laboratory of Molecular Immunology and Antibody Engineering, Jinan University, Guangzhou 510632, PR China

HIGHLIGHTS

- A lateral flow assay based on FRET has been developed.
- The lowest detection limitation of the FQLFA for Rac is 0.16 ng mL^{-1}.
- The FQLFA provide a positive correlation fluorescence signal.
- Providing the evidence of FRET on FQLFA strip.
- Simplifying the process of FQLFA strip.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 25 May 2014
Received in revised form 10 October 2014
Accepted 4 November 2014
Available online xxx

Keywords:
Positive readout
Fluorescent quenching
Ractopamine
Lateral flow sensors

ABSTRACT

A fluorescent polymer dots positive readout and sensitive lateral flow assay (LFA) based on fluorescent quenching has been developed to detect ractopamine (Rac), a chemical residue in food, harmful to human health. Compared with traditional LFA strips, these fluorescent quenching LFA (FQLFA) strips provide a positive correlation method that allows users to obtain results from a weak fluorescent signal. The immunoassay strip scheme is based on the fact that fluorescent polymer dots (FPDs) in close proximity to gold nanoparticles (AuNPs) represent a strong fluorescent quenching. We show that the FQLFA strips can be used as a source to quantitatively analyze Rac in phosphate buffers (PB), swine urine and muscle tissue samples. The lowest detection limitation of the FQLFA was 0.16 ng mL^{-1}. Our results indicated that this novel scheme was more suitable for rapid detection of small molecules.

1. Introduction

Ractopamine hydrochloride (Rac-HCl) is a phenethanolamine member of the β-adrenergic agonists. When Rac-HCl is incorporated into the diet for livestock, growth performance will increase and fatty tissue will decrease. Its chemical structure is shown in Fig. 1.

However, meat products obtained from livestock that consume these compounds may pose potential risks linked to adverse effects that can affect cardiovascular and central nervous systems [1]. The development and application of an effective analytical method for routine monitoring of Rac residue is therefore necessary [2]. Gold-based LFA strips are easy to use, however, the signal is inversely correlated with the concentration of Rac and the strips show less sensitivity to analytes. The detected Rac limit of commercially gold-based LFA strips and highly sensitive gold-based LFA strips is reported as 5 ng mL^{-1} and 1 ng mL^{-1}, respectively [3].

Abbreviations: FRET, fluorescence resonance energy transfer; LFA, lateral flow assay; Rac, ractopamine; Rac-HCl, ractopamine hydrochloride; McAbs, monoclonal antibody; FQLFA, fluorescent quenching LFA; FPDs, fluorescent polymer dots; AuNPs, gold nanoparticles; PB, phosphate buffer; NC, nitrocellulose; Ab-AuNPs, antibody-labeled AuNPs; LDL, lowest detection limit.

* Corresponding author. Tel.: +86 20 85227003; fax: +86 20 85227003.
E-mail address: tyjaq7926@163.com (Y. Tang).

[1] These two authors contributed equally to this work.

http://dx.doi.org/10.1016/j.aca.2014.11.005
0003-2670/

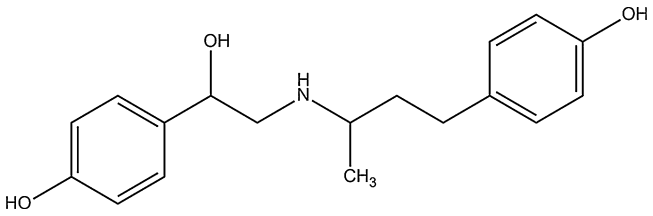


Fig. 1. Chemical structure of Ractopamine hydrochloride.

Fluorescent quenching has been applied in many bioassays such as real-time PC [4,5] and some novel fluorescent quenching immunoassays [6–9]. FRET is a non-radiative process that depends on transferring energy from an excited state donor D to a proximal ground state acceptor A, through long-range dipole–dipole interactions. The excitation wavelength of acceptor A must overlap the emission wavelength of donor D so that energy of the donor can be transferred to the acceptor. However, the acceptor does not necessarily have to remit the energy fluorescently itself (i.e. dark quenching) [10].

FPDs work as donors and AuNPs work as acceptors in our method. When donor FPDs are given a 365 nm excitation wavelength from UV light, the energy transfers from donor FPDs to acceptor AuNPs and is totally absorbed by the AuNPs at a suitable distance (Fig. 2).

AuNPs, widely used in LFA [11,12], represent a class of materials having a high extinction coefficient and a broad visible range absorption. AuNps are highly efficient fluorescent quenchers and frequently used because of their extremely strong absorption ability [13]. Au atoms on the surface possess unoccupied orbitals, which facilitate nucleophiles to donator electrons [14,15], FRET between AuNPs and FPD donors can occur within a restricted distance [16]. For convenience and rapid use, the use of LFA for β-adrenergic agonist detection has been explored [3]. AuNP-based LFA strips have been frequently used, due to the choice of materials and reagents, preparation of AuNP tracers, and release and capillary action of the AuNP tracers. Competitive immunoassay is a successful method for detecting small molecules such as β-adrenergic agonists. However, the sensitivity of the immunoassay is limited.

Positive-readout fluorescent quenching sensitive LFAs (FQLFA) are one-step tests for rapid identification of various analytes. We previously developed a FPD-labeled FQLFA for heavy metal ions [17]. In this study, we described a novel FQLFA, which is a simple and sensitive biosensor for rapid detection of Rac in PB, swine urine and muscle tissue samples. This method produced signals that positively correlated with detected concentrations of Rac. The unique property of AuNPs rendered visual detection of Rac and AuNPs also worked as FRET acceptors that could make contact with FPD donors through antigen–antibody interactions. Test results were obtained by observing the fluorescence present on a test line through a special reader.

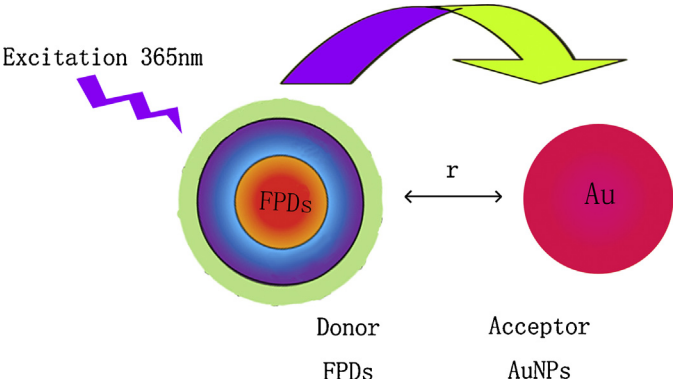


Fig. 2. Schematic of FRET process: when donor FPDs are excited, the excited donor molecules transfer energy to proximal acceptor AuNPs molecules located at an appropriate distance (*r*) from the donor [10].

2. Materials and methods

2.1. Chemical reagents, apparatus, and other materials

Nitrocellulose (NC) membrane (HFB13504), conjugation pad and sample pad were purchased from Millipore (Shanghai, China). Ascites containing anti-ractopamine monoclonal antibody (McAbs) and Rac–BSA antigen were prepared in our laboratory. Ultrapure water was produced using the Milli-Q Ultra Pure System (Millipore, Bedford, MA, USA). FPDs (polystyrene, 50 nm) were purchased from YongRu (Zhuhai, China). All chemicals used were analytical grade or higher.

Multiskan Spectrum safir2 (TECAN, Switzerland), and centrifuge (Beckman, Germany) were supplied by our lab. The platform consisted of motion control with Biostrip Dispenser HGS102, Airjet HGS102, and the programmable strip cutter HGS201 (purchased locally in Shanghai, China). The LaParticle size analysis-laser was provided by Jinan University. FE-SEM (HITACHI S-4800) was supplied by the Analytical and Testing Centre, Guangzhou Institute of Energy Conversion, CAS.

2.2. Preparation of AuNPs [18–20] and Rac McAb-gold complexes [21]

Colloidal AuNPs with a mean particle diameter of 25 nm were produced for this study [22]. Colloidal AuNPs with a mean particle diameter of 25 nm were produced by adding gold chloride with 1% sodium citrate. Super purified water (100 mL) was heated to a boil and 2 mL gold chloride trihydrate solution was added. Next, 3.0 mL of 1% sodium citrate solution was added quickly into the solution and stirred for 20 min. After the color changed, the solution was boiled for another 20 min to complete the reduction of gold chloride, cooled and stored at room temperature.

Three milliliters of purified anti-ractopamine McAbs (30 mg mL^{-1}) were diluted to 100 μL with distilled water and drops were slowly added to 10 mL of colloidal gold solution, which was adjusted to pH 8.5 with 0.25 M K_2CO_3. After incubation for 30 min, 1 mL 10% BSA was added drop by drop. The mixture was incubated for 30 min and then centrifuged at 10,000 rct for 15 min to remove the unconjugated antibodies. After removing the supernatant, the precipitate was re-suspended with PB (0.015 M, pH 7.4) containing 15% (w/v) sucrose, 0.5% (w/v) BSA, and 0.02% (w/v) sodium azide and stored at 4 °C for further use.

2.3. Construction of LFA strips

LFA strips were assembled using the previously described method [23]. 10 μL of BSA–FPD complexes were diluted with 10 μL CB and 20 μL 2 mg mL^{-1} Rac–BSA and then the BSA–FPD complexes were added to the lower side of the NC membrane to form the test (*T*) line. 10 μL of BSA–FPD complexes were diluted with 36 μL CB, which was distributed to the upper side to form the control (C) line. The gold-labeled anti-Rac McAbs were dispensed by an Airjet HGS102 onto the glass fiber pad. The sample pad, conjugate pad, NC membrane and absorbent pad were laminated and glued onto the PVC plate. The LFA strips were cut into 3.8 mm wide and 60 mm long strips, using the programmable HGS201 strip cutter.

2.4. Preparation of Rac in PB

Samples of Rac diluted with PB were prepared in the lab and stored at 4 °C. Rac (0.1 mg) was dissolved in 1 mL of PB solution.

Please cite this article in press as: C.Y. Shi, et al., A fluorescent polymer dots positive readout fluorescent quenching lateral flow sensor for ractopamine rapid detection, Anal. Chim. Acta (2014), http://dx.doi.org/10.1016/j.aca.2014.11.005