Biosensors and Bioelectronics 50 (2013) 62–65

Contents lists available at SciVerse ScienceDirect

Biosensors and Bioelectronics

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

Short communication

Rapid and sensitive detection of β-agonists using a portable fluorescence biosensor based on fluorescent nanosilica and a lateral flow test strip

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ARTICLE INFO

Article history:
Received 13 May 2013
Received in revised form
6 June 2013
Accepted 7 June 2013
Available online 15 June 2013

Keywords:
Fluorescence biosensor
Fluorescent nanosilica
Strip
Clenbuterol

ABSTRACT

A portable fluorescence biosensor with rapid and ultrasensitive response for Clenbuterol (CL) has been built up with fluorescent nanosilica and a lateral flow test strip. Quantitative detection of CL was realized by recording the fluorescence intensity of fluorescent nanosilica captured on the test line. The sensing results indicated that the sensitivity of the fluorescent nanosilica-based strip was better than that of conventional colloidal gold-based strips. The visual limit of detection of the strip for qualitative detection was 0.1 ng/mL while the LOD for quantitative detection could down to 0.037 ng/mL by using fluorescence biosensor. The recoveries of test samples were from 89.3% to 97.7%. The assay time for CL detection was less than 8 min, suitable for rapid testing on-site.

1. Introduction

Clenbuterol (CL), a representative of the class of synthesized β-adrenergic agonists typically employed as a bronchial, was found to improve growth rate, reduce fat deposition and increase protein accretion (Kuiper et al., 1998; Mitchell and Dunnavan, 1998). However, once the animals are fed with CL, the reside may remain in the meat and liver for a long time as a result of its long half-life, so it may enter the body and distribute throughout the body and result in serious harmful health problems to human such as cardiovascular and central nervous diseases (Martinez-Navarro, 1990). Hence, the use of CL in livestock is banned in many countries including China, the United States, and many European countries (Tan et al., 2009). The main analytical methods for CL detection are enzyme-linked immunoassay (Ren et al., 2009; Zhang et al., 2012; Zheng et al., 2009), liquid chromatography (Freire et al., 2009; Mostafa et al., 2009; Zhang et al., 2011), gas chromatography (Wang et al., 2010; Zhao et al., 2010), electrochemical immunosensors (He et al., 2009; Li et al., 2012), surface plasmon resonance immunosensors (Liu et al., 2011; Wang et al., 2012) and other methods (Kong et al., 2009; Qu et al., 2011). Although these conventional strategies exhibit promising results for sensitive detection of CL, there are still some hindrances including incubation and washing steps before analysis, expensive instrument, long operation times, and tedious sample preparation. The lateral flow test strip (LFTS), also called a dry-reagent strip biosensor has been well-established diagnostic tool in laboratory. This technology offers additional advantages when compared to the conventional detection methods: rapid, simple and cost-effective. The most widely used format of LFTS is the employment of colloidal gold as reporters for colorimetric detection, which was either qualitative or semiquantitative and generally utilized for analyzing residues with relatively high concentrations. In order to meet the requirement of sensitive detection, more labels such as latex (Takanashi et al., 2008), quantum dots (Yang et al., 2011), and up-converting phosphorus technology (Qu et al., 2009) have been employed in LFTS development, but no marked improvement in performance has been achieved. Both the colloidal gold and quantum dots based LFTS have been applied to the detection of

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http://dx.doi.org/10.1016/j.bios.2013.06.022

CL with the limit of detection (LOD) by the naked eye 1 ng/mL and 20 ng/mL, respectively (Zhang et al., 2006; Luo et al., 2011). Some uncertain factors led to this undesirable LOD of quantum dots based LFTS. Consequently, a novel label with immense brightness and ideal photostability is highly desirable for FLFTS applications in CL analysis with ultralow concentrations.

During the past decades, lanthanide chelates have been receiving increasing attention because of their applications as luminescent probes for highly sensitive time-resolved fluoroimmunoassay (TR-FIA), fluorescence microscopy bioimaging, and other analytical techniques (Deng et al., 2010; Oh et al., 2011). They have specific luminescent properties that conventional organic dyes and quantum dots do not have, such as large Stokes shifts, sharp emission profiles, very high levels of brightness, excellent stability against photobleaching and long luminescence lifetimes. The β-diketonate chelate 4,4′-bis(1″,1″,1″,2″,2″,3″,3″-heptafluoro-4″,6″-hexanedione-6″-yl) chlorosulfo-*o*-terphen -yl (BHHCT)–Eu (III) is one of the lanthanide labels that has been proven to give high sensitivity in time resolved fluoroimmunoassay (Xu and Li, 2007; Yuan et al., 1999).

In this work, we report, for the first time, on the development of an optical based biosensors of competitive format which employ a fluorescent nanosilica labeled antibody probe as a reporter for the onsite detection of CL residue in urine samples. We introduced Eu(III)–BHHCT into porous silica nanoparticles to form the stable fluorescent nanosilica with desirable luminescence properties. Due to the advantages derived from lanthanide chelates and LFTS, a rapid, sensitive, specificity, and one-step strategy has been developed for CL analysis. Experimental results demonstrate that fluorescent nanosilica-based LFTS has an excellent ability for quantitative analysis of trace amounts of CL.

2. Material and methods

2.1. Reagents and materials

Clenbuterol hydrochloride (CL, ≥95%), β-diketonate chelate 4,4′-bis (1″,1″,1″,2″,2″, 3″,3″-heptafluoro-4″,6″-hexanedione-6″-yl)-chlorosulfo-*o*-terphenyl (BHHCT, ≥90%), Tetraethyl orthosilicate (TEOS, 99.999%), (3-Aminopropyl) trimethoxysilane (APTMS, 97%), Europium(III) chloride hexahydrate ($EuCl_3{\cdot}6H_2O$, 99.9%) and bovine serum albumin (BSA) were purchased from Sigma-Aldrich. Dextran (Mr 500,000) was purchased from Fluka. Staphylococcal Protein-A (SPA, ≥98%) was purchased from ProSpec. The nitrocellulose membrane (SHF-1200420), glass fiber (GF-CP20300), and absorbent paper (C048) was purchased from Millipore. The monoclonal antibody (McAb) against CL was produced in the Key Laboratory of Animal Immunology of the Chinese Ministry of Agriculture.

2.2. Preparation of fluorescent nanosilica

Bare silica nanoparticles were synthesized by a reverse microemulsion method. Amino groups were introduced to the surfaces of these particles by treatment with (3-aminopropyl) trimethoxylsilane (APTMS) (Wang et al., 2008). The aminated nanoparticles were characterized by fourier infrared spectrometer and transmission electron microscopy (TEM) (Fig. S1 and S2) and used for coating with Eu(III)–BHHCT. Typically, 1 mL of 1 mg/mL BHHCT solution was added to 1 mL of 30 mg/mL aminated silica nanoparticles suspension and stirring for 2 h. The nanoparticles were washed with ethanol and resuspended in 1 mL of 0.05 M Tris–HCl buffer (pH 7.8), followed by adding 50 μL of 0.04 M $EuCl_3$. After stirring for 30 min, the nanoparticles were washed and resuspended in 1 mL of ethanol and treated sequentially with TEOS, APTMS, BHHCT and $EuCl_3$ as above for another four times. The final product was resuspended in ethanol and stored at 4 °C before use. The fluorescence spectrum of the Eu(III)–BHHCT-coated silica nanoparticles was obtained using a Cary Eclipse fluorescence spectrophotometer (Fig. S3).

2.3. Preparation of fluorescent nanosilica-anti-CL McAb probe

A Eu(III)–BHHCT-coated silica nanoparticles-anti-CL McAb conjugate was prepared by an improved periodate oxidation method. Briefly, 250 mg of dextran was added to 5 mL of 0.5 M freshly prepared $NaIO_4$ solution to react overnight; the oxidized dextran was dialyzed against water, then mixed with 5 mg of fluorescent nanosilica suspended in 50 μL, 25 mM carbonate buffer (CBS, pH 9.5). After shaking for 3 h, the nanoparticles were washed and resuspended in 50 μL of CBS and then mixed with 100 μL of optimal anti-CL McAb. After a 12 h reaction, 150 μL of 0.5 M $NaBH_3CN$ was added and the mixture was shaken for another 8 h at 4 °C. The antibody-linked nanoparticles were blocked (10 mM Tris–HCl buffer containing 2% BSA, 4% sucrose, and 1% glycine, pH 7.8), rinsed by centrifugation twice and stored at 4 °C.

2.4. Fabrication of fluorescent nanosilica-based FLFTS

The fluorescent nanosilica-based FLFTS is composed of a sample application pad, conjugation pad, nitrocellulose membrane, absorption pad, and a backing card as shown in Fig. 1A. Both the sample pad (15 mm × 30 cm) and conjugation pad (7 mm × 30 cm) were made from glass fiber. The conjugation pad was prepared by dispensing a desired volume of fluorescent nanosilica labeled McAb onto the glass fiber pad using an XYZ Biostrip Dispenser, followed by drying at room temperature before stored at 4 °C. The nitrocellulose membrane (2 × 30 cm) was spotted using the same dispenser with the optimal CL–BSA and SPA applied in the test and control lines, leaving a 0.5 cm space

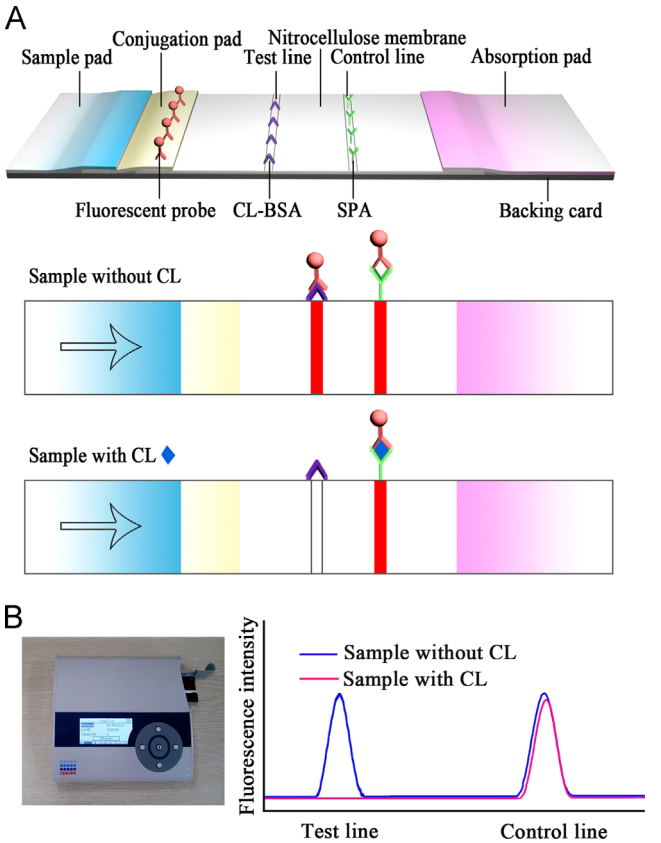

Fig.1. (A) Schematic diagram of the principle for the detection of fluorescent nanosilica-based strip and (B) the test strip reader and the typically results.