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Short communication

A novel fluorescence-quenching immunochromatographic sensor for detection of the heavy metal chromium

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ABSTRACT

A novel fluorescence quenching immunochromatographic sensor (ICS) was developed for detecting chromium (Cr^{3+}) within 15 min utilizing the fluorescence quenching function of gold nanoparticles (AuNPs). The sensor performed with a positive readout. When the low concentrations of Cr^{3+} samples were applied, detection signals of the test line (T line) were quenched, whereas when higher concentration Cr^{3+} samples (1.56 ng/mL) were applied, the detection signal of the T line appeared. The detection signal intensity of the T line increased with increasing concentrations of Cr^{3+}. The low detection limit of developed fluorescence quenching ICS was 1.56 ng/mL. The fluorescence quenching ICS has a linear range of detection of Cr^{3+} comprising between 6.25 ng/mL to 800 ng/mL. The recoveries of the fluorescence quenching ICS to detect Cr^{3+} in tap water ranged from 94.7% to 101.7%. This result indicated that the developed sensor gave higher sensitivity and reliable reproducibility. It could provide a general detection method for small analyte in water samples.

1. Introduction

Competitive immunoassays represent successful methods for detecting small analytes, widely used in medical diagnostic processes (Kauter et al., 2000), and environmental (Zhou et al., 2011) and agricultural (Tang et al., 2010) contaminations monitoring. In the currently most applied competitive immunoassay, the signal intensity during the test is inversely related with the concentration of analytes. This makes the result not intuitive, easily resulting in miscalculation for users, especially non-professionals. Furthermore, in the traditional competitive immunoassay, the concentration of analytes is commonly obtained through quantification of the decreasing signal. It is now known that the sensitivity of the competitive immunoassay is its main limitation. A novel competitive immunoassay that detects the signal intensity positively correlated with the concentrations of analyte could make the signal recognizable with the immune response. This would make a significant improvement on the sensitivity and user-friendliness of competitive immunoassays. Due to convenience and rapidity, the use of immunochromatographic sensors (ICS) for small analytes detection has been largely used (Mirasoli et al., 2012; Wang et al., 2011a; Zou et al., 2010). Gold nanoparticles (AuNPs) (Liu et al., 2012; Zhou et al., 2009) and fluorescence substances are the most common tracers of ICS. Long-term development and research have made AuNPs-based ICS a mature system in the choice of materials and reagents, preparation of AuNPs tracer, and the study of the release and capillary action of AuNPs tracer. However, AuNPs-based ICS strips have low sensitivity, principally attributed to the limited signal amplification. Compared with AuNPs-based ICS, fluorescent ICS are characterized by a high sensitivity and an accurate quantitative detection. Commonly used tracers of fluorescent ICS are fluorescent dyes (Kim et al., 2003; Oh et al., 2009) and latex fluorescence nanoparticles (FNPs)(Khreich et al., 2010). The traditional organic fluorescent dyes such as FITC are known to be not photo-stable and have relatively low fluorescence intensities. The FNPs, due to the high intensity of their fluorescent signal, excellent photostability and high conjugation efficiency (Bonacchi et al., 2011; Yan et al., 2007), may alleviate the drawbacks associated with the low sensitivity of the conventional AuNPs and organic fluorescent dye-based ICS. However, because of their large size, and tendency to agglomerate, it can easily stick to the nitrocellulose (NC) membrane. The above drawbacks have prevented their effective application in ultrasensitive biochemical analysis.

Fluorescence quenching technique has been widely used in various fields, including aptamers based sensor (Wang et al., 2011b), nucleic acid testing (Didenko, 2001), immunoassays (Peng et al., 2007) and protein interaction studies (Kenworthy, 2001). Fluorescence quenching immunoassays for detecting small analytes are performed with a positive readout of the signal intensity and are positively correlated with the concentration of analyte (Peng et al., 2007). Most materials used in fluorescence quenching are organic fluorescence dyes, fluorescent proteins, AuNPs and quantum dots (Sapsford et al., 2006).

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AuNPs are increasingly used in fluorescence quenching-based assays, (Maxwell et al., 2002; Oh et al., 2005, 2006) mostly because of their exceptional quenching ability. The main advantages of AuNPs-based fluorescence quenching sensors are the lower background signal, improved sensitivity, and ability to label the AuNPs with multiple biological molecules. Based on these considerations, here we investigated a novel analytical concept to detect small analytes by AuNPs-based fluorescence quenching ICS. High chromium absorptions *in vivo* can result in various diseases, including fibro-proliferative diseases, airway hypersensitivity, lung cancer, nasal cancer, and other types of tumors. For this reason, we choose the trivalent chromium (Cr^{3+}) as a detection analyte.

2. Material and methods

2.1. Materials

Fluorescence nanoparticles (FNPs) were purchased from Suzhou Biocompass Biotech (Suzhou, China). Chromium chloride (99.99%) was obtained from Aldrich Chemical (Milwaukee, WI, USA). Other powder metals were purchased from Merck Chemical (Darmstadt, Germany). Nitrocellulose (NC) membrane (HFB13504), conjugation pad, and sample pad were purchased from Millipore (Shanghai, China). Plastic backing and absorbent pad were purchased from a local market (Shanghai, China). Anti-Cr^{3+}-EDTA monoclonal antibodies (mAb) and BSA-Cr^{3+}-EDTA were generated in our laboratory.

2.2. Equipment

Transmission electron microscope (TEM, Philips, Holland), ultraviolet–visible spectrophotometer (Shimadzu, Japan), centrifuge (Beckman, Germany), and inductively coupled plasma atomic emission spectrometer (ICP-AES) (Thermo Fisher, America) were used. The platform consists of motion control with a Biostrip dispenser HGS102, and the programmable strip cutter HGS201 (purchased locally in Shanghai, China).

2.3. Preparation of the fluorescence-quenching ICS

The preparation of AuNPs and mAb-gold quencher were performed according to our previous study (Liu et al., 2012). BSA-Cr^{3+}–EDTA-FNPs and BSA-FNPs were prepared as follows: 100 μL FNPs was dispersed in 400 μL of distilled water. After 60 μL, 100 mg/mL EDC and 40 μL 100 mg/mL NHS were added, the solutions were incubated at room temperature for 30 min. The resulting solutions were centrifuged at 9000 rpm for 2 min at 4 °C and then the supernatant was discarded, and the process was repeated three times. The resulting pellet was suspended in 500 μL (pH 7.2) PBS and then mixed with 100 μL 0.1 mg/mL BSA-Cr^{3+}-EDTA. The resulting solution was incubated at room temperature for 2 h and then 100 μL 100 mg/mL BSA was used to block excess carboxyls of FNPs for 1 h. The resulting solutions were centrifuged at 9000 rpm for 2 min at 4 °C and the supernatant was discarded to eliminate the unbounded BSA, and this was then repeated three times. The pellet was suspended in 200 mL 0.01 M pH 7.2 PBS buffer and then stored at 4 °C in the dark with no further purification before use. For preparation of BSA-labeled FNPs, all steps were the same except BSA-Cr^{3+}-EDTA was replaced with BSA. The fluorescence quenching ICS strips were prepared as follows: BSA-Cr^{3+}–EDTA-FNPs and BSA-FNPs were diluted 300-fold with 0.01 M, pH 7.2 PBS buffer and then the BSA-Cr^{3+}–EDTA-FNPs and BSA-FNPs were dispensed on the special area of NC membrane, designated as the test line (T-line) and calibration line (C-line), by using an automatic dispenser with volume of 1 μL/cm. To improve the combined efficiency between mAb-gold nanoparticles and labeled FNPs on the T-line, phosphate-buffered saline (PBS) for diluting BSA-Cr^{3+}-EDTA-FNPs was added to 0.05 mg/mL BSA-Cr^{3+}-EDTA. The sample pad was pretreated by 0.01 M pH 7.2 PBS buffer containing 0.5% (w/v) BSA and 2% Triton X-100. The mAb-gold quencher was dispensed on the conjugate pad by using an automatic dispenser with different volumes. After pads were dried in 37 °C for 2 h, all fluorescence-quenching ICS components were assembled with 2 mm overlaps. These stacks were then cut into test strips and then placed in plastic housings.

2.4. The performance of the fluorescence-quenching ICS

0 μL Cr^{3+} solution with series of concentrations of analyte in distilled water containing 50 nM EDTA-Na_2 was applied to the sample pad. After 15 min, the fluorescence-quenching ICS were photographed and analyzed using the free image analysis software Image J (http://rsb.info.nih.gov/ij/).

2.5. Detection of Cr^{3+} in tap water

To detect the Cr^{3+} in tap water samples, a series of Cr^{3+} concentrations in tap water with 50 nM EDTA-Na_2 were applied to the fluorescence quenching ICS as mentioned above.

2.6. Specificity of the fluorescence quenching ICS

To evaluate the specificity of the fluorescence quenching ICS, metal ions Al^{3+}, Ca^{2+}, Fe^{3+}, Mg^{2+}, Ni^{2+}, Pb^{2+}, Cd^{2+}, Co^{2+}, Cu^{2+}, and Fe^{2+} were chosen as controls each with concentrations of 400 ng/mL.

3. Results and discussion

3.1. The principle of the fluorescence-quenching ICS

The low-molecular-weight Cr^{3+} is too small to be recognized by the antibody. EDTA-Na_2 was therefore selected to chelate Cr^{3+} and form a specific hapten. To prepare a complete antigen and coat to the nitrocellulose membrane (NC membrane), iEDTA, a highly effective bi-functional chelating agent, was used to conjugate Cr^{3+} and carrier proteins (Liu et al., 2011). As shown in Fig. 1a, the fluorescent quenching ICS used in this study consists of five components (from top to bottom): (a) a sample pad for applying samples; (b) a conjugate pad for loading the anti-Cr^{3+}-EDTA mAb-labeled gold nanoparticles (mAb-gold quencher); (c) a 25 mm NC membrane acting as the chromatography matrix, (d) an absorbent pad serving as the liquid sink, and (e) a plastic backing for supporting all the components. Coating antigen BSA-Cr^{3+}-EDTA-labeled FNPs (BSA-Cr^{3+}-EDTA-FNPs) and BSA labeled FNPs (BSA-FNPs) were dispensed on the NC membrane as the T-line and C-line, respectively (Fig. 1a).

The principle of the fluorescence-quenching ICS (Fig. 1) defines as negative sample (no analyte) that is applied to the sample pad. The liquid sample will migrate toward the other end of the test strip with capillary action. As the liquid sample migrates into the conjugation pad, the mAb-gold quencher migrates along. When these mixtures reaches the T-line zone, the optimized amount of mAb-gold quencher binds to the coating antigen and then the fluorescence signal of the T-line will be quenched (Fig. 1b). When a certain amount of positive sample solution is applied to the sample pad, analyte Cr^{3+}-EDTA will bind the mAb-gold quencher first. Then, the amount of mAb-gold quencher bound on T-line will be reduced and the fluorescence signal of T-line will be recovered (Fig. 1c), whereas fluorescence signal intensity of the C-line remains constant (Fig. 1b-c). The detection signal intensity of the