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Short communication

Magnetic nanogold microspheres-based lateral-flow immunodipstick for rapid detection of aflatoxin B_2 in food

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ABSTRACT

A novel membrane-based lateral-flow immunodipstick assay was developed for the fast screening of aflatoxin B_2 (AFB_2) as a model compound in food samples. The detector reagent consisted of magnetic nanogold microspheres (MnGMs) with nano-Fe_2O_3 particles as core and gold nanoparticles as shell, and bio-functionalized with monoclonal *anti*-AFB_2 antibodies. Manually spotted AFB_2–bovine serum albumin conjugates (AFB_2–BSA) and goat *anti*-mouse IgG on nitrocellulose membrane were used as test and control lines, respectively. As the major advantage, experimental results indicated that the visual detection limit (cutoff value) of the MnGM-based dipstick immunoassay with 0.9 ng/ml AFB_2 was about threefold lower compared to a conventional immunodipstick test using gold nanoparticles as detection reagent. Qualitative results (yes/no) could be obtained within 15 min without expensive equipment. The assay was evaluated with AFB_2 spiked or naturally contaminated samples ($n = 8$), including peanuts, hazelnuts, pistacia and almonds, receiving excellent correspondance with results from high performance liquid chromatography (HPLC). Most importantly, the assay gave no false negative results. By controlling the target antibody this assay can be easily extended for use with other food relevant toxins and thus represents a versatile detection method.

1. Introduction

An ultrasensitive and simple method for detecting and quantifying mycotoxins is essential for food safety monitoring (Kralj Cigic and Prosen, 2009; Krska et al., 2008; Murphy et al., 2006; Turner et al., 2009; Urraca et al., 2008; Van Egmond et al., 2007). Despite many advances in this field, simple and inexpensive approaches that do not require tedious and time-consuming sample preparation and cleanup steps for on-site (pre)-screening of mycotoxins are rare. Most importantly, immunochemical methods are to mention which are increasingly become routine methods. Generally, they are performed as enzyme-linked immunosorbent assay (ELISA) tests on microtiter plates. Alternatively, immunosensors with electrochemical or optical transduction are developed (Goryacheva et al., 2007; Prieto-Simón et al., 2007; Sapsford et al., 2006).

Unbeatable as to simplicity and speed of the technique, membrane-based immunoassays such as lateral-flow devices (LFDs) and flow-through format tests enjoy great popularity, first of all for point-of-care testing. The qualitative or semi-quantitative determination of mycotoxins with, for example, a one-step test can be performed within a few minutes without the need of instrumentation and additional chemicals. Furthermore, results are interpretable by non-specialists (Li et al., 2009; Lin et al., 2008). However, LFDs often suffer from low signal intensity and poor quantitative discrimination of the color-formation reaction based on label accumulation (Xia et al., 2009). Therefore, for successful development of user-friendly tests with sufficient sensitivity, the type of label is very important because LFDs are basically designed for visual inspection (Liu et al., 2008). Generally, antibodies are "stained" by coupling to latex particles (blue) or colloidal gold (pink). The latter has been extensively applied due to its inherent advantages, such as easy preparation and good biocompatibility (Ambrosi et al., 2007). Concerning mycotoxins, several papers became known which report on the development of LFDs for T-2 toxin, zearalenone, deoxynivalenol, fumonisin B_1, and aflatoxin B_1, employing colloidal gold-labeled antibodies (Delmulle et al., 2005; Kolosova et al., 2007, 2008; Molinelli et al., 2008; Wang et al., 2006).

The most common principle of LFDs is to move unbound labeled antibodies along the membrane and allowing it to bind a mycotoxin–protein conjugate, which had been immobilized on the

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membrane surface at the test zone. The intensity of the appeared color is therefore indirectly proportional to the concentration of the mycotoxin in the sample. If used for determining a qualitative level (cutoff), a positive sample with a concentration higher than the cutoff will result in no visual line in the test zone. Alternatively, a visible line is formed with a negative sample, i.e. a concentration below the cutoff level.

The emerging research field of nanotechnology, the process to generate and manipulate nanomaterials, provides exciting new possibilities for advanced development of new labels for bioanalytical applications. The present paper, as a matter of principle, describes the development of a new rapid one-step lateral-flow immunoassay for the screening of aflatoxin B_2 in food samples. For the first time, multifunctional magnetic nanogold microspheres were used for labeling monoclonal *anti*-AFB_2 antibodies to improve the sensitivity of the immunoassay-based strip test.

2. Experimental

2.1. Chemicals

Monoclonal *anti*-AFB_2 antibody (clone 3A7), AFB_2 standard solution (0.1721 g/l in acetonitrile) and AFB_2–BSA conjugate were prepared and characterized in our laboratory as described recently (Cervino et al., 2008). Anti-mouse IgG (whole antiserum, produced in goat), Tween 20, gold(III) chloride trihydrate ($HAuCl_4{\cdot}3H_2O$, 99.9% metals basis), sodium citrate tribasic dihydrate ($Na_3C_6H_5O_7{\cdot}2H_2O$), chitosan, and bovine serum albumin (BSA, 96–99%) were purchased from Sigma (St. Louis, MO, USA). Gold colloids (8 nm in diameter) were synthesized according to our previous report (Tang et al., 2005). Cellulose absorbent paper and nitrocellulose membrane (PROTRAN BA 83, pore size, 0.2 μm) were provided from Whatman GmbH (Dassel, Germany). 0.1 M phosphate-buffered saline (PBS, pH 7.4) was prepared by adding 12.2 g K_2HPO_4, 1.36 g KH_2PO_4, and 8.5 g NaCl in 1000 ml deionized water. All other chemicals mentioned were of analytical grade. Peanut standards were prepared as follows: 5.0 g of ground blank peanut samples were extracted with 37.5 ml MeOH/water (80:20, v/v) with stirring for 1 h and followed by filtration. 20 ml of the extract were diluted into 60 ml of water. Finally, standard samples were prepared by spiking aliquots of AFB_2 standards into different volumes of diluted extract.

2.2. Preparation of magnetic nanogold microspheres (MnGMs)

Nano-Fe_2O_3 particles (cores) were prepared by co-precipitation of $Fe^{(II)}$ and $Fe^{(III)}$ chlorides (Fe^{II}/Fe^{III} ratio of 0.5) in alkaline solution as described in the literature (Fremy and Usleber, 2003; Kang et al., 1996). Synthesis of the MnGMs was as follows: 0.5 g of Fe_2O_3 nanoparticles were initially added into 20 ml NaCl solution (0.5 M) containing 0.025 M sodium dodecyl sulphate (SDS), and stirred for 4 h. After washing with water, the SDS–Fe_2O_3 composites were redispersed in 20 ml acetate buffer (pH 3.5, 0.2 M) to which 2 ml chitosan solution (0.5%, w/w) in 1% acetic acid was added and stirred for 8 h. Excess SDS and chitosan were removed in the supernatant fraction after centrifugation. After washing 3 times with 0.002 M acetate buffer (pH 3.5) and ultra-pure water alternately, the chitosan on the surface of Fe_2O_3 nanoparticles was cross-linked with 2.5% glutaraldehyde (pH 4.0) at room temperature for 4 h. The products were enriched with the aid of an external magnet. Afterward, the chitosan/Fe_2O_3 nanocomposites obtained were added to 16-nm gold colloids, and shaken slightly for 4 h at room temperature (RT) to make the gold nanoparticles assemble on the surface of the formed nanocomposites. Finally, the formed MnGMs were obtained by magnetic separation, and stored at 4 °C when not in use.

2.3. Conjugation of anti-AFB_2 with MnGMs

A solution containing 2.0 g of MnGMs was initially sonicated for 5 min, and then the mixture was centrifugated. After washing 3 times with PBS, the precipitate was resuspended in 10 ml of PBS and the pH adjusted to 9.5 using 10% (w/v) K_2CO_3. For labeling, 50 μl of *anti*-AFB_2 (0.646 μg/ml) was added to the mixture, and incubated for 12 h at RT with light stirring to allow antibodies adhesion to the gold nanoparticle surface. The synthesized *anti*-AFB_2–MnGMs were collected from solution by magnetic separation, and stored in 10 ml of PBS at 4 °C when not in use. The procedure is schematically shown in Fig. 1a. For comparison, gold nanoparticle-labeled *anti*-AFB_2 antibodies were prepared as described in the literature (Li et al., 2009).

2.4. Fabrication of the immunodipstick

The immunodipstick is schematically illustrated in Fig. 1b (left). It was prepared with a nitrocellulose membrane (0.3 cm × 2.0 cm). At a distance of 0.5 cm from the top of the membrane, a band of secondary antibodies (undiluted goat *anti*-mouse IgG) was manually spotted as control line at a volume of 1.0 μl/cm membrane width, and analogously, AFB_2–BSA conjugate (1.4 mg/ml) was applied as detection line at a distance of 1.0 cm from bottom. After the membrane was dried for 1 h at RT, it was blocked with BSA (2.0%, w/v) in PBS for 30 min on a shaker. It was then washed 3 times with PBS and again dried for 1 h at RT. The as-prepared membrane and an absorption pad (0.3 cm × 3.0 cm) were assembled onto a plastic backing card. The formed immunodipstick was stored under dry conditions at RT when not in use.

2.5. Immunodipstick procedure

The assay procedure is illustrated in Fig. 1c. The AFB_2 standard sample or diluted extract (100 μl) was initially added into a low-binding polypropylene microplate well (Greiner, art. no. 655201), and then 100 μl of detector reagent containing 80 μl of *anti*-AFB_2–MnGMs and 20 μl of Tween 20 solution (5%, w/v, in PBS) was added to the well. After shaking for 5 min on a minishaker (MS1, IKA, Taquara, Brasil), the dipstick was put into the well, and the liquid moved up the membrane with the aid of absorbent pad. When reaching the test line, *anti*-AFB_2–MnGMs reacted with the AFB_2–BSA conjugate on the membrane, and formed a pink line. When the AFB_2 concentration in the sample solution was above a certain value, all of the *anti*-AFB_2 antibodies on the surface of the nanocomposites were occupied and therefore, MnGMs passed through the test line. After reaching the control line, the immobilized *anti*-mouse IgG could react with the *anti*-AFB_2–MnGMs to form a second pink line. If no control line was present, the test was considered to be invalid. The color was recorded ~10 min after the dipstick was inserted into the well. Fig. 1b (right) shows schematically the results of negative and positive samples as well as invalid tests.

3. Results and discussion

3.1. Construction and detection principle of the MnGM-based immunodipstick

In the present work, Fe_2O_3 nanoparticles not only acted as a substrate for the assembly of gold nanoparticles, but also enabled the rapid separation and purification of bio-nanocomposites after synthesis. Chitosan, as a derivative of the natural polysaccharide chitin, is known for its biocompatibility and metal-binding capacity. The main advantage of chitosan over chitin is that it is soluble in dilute acid solutions through protonation of amine groups (Burke et al.,