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A nanoparticle-based bio-barcode assay for ultrasensitive detection of ricin toxin

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ABSTRACT

The ultrasensitive bio-barcode amplification assay (BCA) technique was developed for the specific detection of the A chain of ricin toxin. The target antigen A chain was first captured by gold nanoparticles (GNPs) coated with polyclonal antibodies. Magnetic microparticles (MMPs) coated with A chain monoclonal antibody were then added to form a sandwich immuno-complex. After the immuno-complex was formed, signal DNA annealed to DNA strands covalently bound to the GNPs were released by heating and characterized by PCR and real-time fluorescence PCR. A detection limit of 1 fg/ml was measured for A chain, six orders of magnitude more sensitive than that of conventional antigen-capture ELISA. The coefficient of variation (CV) of intra-assay and inter-assay ranged from 3.39% to 6.84%. The BCA can detect the A chain in milk and water mimic samples. In the following work it is demonstrated that this assay is a highly sensitive method for the detection of ricin proteins that could be adapted to measure other proteins.

1. Introduction

Ricin is one of the most toxic and easily obtainable plant toxin and is isolated from the seeds of castor bean *Ricinus communis* (Roberts and Smith, 2004). The ricin toxin is a heterodimer comprised of a 32 kDa A chain and a 34 kDa B chain connected by interchain disulfide bond (Stine et al., 2005). The B chain is a lectin which binds specifically to galactosyl residues on the cell surface and appears to trigger the endocytic uptake of ricin. The A chain is separated from the B chain in the cytoplasm and enzymatically inactivates the 60S ribosomal subunit, disrupting protein synthesis. Ricin is the only protein toxin listed under the Schedule 1 category of the Chemical Weapons Convention (CWC) (Atlas, 2002). Intake of trace amounts of ricin results in diarrhea, vomiting, septic shock, pyrogenicity, and even death. The U.S. Centers for Disease Control (CDC) gives a possible minimum amount of 500 μg for the lethal dose of ricin for human if exposure is from injection or inhalation.

Conventional analytical methods including radioimmunoassay (Godal et al., 1981), enzyme-linked immunosorbent assay (ELISA) (Poli et al., 1994), fluorescence-based fiber optic immunoassay (Narang et al., 1997) aptamer microarrays (Kirby et al., 2004), mass sensitive biosensor (Tran et al., 2008), microelectrochemical biosensors (Guglielmo-Viret and Thullier, 2007), electrochemiluminescent (ECL) (Garber and O'Brien, 2008), quantitative polymerase chain reaction (PCR) assay (Melchior and Tolleson, 2010), amperometric immunosensor (Suresh et al., 2010), silica coating magnetic nanoparticle-based silver enhancement immunoassay (Zhang et al., 2010) are effective for ricin detection. However these analytical methods are time-consuming or low sensitive. The bio-barcode amplification (BCA) assay has become a powerful analytical tool for the detection of both protein and nucleic acid targets (Agasti et al., 2010; Nam et al., 2004; Fournier-Wirth and Coste, 2010). Here,

Abbreviations: BCA, bio-barcode amplification assay; GNPs, gold nanoparticles; MMPs, magnetic microparticles; ELISA, enzyme-linked immunosorbent assay; PCR, polymerase chain reaction.

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we report that the BCA (Fig. 1) using SYBR-Green real-time PCR as a readout can be used to detect trace amounts of the ricin A chain.

2. Materials and methods

2.1. Reagents and materials

Ricin, Camphorin protein and ebulitins protein were purchased from NOVASYGEN (Beijing, China). The monoclonal antibody (mAb) was purchased from Affinity Bioreagents (U.S.A.). Ricin A chain antigen-specific Ab was purchased from Abcam (England). Tosyl activated-functionalized magnetic microparticle (MMP) was purchased from Invitrogen (U.S.A.). Taq™ was obtained from TaKaRa (Dalian, China). RealMasterMix and SYBR solutions were from TIANGEN (Beijing, China). iCycler Detection System was bought from Bio-Rad (U.S.A.).

2.2. Preparation of magnetic microparticles probe

For preparation of the magnetic microparticles (MMPs) probe, the monoclonal antibodies (mAb) (Affinity Bioreagents, U.S.A.) specific to ricin A chain were bound to 100 μl tosyl activated-functionalized magnetic microparticles (MMPs) (30 mg/ml aqueous solution, 2×10^9 beads/ml, 2.8 μm diameter) (Invitrogen, U.S.A.) according to the manufacturer's protocol.

2.3. Preparation of gold nanoparticles probe

The following DNA strands were synthesized: complementary probe NP, 5′-TAC GAG TTG AGA CCG TTA AGA CGA GGC AAT CAT GCA ATC CTG AAT GCG A_{10}-$(CH_2)_6$-SH-3′; barcode, 5′-CGC ATT CAG GAT TGC ATG ATT GCC TCG TCT TAA CGG TCT CAA CTC GTA-3′ (Georganopoulou et al., 2005). The gold nanoparticles (GNPs) (30 nm diameter) were synthesized using a chemical reduction method (Hill and Mirkin, 2006). And then the GNPs (1 ml) were incubated with 5 μg of ricin A chain antigen-specific Ab (Abcam, England) in a basic aqueous solution (pH 9.5) for 30 min. The particles were subsequently modified with thiolated DNA complementary to the barcode DNA (final concentration, 2 μM) by slow salt aging (40 h) to a final concentration of PBS (0.1 M NaCl in 0.01 M of phosphate buffer, pH 7; denoted as PBS unless indicated otherwise). Unbound thiolated DNA was removed by several rounds of centrifugation (10,000 × g for 30 min) and rinsing in PBS. The barcode DNA was added (2 μM) to the modified GNPs solution and allowed to hybridize at room temperature for 4 h. The nanoparticles probes (NPs) were centrifuged at 14,000 × g for 30 min, and the supernatant removed. The particles were resuspended in 0.1 M PBS, and the procedure was repeated 3 times.

2.4. Bio-barcode amplification assay

2.4.1. Formation of the sandwich structure

In the BCA, 10 μl of ricin (NOVASYGEN, Beijing, China) at different concentrations were added to 50 μl of MMPs (5 mg/ml solution) and incubated for 1 h at 37 °C with vigorous stirring. After magnetically immobilizing the MMPs, unbound antigens were removed by repeated washing with PBS. Washed MMPs were incubated with 50 μl of NPs (0.1 nM) for 30 min at 37 °C with vigorous stirring. The NP-bound MMP complexes were then magnetically separated and washed four times with 500 μl of PBS. In the final step of the assay, 50 μl of H_2O was added and the solutions were vigorously stirred for 30 min at 60 °C to allow for dehybridization of the target DNA. The beads were then separated from the supernatant magnetically, and the barcode DNA collected for quantification by PCR and real-time PCR methods.

2.4.2. Detection of barcode DNA by PCR

PCR was performed in a final volume of 20 μl containing PCR buffer (10 mM Tris–HCl pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$), 0.2 mM of dNTP mix (TaKaRa, Dalian, China), 20 pmol of forward primer (5′ CGC ATT CAG GAT TGC ATG AT 3′), 20 pmol of reverse primer (5′ TAC GAG TTG AGA CCG TTA AG 3′), 1.2 U Taq™ (TaKaRa, Dalian, China) and 1 μl the barcode DNA solution under the following conditions: 94 °C for 5 min, followed by 35 cycles of 94 °C for 20 s, 58 °C for 10 s and 72 °C for 10 s, and a final incubation at 72 °C for 3 min.

2.4.3. Detection of barcode DNA by real-time fluorescence PCR

Real-time fluorescence PCR detection was conducted in a reaction mixture (20 μl) containing RealMasterMix (TIANGEN, Beijing, China), SYBR solution (TIANGEN, Beijing, China), 20 pmol of both above primers and 1 μl of the barcode DNA solution. Reactions were carried out at 94 °C for 5 min followed by 45 cycles of 94 °C for 20 s, 58 °C for 10 s and 72 °C for 10 s, and a final incubation at 72 °C for

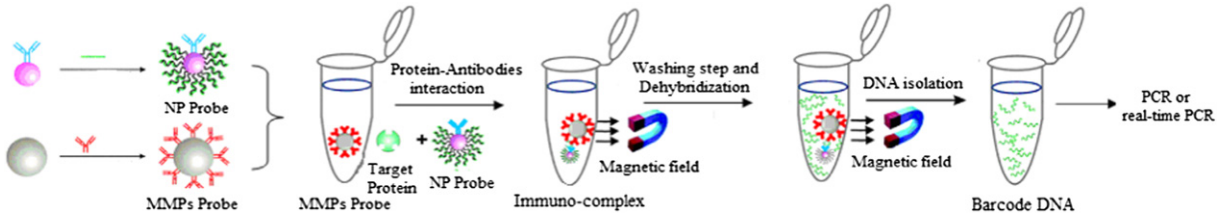

Fig. 1. The barcode assay method. The assay uses MMPs probe functionalized with mAbs that recognize and bind target protein (ricin toxin). The ricin toxin is then functionalized with NP probe that has been modified with ricin A chain antigen-specific Ab and signal DNAs. After washing while using a magnet to immobilize the MMPs probe, a dehybridization step releases hundreds of barcode DNA strands and then using PCR or real-time PCR to detect the barcode DNA strands.