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Detecting clothianidin residues in environmental and agricultural samples using rapid, sensitive enzyme-linked immunosorbent assay and gold immunochromatographic assay

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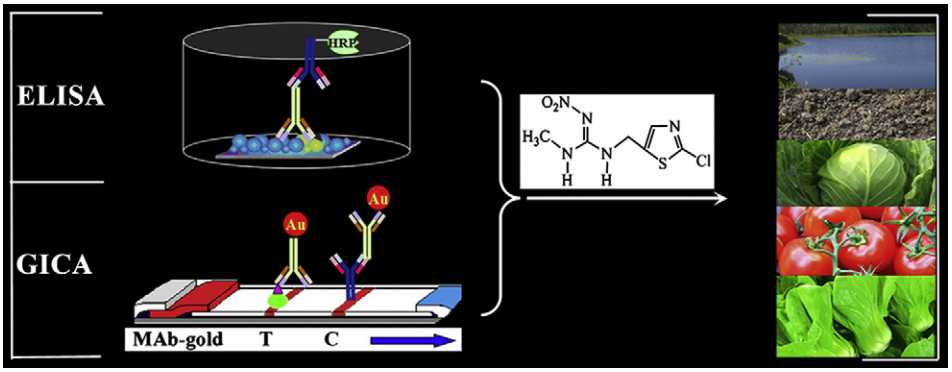
HIGHLIGHTS

- An ELISA and a GICA were developed and applied to the determination of clothianidin.
- The results for authentic samples correlated well with those obtained by HPLC.
- GICA showed significant improvements in rapidity, portability, and on-site detection.

GRAPHICAL ABSTRACT



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ABSTRACT

Two rapid, sensitive immunoassays based on monoclonal antibody for detecting clothianidin were developed and applied in agricultural samples: a quantitative enzyme-linked immunosorbent assay (ELISA) and a semi-quantitative gold immunochromatographic assay (GICA). Under optimal conditions, the half-maximal inhibition concentration (IC_{50}) and the limit of detection (LOD, IC_{10}) of clothianidin were 25.6 and 3.8 ng mL^{-1} for ELISA. GICA using colloidal gold-MAb probe had a visual detection limit of 8 ng mL^{-1}, and the results can be judged by the naked eye within 10 min. The cross-reactivities of the immunoassays with its analogues were negligible except for that with dinotefuran. For the spiked agricultural samples, recoveries of 78.0 to 114.5% with relative standard deviations (RSDs) of 3.2 to 12.8% were achieved for ELISA and further evaluated by GICA. Furthermore, the results of ELISA and GICA for the authentic samples correlated well with those obtained by HPLC. Overall, the proposed ELISA and GICA are satisfactory for rapid, sensitive, and quantitative/semiquantitative detection of clothianidin residues in agricultural samples.

1. Introduction

Immunoassays are now well-established techniques for the determination of trace levels of small molecule analytes. They are proven to be rapid, sensitive, and cost-effective analytical tools for the monitoring of environmental and agricultural contaminants (Li and Li, 2000; Silva

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et al., 2014). The most commonly used enzyme-linked immunosorbent assays (ELISAs) are performed with primary or secondary antibodies that are labeled with enzymes to generate the reporter signal (Wanatabe et al., 2011; Zhang et al., 2008). A gold immunochromatographic assay (GICA) using gold nanoparticles as a detection marker is a significant one-step assay for on-site detection. Due to the remarkable advantages of rapidity, simplicity, portability and the ability to be used for on-site detection, GICA has gained increasing interest and has been widely applied in various fields of disease diagnosis, food safety, and environment monitoring (Li et al., 2013a; Shim et al., 2013; Zhou et al., 2013). By using ELISA and GICA in combination, screening and semiquantitative and quantitative determination might be achieved for target analytes.

Clothianidin is a member of the neonicotinoid insecticides, which has been widely applied in vegetables and other field crops for the long-term control of a wide variety of pests with excellent efficacies (Motohiro and John, 2005; Uneme, 2011). As a result, clothianidin can be present in river water, soil and agricultural products. To protect consumers from risks related to clothianidin residue, the monitoring of clothianidin is of great importance, and the maximum residue limits (MRLs) of clothianidin have been established. In the USA, the MRLs of clothianidin were set at 10 ng g^{-1} for sorghum and cotton, 20 ng g^{-1} for *Beta vulgaris*, 50 ng g^{-1} for potato, and 600 ng g^{-1} for grape (Li et al., 2012). There is no suggested MRL for clothianidin in China. Several instrument-based detection methods for clothianidin have been developed including gas chromatography (GC) (Li et al., 2012) and high performance liquid chromatography (HPLC) (Hou et al., 2011; Kim et al., 2012), which are characterized by high precision and sensitivity. Unfortunately, they are infeasible for large-scale and on-site fast analyses because they always require tedious sample preparation prior to instrumental analysis (Hennion and Barcelo, 1998; Van Emon et al., 2013). As mentioned earlier, immunoassays are increasingly considered as alternative or complementary methods for residue analysis and have significant advantages.

Polyclonal antibody-based ELISAs have been applied to the determination of clothianidin in our previous studies (Li et al., 2013b). In this study, the monoclonal antibody (MAb) was produced and used to develop ELISA and GICA, resulting in realizing quantitative and semiquantitative detection for clothianidin in agricultural samples. The immunochromatographic strips were prepared using the MAb-nanogold probe, which has been optimized and evaluated carefully. Moreover, the immunoassays have been applied to authentic samples and confirmed by HPLC. To our knowledge, this is the first report of successful semiquantitative detection of clothianidin using a GICA.

2. Materials and methods

2.1. Reagents and equipments

Clothianidin and the pesticide standards used for cross-reactivity studies were supplied by Jiangsu Pesticide Research Institute (Jiangsu, China). Bovine serum albumin (BSA), ovalbumin (OVA), horseradish peroxidase (HRP)-labeled goat anti-mouse IgG and goat anti-mouse IgG were purchased from Sigma-Aldrich (St. Louis, MO, USA). Chloroauric acid ($HAuCl_4$), trisodium citrate, PEG 20000 and other chemical reagents were all supplied by Aladdin (Shanghai, China). The microtiter plates used were 96-well transparent microplates (Nunc, Roskilde, Denmark). Nitrocellulose (NC) membranes, glass-fiber conjugate pads, sample pads, and absorbent pads were provided by Millipore (Bedford, MA, USA). BLAB/c female mice were obtained from the Center of Comparative Medicine of Yangzhou University (Yangzhou, China). All animals used in this study and animal experiments were approved by the Committee of Laboratory Animal Management of Jiangsu Province. The license number was SYXK (SU) 2002-0045.

The test strips were prepared using a XYZ-3000 dispensing platform and a CM4000 guillotine cutter (BioDot, Irvine, CA, USA). The diameters of colloidal gold particles were scanned by an H-7650 transmission electron microscope (Hitachi, Tokyo, Japan). Milli-Q purified water was obtained from a Milli-Q purification system (Millipore, Bedford, MA, USA). ELISA was detected using a SpectraMax M5 (Molecular Devices, Sunnyvale, CA, USA). The results of ELISA and GICA were validated with an Agilent 1260 HPLC equipped with an ultraviolet detector (Agilent, Wilmington, DE, USA).

2.2. Preparation of antigens and monoclonal antibody

The chemical synthesis and identification of clothianidin hapten (1-[2-(2-carboxyethylthio)-1,3-thiazol-5-ylmethyl]-3-methyl-2-nitroguanidine) were performed as described in a previous report (Li et al., 2013b). Subsequently, the hapten was conjugated to BSA and OVA to produce an immunogen and coating antigen by the activated ester method (Fang et al., 2011). The anti-clothianidin monoclonal antibody (MAb) was prepared according to the classic hybridoma technology (Wang et al., 2011). Firstly, BALB/c mice (six weeks old) were immunized with the immunogen by intraperitoneal injection. The mouse showing the highest anti-clothianidin activity was selected to be spleen donors. Secondly, the hybridoma cells were acquired by fusion of the spleen cells isolated from the selected mouse with SP2/0 myeloma cells. Hybridoma cells secreting highly specific and sensitive anti-clothianidin antibodies were selected with the limiting dilution method and expanded. Finally, the selected clones were used for MAb production by ascite growth. The ascite fluids were collected and purified by saturated ammonium sulfate precipitation, and purified MAb was stored at −20 °C. From determination with the IsoQuick™ Kit for mouse monoclonal isotyping (Sigma, USA), the MAb belonged to the IgG3 subclass with a kappa light chain.

2.3. Indirect competitive ELISA protocol

For the ic-ELISA, the detection procedure was carried out as previously described (Gui et al., 2009). The concentrations of antibody and coating antigen were optimized by checkerboard titration. The working solutions, which contained a series of methanol contents, Na^+ and pH values were studied to evaluate the sensitivity of the ELISA. Additionally, the cross-reactivities (CRs) for compounds structurally related to clothianidin (Fig. S1, see Supplementary material) were determined under the optimized ELISA conditions.

2.4. Preparation of the colloidal gold

Colloidal gold was prepared according to the method of Zhang et al. (2011) with modifications. Under stirring conditions, a 1% $HAuCl_4$ solution (1 mL) was added to 100 mL of Milli-Q purified water and heated to boiling. Then, 2 mL of 1% trisodium citrate solution was added rapidly under constant stirring. The solution was boiled for another 5 min after the mixture's color changed from deep blue to brilliant wine red. After cooling to room temperature, the dispersion and diameters of the colloidal gold were checked with a transmission electron microscope.

2.5. Preparation of MAb-nanogold probes

The preparation of MAb-nanogold probes was the same as described by Hua et al. (2010). The MAb was dissolved in Milli-Q purified water and dialyzed against 0.9% NaCl solution. The pH value of the colloidal gold solution was adjusted to 8.2 with 0.2 mol L^{-1} K_2CO_3. The lowest amount of MAb for stabilizing colloidal gold was determined by salt-induced precipitation experiment. More than 10% of the lowest amount of MAb was added rapidly into 20 mL of colloidal gold solution with quick stirring, and then it was incubated for 1 h. Subsequently, 2 mL of 10% BSA solution was added to block excess reactivity of colloidal gold