Biosensors and Bioelectronics 42 (2013) 170–176

Contents lists available at SciVerse ScienceDirect

Biosensors and Bioelectronics

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

Development of an immunochromatographic assay based on carbon nanoparticles for the determination of the phytoregulator forchlorfenuron

Celia Suárez-Pantaleón [a], Jan Wichers [b], Antonio Abad-Somovilla [c], Aart van Amerongen [b,*], Antonio Abad-Fuentes [a,**]

[a] Department of Biotechnology, IATA-CSIC, Agustí Escardino 7, 46980 Paterna, València, Spain
[b] Biomolecular Sensing & Diagnostics, Wageningen UR Food & Biobased Research, BornseWeilanden 9, P.O. Box 17, 6700 AA Wageningen, The Netherlands
[c] Department of Organic Chemistry, Universitat de València, Doctor Moliner 50, 46100 Burjassot, València, Spain

ARTICLE INFO

Article history:
Received 31 July 2012
Received in revised form
24 October 2012
Accepted 1 November 2012
Available online 7 November 2012

Keywords:
CPPU
Hapten
Immunoreactive strips
Lateral flow immunoassay
Carbon nanoparticles
Immunosensing

ABSTRACT

Rapid analytical methods enabling the determination of diverse targets are essential in a number of research areas, from clinical diagnostics to feed and food quality and safety. Herein, the development of a quantitative immunochromatographic assay for the detection of the synthetic phytoregulator forchlorfenuron (CPPU) is described. The competitive lateral flow immunoassay (LFIA) was based on the immobilization onto a nitrocellulose membrane of an ovalbumin–CPPU conjugate (test line) and on the use of an immunodetection ligand consisting of carbon nanoparticles labeled with an anti-CPPU monoclonal antibody through interaction with a secondary antibody. The presence of CPPU in horticultural samples was visually interpreted by the decrease in the black signal intensity of the test line, according to the competitive character of the format. The quantitative determination of the analyte was easily performed by a two-step procedure consisting of flatbed scanning of the strips followed by computer-based image analysis of the pixel gray volumes of the test lines. Under optimized conditions, the immunochromatographic test afforded a limit of quantification in buffer of 89 ng/L. The accuracy of the strip test was assessed by the analysis of fruit samples with incurred residues, and the obtained results were compared with those derived from two reference methods, ELISA and HPLC. The LOQ of the CPPU-specific LFIA in kiwifruits and grapes was established at 33.4 μg/kg. The excellent analytical performance of the developed strip test demonstrates the potential of immunochromatographic assays for the quantitative monitoring of small organic molecules in complex matrices.

© 2012 Elsevier B.V. All rights reserved.

1. Introduction

Forchlorfenuron (Fig. 1S), also referred to as CPPU (1-(2-chloro-4-pyridyl)-3-phenylurea), is a highly effective synthetic plant growth regulator (Takahashi et al., 1978) applied worldwide to increase fruit size, especially of kiwifruits and table grapes (Iwahori et al., 1988; Nickell, 1986). The intensive use of agrochemicals may lead to the presence of residues in foodstuffs when commodities reach the market, an issue of concern and high priority to both public authorities and the general population. Accordingly, the use of agrochemicals in different crops is strictly regulated by international organizations and national governments. With the aim of protecting public health and the environment, maximum residue limits (MRLs) fixing the highest acceptable amount of a particular chemical in a certain crop have been established. For CPPU in particular, MRLs have been set up from 10 to 100 μg/kg, depending on the country and the target horticultural commodity. To ensure the effectiveness of the monitoring systems in place for risk management in food, the implementation of reliable, fast, and user-friendly analytical methods for the traceability of xenobiotics is highly desirable.

The analysis of chemical residues and contaminants has been traditionally dominated by the instrumental techniques. Chromatographic methods for CPPU determination based on high-performance liquid chromatography (HPLC) coupled to different detection systems have been reported (Hu and Li, 2006; Valverde et al., 2010). Although highly accurate, reproducible, and sensitive, chromatographic methods are often time-consuming, expensive, and laboratory-oriented as they require well-trained personnel and sophisticated instrumentation (Lee and Kennedy, 2001). Immunoanalytical tools are deemed highly sensitive, selective, rapid, and cost-effective methods, thus complementing chromatographic analysis. In addition, they are especially well-suited for high-throughput screening in difficult matrices without extensive sample pre-treatment (Knopp, 2006). Moreover, antibodies can be easily integrated into a

* Corresponding author. Tel.: +31 317480164; fax: +31 317483011.
** Corresponding author. Tel.: +34 963900022; fax: +34 963636301.
E-mail addresses: aart.vanamerongen@wur.nl (A. van Amerongen), aabad@iata.csic.es (A. Abad-Fuentes).

0956-5663/$ - see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.bios.2012.11.001

variety of automated systems, from liquid handling workstations to immunosensor-based analytical platforms (Bange et al., 2005). In previous papers, the production of monoclonal and polyclonal antibodies (mAb and pAb, respectively) for CPPU was described (Suárez-Pantaleón et al., 2008, 2010, 2011), and enzyme-linked immunosorbent assays (ELISAs) were developed and successfully applied to the detection of CPPU in fruit samples. Even though ELISA certainly is one of the most widespread and popular kind of immunoassays, immunochromatographic assays are better suited for on-site rapid applications. The large number of scientific publications in the diagnostic (Andreo et al., 2006; Mens et al., 2008), medical (Lin et al., 2008; Omidfar et al., 2012; Yang et al., 2011), veterinary (Noguera et al., 2011), drug of abuse (Gandhi et al., 2009), environmental (Blažková et al., 2009; Kim et al., 2003; Zhou et al., 2010), and food safety areas (Anfossi et al., 2011; Aldus et al., 2003; Tang et al., 2009; Zhang et al., 2011; Zhou et al., 2009), well illustrates the prominent position that immunochromatographic methods have attained in the analytical field as portable point-of-care devices in recent years (Posthuma-Trumpie et al., 2008).

Strip-based tests were originally designed for sample screening, thus efficiently reducing analytical costs because only non-compliant samples would be submitted to further determination by confirmatory methods. At present, there is an urgent need for strip-based tests meeting requirements of robustness and accuracy while keeping simplicity and affordability. Accordingly, the development of quantitative immunochromatographic tests is gaining increasing attention. Successful examples have been described for the analysis of proteins (Kim et al., 2006; van Amerongen et al., 1994; Wei et al., 2011), nucleic acids (Blažková et al., 2011; He et al., 2011; Noguera et al., 2011; van Amerongen and Koets, 2005), and small organic molecules (Blažková et al., 2010; Campbell et al., 2007; Hua et al., 2010; Kaur et al., 2007; Omidfar et al., 2010; Mirasoli et al., 2012; O'Keeffe et al., 2003; Wang et al., 2011; Xie et al., 2009).

Based on the characterization by ELISA of previously produced immunoreagents, the mAb p6♯42, with a remarkable affinity and specificity to the synthetic cytokinin CPPU, was selected for the development of a rapid strip test. In the present work, a mAb-based LFIA using carbon nanoparticles as label is presented for the quantitative and rapid detection of CPPU. The analytical performance of the one-step strip test was assessed by determining CPPU in incurred kiwifruit and grape samples, using ELISA and HPLC as reference methods.

2. Materials and methods

2.1. Reagents

Forchlorfenuron [1-(2-chloro-4-pyridyl)-3-phenylurea, CPPU] (CAS Registry No. 68157-60-8, MW 247.7 g/mol) and 1-(4-pyridyl)-3-phenylurea (PPU) were from Sigma-Aldrich (Madrid, Spain). Stock solutions of CPPU (100 mM) and PPU (100 mM) were prepared in anhydrous N,N-dimethylformamide and methanol, respectively, and they were stored at −20 °C. Ortho-phenylenediamine (OPD) was from Sigma-Aldrich (Madrid, Spain). Spezial Schwarz 4 carbon nanoparticles were acquired from Degussa AG (Frankfurt, Germany). Bovine serum albumin (BSA) was purchased from Sigma-Aldrich (Zwijndrecht, The Netherlands). Nitrocellulose Hi-Flow Plus membrane HF090 (capillary flow time of 90 s/4 cm) was from Millipore (Molsheim, France). Absorption pads (filter paper 2886) were purchased from Schleicher & Schuell (Middlesex, UK).

The synthesis of haptens p6 and p2, the preparation of capture conjugates OVA–p6 and OVA–p2, and the production of mAb p6♯42 have been previously described (Suárez-Pantaleón et al., 2008). Cross-reactivity studies with the mAb p6♯42 showing the high specificity of the anti-CPPU antibody have been previously reported (Suárez-Pantaleón et al., 2011). The chemical structure of CPPU and haptens is shown in Fig. 1S. Polyclonal goat anti-mouse IgGFcγ fragment specific immunoglobulins (GAM) and polyclonal donkey anti-goat IgG (H+L) immunoglobulins (DAG) were from Jackson Immunoresearch Europe (Sanbio, Uden, The Netherlands). Polyclonal rabbit anti-mouse immunoglobulins labeled with peroxidase (RAM-HRP) were from Dako (Glostrup, Denmark). 96-well Costar flat-bottom high-binding polystyrene microplates were from Corning (Corning, NY, USA).

2.2. Instrumentation

Capture conjugates (CC) were immobilized onto the membranes using a TLC-spotter Linomat IV (Camag, Muttenz, Switzerland). Membranes were cut into strips using a Bio-Dot CM4000 cutter (Biodot Inc., Irvine, CA, USA). Scanning of the strips was carried out with an Epson 3200 Photo scanner (Seiko Epson, Nagano, Japan). Measurements of the pixel gray volume of the test and control lines were performed using the TotalLab image analysis software (Nonlinear Dynamics, Newcastle, UK).

2.3. Buffers

(1) *Coupling buffer*: 5 mM sodium borate buffer, pH 8.8; (2) *Washing solution*: 5 mM sodium borate buffer, pH 8.8, 1% (w/v) BSA, 0.02% (w/v) NaN_3; (3) *Storage buffer*: 100 mM sodium borate buffer, pH 8.8, 1% (w/v) BSA, 0.02% (w/v) NaN_3; (4) *Spraying buffer*: 5 mM sodium borate buffer, pH 8.8; (5) *Running buffer*: 100 mM sodium borate buffer, pH 8.8, 1% (w/v) BSA, 0.05% (v/v) Tween 20, 0.02% (w/v) NaN_3.

2.4. Development of the LFIA

2.4.1. Preparation of carbon nanoparticles—secondary antibody detection conjugate (DC)

For the preparation of the DC, carbon nanoparticles were labeled with GAM immunoglobulins (O'Keeffe et al., 2003). A 1% (w/v) suspension of carbon was prepared in demineralized water by sonication. Then, 100 μL of coupling buffer containing 175 μg of GAM were added dropwise to 500 μL of a 5-fold dilution of carbon (0.2%, w/v) in coupling buffer. After overnight incubation at 4 °C under gentle stirring, the solution was washed four times with washing solution by centrifugation (13600*g*; 15 min). Then, the DC was reconstituted in storage buffer at a final concentration of carbon of 0.2%, and stored at 4 °C. Before running experiments, the working dilution of the GAM–carbon conjugate was sonicated for 10 s.

2.4.2. Immobilization of reagents onto nitrocellulose membranes

Capture conjugates OVA–p6 and OVA–p2 diluted in spraying buffer were immobilized onto plastic-backed membranes at 3 cm from the origin forming the test line (TL). The final concentration of CC on the TL was 62.5, 250 or 1000 ng/strip. DAG immunoglobulins (80 ng/strip), also diluted in spraying buffer, were immobilized onto the nitrocellulose at 3 mm above the TL to form the control line (CL). After spraying, the membranes were dried overnight at 37 °C. Then, the nitrocellulose membranes were placed on a second plastic backing and an absorbance pad was applied at the upper part of the membranes. Subsequently, the membranes were cut into 0.5 × 5 cm strips and they were stored in sealed plastic laminated aluminum bags with a desiccation pad at room temperature until use.

2.4.3. LFIA procedure and signal processing

For convenience, microtiter plate wells instead of plastic housings or tubes were used as containers for running the

Download English Version:

https://daneshyari.com/en/article/867090

Download Persian Version:

https://daneshyari.com/article/867090

Daneshyari.com