Trends in Food Science & Technology 71 (2018) 181–187

Contents lists available at ScienceDirect

Trends in Food Science & Technology

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

Review

Non-competitive immunoassay for low-molecular-weight contaminant detection in food, feed and agricultural products: A mini-review

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ARTICLE INFO

Keywords:
Anti-metatype antibody
Hapten
Immunosensor
Non-competitive immunoassay
Immunocomplex antibody
Open sandwich immunoassay

ABSTRACT

Background: Immunoassays have gained considerable attention in safety assurance for food, feed and agricultural products. Generally, immunoassays are presented either in a competitive or non-competitive, sandwich-type format, and the former is extensively employed for low-molecular-weight contaminants, which usually bear one accessible epitope. Theoretically, non-competitive, sandwich-type immunoassays have higher sensitivity, precision and linearity. However, the analyte to be measured in such a format must be large enough to have at least two epitopes to be captured. It is not feasible to detect low-molecular-weight contaminants through conventional non-competitive sandwich-type immunoassay. Consequently, there is a trend to develop new types of sensitive non-competitive immunoassays for low-molecular-weight contaminants.

Scope and approach: This article reviews the progress in non-competitive immunoassays for low molecular weight contaminants in food, feed and agricultural products, including the principles, applications and suggested perspectives for this field.

Key findings and conclusions: *Anti*-metatype antibody-based immunoassays are the most promising method, but dissociation of the antibody-hapten complex might be a challenge, and therefore more in-depth research should be focused on preparation of new formats of the antibody-hapten complex. Meanwhile, strategies for direct non-competitive detection or aimed at the simultaneous detection of different targets would be especially desirable besides focusing on improving the sensitivity and specificity of the detection.

1. Introduction

Low molecular weight contaminants (or hapten molecules) with a molecular weight less than 1000 Da, such as pesticides, veterinary drugs and mycotoxins, in food, feed and agricultural products, pose potential risks to food safety (Guo, Feng, Fang, Xu, & Lu, 2015; Malarkodi, Rajeshkumar, & Annadurai, 2017). Therefore, the sensitive detection of these contaminants is of great significance, and a number of methods have been developed for their successful measurement. Thin-layer chromatography (TLC) (Kotzybik et al., 2016; Li et al., 2016), gas chromatography-mass spectrometry (GC-MS) (Lee et al., 2017; Lin et al., 2015), and high-performance liquid chromatography (HPLC) with ultraviolet (de Figueiredo et al., 2015; de Lima, Vieira, Martins, Boralli, Borges, & Figueiredo, 2016), fluorescence (Smith, Francis, Johnson, & Gaskill, 2017; Rahman et al., 2017) or mass spectrometry detection (Santos & Ramos, 2016; Trevisan, Owen, Calatayud-Vernich, Breuer, & Picó, 2017), and immunoassays (Hu et al., 2017; Spinks, 2000) are widely employed. Among them, immunoassays have gained substantial attention due to their rapid, sensitive and high-throughput screening ability (Zhang et al., 2017; Yang, Zhang, Chen, He, & Hu, 2017).

As is well known, immunoassays can be categorized into competitive and non-competitive formats. Theoretically, the latter has a higher sensitivity, precision and linearity (Islam et al., 2011). However, the detection of low molecular weight contaminants is normally based on the competition principle (Anfossi, Di Nardo, Giovannoli, Passini, & Baggiani, 2015; Liu, Ye, Chen, Wang, & Chen, 2015), as in most other immunochemical detection of small molecules (Suzuki, Munakata, Morita, Shinoda, & Ueda, 2007). A non-competitive immunoassay, such as a conventional, sandwich-type immunometric assay, is widely used to determine analyte concentrations with high sensitivity and selectivity. However, the analyte to be determined should have at least two accessible epitopes (Janssen et al., 2015; Kobayashi & Oyama, 2011). Consequently, low molecular weight contaminants in food, feed

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https://doi.org/10.1016/j.tifs.2017.11.014
Received 19 August 2016; Received in revised form 20 August 2017; Accepted 19 November 2017
Available online 23 November 2017
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Table 1
Comparison of six types of non-competitive immunoassays for low molecular weight analytes in food, feed and agricultural products.

Type	Methodology	Advantage	Disadvantage	Analyte	Reference
Open sandwich immunoassay	VH-VL association can be enhanced by the presence of antigen	The possibility of obtaining immunoreagents as fusion proteins; Selection of VH/VL pairs using libraries	Selecting VH/VL pairs for hapten	bisphenol A zearalenone estradiol	Aburatani et al., 2003 Suzuki et al., 2007 Liu, Yang, et al., 2012
Anti-metatype antibody-based immunoassay	*Anti*-metatype antibodies are specific for the conformation of the bound antibody	Selection of suitable immunoreagents is facilitated by the use of libraries	Preparation of *anti*-metatype antibodies	morphine clomazone HT-2 toxin	Pulli, Höyhtyä, Söderlund, & Takkinen, 2005 Rossotti et al., 2010 Arola et al., 2016
Idiometric assay	α-Ab1 preferentially binds to the hapten-occupied Ab1	No obvious advantage	Preparation of the first antibody and *anti*-idiotypic antibodies	estradiol estradiol	Barnard & Kohen, 1990 Barnard et al., 1991
Giraudi's method	Blocking free sites of capture antibody by a "blocking reagent"; which is more strongly bound than the analyte to the immobilized antibody	No need to produce more than one antibody	Preparation of blocking reagent	aflatoxins cortisol	Acharya & Dhar, 2008 Anfossi et al., 2002
Special separation	No uniform methodology	No need to produce more than one antibody	Design of separation strategy; an extra separation process is needed	DDT AFB1	Anfossi et al., 2004 Saha et al., 2013
Direct non-competitive immunosensor	At the time of antibody-hapten interaction, measuring changes in optical, mass, electrochemical, and other signals.	Rapidity and simplicity as no label is required	High-tech instrument and operators are needed	ochratoxin A ochratoxin A carbofuran	Khan & Dhayal, 2009 Li et al., 2012 Dai et al., 2017

and agricultural products are not suitable for detection through conventional, sandwich-type immunoassays. In the past few years, researchers have proposed several types of non-competitive immunoassays for low molecular weight contaminants, and a list of frequently used formats is shown in Table 1. Existing approaches for non-competitive immunoassays for low molecular weight molecules have been previously reviewed (Fan & He, 2012; Li & Deng, 2016). This review seeks to present an update of the state-of-the-art and to focus on advances in non-competitive immunoassays for low molecular weight contaminants in food, feed and agricultural products.

2. Non-competitive immunoassays for low molecular weight contaminants in food, feed and agricultural products

2.1. Open sandwich immunoassay

Open sandwich enzyme-linked immunosorbent assay (OS-ELISA), was first proposed by Ueda (Ueda et al., 1996). OS-ELISA was based on the phenomenon that the heavy-chain variable region (VH)/light-chain variable region (VL) association could be enhanced by the presence of the analyte. Since the advent of this method, several studies detecting low molecular weight contaminants in food, feed and agricultural products, such as Gonyautoxin (Hara, Dong, & Ueda, 2013), estradiol (Liu, Eichenberger, Fujioka, Dong, & Ueda, 2012), benzaldehyde (Shirasu et al., 2009) and zearalenone (Suzuki et al., 2007), have been reported. Generally, the VH or VL fragment was immobilized into ELISA plate wells, and the analyte and enzyme-VL or enzyme-VH fusion protein were added, followed by the enzymatic reaction (i.e.: color development) and signal detection. As is true for traditional sandwich-type immunoassays, the detected signal was proportional to the content of the analyte. Fig. 1 illustrates a schematic diagram of OS-ELISA with maltose binding protein as the immobilization ligand and alkaline phosphatase as the signal reporter. The fusion proteins comprised of VH/VL and an immobilization or reporter protein have been used extensively, since such strategy shortened the incubation time and facilitated the detection process.

Apparently, the VH and VL fragments are needed for developing OS-ELISA, and they were originally obtained by cloning and measuring the expression of relative genes from monoclonal antibodies. However, whether the antibody used have suitable properties, such that the VH/VL interaction would become fairly strong along with the addition of the analyte, can only be decided after measuring the VH/VL interaction, and this represents a major limitation for OS-ELISA. Therefore, Aburatani et al. (2003) devised a phage-based "split-Fv system", a filamentous phage p7-p9 display system individually displaying VH and VL fragments as a functional Fv on the tip of the phage to ensure fast and effective selection of Fv fragments suitable for OS-ELISA. The system was successfully used for selecting VH/VL fragments for bisphenol A, and a limit of detection (LOD) of 1 ng/mL was found. Likewise, by using VH/VL genes cloned from hybridomas, Suzuki et al. (2007) developed an open sandwich phage ELISA to detect the estrogenic mycotoxin zearalenone. To confirm the result obtained with the split-Fv phage display, the author performed OS-ELISA with the purified proteins (VH-maltose binding protein for immobilization and VL-alkaline phosphatase for color development) achieving a lower detection limit (approximately 0.1 ng/mL) together with a wider working range compared with a competitive ELISA performed with the split-Fv phage or the original monoclonal antibody. Likewise, Dong, Ihara, & Ueda (2009) developed a strategy based on a phagemid vector in which two identical restriction sites were incorporated into both ends of a human constant region domain. After selection of the M13 phage displaying a Fab fragment, the vector was easily converted to the vector able to produce the VH-displaying phage and the light chain simultaneously in the culture supernatant. Subsequently, they could be directly used for OS-ELISA. By using the strategy proposed by Dong et al. (2009), Hara et al. (2013) employed an OS-ELISA to detect GTX2/3

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