Advanced Materials Research *Online: 2012-07-26*
ISSN: 1662-8985, Vols. 550-553, pp 1911-1914
doi:10.4028/www.scientific.net/AMR.550-553.1911

Detection of Zearalenone by Chemiluminescence Immunoassay in Corn Samples

Yuan-Kai WANG[a], Qi ZOU[b], Jian-He SUN[c], Heng-an WANG[d] and Ya-Xian YAN[*e]

Key Laboratory of Veterinary Biotechnology, School of Agriculture and Biology, Shanghai Jiao Tong University, Shanghai 200240, China

[a]email: grand1986stone@yahoo.com.cn, [b]email: 179021491@qq.com, [c]email: sunjhe@sjtu.edu.cn, [d]email: hawang@sjtu.edu.cn, [e]email: yanyaxian@sjtu.edu.cn

Keywords: Zearalenone, Polyclonal antibody, Chemiluminescence, Enzyme-linked immunosorbent assay (ELISA)

Abstract. A chemiluminescence immunoassay with polyclonal antibody for detecting zearalenone in corn samples was developed. The optimized reaction conditions include the concentration of coating antigen (0.253 μg/ml), the polyclonal antibody (1:7500) and HRP labeled goat anti-mouse antibody (1:40000), the competition time (60 min), pH of dilution buffer (7.4), the concentration of methanol (10%) and the ratio of luminol to ρ-iodophenol (2:5) were all investigated. Based on optimum conditions, the chemoluminscence ELISA detect range was f 0.05 - 54.34 ng/ml, with IC50 was 2.93 ng/ml, and the limit of detection was 0.02 ng/ml. The chemiluminescence immunoassay shows a good agreement with commercial ELISA kit for detection zearalenone in the spiked samples. The chemiluminescence immunoassay is easier in pretreating, and more sensitive when compared with other detection methods include chromatography methods and immunoassays.

Introduction

Zearalenone (ZEN) is a mycotoxin, which induced by *Fusarium* as the secondary metabolite. It mainly contaiminated grain, include maize and corn. ZEN had strongly reproductive toxicity, teratogenicity and immune toxicity. It could induce estrogen syndrome of mammal, include miscarriage and stillbirth. Therefore, ZEN had a serious impact on the livestock industry of the world. Most of the countries in the world have the strict rules for the content of zearalenone in cereal or food nowadays. For example, ZEN in cereals cannot exceed 50 ng/g in Australia and 100 ng/g in Italy, and must be less than 200 ng/g in vegetable oil and cereals in French.

The quantitative detection methods for ZEN are often divided into two parts. On the one hand, the chromatography methods, which include thin layer chromatography, gas chromatography-mass spectrometry and high performance liquid chromatography. These methods have sensitive and reliable results, but expensive and have complex procedures. So these are limited in on-site applications. On the other hand, the immunoassays, like enzyme-linked immunosorbent assay (ELISA) [1], are rapidly, accurately, more easily and at a lower cost compared to chromatography methods. But with the attentions of the society for food safety, some types of materials like baby foods need highly sensitive detection methods, and ELISA could not solve this problem very well. Chemiluminescence immunoassay (CLIA) is a highly sensitive detection methods which formed by the ELISA and chemiluminescence. It has the advantages of ELISA, but more sensitive and wider detection linear range. Many researchers had focus on the CLIA, and found its superiority in sensitivity [2-4], but only a few for mycotoxins detection [5].

In this study, a highly sensitive CLIA against ZEN was prepared. The reaction conditions of the CLIA were optimized to acquire better performances. This study provides an effective way for the rapid trace detection of zearalenone.

Materials and Methods

Materials. Bovine serum albumin (BSA), ZEN, deoxynivalenol (DON), aflatoxin B1 (AFB1), fumonisin B1 (FB1), O-carboxymethyloxime (CMO), N-hydroxysuccinimide (NHS), 1,3-dicyclohexylcarbodiimide (DCC) and ρ-iodophenol were purchased from the Sigma Chemical Co. (St. Louis, USA); ovalbumin (OVA), nonfat dried milk, luminol were from Sangon Biotech (Shanghai, China); polyclonal antibody against ZEN were prepared in our laboratory; horseradish peroxidase (HRP) labeled goat anti-rabbit antibody was from ICL (Portland, USA); commercial ELISA kit against ZEN were purchased from R-Biopharm (Darmstadt, Germany); ZEN-free corn samples were provided by the Shanghai Entry-Exit Inspection and Quarantine Bureau.

Equipment. Chemiluminescence microtiter plates were purchased from Greiner Bio (Würzburg, Germany); chemiluminescence signal detector (infinite F200) was from TECAN (Männedorf, Switzerland).

Synthesis of ZEN conjugates. ZEN conjugates (ZEN-BSA and ZEN-OVA) were synthesized according to the published reports [6]. ZEN and CMO were synthesized firstly. Then DCC and NHS were added into the mixture. The conjugate were dialyzed using 10 mM phosphate buffered saline (PBS) at last, and stored in 4℃.

Preparation of antibody. The New Zealand white SPF rabbits were immunized by ZEN-BSA (1 mg/ml) and Freund's adjuvant six times to prepare ploYclonal antibody against ZEN. The sera were collected and purified by affinity chromatography instruments. The cross-reactivity of the antibody was investigated by indirect competitive ELISA (IC-ELISA), which DON, AFB1, FB1 were added as competitive antigens.

Development of CLIA. The concentrations of coating antigen (ZEN-OVA) and polyclonal antibody were optimized by checkerboard titration. ZEN-OVA (1 mg/ml) were diluted by carbonate buffer (0.05M, pH 9.6) to 1/500, 1/1000, 1/2000, 1/4000, and 100 μl coating antigen were added to microtiter plate overnight at 4ºC. The plate were washed three times and blocked by 5% nonfat dried milk 2h at 37℃. The polyclonal antibody were diluted by PBS (0.01M, pH 7.4) to 1/2500, 1/5000, 1/7500, 1/10000, and mixed with same volume competitive antigens. The mixture were incubated 1h at 37℃, and added to the plate after wash three times. Different concentration of HRP labeled goat anti-rabbit antibody (1/10000, 1/20000, 1/30000, 1/40000, 1/50000, 1/60000, 1/70000, 1/80000) were added to the plate and incubated 1h at 37℃. At last, a mixture of luminol and ρ-iodophenol (150 μl) were added to the plate, and determined by chemiluminescence signal detector immediately. The results were expressed in relative light units (RLU), and RLUmax and IC50 were used to analyze the optimum concentrations in CLIA.

Optimization of CLIA. In order to acquire better performances of CLIA, the competition time (15, 30, 45, 60 min), pH of buffer (4.0, 5.7, 7.4, 9.0), the concentration of methanol in buffer (5%, 10%, 20%, 30%) and different ratio of luminol to ρ-iodophenol (1:10, 1:5, 2:5, 4:5) in CLIA were evaluated. RLU/RLUmax was used to analyze the optimum conditions of CLIA.

Sample preparation and recovery studies. The spiked corn samples were extraction as follows: 10 g of sample was extracted with 100 mL methanol: water (70:30) for 15 min at room temperature on a horizontal shaker. The supernatant was filtered through rapid qualitative filter paper and diluted seven times with 10 mM PBS. 50 μL diluted solutions were mixed with an equal volume of polyclonal antibody and incubated for 60 min. The samples were also determined by the commercial ZEN ELISA kit which was used as reference method.

Results

Development of CLIA. The conjugates of ZEN were evaluated by ultraviolet wavelength scanning. The results showed the maximal absorption peaks of ZEN, OVA, and ZEN-OVA were different, and ZEN, BSA and ZEN-BSA were also different. These results indicated the conjugates were synthesized successfully. The concentrations of coating antigen (1/4000, 0.25μg/ml), polyclonal antibody (1/7500) and HRP labeled goat anti-rabbit antibody (1/40000) were chosen according to the lowest IC50 or RLUmax. The results were shown in Fig. 1-2.

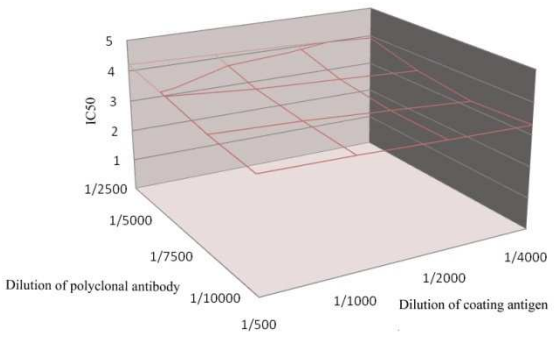

Fig. 1. Optimization of polyclonal antibody and coating antigen

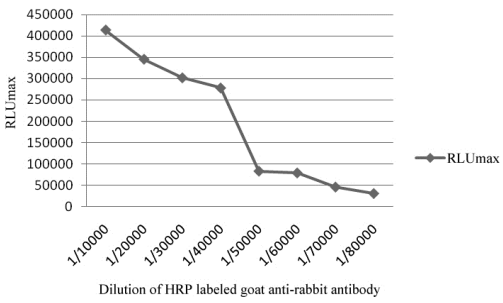

Fig. 2. Influences of HRP labeled goat anti-mouse antibody

Optimization of CLIA. The competition time (60 min), pH of buffer (7.4), influence of methanol in buffer (10%) and different ratio of luminol to ρ-iodophenol (2:5) in CLIA were chosen to optimize the detection limit and range of CLIA (Fig. 3-6). The standard curve of CLIA was

established (Fig. 7). The IC50 was 2.93 ng/ml, the detection range was 0.05-54.34ng/ml, and the detection limit was 0.02 ng/ml. The detection limit was significantly lower than other immunoassay which determination of ZEN [1, 7].

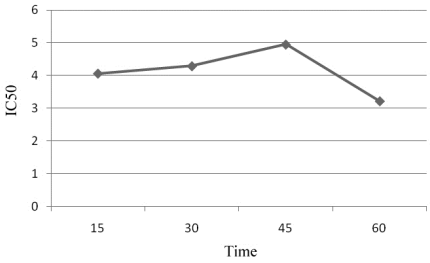

Fig. 3. Influences of different competition time in CLIA

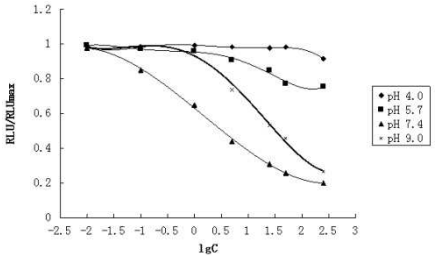

Fig. 4. Standard curves of different pH of buffer in CLIA

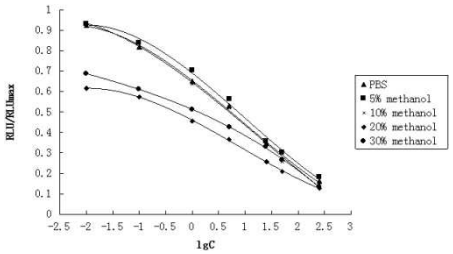

Fig. 5. Standard curves of different concentrations of methanol

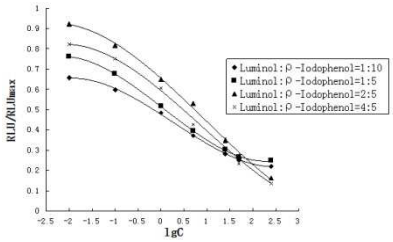

Fig. 6. Standard curves of different ratio of luminol to ρ-iodophenol

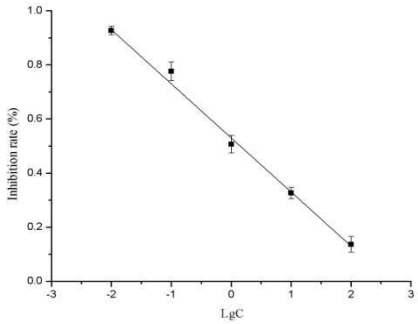

Fig. 7. Standard curve of CLIA against ZEN

Assessment of the assay reproducibility and cross-reactivity. The intra-assay and inter-assay reproducibility were evaluated by determination of five concentrations of FB1 three times in three different days. The variation of percent inhibition and deviation of mean values were 2.1, 1.4, 5.3, 2.0, 1.8% and 2.3, 4.1, 5.9, 8.4, 10.5%. The specificity results showed DON, AFB1 and FB1 didn't have cross-reactivity in CLIA.

Recovery studies. The three different concentrations of spiked samples were determined by CLIA and commercial ZEN ELISA kit. The results were showed in Table. 1. These results indicated the CLIA and commercial ZEN ELISA kit could detect ZEN well, and two immunoassays show a good agreement.

Table. 1. Detection of spiked samples by CLIA and ELISA kit for ZEN

Samples	Spiked (μg/kg)	CLIA		ELISA kit	
		Founded (μg/kg)	Recovery (%)	Founded (μg/kg)	Recovery (%)
1	20	19.1	95.5%	18.4	92.0%
2	50	48.2	96.4%	51.8	103.6%
3	80	81.9	102.4%	82.3	102.9%

Conclusions

In this study, a high sensitive chemiluminescence immunoassay was prepared. The IC50 was 2.93 ng/ml, the detection range was 0.05-54.34ng/ml, and the detection limit was 0.02 ng/ml. The detection range and limit were significantly lower than other immunoassay. This chemiluminescence immunoassay had a good correlation with commercial ELISA kit for detection ZEN in the spiked samples.

Acknowledgements

This work was financially supported by the Chinese National Programs for High Technology Research and Development (No. 2007AA10Z424).

References

[1] N. A. Burmistrova, I. Y. Goryacheva, E. Y. Basova, A. S. Franki, D. Elewaut, K. Van Beneden, D. Deforce, C. Van Peteghem and S. De Saeger: Anal Bioanal Chem. Vol. 395 (2009), p. 1301

[2] R.S. Chouhan, K.V. Rana and C.R. Suri: J AOAC Int. Vol. 93 (2010), p. 28

[3] X. Huang and J. Ren: Anal Chim Acta. Vol. 686 (2011), p. 115

[4] F. Liu, Y. Li and C. J. Song: Anal Chem. Vol. 82 (2010), p. 7758

[5] Y. Quan, Y. Zhang, S. Wang, N. Lee and I. R. Kennedy: Anal Chim Acta. Vol. 580 (2006), p. 1

[6] D. Thouvenot and R. F.Morfin: Appl Environ Microbiol. Vol. 45 (1983), p. 16

[7] M. Schmidt-Heydt and R.Geisen: Int J Food Microbiol. Vol. 117 (2007), p. 131