Novel Mycotoxin Collection System using Magnetic Nanoparticles for Determination of Aflatoxin B1 and Zearalenone in Feed

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Abstracts

This study was conducted to develop a novel tool to collect mycotoxins, Alfatoxin B1 (AFB1) and Zearalenone (ZEN), which often are contaminated in feed. The novel tool utilized monoclonal antibodies (Mab) against AFB1 (kjAFB) or ZEN (kkZEN), which were produced in our laboratory, and magnetic nanoparticle (MNP) synthesized for this study. The condition for coupling of Mab to MNPs was optimized, and the mycotoxin binding capacity of Mab coupled MNPs was determined. The Mab coupled MNPs demonstrated remarkable efficiency of recovery with faster and easier procedure than commercial immunoaffinity chromatography (IAC) method.

Keywords: Aflatoxin B1, Zearalenone, Mab, Magnetic nanoparticle, immunoaffinity chromatography.

Introduction

A common method for determination of mycotoxins contaminated in feeds combines immunoaffinity chromatography (IAC) and high-performance liquid chromatography (HPLC) with UV detection[1,2]. However, the IAC method to collect mycotoxins is expensive and time-consuming. Moreover, its collection efficiency has failed to provide complete satisfaction for users. The present study was conducted to develop the better collection system utilizing magnetic nanoparticle (MNP), which is synthesized for the present study and featured with easy collection by magnetism and fast dispersibility by tiny size.

Materials and Methods

MNP was synthesized by a slight modification of the literature method,[3] and the surface of MNP was coated with silica (SiO_2) by a polyvinylpyrrolidone(PVP)-mediated method to generate MNP@SiO_2 as previously reported in the literature.[4] Finally, the surface of MNP@SiO_2 was further modified with aminoorganosilanes in order to introduce amino-terminal groups for simple and easy coupling with biomolecules.

Monoclonal antibodies, which were kj-AFB and kk-ZEN against aflatoxin B1 (AFB1) and zearalenone (ZEN), respectively, were produced in our laboratory, and covalently coupled with the synthesized MNP.

The optimal condition for coupling between Mabs and MNP was determined by reacting Mab at 50, 100, 200ug with MNP at 2mg. Firstly, a function group of MNP, -NH2, was covalently reacted with one of hydroxyl groups (-OH) of glutaraldehyde for 15min at room temperature. In the next step, the other hydroxyl group of glutaraldehyde bound to MNP was covalently bound to amine group of Mab.

The Mab coupled MNPs, C-AFB1 Mab/MNP and C-ZEN Mab/MNP, were reacted with mycotoxins, AFB1 and ZEN, to assess capacity for mycotoxin collection. 10ppb of AFB1 and 50ppb of ZEN were spiked into corn and feed (commercial product X), which did not contain detectable level of AFB1 or ZEN under the HPLC condition of this study. Selective reaction and harvest for Mab coupled MNP were carried out easily by re-suspension and magnetic separation, while collection of mycotoxins using IAC (Neogen, Lincoln, NE, USA) was conducted following manufacturer’s protocol. The amount of collected mycotoxins was quantitated by high-performance liquid chromatography (HPLC) column.

The recovery efficiency was evaluated using a formula ([amount of collected mycotoxins/ amount of spiked mycotoxins] x 100) to compare AFB1 or ZEN Mab coupled MNP with commercial AFB1 or ZEN-IACs.

Results and Discussion

The optimal amount of covalently coupled Mab to MNPs, C-AFB1 Mab/MNP and C-ZEN Mab/MNP, was shown in Table 1. Among the three different condition of amounts of Mab bound to 2mg of MNP, we selected 100ug Mab:2mg MNP for both mycotoxins because the condition used the less amount of Mab and showed similar level of mycotoxin binding capacity to 200ug Mab:2mg MNP. In the condition, the amount of bound Mab was 42.16ug and 40.39ug to 1mg of MNP for AFB1 and ZEN, respectively. C-AFB1 Mab/MNP and C-ZEN Mab/MNP showed individual binding capacity of 1.03ng and 2.24ng per 1ug Mab, respectively (Table 1).

In application for collection of mycotoxins (10ppb for AFB1 and 50ppb for ZEN) spiked into corn and feed (product X) under the above coupling condition, 45ug C-AFB1 Mab/MNP and 40.0ug C-ZEN Mab/MNP were determined to recover all mycotoxins and used for evaluation of recovery efficiency. Comparison of recovery efficiency showed that C-AFB1/MNP and AFB1-IAC were 90.28% and 83.7% in corn, respectively, and were 84.06% and 77.96% in product X, respectively, while C-ZEN/MNP and ZEN-IAC were 99.9% and 86.0% in corn, respectively, and 93.2% and 83.9% in product X, respectively (Table 2).

The results demonstrated that the novel method had remarkable efficiency of the collection system using nanoparticles for mycotoxin from feed, when compared to IAC method.

Conclusion

It is suggested that the novel collection system would be a great tool to monitor and regulate mycotoxin contaminated in feeds.

Table.1 The optimal amount of Mab coupled to Magnetic nanoparticle for effective toxin determination

Mab coupled MNP	Amount of MNP for coupling (mg)	Amount of Mab for coupling (ug)	Amount of coupled Mab (ug)	Amount of Mab coupled per 1 mg MNP (ug)	Amount of mycotoxins per 1ug of Mab (ng)
C-AFB1 Mab/MNP	2	200	189.80	94.90	1.18
		100	84.32	42.16	1.03
		50	33.58	16.89	0.06
C-ZEN Mab/MNP	2	200	189.65	99.83	2.50
		100	40.39	40.39	2.24
		50	30.13	15.07	1.83

Table.2 Toxin recovery from spiked samples (n=3) by covalently Mab coupled Magnetic Nanoparticle or immunoaffinity column

Toxin	Collection type	Amount of spiked toxin (ng/ml)	Amount of recovered toxin (Mean±SD) (ng/ml)	Recovery Rate *(Mean±SD) (%)
AFB1	C-AFB1 Mab/MNP	Corn	9.02±1.0	90.26±1.0
		Product X	8.40±0.1	84.06±2.02
	IAC	Corn	41.86±4.98	83.72±4.98
		Product X	7.79±0.96	77.96±9.98
ZEN	C-ZEN Mab/MNP	Corn	49.66±0.4	99.9±0.4
		Product X	4.66±2.08	93.2±0.4
	IAC	Corn	43.0±0.7	86.0±0.7
		Product X	41.95±0.93	83.9±0.8

*(amount of recovered toxin/amount of spiked toxin) x 100

References

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