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Development of a rapid method for the quantitative determination of deoxynivalenol using Quenchbody

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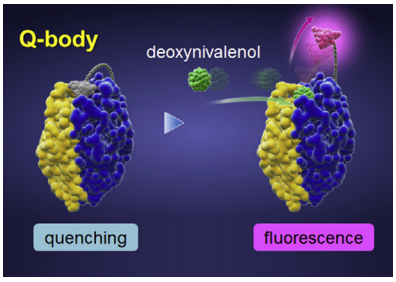
HIGHLIGHTS

- A rapid method for quantitation of DON using Q-body has been developed.
- A recovery test using the anti-DON Q-body was performed.
- The concentrations of DON in wheat were quantitated by the anti-DON Q-body.

GRAPHICAL ABSTRACT



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ABSTRACT

Quenchbody (Q-body) is a novel fluorescent biosensor based on the antigen-dependent removal of a quenching effect on a fluorophore attached to antibody domains. In order to develop a method using Q-body for the quantitative determination of deoxynivalenol (DON), a trichothecene mycotoxin produced by some *Fusarium* species, anti-DON Q-body was synthesized from the sequence information of a monoclonal antibody specific to DON. When the purified anti-DON Q-body was mixed with DON, a dose-dependent increase in the fluorescence intensity was observed and the detection range was between 0.0003 and 3 mg L^{-1}. The coefficients of variation were 7.9% at 0.003 mg L^{-1}, 5.0% at 0.03 mg L^{-1} and 13.7% at 0.3 mg L^{-1}, respectively. The limit of detection was 0.006 mg L^{-1} for DON in wheat. The Q-body showed an antigen-dependent fluorescence enhancement even in the presence of wheat extracts. To validate the analytical method using Q-body, a spike-and-recovery experiment was performed using four spiked wheat samples. The recoveries were in the range of 94.9–100.2%. The concentrations of DON in twenty-one naturally contaminated wheat samples were quantitated by the Q-body method, LC-MS/MS and an immunochromatographic assay kit. The LC-MS/MS analysis showed that the levels of DON contamination in the samples were between 0.001 and 2.68 mg kg^{-1}. The concentrations of DON quantitated by LC-MS/MS were more strongly correlated with those using the Q-body method ($R^2 = 0.9760$) than the immunochromatographic assay kit ($R^2 = 0.8824$). These data indicate that the Q-body system for the determination of DON in wheat samples was successfully developed and Q-body is expected to have a range of applications in the field of food safety.

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1. Introduction

Deoxynivalenol (DON) is a trichothecene mycotoxin produced by some species of *Fusarium*, such as *Fusarium graminearum* and *Fusarium culmorum*. Because these fungi have a large geographical distribution, contamination of DON in agricultural products, including wheat, barley and maize, has occurred worldwide [1–4]. The studies about the toxicity of DON using experimental animals has showed that DON causes vomiting, the loss of appetite, lower body weight gains and immunosuppression [5,6]. Some epidemiological studies indicate that human exposure to DON induces acute symptoms, such as diarrhea, headache, nausea and vomiting. Outbreaks of mycotoxicoses associated with the consumption of cereals contaminated with trichothecenes have been reported in Asian countries [7]. In order to prevent the intake of DON, regulatory or guideline limits for DON in food have been set in some counties in the late 1990s. Japan has set a provisional regulatory limit of 1.1 mg kg^{-1} of DON for wheat in 2002. In the EU, the maximum limits are set between 200 μg kg^{-1} for processed cereal-based foods and baby foods for infants and young children and 1750 μg kg^{-1} for unprocessed durum wheat, oats and maize, while the USA has proposed a guidance level of 1000 μg kg^{-1} for DON in final wheat products [8].

Many analytical methods for the determination of DON in cereals have been developed. In order to quantitate a small amount of DON accurately, instrumental analytical techniques using HPLC, GC–MS, LC–MS or LC–MS/MS have been recently reported [9–12]. A method using a multifunctional clean-up column coupled with HPLC has been validated by an interlaboratory study and is used as the official analytical method for the determination of DON contamination in wheat in Japan [13]. An HPLC method with immunoaffinity column cleanup and UV detection for determination of DON in cereal and cereal products has been adopted by the European Committee for Standardization (EN 15891:2010). For rapid screening of DON contamination in food, several methods based on immunochromatographic (IC) assay, enzyme-linked immunosorbent assay (ELISA), a surface plasmon resonance immunoassay and a fluorescence polarization immunoassay have been developed [14–19]. Some analytical kits based on IC assay and ELISA for DON detection in cereals are commercially available. In Japan, ELISA has been adopted as an official method for screening of DON in wheat. In the case that the measured value by ELISA is above the cut-off value, 0.7 mg kg^{-1}, the concentration of DON in the sample is required to be quantitated by the official instrumental method. It is recommended to take technical training before using the ELISA kit because experience is essential for accurate and reliable testing. Therefore, in order to perform more efficient screening of DON in foods, development of a method which enables rapid quantitation of DON without requiring a specific skill is needed.

Quenchbody (Q-body) is a novel fluorescent biosensor based on the antigen-dependent removal of a quenching effect on a fluorophore attached to antibody domains [20–22]. In the absence of a target molecule of Q-body, the fluorescence of Q-body is quenched by the interaction between a fluorophore and conserved tryptophan residues in antibody variable region, whereas, in the presence of a target molecule, a conformational change of Q-body by antigen–antibody interaction causes fluorescence emission from the unquenched fluorophore. This reaction occurs just after mixing Q-body and antigen, and the result can be obtained only by measuring the fluorescence without some time-consuming steps, such as washing and soaking. By designing the antigen biding site in Q-body, detection systems targeting a variety of compounds have been developed until now. For example, bisphenol A, osteocalcin and some drugs, such as morphine and cocaine, have been successfully quantified by using Q-body system [20].

The aim of this study was to develop a rapid method for quantitation of DON using Q-body. Q-body for analysis of DON was designed from sequence information of a monoclonal antibody specific to DON, and a recovery test using spiked wheat samples was performed in order to validate the analytical method using the Q-body. The concentrations of DON in naturally contaminated wheat samples were quantitated by three different methods, the Q-body system, IC assay kit and LC-MS/MS, and the usefulness of the Q-body system was evaluated by comparing the results.

2. Materials and methods

2.1. Materials

The stock solution of DON (100 mg L^{-1}) was prepared by dissolving crystalline DON (Sigma–Aldrich, St. Louis, MO, USA) in distilled water. The stock solution was diluted with distilled water to make the standard solutions of DON. LC/MS-grade acetonitrile and water were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). The naturally contaminated wheat samples were imported from USA, Australia or Canada. They were supplied by an import inspection agency in 2012 and 2013.

2.2. Vector construction

Vectors for anti-DON Q-body expression were constructed according to a previous report [21]. The details are as follows. The PCR primers used in this study are shown in Supplementary Material 1. An expression vector pROX-DON-VH-CH1, harboring a T7 promoter-controlled heavy-chain Fd (VH–CH1) gene of anti-DON antibody [23] fused with an N-terminal ProX tag and a C-terminal FLAG tag, was generated by splice-overlap-extension (SOE) PCR. The VH chain was combined by SOE PCR with specific primers (Don-H-P1, Don-H-P2, Don-H-P3, Don-H-T1, Don-H-T2, Don-H-T3, Don-H-P and Don-H-T). CH1 was amplified using CH1–P and CH1-FLAG-T from a vector, pCHCκ, harboring the mouse IgG1 CH1 gene and Cκ gene. pCHCκ was prepared by an artificial gene synthesis service (Funakoshi, Japan). The vector has the CH1 and Cκ sequences, AKTTPPSVYPLAPGSAAQTNSMVTLGCLVKGYFPEPVTVT WNSGSLSSGVHTFPAVLESDLYTLSSSVTVPSSPRPSETVTCNVAHPASST KVDKKIVPRDC and ADAAPTVSIFPPSSEQLTSGGASVVCFLNNFYPK-DINVKWKIDGSERQNGVLNSWTDQDSKDSTYSMSSTLTLTKDEYERHN SYTCEATHKTSTSPIVKSFNRNEC. The amplified VH and CH1 genes were linked by SOEPCR with 5′ GS-primer and CH1-FLAG-T, and the construct was cloned into the NcoI- and BamHI-digested pROX-FL92 (ProteinExpress, Chiba, Japan) using the In-Fusion PCR cloning kit (Clontech, USA). In addition, an expression vector pROX-DON-VL-Cκ harboring a T7 promoter-controlled light-chain (VL-Cκ) gene of anti-DON antibody fused with an N-terminal ProX tag containing an amber codon (ATG TCT AAA CAA ATC GAA GTA AAC TAG TCT AAT GAG) and a C-terminal His tag was constructed by SOE PCR. The VL chain was combined by SOE PCR with primers (Don-L-P1, Don-L-P2, Don-L-P3, Don-L-T1, Don-L-T2, Don-L-T3, Don-L-P and Don-L-T). The mouse Cκ gene was amplified using the 5′-primer (Cκ Pv3) and 3′-primer (Cκ+C-His_T) from pCHCκ. The amplified VL and Cκ genes were linked by SOE PCR using 5′ 2 GS-primer and Cκ+C-His_T, and the construct was then cloned into NcoI- and SmaI-digested pROX-FL92 using the In-Fusion PCR cloning kit.

2.3. Cell-free co-transcription/translation

Cell-free co-transcription/translation and purification to obtain the anti-DON Q-body solution were conducted as previously described [21]. TAMRA-C6-tRNA was synthesized as previously described [20]. The incorporation of TAMRA-C6-AF into the N-

terminal region of Fab was performed using a cell-free transcription/translation system. The reaction mixture (60 μL) consisted of 41.1 μL of the reaction mix, 4.5 μL of amino acid mix, 0.4 μL of 120 mM glutathione (GSSG/GSH = 10/1), 4 μL of distilled water, 4 μL of plasmid DNAs (50 ng μL^{-1}) and 6 μL of aminoacyl-tRNA (1440 pmol). All the reagents used, with the exception of the plasmid and tRNA, were provided in Musaibou-kun kit (Taiyo Nippon Sanso Corporation, Japan). The reaction mixture was incubated at 37 °C for 2 h and subsequently incubated at 4 °C for 16 h. Tandem affinity purification with anti-Flag- and nickel-affinity chromatography was then performed. First, the reaction mixture (60 μL) was incubated with 20 μL of Flag M2 affinity gel. After incubation at room temperature for 15 min, the column was washed three times with wash buffer (20 mM phosphate, 0.5 M NaCl, 0.1% polyoxyethylene lauryl ether, pH 7.4). The bound proteins were subsequently eluted with two 200-μL volumes of wash buffer containing 100 μg mL^{-1} of Flag peptide. The eluted proteins were then applied to a His Spin Trap Column (GE Healthcare, Piscataway, NJ, USA). After incubation at room temperature for 15 min, the column was washed three times with wash buffer containing 60 mM imidazole. The bound proteins were then eluted with two 200-μL volumes of wash buffer containing 0.5 M imidazole. The eluate was subsequently passed through an UltraFree-0.5 centrifugal device (Millipore, Billerica, MA) and equilibrated with phosphate buffered saline supplemented with Tween-20 (PBST, 10 mM phosphate, 137 mM NaCl, 2.7 mM KCl, 0.05% Tween 20, pH 7.4) to concentrate the protein in the buffer. The concentration of the labeled Fab protein was determined by comparing the fluorescence intensities of a known concentration of free TAMRA dye (Anaspec, Fremont, CA) and the sample under denaturing conditions in 7 M GdnHCl, 100 mM DTT, pH 7.4.

2.4. Quantitation of DON by the Q-body method

The purified anti-DON Q-body (16 nM, 30 μL) in PBST containing 0.2% BSA was mixed with 30 μL of the standard solution of DON, and the fluorescence intensity was measured at 580 nm with excitation set at 530 nm on a SpectraMax Paradigm Multi-Mode reader (Molecular Devices, USA). The EC_{50} values were calculated using curve fitting of the observed fluorescence intensities at the maximum emission wavelength and employing a sigmoidal dose–response model based on the ImageJ software (http://rsbweb.nih.gov/ij/). All the fluorescence intensities were normalized by setting the intensity of each sample at the zero dose (without antigen) as one, at the maximum emission wavelength, unless otherwise stated. The limit of detection was assessed by analyzing a blank sample 6 times and calculating the 3.3 SD (standard deviation) limit. For the quantitation of DON in wheat, 10 g of the wheat sample was extracted with 40 mL of distilled water and then shaken for 10 min. After centrifugation (12,000 *g*, 10 min), the supernatant was diluted four times by distilled water and 30 μL of the dilution was mixed with the purified anti-DON Q-body (16 nM, 30 μL) in PBST containing 0.2% BSA, and the fluorescence intensity was measured. The water extract from wheat was prepared from DON-negative wheat (batch number: D-W-100; Trilogy Analytical Laboratory Inc., Washington, MO, USA). For the spike-and-recovery experiment, the DON-negative wheat was spiked at four concentrations (0.5, 1.0 2.0, and 5.0 mg kg^{-1}). The concentration of DON in wheat was calculated from a calibration curve, which was created by plotting the fluorescence intensity of eight standard solutions at different concentrations (0.0001, 0.0003, 0.001, 0.003, 0.01, 0.03, 0.1, 0.3, 1.0 and 3.0 mg L^{-1} final concentrations).

2.5. LC-MS/MS analytical method

The LC-MS/MS method was developed and validated in our previous study [24]. Twenty-five grams of the wheat sample was extracted with 100 mL of distilled water and then shaken for 30 min. After centrifugation (3000 *g*, 10 min), the supernatant was collected. Aliquots of 10 mL of the supernatant were diluted with 50 mL of phosphate-buffered saline (PBS). After the diluted extract was filtered through a glass fiber filter GA-55 (Toyo Roshi Kaisha, Japan), 6 mL of the filtrate was applied to a DON-NIV WB immunoaffinity column (VICAM, Milford, MA, USA). The column was washed with 10 mL of PBS followed by a wash with 10 mL of distilled water. Toxins were eluted using 0.5 mL of methanol followed by 1.5 mL of acetonitrile. The eluate was dried under nitrogen at 40 °C. The residue was dissolved in 0.25 mL of 10% acetonitrile in water. LC-MS/MS analyses were performed with a 3200 Q TRAP LC-MS/MS system (AB Sciex, Foster City, CA, USA) equipped with an ESI source and an LC-20A series high performance LC system (Shimadzu Corp., Kyoto, Japan). The column used was a 150 mm × 2.1 mm i.d., 3 μm, Inertsil ODS-3 (GL Sciences, Inc., Tokyo, Japan). Chromatographic separation was achieved at 40 °C using a gradient elution of 5–73% acetonitrile in water from 0 to 8 min and followed by an isocratic elution of 90% acetonitrile in water from 8 to 10 min at a flow rate of 0.2 mL min^{-1}. The ESI source was operated at 400 °C in the negative ionization mode. The following multiple reaction monitoring transitions were used: DON, 295 $[M - H]^-$ to 265 (quantifier ion: collision energy, −12 eV) and 138 (qualifier ion: collision energy, −28 eV). The other conditions were described in our previous report [24].

2.6. Immunochromatographic assay

The DON-V kit (VICAM) was used for the immunochromatographic (IC) assay. The assay was performed according to the manufacturer's protocol. The extraction was performed similarly to the Q-body method. Aliquots of 100 μL of the extract were diluted with 1.0 mL of distilled water. One hundred microliters of the diluted solution was mixed with 100 μL of the provided diluent, and 100 μL of the mixture was applied to the DON-V test strip. The strip was incubated at 25 °C for 3 min, and the signal was quantitated by a Vertu Lateral Flow Reader (VICAM).

3. Results and discussion

In order to confirm the antigen-dependent fluorescence enhancement of the synthesized Q-body for DON, the Q-body was mixed with the standard solutions of DON (0.0001, 0.0003, 0.001, 0.003, 0.01, 0.03, 0.1, 0.3, 1.0 and 3.0 mg L^{-1} final concentrations) and the fluorescence emission was measured (Fig. 1). The fluorescence intensities increased dose-dependently at the concentrations between 0.0003 and 3 mg L^{-1}. The relative 3.9-fold intensity was reached at a concentration of 3 mg L^{-1}, as compared with the basal intensity without DON, and the EC_{50} value was 0.03 mg L^{-1}. The coefficients of variation were 7.9% at 0.003 mg L^{-1}, 5.9% at 0.03 mg L^{-1} and 13.1% at 0.3 mg L^{-1}, respectively. The limit of detection was 0.006 mg kg^{-1} for DON in wheat. The concentration of DON in the wheat sample is 32-fold of the measured value because 10 g of the wheat sample was extracted with 40 mL of distilled water and the extract was diluted four times and the dilution was mixed with equal volume of the Q-body solution. The influence of the wheat matrix on the fluorescence increase was evaluated by mixing the Q-body and DON with or without the water extracted from the wheat. A dose-dependent increase of the fluorescence intensity was observed under both conditions (Supplementary Material 2). The water extracted from wheat significantly decreased the fluorescence increase of the Q-body, but the decreasing rate of the intensity was less than 1%. In order to evaluate cross-reactivity of the anti-DON Q-body against other

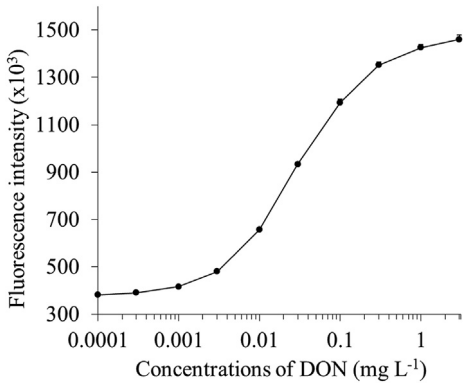

Fig. 1. The dose-dependent fluorescence enhancement of anti-DON Q-body. The standard solutions of DON were mixed with anti-DON Q-body and the fluorescence intensities were measured. Data are presented as the mean ± SD (n = 6).

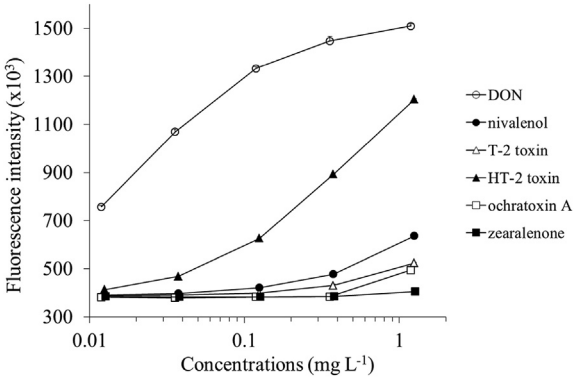

Fig. 2. Evaluation of cross-reactivity of anti-DON Q-body against mycotoxins occurring in wheat. The standard solutions of DON (○), nivalenol (●), T-2 toxin (△), HT-2 toxin (▲), ochratoxin A (□) or zearalenone (■) were mixed with anti-DON Q-body and the fluorescence intensities were measured (n = 3).

mycotoxins in wheat, the Q-body was mixed with nivalenol, T-2 toxin, HT-2 toxin, ochratoxin A or zearalenone and the fluorescence emission was measured (Fig. 2). The Q-body was most specific for DON (EC_{50} 0.03 mg L^{-1}) followed by HT-2 toxin (EC_{50} 0.55 mg L^{-1}). The EC_{50} values of other compounds were more than 1 mg L^{-1}. These results indicated that the Q-body method for DON was successfully constructed and can be used for the quantitation of DON. Because the wheat matrix hardly affected the fluorescence increase of the Q-body, purification steps were considered to be unnecessary for the determination of DON in wheat by the Q-body.

An analysis of the blank wheat sample and the spiked samples containing DON at four concentrations (0.5, 1.0 2.0, 5.0 mg kg^{-1}) in six replicates was performed to validate the method using Q-body. DON was extracted from the samples by water. The extract was diluted by distilled water and the dilution was mixed with Q-body and the emitted fluorescence was measured. The concentrations of DON in the samples were calculated by comparing the fluorescence intensity of the samples with that of the standard solutions. The calculated recoveries are shown in Table 1. The recoveries from the spiked samples were in the range of 94.9–100.2%. When the concentrations of DON in the spiked samples were quantitated by an IC kit, the recoveries were between 75.7 and 89.1%. This result showed that the Q-body could be used for the quantification of DON in wheat without any purification steps, and had the same capabilities at a commercially available IC kit.

Table 2
DON concentrations (mg kg^{-1}) in naturally contaminated wheat samples measured by LC-MS/MS, the Q-body system and an immunochromatographic kit.

Sample	LC-MS/MS		Q-body		IC kit	
	Conc.	p or n[a]	Conc.	p or n	Conc.	p or n
1	0.001	n	<0.007	n	<0.25	n
2	0.002	n	0.01	n	<0.25	n
3	0.07	n	0.05	n	<0.25	n
4	0.09	n	0.07	n	<0.25	n
5	0.23	n	0.19	n	<0.25	n
6	0.28	n	0.24	n	<0.25	n
7	0.29	n	0.30	n	<0.25	n
8	0.38	n	0.33	n	0.28	n
9	0.46	n	0.37	n	0.45	n
10	0.46	n	0.39	n	0.28	n
11	0.51	n	0.42	n	0.37	n
12	0.52	n	0.41	n	0.26	n
13	0.65	n	0.48	n	0.37	n
14	0.70	n	0.62	n	0.45	n
15	0.77	p	0.72	p	0.52	n
16	0.82	p	0.77	p	0.71	p
17	0.88	p	0.76	p	0.75	p
18	0.90	p	0.81	p	0.66	n
19	1.84	p	1.59	p	1.26	p
20	1.90	p	1.74	p	1.49	p
21	2.68	p	1.92	p	3.55	p

Abbreviation: IC, immunochromatographic; Conc., concentration.
[a] "p" means positive (more than 0.70 mg kg^{-1}) and "n" means negative (equal to or less than 0.70 mg kg^{-1}).

The DON concentrations in the naturally contaminated wheat samples were quantitated by three methods, LC-MS/MS, the Q-body assay system and the IC assay kit for the comparison of the analytical methods (Table 2). The values greater than 0.7 mg kg^{-1}, a cut-off value for the screening of DON in Japan, were considered to be positive (p) and those equal to or less than 0.7 mg kg^{-1} were regarded as negative (n). Fourteen samples (No. 1–14) were negative and 7 samples (No. 15–21) were positive in the both LC-MS/MS and the Q-body assay system, and the concordance rate of the results between the two methods was 100%. On the other hand, the concordance rate of the results between the LC-MS/MS analysis and the IC assay kit was 90%. The values measured by LC-MS/MS and the Q-body assay system or the IC assay kit were plotted (Fig. 3a and b). The concentrations of DON quantitated by LC-MS/MS were more strongly correlated with those using the Q-body assay system (R^2 = 0.9760) than the IC assay kit (R^2 = 0.8824). These results suggested that the Q-body assay system for DON has an equivalent performance to the widely used, commercially available IC assay kit.

Table 1
Spiking concentrations and the recovery of DON for validation of the methods.

Spiking conc. (μg kg^{-1})	Recovery (%)			
	Q-body		Immunochromatographic assay	
	Mean	RSD	Mean	RSD
500	96.1	1.5	82.3	19.2
1000	94.9	1.3	75.7	12.5
2000	100.2	3.8	86.4	6.9
5000	98.3	2.8	89.1	8.9

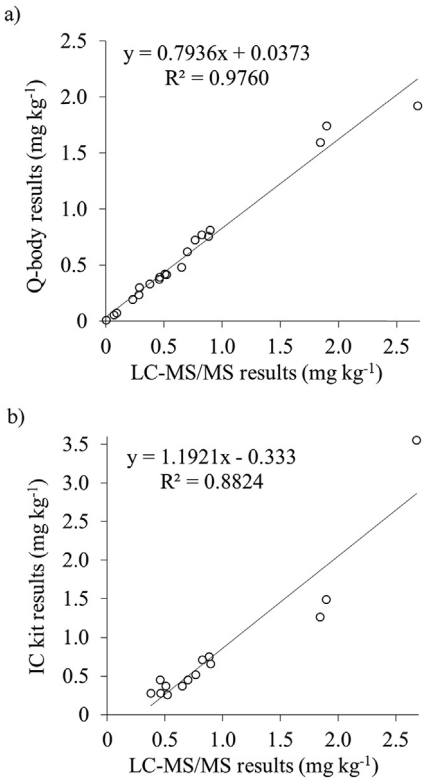

Fig. 3. Comparison of the concentrations of DON in the naturally contaminated wheat samples using LC-MS/MS and the Q-body assay system (a) or an immunochromatographic (IC) kit (b).

4. Conclusion

The results of a spike-and-recovery experiment and the comparison of the quantitative values of DON in the wheat samples indicate that the Q-body assay system has an adequate capacity to determine DON levels in wheat. This assay system is easier to use than the IC assay and ELISA kits, thus making it possible to perform rapid measurements. DON contaminates various types of foods and feedstuffs. In order to prove its practicality, whether the Q-body assay system is also applicable to other samples in addition to wheat is now being evaluated. Contamination with not only DON, but also various toxic compounds in foods is a significant concern from the viewpoint of food safety, and the development of easy and rapid methods for detecting contaminants in foods can contribute to alleviating the problem of food poisoning. Because the Q-body assay system can target a wide variety of compounds by simply altering the antigen binding, this system is therefore expected to have a wide range of applications in the field of food safety.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.aca.2015.07.020.

References

[1] R.A. Canady, R.D. Coker, S.K. Egan, R. Krska, T. Kuiper-Goodman, M. Olsen, J. Pestka, S. Resnik, J. Schlatter, Deoxynivalenol. Safety Evaluation of Certain Mycotoxins in Food, in: WHO Food Addit. Ser. 47, 2001, pp. 420–555.
[2] J.C. Larsen, J. Hunt, I. Perrin, P. Ruckenbauer, Workshop on trichothecenes with a focus on DON: summary report, Toxicol. Lett. 153 (2004) 1–22.
[3] European Food Safety Authority, Deoxynivalenol in food and feed: occurrence and exposure, EFSA J. 11 (2013) 3379.
[4] T. Yoshinari, H. Takeuchi, K. Aoyama, M. Taniguchi, S. Hashiguchi, S. Kai, M. Ogiso, T. Sato, Y. Akiyama, M. Nakajima, S. Tabata, T. Tanaka, E. Ishikuro, Y. Sugita-Konishi, Occurrence of four *Fusarium* mycotoxins, deoxynivalenol, zearalenone, T-2 toxin, and HT-2 toxin, in wheat, barley, and Japanese retail food, J. Food Prot. 77 (2014) 1940–1946.
[5] P. Sobrova, V. Adam, A. Vasatkova, M. Beklova, L. Zeman, R. Kizek, Deoxynivalenol and its toxicity, Interdiscip. Toxicol. 3 (2010) 94–99.
[6] J.J. Pestka, A.T. Smolinski, Deoxynivalenol: toxicology and potential effects on humans, J. Toxicol. Environ. Health B Crit. Rev. 8 (2005) 39–69.
[7] M. Peraica, B. Radić, A. Lucić, M. Pavlović, Toxic effects of mycotoxins in humans, Bull. WHO 77 (1999) 754–766.
[8] FAO (Food and Agriculture Organization), Worldwide Regulations for Mycotoxins in Food and Feed in 2003, FAO Food and Nutrition Paper 81, FAO/UN, Rome, Italy, 2004.
[9] T. Tanaka, A. Yoneda, S. Inoue, Y. Sugiura, Y. Ueno, Simultaneous determination of trichothecene mycotoxins and zearalenone in cereals by gas chromatography-mass spectrometry, J. Chromatogr. A 882 (2000) 23–28.
[10] S.J. MacDonald, D. Chan, P. Brereton, A. Damant, R. Wood, Determination of deoxynivalenol in cereals and cereal products by immunoaffinity column cleanup with liquid chromatography: interlaboratory study, J. AOAC Int. 88 (2005) 1197–1204.
[11] K. Aoyama, H. Akashi, N. Mochizuki, Y. Ito, T. Miyashita, S. Lee, M. Ogiso, M. Maeda, S. Kai, H. Tanaka, H. Noriduki, H. Hiraoka, T. Tanaka, E. Ishikuro, Y. Itoh, T. Nagayama, M. Nakajima, S. Naito, Y. Sugita-Konishi, Interlaboratory study of LC-UV and LC-MS methods for the simultaneous determination of deoxynivalenol and nivalenol in wheat, Shokuhin Eiseigaku Zasshi 53 (2012) 152–156.
[12] T. Yoshinari, T. Tanaka, E. Ishikuro, M. Horie, T. Nagayama, M. Nakajima, S. Naito, T. Ohnishi, Y. Sugita-Konishi, Inter-laboratory study of an LC-MS/MS method for simultaneous determination of deoxynivalenol and its acetylated derivatives, 3-acetyl-deoxynivalenol and 15-acetyl-deoxynivalenol in wheat, Shokuhin Eiseigaku Zasshi 54 (2013) 75–82.
[13] Y. Sugita-Konishi, T. Tanaka, S. Tabata, M. Nakajima, M. Nouno, Y. Nakaie, T. Chonan, M. Aoyagi, N. Kibune, K. Mizuno, E. Ishikuro, N. Kanamaru, M. Minamisawa, N. Aita, M. Kushiro, K. Tanaka, K. Takatori, Validation of an HPLC analytical method coupled to a multifunctional clean-up column for the determination of deoxynivalenol, Mycopathologia 161 (2006) 239–243.
[14] A.Y. Kolosova, L. Sibanda, F. Dumoulin, J. Lewis, E. Duveiller, C. Van Peteghem, S. De Saeger, Lateral-flow colloidal gold-based immunoassay for the rapid detection of deoxynivalenol with two indicator ranges, Anal. Chim. Acta 616 (2008) 235–244.
[15] A.L. Albert, P.D. Champoux, A.H. Davis, QuickTox kit for QuickScan DON (vomitoxin), J. AOAC Int. 96 (2013) 1006–1016.
[16] Z. Dzuman, M. Vaclavikova, I. Polisenska, Z. Veprikova, M. Fenclova, M. Zachariasova, J. Hajslova, Enzyme-linked immunosorbent assay in analysis of deoxynivalenol: investigation of the impact of sample matrix on results accuracy, Anal. Bioanal. Chem. 406 (2014) 505–514.
[17] A. Lupo, C. Roebuck, K. Settimo, A. Quain, J. Kennedy, M. Abouzied, Validation study of a rapid ELISA for detection of deoxynivalenol in wheat, barley, malted barley, corn, oats, and rice. performance tested method 090901, J. AOAC Int. 93 (2010) 600–610.
[18] T. Kadota, Y. Takezawa, S. Hirano, O. Tajima, C.M. Maragos, T. Nakajima, T. Tanaka, Y. Kamata, Y. Sugita-Konishi, Rapid detection of nivalenol and deoxynivalenol in wheat using surface plasmon resonance immunoassay, Anal. Chim. Acta 673 (2010) 173–178.
[19] C. Maragos, Fluorescence polarization immunoassay of mycotoxins: a review, Toxins 1 (2009) 196–207.
[20] R. Abe, H. Ohashi, I. Iijima, M. Ihara, H. Takagi, T. Hohsaka, H. Ueda, "Quenchbodies": quench-based antibody probes that show antigen-dependent fluorescence, J. Am. Chem. Soc. 133 (2011) 17386–17394.
[21] R. Abe, H.J. Jeong, D. Arakawa, J. Dong, H. Ohashi, R. Kaigome, F. Saiki, K. Yamane, H. Takagi, H. Ueda, Ultra Q-bodies: quench-based antibody probes that utilize dye-dye interactions with enhanced antigen-dependent fluorescence, Sci. Rep. 4 (2014) 4640.
[22] H. Ueda, J. Dong, From fluorescence polarization to Quenchbody: recent progress in fluorescent reagentless biosensors based on antibody and other binding proteins, Biochim. Biophys. Acta 1844 (2014) 1951–1959.
[23] C.M. Maragos, L. Li, D. Chen, Production and characterization of a single chain variable fragment (scFv) against the mycotoxin deoxynivalenol, Food Agric. Immunol. 23 (2012) 51–67.
[24] T. Yoshinari, S. Sakuda, K. Furihata, H. Furusawa, T. Ohnishi, Y. Sugita-Konishi, N. Ishizaki, J. Terajima, Structural determination of a nivalenol glucoside and development of an analytical method for the simultaneous determination of nivalenol and deoxynivalenol, and their glucosides, in wheat, J. Agric. Food Chem. 62 (2014) 1174–1180.