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Development of Direct Competitive Enzyme-Linked Immunosorbent Assay for the Determination Cadmium Residue in Farm Produce

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Abstract Cadmium, a toxic heavy metal, poses a significant threat to human health. Currently, the methods for detecting cadmium residue in farm produce need expensive equipment, intensive labor, and much time to finish one detection. In this study, a direct competitive enzyme-linked immunosorbent assay (DC-ELISA) based on a cadmium-chelate-specific monoclonal antibody has been developed. The DC-ELISA showed an IC_{50} of 2.30 μg/L with a detection limit of 0.20 μg/L for cadmium. The assay has been demonstrated to be highly specific since the monoclonal antibody showed little or no cross-reactivity with all tested metal chelates which include Cd^{2+}, Pb^{2+}, Hg^{2+}, Zn^{2+}, Na^{+}, Ca^{2+}, Fe^{3+}, Mg^{2+}, Mn^{2+}, Cu^{2+}, Al^{3+}, Co^{2+}, Cr^{2+}, Ni^{2+}, Sn^{2}, and K^{+}. The assay showed that a mean recovery ranged from 100.47% to 103.86%, and the coefficients of variations for intra- and inter-assay were 1.73–7.14% and 3.63–6.81%, respectively. Then, several farm produces including wheat flour, apple juice, rice flour, and tea were analyzed for cadmium residue with DC-ELISA and graphite furnace atomic absorption spectroscopy (GFAAS). The correlation coefficient between the DC-ELISA and GFAAS was 0.99. It was demonstrated that the DC-ELISA can be used as a simple and economic method to detect and quantitate cadmium residue in farm produce.

Keywords Cadmium · Hapten · Chelator · Monoclonal antibody · ELISA · Farm produce

Introduction

Cadmium is a toxic element exerting profound adverse effects on many life processes and has a biological half-life more than 10 years once accumulated in human body [1, 2].

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Depending upon the route of exposure, high accumulation of cadmium can cause the damage of kidney or lung. Thus, rapid detection of cadmium residue is critical for farm industry and human health [3, 4].

Currently, the methods for detecting cadmium in water, vegetables, and many other farm products include graphite furnace atomic absorption spectroscopy (GFAAS) and inductively coupled plasma atomic emission spectroscopy [5, 6]. Although these analytical methods are sensitive and reliable, they are labor-intensive, time-consuming, and expensive to use. Immunoassays such as enzyme-linked immunosorbent assays (ELISA) have recently emerged as an alternative to the traditional methods because they are usually less time-consuming, inexpensive, simple, specific, and reasonably portable [7].

Based on a cadmium-chelate-specific monoclonal antibody, an indirect ELISA has been developed for measuring cadmium in environmental samples [8, 9], but the limit of detection of cadmium were quite different, from 7 μg/L to lower detection limit 0.313 μg/L. It was owing to different antibody titers. Darwish et al. [10, 11] developed and validated a direct ELISA for measurement of cadmium in environmental water samples and human serum; the assay limit of detection was 0.3 and 0.24 μg/L, respectively, but the correlation coefficient of the results between GFAAS and direct ELISA was 0.98 and 0.984, respectively, meaning that this immunoassay was not so stable. Although indirect and direct ELISA has been developed for cadmium detection, their application was limited in two liquid samples which were water and serum, and they did not extend the application further in other samples.

In this study, based on the monoclonal antibody highly specific against cadmium, a direct competitive enzyme-linked immunosorbent assay (DC-ELISA) was developed. It demonstrated that this assay is sensitive and specific and therefore has a great potential to be utilized in detecting cadmium residues in several farm produce which include liquid and solid samples.

Materials and Methods

Materials

All metal ions were under atomic absorption standards. Cd^{2+}, Pb^{2+}, Hg^{2+}, Zn^{2+}, Na^{+}, Ca^{2+}, Fe^{3+}, Mg^{2+}, Mn^{2+}, Cu^{2+}, Al^{3+}, Co^{2+}, Cr^{2+}, Ni^{2+}, Sn^{2+}, and K^{+} (1,000 μg/mL in 2% HNO_3) were purchased from National Research Center of Standard Materials (Beijing, China). 1-(4-Isothiocyanobenzyl) ethylenediamine-*N*,*N*,*N*′,*N*′-tetraacetic acid (ITCBE) was purchased from Dojindo Laboratories (Shanghai, China). Centricon-30 micro-concentrators were products of Millipore Co. (Bedford, MA, USA). Bicinchoninic acid protein assay kit and TNBSA were purchased from Pierce Co. (Rockford, IL, USA). Immunoglobulin M from mouse, bovine serum albumin (BSA), keyhole limpet hemocyanin (KLH), horseradish peroxidase (HRP), ethylenediaminetetraacetic acid (EDTA), tetramethyl benzidine (TMB) and ultrapure nitric acid were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Wheat, apple juice, rice, and tea were purchased from a supermarket in Guangzhou, Guangdong province.

Preparation of MAb Against Cd-EDTA and Cd-ITCBE-HRP Conjugates

The monoclonal antibody (3A9D9H4) against Cd-EDTA was generated by fusing SP2/0 mouse myeloma cells with spleen cells from a BALB/c mouse immunized with Cd-ITCBE-KLH.

Monoclonal antibody MAb 3A9D9H4 was screened by two coating antigens (Cd-ITCBE-BSA and ITCBE-BSA). This antibody has been prepared, isolated, and purified in our laboratory.

Cadmium and ITCBE in PBS (0.15 M, pH 10.0) were mixed at an equal molar ratio. The pH of the mixture was adjusted to 7.4 with Tris and the solution was incubated for 24 h at 25 °C. An equal amount of HRP was then added to Cd-ITCBE solution and pH of the solution was adjusted to 9.0 with Tris before another 24 h of incubation at 25 °C. The small unconjugated molecules were removed by buffer exchange with centricon-30 micro-concentrators. The conjugates were washed with HEPES (0.1 M, pH 7.4) [12–14].

Optimal Concentrations of Antibody and Enzyme-Labeled Antigen

The optimal concentrations of MAb3A9D9H4 and Cd-ITCBE-HRP were determined by a checkerboard assay [9, 10]. MAb3A9D9H4 was diluted into HEPES-buffered saline (HBS; 137 mM NaCl, 3 mM KCl, and 10 mM HEPES, pH 7.4) at concentrations of 0.2, 0.4, 0.8, 1.6, and 2.4 μg/mL and coated onto microwell plates by incubation at 4 °C overnight. After washing with HBS-Tween (274 mM NaCl, 6 mM KCl, 20 mM HEPES, and 0.05% Tween-20, pH 7.4) and blocking with 1% BSA in HBS, Cd-ITCBE-HRP was serially diluted at concentrations of 2, 4, 8, 16, 32, 64, 128, and 256 μg/mL in HBS through the wells of the plates and allowed to incubate in the microwells. After 1 h at 37 °C, the plates were washed again and incubated with TMB. The enzymatic reaction was stopped by 2 M H_2SO_4 and absorbance was measured at 450 nm.

Optimal Concentration of EDTA

After checkerboard titration, MAb3A9D9H4 was coated onto microwell plates with optimal concentration. After washing and blocking, Cd-ITCBE-HRP was diluted to its optimal concentration in HBS which contained EDTA at concentrations of 0.975, 1.95, 3.9, 7.8, 15.6, 31.25, and 62.5 mM and 0.125, 0.25, 0.5, and 1 M. The mixture of Cd-ITCBE-HRP and EDTA was added to the wells. After incubation for 1 h at 37 °C, the plates were washed again and incubated with TMB. The enzymatic reaction was stopped by 2 M H_2SO_4 and absorbance was measured at 450 nm.

Direct Competitive Enzyme-Linked Immunosorbent Assay

Microwell plates were coated with MAb3A9D9H4 at 4 °C overnight, washed three times with HBS-Tween, and then blocked with 1% BSA in HBS. Then, Cd-ITCBE-HRP was diluted to its optimal concentration in HBS. Cadmium with different concentration (200, 100, 50, 25, 12.5, 6.25, 3.125, 1.56, 0.8, 0.4, 0.2, 0.1, 0.05, and 0.025 μg/L) was added to HBS containing 8 mM EDTA and then incubated in 37 °C for 1 h. Then, the hapten Cd-EDTA was mixed with equal volume of Cd-ITCBE-HRP. The mixture was added to microwells. After incubation for 1 h at 37°C, the plates were washed three times. Then, TMB was added to each well and the plates were incubated at 37 °C for 30 min. The enzymatic reaction was stopped by 2 M H_2SO_4 and absorbance was measured at 450 nm.

Determination of Cross-Reactivity

The cross-reactivity for several metal ions including Cd^{2+}, Pb^{2+}, Hg^{2+}, Zn^{2+}, Na^{+}, Ca^{2+}, Fe^{3+}, Mg^{2+}, Mn^{2+}, Cu^{2+}, Al^{3+}, Co^{2+}, Cr^{2+}, Ni^{2+}, Sn^{2+}, or K^{+} was tested using the DC-ELISA [15, 16]. Metal ions with different concentrations which ranged from 0.01 to 10^5 μg/L were mixed

with 5 mM EDTA overnight to form hapten metal chelate. After coating and blocking, hapten metal chelate was mixed with equal volume of Cd-ITCBE-HRP and the mixture was added to wells. After 1 h at 37°C, the plates were washed again and incubated with TMB. The enzymatic reaction was stopped by 2 M H_2SO_4 and absorbance was measured at 450 nm. Values for IC_{50} were those that gave the best fit to the following equation: $A=A_0-[Cd]\ (A_0-A_1)/IC_{50}+[Cd]$ [17]. A is the signal at a definite known concentration of cadmium, A_0 is the signal in the absence of cadmium, A_1 is the signal at the saturating concentration of cadmium, and IC_{50} is the cadmium concentration that produces a 50% inhibition of the signal. The cross-reactivity values were calculated as % $CR=100\times[IC_{50}$ (Cd-EDTA)/IC_{50} (other metal-chelate)] [11].

Analysis of Several Farm Produce

Wheat, rice, and tea were ground and then added to ultrapure nitric acid overnight. Apple juice was directly added to nitric acid overnight. The mixture was heated to boiling until solved completely. The liquid were centrifuged for 10 min at 8,000 rpm. The supernatants were then mixed (9:1) with 10× HBS. The pH of solution was adjusted to 7.4 with saturated Tris. The solution after pretreatment was analyzed by DC-ELISA and GFAAS. The coefficient correlation of results between DC-ELISA and GFAAS was calculated with excel.

Results and Discussion

Preparation of Monoclonal Antibody 3A9D9H4

Cadmium, like most other small and simple inorganic molecule, is a hapten which is non-immunogenic. In this study, ITCBE as a bifunctional chelator was used for conjugating carried protein and chelating cadmium ion. Therefore, hapten Cd-ITCBE was conjugated to KLH as an immunogen for mouse immunization and Cd-ITCBE was conjugated with BSA as a coating antigen. ITCBE-BSA was used for screening against non-specific cell clones. The successfully preparation of the monoclonal antibody against cadmium chelate is the first critical step for establishing immunoassay method for cadmium chelate. After immunization of Balb/c mice, hybridoma preparation, and positive cloning, we had screened several monoclonal antibody special against cadmium chelate. One of them, MAb 3A9D9H4, was used in the following study.

Optimal Concentrations of Antibody and Enzyme-Labeled Antigen

The purity of enzyme-labeled antigen was determined with sodium dodecyl sulfate–polyacrylamide gel electrophoresis as described [18]. To establish DC-ELISA, the optimum concentrations of MAb 3A9D9H4 and enzyme-labeled antigen Cd-ITCBE-HRP were determined by checkerboard titration. As shown in Fig. 1, at 0.8 μg/mL of MAb 3A9D9H4 in HBS, increasing the concentration of Cd-ITCBE-HRP from 32 to 256 μg/mL did not significantly change the absorbance which ranged from 0.997 to 1.146. In addition, at a fixed concentration of 32 μg/mL for Cd-ITCBE-HRP, dilution of MAb 3A9D9H4 from 2.4 to 0.2 μg/mL resulted in a decrease of absorbance from 1.301 to 0.384. These results thus indicated that the optimal concentrations for MAb 3A9D9H4 and Cd-ITCBE-HRP in DC-ELISA were 0.8 and 32 μg/mL, respectively.

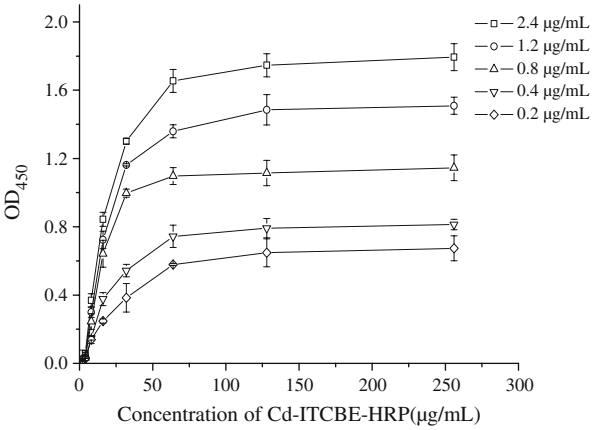

Fig. 1 Optimal concentrations of coating antibody and enzyme-labeled antigen for DC-ELISA as determined by a checkerboard assay. Each *point* represented the mean of triplicate±SD. MAb3A9D9H4 was coated onto microwell plates with concentration of 0.2, 0.4, 0.8, 1.6, and 2.4 μg/mL. Cd-ITCBE-HRP was serially diluted at concentrations of 2, 4, 8, 16, 32, 64, 128, and 256 μg/mL. The *symbol on the right side* represented the concentration of coating antibody MAb 3A9D9H4. *Square*, 2.4 μg/mL; *circle*, 1.2 μg/mL; *triangle*, 0.8 μg/mL; *inverted triangle*, 0.4μg/mL; *diamond*, 0.2 μg/mL. *OD* optical density

Optimal Concentration of EDTA

After checkerboard titration, the optimum concentration of EDTA was determined as described in "Materials and Methods". As shown in Fig. 2, when the concentration of EDTA was increased from 0.975 to 1000 mM, the OD_{450} of immunoassay was decreased from 0.998 to 0.164. This effect is due to the inhibitory effect of EDTA on the enzyme activity of horseradish peroxidase (data not shown), not the binding interaction between the antibody and metal-free EDTA. In the following analysis, unless otherwise indicated, the concentration of EDTA used in DC-ELISA was 8 mM and the corresponding OD_{450} was between 0.7 and 0.8.

The concentration of EDTA used in DC-ELISA was an important influencing factor. If the concentration of EDTA used in DC-ELISA was too high, the inhibitory effect of EDTA on the enzyme activity was increased and the OD_{450} of immunoassay became low. If the concentration of EDTA was low, the amount of metal ions chelated by EDTA would be limited and some cadmium residue in sample could not be chaleted by EDTA, so the result

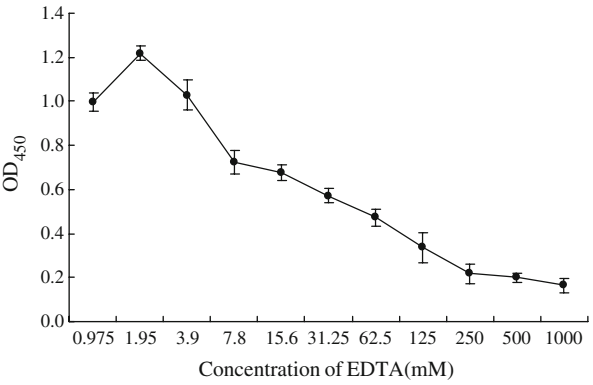

Fig. 2 Optimal concentration of EDTA as determined in direct ELISA. Each *point* represented the mean of triplicate±SD. 0.8 μg/mL of MAb 3A9D9H4 was coated onto microwell plates and Cd-ITCBE-HRP was diluted to 32 μg/mL in HBS which contained different concentration of EDTA at concentrations of 0.975, 1.95, 3.9, 7.8, 15.6, 31.25, and 62.5 mM and 0.125, 0.25, 0.5, and 1 M. *OD* optical density

of DC-ELISA would be inaccurate. Considering the two aspects described above, 8 mM EDTA was chosen for use in DC-ELISA. Under this concentration, all metal ions in samples could be chelated completely by the EDTA.

Sensitivity of DC-ELISA

The sensitivity of DC-ELISA for cadmium detection was determined. As shown in Fig. 3, quantification of cadmium ion was carried out by DC-ELISA with MAb 3A9D9H4. The IC_{50} value, the hapten concentration causing 50% inhibition, was 2.30 μg/L and the detection limit was 0.20 μg/L. These results indicated that MAb 3A9D9H4-based DC-ELISA for cadmium detection was highly sensitive and could be used for detecting cadmium residue in samples.

Before DC-ELISA, an indirect competitive ELISA (IC-ELISA) based on MAb 3A9D9H4 was established (data not shown). The IC_{50} and the detection limit of IC-ELISA were 42.6 and 2.56 μg/L, respectively. Compared to DC-ELISA, the IC_{50} and the detection limit of IC-ELISA were a little higher. One reason was that less procedure and reagent was needed in DC-ELISA than in IC-ELISA. The sensitivity and detection limit of ELISAs were easily influenced by the reagents used in reaction system. The more kinds of reagents used in ELISA, the more were the negative effects. The maximal possibility was the principle in two reaction systems. Figures 4 and 5 illustrated the general procedures of DC-ELISA and IC-ELISA. In the two immunoassays, the competitive binding was between Cd-EDTA and Cd-ITCBE-BSA or Cd-ITCBE-HRP. In IC-ELISA, color reaction was formed by addition of enzyme-labeled anti-antibody and TMB substrate. The color reaction in DC-ELISA just needed TMB substrate, and the enzyme-labeled antigen Cd-ITCBE-HRP which could react with both MAb 3A9D9H4 and TMB substrate replaced the function of enzyme-labeled antibody. So the sensitivity of DC-ELISA was higher than IC-ELSA and the detection limit of IC-ELISA was lower.

Recovery and Cross-Reactivity of DC-ELISA

As shown in Table 1, recoveries of cadmium from the wheat flour spiked with different concentrations of cadmium were determined by DC-ELISA based on the values obtained

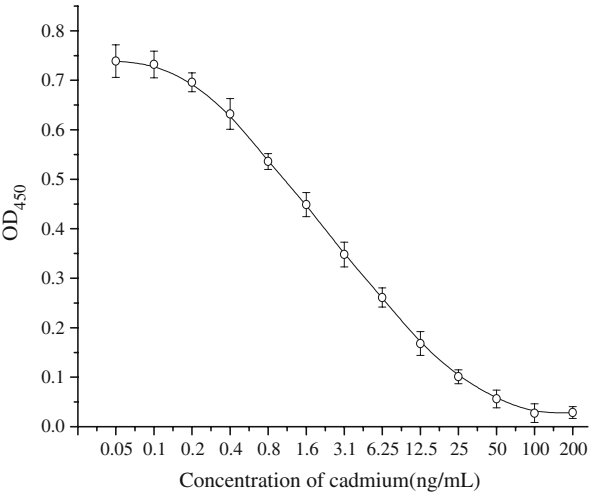

Fig. 3 Standard curve of DC-ELISA for cadmium detection. Each *point* represented the mean of three replicates±SD. 0.8 μg/mL of MAb 3A9D9H4 was coated onto microwell plates and the concentration of Cd-ITCBE-HRP was diluted to 32 μg/mL with HBS. HBS buffer contained 8 mM EDTA which was used to chelate 200, 100, 50, 25, 12.5, 6.25, 3.125, 1.56, 0.8, 0.4, 0.2, 0.1, 0.05, and 0.025 μg/L of cadmium. *OD* optical density

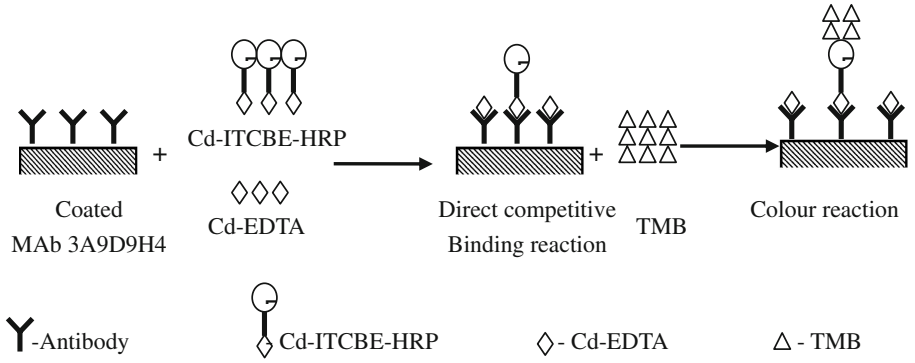

Fig. 4 Schematic diagram of the direct competitive ELISA for detection of Cd-EDTA

from the standard curves. Mean recovery ranged from 100.47% to 103.86% and the coefficient of variability ranged from 4.19% to 8.37%. The mean recovery of cadmium determined by DC-ELISA in wheat flour sample was slightly over 100%. It meant that the detection value of cadmium residue in samples would be slightly higher than the actual value. Under this condition, to reduce the error, it was very necessary that the sample should be determined by DC-ELISA with three replicates.

The specificity of the assay for cadmium was determined in a mixed sample presence of different metal ions (Cd^{2+}, Pb^{2+}, Hg^{2+}, Zn^{2+}, Na^{+}, Ca^{2+}, Fe^{3+}, Mg^{2+}, Mn^{2+}, Cu^{2+}, Al^{3+}, Co^{2+}, Cr^{2+}, Ni^{2+}, Sn^{2+}, or K^{+}) at concentrations of 10^{-2}–10^{5} μg/L. As shown in Table 2, the cross-reactivity exhibited by any of the tested metals was <0.6%. Furthermore, lead and mercury showed 0.54 and 0.47% cross-reactivity, respectively in our study, which were lower than these reported previously [11].

EDTA and ETDA-based chelators form hexacoordinate complexes with most metal ions. The DC-ELSA could determine the concentration of Cd-EDTA, so if the samples contained the analogs of EDTA with hexacoordinate or ETDA-based chelators, there would be some

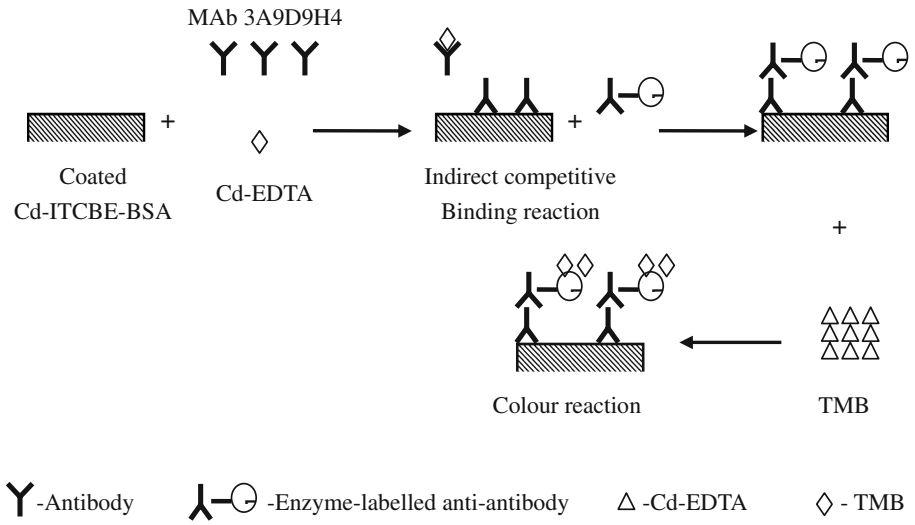

Fig. 5 Schematic diagram of the indirect competitive ELISA for detection of Cd-EDTA

Table 1 Recovery of cadmium from spiked wheat sample.

Cadmium fortified (μg/L)	Cadmium detected (μg/L)	Mean recovery (%, n=3)	CV (%)
5	5.06±0.37	101.36	7.35
10	10.04±0.38	100.47	3.79
20	20.12±0.89	100.60	4.40
40	41.54±1.76	103.86	4.24
60	62.08±2.47	103.47	3.98

Varying concentrations of atomic absorption grade cadmium were added into the wheat which was pretreated as mentioned in "Materials and Methods" to give final cadmium concentrations of 5, 10, 20, 40, and 60 μg/L with the titration of GFAAS. DC-ELISA was performed as described in "Materials and Methods". Each value represented the mean of three replicates±SD. Cadmium fortified and cadmium detected referred to the concentration of cadmium in wheat sample solution detected by GFAAS and DC-ELISA, respectively. Mean recovery referred to the value of [cadmium detected]/[Cadmium fortified]. CV referred to the coefficient of variation of cadmium detected

specific interference to DC-ELISA. Except metal chelates, some kinds of proteins, enzymes, pigments, and organic residues in the samples would show some influence on the specificity and sensitivity of ELISA because of non-specific interference. But these substances mostly would be removed from ELISA system after washing step.

Intra- and Inter-assay to Determine the Reliability of DC-ELISA

It is known that antigen–antibody interaction in immunoassays can be affected by various substances existing in samples with complex matrices. This poses a significant challenge for immunoassays to detect cadmium residue. In this study, samples were ground into flour and dissolved in ultrapure nitric acid in order to reduce matrix interferences. This treatment allowed all metals existing as solid samples, such as wheat, to be converted into a soluble form of nitrate. With addition of EDTA, the metals were chelated and the concentration of metal chelates could then be analyzed by DC-ELISA. Although the dilution ratio of analysis samples from different resources would affect the detection value of cadmium and

Table 2 Cross-reactivity of MAb 3A9D9H4 to different metal chelates.

Metal chelate	IC_{50} (μg/L)	Cross-reactivity (%)
Cd-EDTA	2.30	100
Pb-EDTA	423.26	0.54
Hg-EDTA	493.77	0.47
Mg-EDTA	976.67	0.24
Ca-EDTA	869.19	0.26
Zn-EDTA	897.30	0.26
Na-EDTA	1184.81	0.19
K-EDTA	1347.44	0.17
Mn-EDTA	904.24	0.25
Cu-EDTA	821.92	0.28
Fe-EDTA	853.25	0.27
Al-EDTA	877.42	0.26
Co-EDTA	884.56	0.26
Cr-EDTA	925.92	0.25
Ni-EDTA	948.44	0.24
Sn-EDTA	939.38	0.24

IC_{50} referred to the metal ion concentration that produces a 50% inhibition of the signal. Cross-reactivity was calculated as % CR=100×[IC_{50} (Cd-EDTA)/IC_{50} (other metal-chelate)]

Table 3 Reliability of DC-ELISA for cadmium detection determined by inter- and intra-assay.

Cadmium concentration	Inter-assay (n=6)		Intra-assay (n=6)	
	Cadmium concentration detected (μg/L)	CV (%)	Cadmium concentration detected (μg/L)	CV (%)
5	5.24±0.36	6.81	4.93±0.35	7.14
10	10.16±0.37	3.63	9.96±0.43	4.36
20	20.47±1.03	5.04	19.78±0.61	3.10
40	42.5±1.95	4.60	40.41±0.70	1.73
60	63.08±2.68	4.25	61.09±1.97	3.23

The concentrations of cadmium in fortified wheat samples were determined with DC-ELISA. The inter-assay was assessed by analyzing six replicates of each sample in a single run, and the intra-assay was assessed by analyzing the same sample, as triplicates, in two separate run. Each value represented the mean of three replicates±SD. CV referred to the coefficient of variation

cause little non-specific interference, the determination with three replicates would reduce the interference effectively.

To determine the reproducibility of the assay, the intra- and inter-assay of DC-ELISA were examined with spiked wheat samples. As shown in Table 3, the coefficients of variations for intra- and inter-assay were 1.73–7.14% and 3.63–6.81%, respectively. These data suggested that the DC-ELISA for cadmium detection was reliable.

Comparison DC-ELISA to GFASS

Because GFASS is a conventional method used to detect cadmium in food samples, the results from DC-ELISA for testing samples were compared to GFASS. As shown in Table 4, in experiments detecting cadmium residue in several farm produce, including apple juice, rice flour, wheat flour, and tea, the overall coefficient of variations of DC-ELISA was 6.67–10.19%. The coefficient correlation of results between DC-ELISA and GFAAS was 0.999.

Conclusions

The MAb3A9D9H4 we generated was highly specific, with little or no cross-reactivity to other metal chelates, including Pb-EDTA, Hg-EDTA, Zn-EDTA, and so on. In addition, the MAb 3A9D9H4-based DC-ELISA we have established was capable of detecting cadmium ion as low as 2.30 μg/L. Recoveries of cadmium from wheat samples ranged from 100.47% to 103.86%. Moreover, the reliability of the assay was determined with spiked wheat

Table 4 Comparison of DC-ELISA to GFAAS for analysis of cadmium residue in farm produce.

Samples	DC-ELISA (ppb)	CV (%)	GFAAS (ppb)
Apple juice	3.14±0.32	10.19	3.59±0.09
Rice flour	88.69±5.92	6.67	87.78±5.04
Wheat flour	19.44±1.39	7.15	18.48±4.21
Tea	60.82±6.44	10.59	62.22±4.28

CV referred to the coefficient of variation and ppb referred to the concentration of cadmium in samples; for apple juice, ppb represented μg/L, for the other three samples, ppb represented μg/kg. Each value represented the mean of three replicates±SD

samples, and the coefficients of variations for intra- and inter-assay were 1.73–7.14% and 3.63–6.81%, respectively. The result from the DC-ELISA was compared with that from GFAAS. The coefficient of variations of DC-ELISA was 6.67–10.59% and the overall coefficient correlation of results between DC-ELISA and GFAAS was 0.99. Our results showed that DC-ELISA based on MAb3A9D9H4 we developed could be used to detect cadmium residue in farm produce.

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