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Cadmium Purification and Quantification Using Immunochromatography

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One of the pathways by which cadmium enters human beings is through the consumption of agricultural products. The monitoring of cadmium has a significant role in the management of cadmium intake. Cadmium purification and quantification using immunochromatography were conducted in this study as an alternative means of cadmium analysis. The samples used in this study were rice, tomato, lettuce, garden pea, *Arabidopsis thaliana* (a widely used model organism for studying plants), soil, and fertilizer. The cadmium immunochromatography has been produced from the monoclonal antibody Nx2C3, which recognize the chelate form of cadmium, Cd·EDTA. The immunochromatography can be used for quantification of cadmium in a range from 0.01 to 0.1 mg/L at 20% mean coefficient of variance. A chelate column employing quaternary ammonium salts was used for the purification of cadmium from HCl extracts of samples. Recoveries of cadmium were near 100%, and the lowest recovery was 76.6% from rice leaves. The estimated cadmium concentrations from the immunochromatography procedure were evaluated by comparison with the results of instrumental analysis (ICP-AES or ICP-MS). By comparison of HCl extracts analyzed by ICP-MS and column eluates analyzed by immunochromatography of the samples, the estimated cadmium concentrations were closely similar, and their recoveries were from 98 to 116%.

KEYWORDS: Cadmium; immunochromatography; Nx2C3

INTRODUCTION

The accumulation of cadmium in soil and translocation into plants and hence the food chain pose a critical problem worldwide. Dietary intake of cadmium from plants and animals grown on polluted soil results in various human diseases (*1*). Cadmium inputs to soil are through various sources such as fertilizer, industrial waste, mine drainage, and soil amendments (*2*). Monitoring of cadmium fluxes in agricultural systems is needed for the proper management of cadmium in the food chain.

The most frequently used methods in cadmium analysis at present are atomic absorption spectroscopy (AAS), inductively coupled plasma atomic emission spectrometry (ICP-AES), and inductively coupled plasma-mass spectroscopy (ICP-MS). These methods are sensitive and accurate, but they are also time-consuming and require sophisticated equipment, generally in a laboratory setting. In cases where suitable antibodies and extraction protocols are available or can be developed, immunoassays offer a simple, fast, cost-effective alternative (*3*−*7*).

At present, all antibodies specific for cadmium recognize a chelated form of cadmium, that is, Cd·EDTA (*8*−*11*). A one-step competitive immunoassay employing an anti-Cd·EDTA antibody has been reportedly applied for the detection of cadmium in environmental water (*12*) and human serum (*13*). We reported an immunochromatography study employing an anti-Cd·EDTA antibody, which could be used for the quantification of cadmium in the range from 0.01 to 0.1 mg/L at 20% mean coefficient of variance (*10*, *14*). Although the anti-Cd·EDTA antibody was highly specific to Cd·EDTA, a cadmium purification column was needed for the measurement of cadmium in extracts of rice grain as determined by immunochromatography because the concentrations of coextracted metals, zinc, manganese, copper, and iron exceeded the level that could be disregarded by the specificity of the antibody (*10*, *14*, *15*). The present study describes the validation of the chelate column using extracts of soil, fertilizer, rice, and some plant organs. The results of immunochromatography for the purified cadmium are also described here.

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MATERIALS AND METHODS

Reagents and Antibody. 1-(4-Isothiocyanobenzyl) ethylenediamine-*N*,*N*,*N*′,*N*′-tetraacetic acid (isothiocyanobenzyl-EDTA) was obtained from Dojindo (Kumamoto, Japan). Ovalbumin (OVA) were obtained from Sigma-Aldrich (H7017, A9647, and A2512; St. Louis, MO). Cd·EDTA−protein conjugates were prepared as described by Darwish and Blake (*12*). The Nx2C3 antibody that recognizes Cd·EDTA complexes was prepared from a mouse hybridoma (*10*). It was deposited at the International Patent Organism Depository, National Institute of Advanced Industrial Science and Technology (Tsukuba, Japan) as deposition no. FERM P-19703. A gold particle-labeled antibody was prepared as described by Paek et al. (*16*). and 80 ng aliquot of the antibody was freeze-dried in a glass vial.

Plant Materials and Treatments. Fifty Japonica rice grain samples were harvested from different paddy fields. The fields were located within the same region in Japan. Ten rice leaf samples (*Oryza sativa*) were harvested from different pods. These grains and leaves were selected to be representative of different cadmium concentrations based on a previous survey (data unpublished). Each rice grain sample and each rice leaf sample were milled with a coffee mill for approximately 60 s. For preparing HCl extracts of the rice grains, 2 g of the milled rice grain was mixed with 20 mL of 0.1 M HCl. After 1 min of shaking, the extracts were filtered with filter paper (Qualitative no. 2, Advantec Toyo, Tokyo, Japan). For preparing HCl extracts of the rice leaves, 1 g of milled sample was mixed with 20 mL of 0.1 M HCl, shaken for 1 h, and then filtered. For preparing completely digested solution, 0.5 g of the milled sample was mixed with 8.0 mL of HNO_3. After 5−6 h of incubation at 130 °C, the solution volume was adjusted to 50 mL with ultrapure water.

Immature tomatoes were harvested from the tomato plants watered by 1 mg/kg cadmium-contaminated water for 2 months before harvesting. Tomatoes were crushed by a juicer with distilled water 2 times the weight of the tomatoes. For preparing HCl extracts, 36 mL of juice was transferred to a new 50 mL conical tube and mixed with 4 mL of 1.0 M HCl. After an hour of shaking, supernatants were harvested by centrifugation. For preparing completely digested solution, 0.5 mL of juice was transferred to a new test tube and mixed with 6.0 mL of concentrated HNO_3. After overnight incubation at 130 °C, the solution volume was adjusted to 50 mL with distilled water.

Tomato leaves were harvested from the tomato plants watered by 1 mg/kg cadmium-contaminated water for 5 days before harvesting. Tomato leaves were twice washed with distilled water and dried with paper (Kimwipes, Kimberly-Clark). Tomato leaves were frozen in liquid nitrogen and ground with a mortar and pestle. Liquid nitrogen was added as necessary during the grinding. For preparing HCl extracts, 1.57 g of tomato leaf powder was mixed with 12 mL of 0.1 M HCl. After 1 h of shaking, supernatants were taken by centrifugation. For preparing a completely digested solution, 0.5 mL of leaf juice was transferred to a new test tube and mixed with 5.0 mL of HNO_3. After 6 h of incubation at 130 °C, the solution volume was adjusted to 50 mL with ultrapure water.

Lettuce leaves were harvested after being watered by 1 mg/kg cadmium-contaminated water for a week before harvesting. Thirty grams of lettuce leaves was blended with 90 mL of distilled water by a juicer for 1.5 min (lettuce/water = 1:3). For preparing HCl extracts, 10 mL of 1.0 M HCl was added into the lettuce juice. After 1 h of shaking, the solution was filtered by the paper wipes and filtration paper. For preparing completely digested solution, 1 mL of the solution was transferred to a new test tube and mixed with 10 mL of HNO_3. After 6 h of incubation at 130 °C, the solution volume was adjusted to 50 mL with ultra pure water.

Arabidopsis thaliana were grown in cadmium-contaminated agar medium. There were three *Arabidopsis* samples, A, B, and C, which were grown in 40, 60, and 80 μM cadmium-contaminated agar medium, respectively. The *Arabidopsis* specimens were separated into root and terrestrial parts. The terrestrial parts were washed twice with distilled water and then dried with paper wipes. The plants were frozen by adding liquid nitrogen and ground with a mortar and pestle. Liquid nitrogen was added as necessary during the grinding. For preparing HCl extracts, the plant powder was transferred into a new test tube, weighed, and mixed with 12 mL of 0.1 M HCl. After 1 h of shaking, supernatants were taken by centrifugation. For preparing completely digested solution, 1 mL of plant juice before centrifugation was transferred to a new test tube and mixed with 5.0 mL of HNO_3. After 6 h of incubation at 130 °C, the solution volume was adjusted to 50 mL with ultrapure water.

Garden pea samples were bought from a local supermarket. The peas weighed 60 g and were blended by a juicer with 120 mL of distilled water for 1 min. For preparing HCl extracts, 90 mL of pea juice was mixed with 10 mL of 1 M HCl. After an hour of shaking, the cadmium concentrations were varied to be 0, 20, 30, 40, and 60 μg/L by the addition of $CdCl_2$ solution. The solutions were filtered by the paper wipes and filtration paper. For preparing a completely digested solution, 1 mL of the solution was transferred to a new test tube and mixed with 10 mL of HNO_3. After 6 h of incubation at 130 °C, the solution volume was adjusted to 50 mL with ultrapure water.

Soil Materials and Treatment. Soil samples were collected from paddy fields in the same village in Japan. Seventy-four soil samples were selected to be of different cadmium concentrations, on the basis of the results of a previous survey (data unpublished). HCl extracts were prepared according to the methods specified in the attached Table 2 of Japanese Ministry Ordinance 47, Ministry of Agriculture and Forestry (Japan), 1971. Briefly, 50 mL of 0.1 M HCl was added to 10 g of soil and then shaken for an hour and filtered with the filter paper (Qualitative no. 2, Advantec Toyo). Fourteen randomly selected samples of the 74 samples were used for the various metal concentration analyses.

Fertilizer Materials and Treatment. Fertilizers certified as "standard material" were purchased from the Food and Agricultural Materials Inspection Center, Saitama, Japan. Fertilizer A is FFIS-A-06, which is a chemical fertilizer, and its certified compositional elements are 10.34% total N, 13.73% citric acid soluble P, 12.06% water-soluble K, and 1.94 mg/kg total Cd. Fertilizer B is FFIS-B-06, which is a chemical fertilizer, and its certified components are 8.20% ammonia N, 9.25% citric acid soluble P, 5.48% water-soluble K, and 2.49 mg/kg total Cd. For preparing HCl extracts, 20 mL of 0.1 M HCl was added to 1 g of fertilizer. After 1 h of shaking, supernatants were harvested by centrifugation.

Immunochromatography Format. The format for cadmium immunochromatography is illustrated in **Figure 1**. Samples were mixed with EDTA and gold particle labeled anti-Cd·EDTA antibody to make complexes before loading onto the sample pad. When loaded, they migrate toward the test line, where conjugates of Cd·EDTA−OVA were immobilized to capture Cd·EDTA free antibodies. On the other hand, Cd·EDTA bound antibodies passed through the test line. Thus, the red band observed on the test line decreased inversely proportionally to the cadmium concentration.

Immunochromatography Measurements. For cadmium concentration measurement by immunochromatography, cadmium in HCl extracts was purified by the quaternary ammonium salts employed in a chelating column that was designed on the basis of Akatsuka's method (*17*). Five milliliter HCl extracts of each sample were passed through the chelating column. The columns were cleaned by passing through 5 mL of 0.1 M HCl. Cadmium was extracted from the column by adding 5 mL of 0.1 M HNO_3. The HNO_3 column eluates were used to further carry out the cadmium measurements by immunochromatography. Twenty microliters of the column eluates was mixed with 380 μL of 50 mM Tris buffer (pH 7.5) containing 0.3 μM EDTA. A hundred microliters of sample solution was added to the freeze-dried antibody (80 ng) labeled with 40 nm gold particles (BB International, New York) and then mixed well with a vortex. For the immunochromatography assay, 70 μL of the sample solution was loaded to the sample pad. After 40 min, the red band that appeared on the test band was read by an immunochromatography reader, DiaScan 30-D (Otsuka Electronics, Osaka, Japan). The sample solution was diluted appropriately until the cadmium concentration from the assay became <0.06 mg/L.

Cadmium Concentration Determination by Immunochromatography Analysis. Each solution of 10, 30, or 60 ppb of $CdCl_2$ in 0.1 M HNO_3 was measured twice, and the color data were used to make a "concentration−color" standard curve by exponential approximation with Microsoft Excel. The equation of the curve was used to calculate the cadmium concentration of each sample.

Instrumental Analysis. The digested samples were stored at 4 °C until they were analyzed using inductively coupled plasma−atomic emission spectroscopy (ICP-AES; SPECTRO CIROS 120, Rigaku Co. Ltd., Japan; RF generator power, 1400 W; plasma gas flow rate, 13 L/min; carrier gas flow rate, 0.9 L/min; analyte lines, Cd 214.438 nm, Zn 213.856 nm; Mn

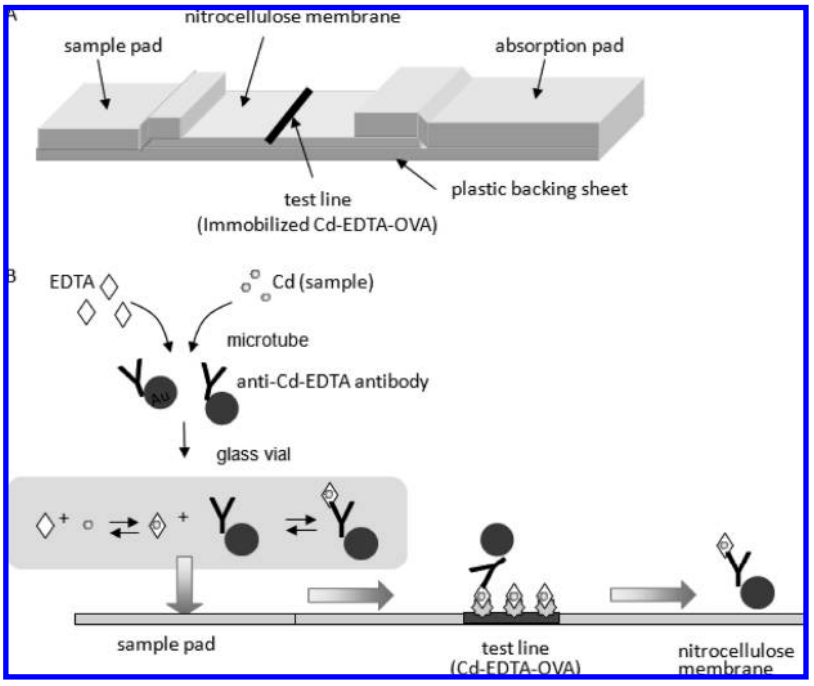

Figure 1. Cadmium immunochromatography system: (**A**) physical layout of the essential components; (**B**) functionality (see Materials and Methods).

257.610 nm, Mg 279.553 nm, Fe 259.940 nm, Cu 324.754). The HCl extract and nitrate-digested samples were stored at 4 °C until they were analyzed using inductively coupled plasma-mass spectrometry (ICP-MS, Thermo Elemental X7, Thermo Fisher Scientific, Yokohama, Japan; generator frequency, 27.12 MHz; RF power, 1.40 kW; plasma gas flow rate, 13.7 L/min; carrier gas flow rate, 0.88 L/min; element *m*/*z*; Mg 24, Mn 55, Fe 56, Cu 65, Zn 66, Cd 111).

RESULTS

Purification of Cadmium. The anti-Cd·EDTA antibody employed in the immunochromatography (**Figure 1**) had specific binding capacity toward Cd·EDTA and low cross-reactivity for other EDTA−metal complexes. For example, the K_d values of Mn·EDTA and Zn·EDTA were, respectively, 139 and 175 times higher than that of Cd·EDTA (*10*). However, the specificity of the antibody was not always sufficient to detect cadmium in natural samples. The excess coexisting metals would act as dominant competitors against cadmium to the antibody and inhibit the accurate detection of cadmium by the immunochromatography. Thus, separation of cadmium from other metals is necessary for the detection of cadmium by the antibody. Akatsuka et al. reported that a chelate column employing quaternary ammonium salts could be used for preconcentration of trace cadmium from seawater (*17*). We evaluated the column for the purification of cadmium from the HCl extracts listed in **Table 1**, which were derived from plants, soils, and fertilizers. These extracts contained the source material in $^1/_{10}$−$^1/_{20}$ w/v (see Materials and Methods). Metal recovery was confirmed using cadmium, copper, iron, magnesium, manganese, and zinc, five metals other than cadmium that were selected because of their common presence in natural samples and their relatively high cross-reactivity with the Nx2C3 antibody.

The results in **Table 1** may be summarized as follows. The lowest cadmium mean extract concentration was 0.01 mg/L from tomato leaves, and the highest cadmium mean concentration of the extract was 0.79 mg/L from *Arabidopsis*. In many cases, recoveries of cadmium were near 100%, and the lowest recovery was from rice leaves at 76.6%. The highest iron mean concentration of the extract was 57 mg/L from paddy soils, and its recovery was 0.2%. The highest iron mean concentration of the column eluates was 3.8 mg/L from rice leaves, and its recovery rate was 76.6%, which was the highest of all of the samples. Plant samples, except for the garden pea, exhibited high recoveries of iron, although the reason is not known. The highest zinc mean concentration of the extract was 2.7 mg/L from fertilizer B, and its recovery was 4.6%. The highest zinc concentration of the eluates was 0.3 mg/L from lettuce, and its recovery was 26.4%; the second was 0.23 mg/L from garden pea, and its recovery was 14.1%. The highest copper mean concentration of the extract was 0.37 mg/L from fertilizer B, with a recovery of 1.2%. The highest magnesium mean concentration of the extract was 107 mg/L from rice grain, and its recovery was 0.1%. The highest manganese mean concentration of the extract was 34 mg/L from rice grain, with a recovery of under 0.01%. In the column eluates, the concentrations of copper, magnesium, and manganese were < 0.12 mg/L (manganese from rice leaves).

Cadmium Determination Using Immunochromatography. Purified cadmium in the column eluates was neutralized and mixed with EDTA and anti-Cd·EDTA antibody for immunochromatography. The cadmium concentration was estimated from the color band on the test line of the immunochromatography result and comparing it with the calibration curve. The estimated cadmium concentrations from the immunochromatography assay were evaluated by comparison with the results of the instrumental analysis (ICP-AES or ICP-MS).

In the test using 50 rice grains, the relationship between findings in the column eluates with immunochromatography and the completely digested sample with ICP-AES showed a high correlation, with a correlation coefficient of 0.97 (**Figure 2**). In a similar comparison of 10 samples of rice leaves, two measurements were also highly correlated, with a correlation coefficient of 0.97 (**Figure 3**).

For the paddy soil test, ICP-AES measurement was carried out with 73 HCl extracts, and then the estimated concentrations were compared with the results of immunochromatographic analysis of the column eluates from each of the samples. The correlation coefficient derived from the comparison was 0.89 (**Figure 4**).

Cadmium-contaminated HCl extracts of the garden pea were prepared by cadmium spiking of the cadmium-free extract

Table 1. Metal Concentrations in Sample Extract and Column Eluate

		mean concentration (mg/L)		
source	element	HCl extract	chelate column eluent	recovery (%)
rice grain (n = 50)	Cd	0.029 ± 0.021	0.029 ± 0.021	101
	Cu	0.16 ± 0.044	<0.005	<3.1
	Fe	0.17 ± 0.069	0.12 ± 0.022	73.8
	Mg	107 ± 5.0	0.070 ± 0.094	0.1
	Mn	2.2 ± 0.68	0.004 ± 0.005	0.2
	Zn	2.1 ± 0.16	0.032 ± 0.013	1.5
rice leaves (n = 10)	Cd	0.050 ± 0.042	0.059 ± 0.051	118
	Cu	0.15 ± 0.12	0.007 ± 0.016	4.6
	Fe	5.0 ± 2.9	3.8 ± 2.5	76.6
	Mg	45 ± 7.0	0.12 ± 0.10	0.3
	Mn	20 ± 13	0.046 ± 0.051	0.2
	Zn	2.2 ± 1.0	0.04 ± 0.024	1.8
tomato (n = 4 ; Cd contaminated = 2 Cd free = 2)	Cd	0.011 ± 0.011	0.012 ± 0.012	111
	Cu	0.14 ± 0.025	0.010 ± 0.002	7.1
	Fe	0.24 ± 0.030	0.046 ± 0.001	19.3
	Mg	59 ± 17	0.038 ± 0.025	0.1
	Mn	0.38 ± 0.091	0.0037 ± 0.006	1.0
	Zn	0.59 ± 0.12	0.034 ± 0.018	12.4
tomato leaves (n = 3; Cd contaminated = 1, Cd free = 2)	Cd	0.010 ± 0.026	0.010 ± 0.025	100
	Cu	0.099 ± 0.019	0.001 ± 0.001	1.0
	Fe	0.35 ± 0.004	0.094 ± 0.093	26.6
	Mg	89 ± 58	0.011 ± 0.011	0.0
	Mn	1.1 ± 0.91	0.001 ± 0.001	0.1
	Zn	0.46 ± 0.067	0.071 ± 0.033	15.5
lettuce (n = 3; Cd contaminated = 1, Cd free = 2)	Cd	0.10 ± 0.11	0.083 ± 0.092	81.4
	Cu	0.14 ± 0.007	0.005 ± 0.000	3.6
	Fe	0.17 ± 0.059	0.040 ± 0.005	23.3
	Mg	59 ± 3.0	0.026 ± 0.0042	0.0
	Mn	1.9 ± 0.20	0.002 ± 0.001	0.1
	Zn	1.2 ± 0.10	0.30 ± 0.001	26.4
Arabidopsis (n = 4)	Cd	0.79 ± 0.70	0.85 ± 0.75	108
	Cu	0.009 ± 0.003	0.001 ± 0.000	13.3
	Fe	0.10 ± 0.057	0.044 ± 0.031	43.1
	Mg	4.8 ± 1.8	0.001 ± 0.001	0.0
	Mn	0.30 ± 0.10	0.001 ± 0.000	0.2
	Zn	0.25 ± 0.086	0.041 ± 0.016	16.3
garden pea (n = 5)	Cd	0.025 ± 0.018	0.032 ± 0.023	128
	Cu	0.037 ± 0.002	0.001 ± 0.000	3.9
	Fe	0.50 ± 0.070	0.034 ± 0.017	6.9
	Mg	37 ± 2.2	0.004 ± 0.002	0.0
	Mn	0.54 ± 0.032	0.001 ± 0.000	0.1
	Zn	1.7 ± 0.11	0.23 ± 0.022	14.1
paddy soil (n = 14)	Cd	0.027 ± 0.004	0.034 ± 0.007	126
	Cu	0.61 ± 0.2	<0.005	<0.8
	Fe	57 ± 47	0.089 ± 0.056	0.2
	Mg	110 ± 20	0.099 ± 0.071	0.1
	Mn	29 ± 12	0.021 ± 0.018	0.1
	Zn	1.3 ± 0.36	0.077 ± 0.10	6.1
fertilizer A (n = 3)	Cd	0.034 ± 0.007	0.044 ± 0.002	127
	Cu	0.006 ± 0.002	0.006 ± 0.005	102
	Fe	3.8 ± 1.7	0.12 ± 0.071	3.2
	Mg	550 ± 74	0.082 ± 0.030	0.0
	Mn	34 ± 2.2	0.011 ± 0.007	0.0
	Zn	0.93 ± 0.22	0.15 ± 0.004	16.4
fertilizer B (n = 3)	Cd	0.078 ± 0.012	0.093 ± 0.007	119
	Cu	0.37 ± 0.035	0.0043 ± 0.002	1.2
	Fe	21 ± 0.63	0.13 ± 0.030	0.6
	Mg	91 ± 7.3	0.021 ± 0.014	0.0
	Mn	2.0 ± 0.089	0.002 ± 0.001	0.1
	Zn	2.7 ± 0.42	0.12 ± 0.070	4.6

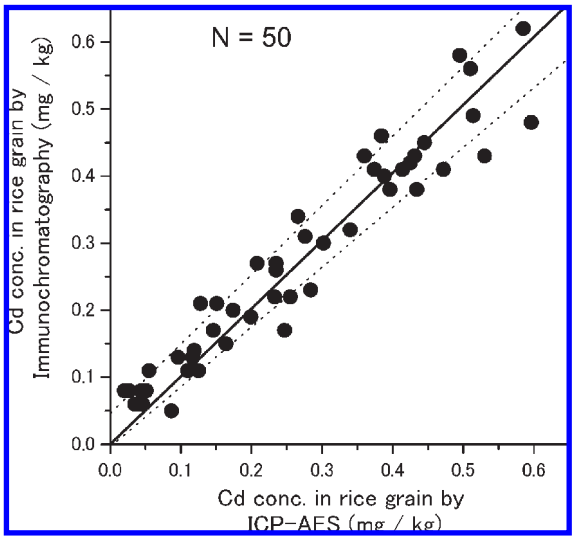

Figure 2. Estimated cadmium concentration in rice by immunochromatography and ICP-AES. The estimated cadmium concentrations from the two forms of measurement are plotted. Dotted lines show 95% confidence intervals.

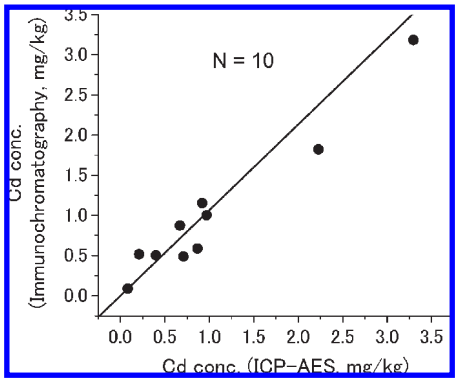

Figure 3. Estimated cadmium concentration in rice leaves by immunochromatography and ICP-AES. The estimated cadmium concentrations from the two measurements are plotted.

(< 0.001 mg/L determined by ICP-MS), and they were applied to the chelate column. The estimated cadmium concentrations of the column eluates by immunochromatography were almost the same amount as the spiked cadmium concentrations (correlation coefficient = 0.99, slope = 0.97; **Figure 5**).

The results of immunochromatography and the instrumental analysis of the other samples are summarized in **Table 2**. Comparison of HCl extracts analyzed by ICP-MS and column eluates analyzed by immunochromatography shows that the estimated cadmium concentrations were close and their recoveries were in a range from 98 to 116%. However, in fertilizers, the cadmium concentration estimated by immunochromatography using column eluates was 40−60% of that of completely digested solution. In the case of lettuce, the cadmium concentration in column eluates estimated by immunochromatography was 1.4 times higher than that estimated by ICP-MS. The reasons for these results will be considered under Discussion.

DISCUSSION

In this study, we measured the cadmium concentration in various agricultural samples pretreated with a chelate column and evaluated these findings by comparison with instrumental analysis.

The first procedural step of the immunochromatography assay was cadmium extraction from the samples. Although we did not

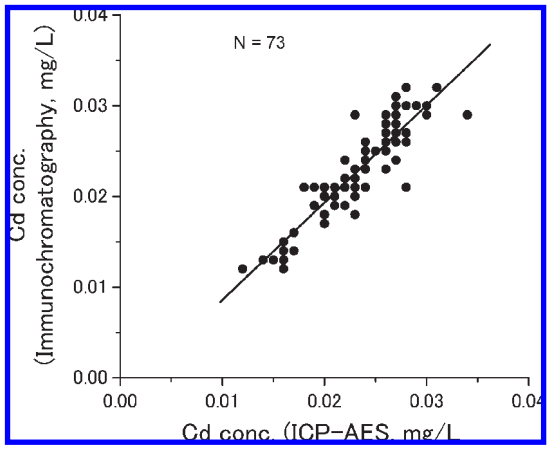

Figure 4. Estimated cadmium concentration in paddy soil by immunochromatography and ICP-AES. The estimated cadmium concentrations from the two measurements are plotted.

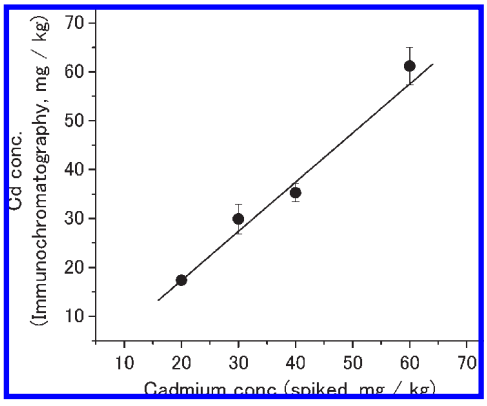

Figure 5. Cadmium recovery test using garden pea extract. The estimated cadmium concentrations by immunochromatography are plotted along with the spiked cadmium concentration. Each data point represents the mean ± SD of three independent determinations.

analyze the total cadmium amounts in the paddy soils, in this study, cadmium could be obtained from the source samples by extraction with 0.1 N HCl, except for fertilizers (**Table 2**). The cadmium recoveries of fertilizers A and B at the HCl extraction step were 40 and 60%, respectively. One of the differences between fertilizers A and B was the concentration of NPK elements (see Materials and Methods). Fertilizer A had high NPK, and fertilizer B had low NPK. Cadmium can form complexes with phosphorus compounds (*18*), and thus the amounts of cadmium extracted with HCl would be inversely related to the concentration of phosphorus compounds in fertilizers.

The second step, cadmium purification from HCl extracts, was important for subsequent immunochromatography measurement, because coextracted metals reacted with the antibody and reduced the color of test line, and it would lead to overestimation of cadmium. One method to decrease the cross-reactivity of the antibody to nontargeted metals is to limit the EDTA concentration (*19*). This method was useful for manganese and calcium, on which the formation constant (K_{ML}) for the EDTA complex was much lower than that of cadmium because such metals bind to EDTA only after all of the cadmium has bound (*19*). In this study, the antibody reacted in the buffer that contained 0.3 μM EDTA in accord with this description. For other metals, however, in which the K_{ML} of the EDTA complex was close to cadmium, this method would not be efficient because these metals bind to EDTA as well as cadmium. Therefore, we employed a column

Table 2. Comparison of Estimated Cadmium Concentrations

	estimated cadmium concentration (mg of cadmium/kg of source)			
		HCl extract	chelate column eluent	
source	completely digested solution	ICP-MS[a]	ICP-MS	immunochromatography[b]
tomato	0.079	0.073 (92.4)	0.08	0.083 ± 0.010
tomato leaves	0.25	0.19 (74.6)	0.18	0.22 ± 0.02
lettuce	0.69	0.89 (129.0)	0.73	1.03 ± 0.25
Arabidopsis A	31.0	31.7 (102.5)	35.0	36.2 ± 5.1
Arabidopsis B	56.7	59.1 (104.1)	63.6	58.1 ± 3.7
Arabidopsis C	69.3	73.4 (105.9)	78.1	84.2 ± 12.5
fertilizer A	1.94	0.78 (40.2)	0.84	0.80 ± 0.11
fertilizer B	2.49	1.72 (69.1)	1.76	1.74 ± 0.27

[a] Values in parentheses indicate % recovery. [b] Each value represents the mean of three replicates ± SD.

proposed by Akatsuka et al. (*17*) to remove the zinc, manganese, copper, and iron that would be major elements in agricultural samples that had reactivity with the antibody. If these metals could not be removed through the column adequately, the cadmium concentration in the column eluates estimated by immunochromatography would be higher than that estimated by instrumental analysis. The column eluates of lettuce contained 0.30 mg/L of zinc, and this may be why the immunochromatography estimated cadmium concentration was 1.41 times higher than that estimated by ICP-MS (**Table 2**). However, the results obtained from green pea measurement showed that estimations of cadmium concentration by immunochromatography were almost correct, although the mean concentration of zinc in the column eluates was 0.23 mg/L (**Figure 5**; **Table 1**). Because the K_d of the antibody to Zn·EDTA was 175 times higher than that to Cd·EDTA (*10*), 0.30 mg/L of zinc would correspond to 0.002 mg/L of cadmium. The level was 70 times lower than the cadmium concentration analyzed by ICP-MS, so that it would be difficult to think that 0.30 mg/L of zinc had any affect on the binding reaction of the antibody to Cd·EDTA. Although the reason why the cadmium concentration of lettuce was overestimated by immunochromatography in this study remains unknown, there was no evidence that the metals remaining in the column eluates affected the measurement by immunochromatography. Thus, it may be concluded that in pretreatment for immunochromatography, determination of the readiness of samples of plants, soil, or fertilizer can be conducted by using the cadmium separation column.

The detection limit of immunochromatography for the cadmium in column eluates was 0.01 mg/L (*10*). Therefore, detection limits of cadmium in the samples depend on the dilution rate during pretreatment. If one needs to detect 0.1 mg/kg cadmium in the sample, the dilution rate is permitted up to 10 times. In this study, we recovered column eluates at the same volume as the column applied solution. In the original paper the column was used for the preconcentration of trace cadmium in seawater (*17*). Therefore, if necessary, cadmium can be concentrated in the column step.

Time-consuming processes in the immunochromatography assay included the column step and waiting for the appearance of the red bands on the test line of the immunochromatography. To shorten the time needed for column treatment, a vacuum manifold was used to treat plural samples simultaneously and, in this case, it took approximately 15 min from the time of applying the samples to recovering eluates. The band appearance took 40 min, but it can be conducted in parallel using up to about 40 samples. In the case of rice grain, HCl extraction needed only 1 min and shaking by hand was able to extract the cadmium adequately; we would estimate 80 rice grain samples could be treated from mill to immunochromatographic measurement per person per day (8 h).

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