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A scFv Antibody-Based Immunoaffinity Chromatography Column for Clean-Up of Bisphenol A-Contaminated Water Samples

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This is a report on the development of immunoaffinity chromatography using a column of silica gel with an immobilized single-chain variable fragment (scFv) antibody specific to bisphenol A (BPA) for cleanup of BPA-contaminated water samples. The BBA-2187 scFv antibody specific to BPA was purified from the periplasmic fractions of the recombinant *Escherichia coli*. After a sample of BPA-contaminated river water was applied to the immunoaffinity column, the background signal intensity observed in high-performance liquid chromatography (HPLC) analysis of the eluates was markedly lower than that observed in HPLC analysis of the eluates from an Oasis HLB cartridge treated with the same sample. The immunoaffinity column efficiently concentrated BPA from actual river water samples with different matrices. Our results demonstrate that the immunoaffinity column with immobilized BBA-2187 scFv antibody is efficient for the cleanup of BPA-contaminated water samples from different sources.

KEYWORDS: Bisphenol A; enzyme-linked immunosorbent assay; immunoaffinity column; single-chain variable fragment antibody

INTRODUCTION

Bisphenol A (BPA) has been used for the production of polycarbonate, as well as epoxy- and phenoxy-resins. It is also used as a stabilizer and an antioxidant for plastics including poly(vinyl chloride). However, BPA binds weakly to human estrogen receptors α and β (*1*, *2*) and shows endocrine-disrupting activity toward the river fish medaka (*Oryzias latipes*) (*3*). In addition, low-dose exposure (2−20 μg kg^{-1}) of BPA to mouse embryos in utero causes adverse health effects (*4*). BPA was detected in 65% of 75 water samples collected from rivers, ponds, seawater, and groundwater in Japan, at a maximum concentration of 0.92 ng mL^{-1} (*5*). Therefore, monitoring BPA in environmental water samples at ppt levels is important.

Gas chromatography/mass spectrometry is generally used for analysis of BPA in environmental water samples; this analytical method requires several steps, including extraction and cleanup of BPA-contaminated water samples for detection of ppt levels of BPA. These steps often include partitioning in an organic solvent and solid-phase extraction (SPE) based on hydrophobic interactions, adsorption, or ion exchange (*6*). Both procedures remove compounds that interfere with analysis of the target compounds from complex sample matrices, but the compounds are not always removed completely, because of lack of specificity and poor removal efficiency. By comparison, immunoaffinity chromatography offers advantages over conventional cleanup and concentration methods because of its molecular recognition and high specificity toward an antigen or a group of structurally related antigens (*7*). The antibodies immobilized on the solid support specifically retain an antigen from a solution passed through the support. The bound antigen is then eluted with a small amount of organic solvent, and the support is regenerated for reuse.

Immunoaffinity columns are useful for extraction of endocrine disruptors and pesticide residues (*8*, *9*). Most of the reported immunoaffinity columns use polyclonal and monoclonal antibodies. Both of these types of antibodies can be difficult to reproduce in large quantities and can interfere with the antigen-binding reaction because of their large molecular size. By comparison, recombinant antibodies can be mass produced by the culture of recombinant bacteria, and recombinant antibodies are smaller than intact antibodies. The successful cloning and

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production of a series of recombinant antibodies including antigen-binding fragments (Fab), single-chain antibodies, and single-chain variable fragments (scFv) in recombinant *E. coli* revealed that they retain specificity and sensitivity for the target antigen (*10*−*12*).

In this study, we developed an immunoaffinity column chromatography method using a scFv antibody specific to BPA for the cleanup of water samples. The scFv antibody derived from the monoclonal antibody BBA-2187, which was the most sensitive of four monoclonal antibodies reported previously (*13*), was immobilized on silica gel. The performance of the immunoaffinity column for purification and concentration of BPA from environmental water samples was compared to the performance of SPE by using high-performance liquid chromatography (HPLC) and enzyme-linked immunosorbent assay (ELISA) techniques.

MATERIALS AND METHODS

Chemicals and Reagents for Column Chromatography. BPA (4,4′-isopropylidenediphenol) was purchased from Kanto Chemical Co. (Tokyo, Japan). The BPA hapten (4-{4-[1-(4-hydroxyphenyl)-1-methylethyl]phenoxy}butyric acid) was provided by Otsuka Chemical Co. (Osaka, Japan). TALON resin and a disposable column for immobilized metal affinity chromatography (IMAC) were purchased from Takara Bio Inc. (Shiga, Japan) and Qiagen GmbH (Hilden, Germany), respectively. A HiTrap Q column for anion-exchange chromatography was purchased from GE Healthcare Bio-Science Corp. (Piscataway, NJ). Wakogel C-300 and glass syringe barrels with Teflon frits for immunoaffinity column chromatography were purchased from Wako Pure Chemical Industries (Osaka, Japan) and Sigma-Aldrich Co. (St. Louis, MO), respectively. An Oasis HLB cartridge column was purchased from Waters Corp. (Milford, MA) for SPE of BPA. A Cosmosil $5C_{18}$-AR-II column (4.5 μm, 150 mm × 4.6 mm i.d.) was purchased from Nacalai Tesque (Kyoto, Japan). Glassware was used for all experiments with BPA.

Large-Scale Production of BBA-2187 scFv Antibody by the Culture of the Recombinant *E. coli* Strain Rosetta (DE3). A colony of the recombinant *E. coli* Rosetta (DE3) (EMD Biosciences, San Diego, CA) harboring the expression plasmid for the BBA-2187 scFv antibody (*13*) was inoculated into 10 mL of SB medium [3.2% (w/v) tryptone, 2% (w/v) yeast extract, and 0.5% (w/v) sodium chloride] containing 25 mg L^{-1} kanamycin and 34 mg L^{-1} chloramphenicol in a test tube. The preculture was incubated for 8 h at 25 °C with shaking at 200 rpm and then transferred to 5 L of SB medium containing both antibiotics at the same concentrations in a water-jacketed fermentor (MBF-1000M, Tokyo Rikakikai Co., Tokyo, Japan). The culture was grown under the following conditions: temperature, 25 °C; stirring speed, 700 rpm; and air supplementation, 50 mL min^{-1}. After 18 h, induction with isopropyl-β-D-thiogalactopyranoside (final concentration, 1 mM) was performed for 4 h.

Purification of BBA-2187 scFv Antibody. The *E. coli* cells in the culture were harvested by centrifugation at 6000*g* for 10 min at 4 °C. The pellets were resuspended in 300 mL of TES buffer (30 mM Tris-HCl, 0.5 M sucrose, and 1 mM ethylenediaminetetraacetic acid, pH 8.0) for a 5 L culture. The cell suspensions were centrifuged at 10000*g* for 15 min at 4 °C. The pellets were resuspended in 300 mL of 5 mM magnesium sulfate for a 5 L culture with stirring. The spheroplasts were centrifuged at 15000*g* for 30 min at 4 °C. The supernatant, which was the osmotically shocked fluid containing periplasmic proteins, was mixed with ammonium sulfate at a final concentration of 60% saturation. The mixture was incubated for 1 h at 4 °C and then centrifuged at 30000*g* for 30 min at 4 °C. The pellets were suspended in 30 mL of extraction/wash buffer (50 mM sodium phosphate, 300 mM sodium chloride, and 10% glycerol, pH 7.0).

BBA-2187 scFv antibody was further purified by the use of IMAC with TALON resin in a disposable column. The affinity media were prepared according to the manufacturer's instructions. The fractions containing scFv antibody were added to 5 mL of the pre-equilibrated resin dissolved in 40 mL of extraction/wash buffer in a 50 mL tube. The resin was gently agitated at a room temperature for 20 min to allow the polyhistidine-tagged scFv antibody to bind to the resin and was then centrifuged at 1000*g* for 5 min. The supernatant was carefully removed. The resin was transferred to a 10 mL disposable column. The column with the resin was washed with 25 mL of extraction/wash buffer, and the trapped protein was eluted with 10 mL of elution buffer (50 mM sodium phosphate, 300 mM sodium chloride, 10% glycerol, and 150 mM imidazole, pH 7.0). The eluate was dialyzed overnight in buffer A (20 mM Tris-HCl, 75 mM sodium chloride, and 10% glycerol, pH 8.0) with a Visking membrane (MWCO14000, Sogo Laboratory Glass Works Co., Kyoto, Japan).

Anion-exchange chromatography was performed on a HiTrap Q column (1 mL) in an ÄKTAprime system (GE Healthcare Bio-Science Corp.). The column was equilibrated with buffer A, and the solution purified by IMAC and dialysis was loaded onto the column at a flow rate of 1.0 mL min^{-1}. After the column was washed with 2 mL of buffer A, the protein fractions containing scFv antibody were eluted with a linear gradient from buffer A to buffer B (20 mM Tris-HCl, 500 mM sodium chloride, and 10% glycerol, pH 8.0). The fractions of periplasm, ammonium sulfate precipitates, and eluates from IMAC and anion-exchange chromatography were subjected to sodium dodecyl sulfate−polyacrylamide gel electrophoresis (SDS-PAGE) (12%) to check the purity of the scFv antibody, and the binding activity of the purified scFv antibody toward BPA was measured by indirect competitive (ic)-ELISA.

Preparation of an Immunoaffinity Column with the scFv Antibody. Wakogel C-300 was modified with aminopropyl groups using 3-aminopropyltriethoxysilane in an aqueous solution, as described in a previous study (*14*). The modified silica gel (0.5 g) was activated with 10 mL of 2.5% glutaraldehyde in 100 mM sodium phosphate (pH 7.2). The mixture was agitated for 1 h at room temperature under reduced pressure. The activated silica gel was washed with 5 mL of distilled water. BBA-2187 scFv antibody solution (5 mL), prepared by ultrafiltration with Amicon Ultra 10K (Millipore Corp., Billerica, MA) and dissolution in phosphate-buffered saline (PBS, 10 mM sodium phosphate and 150 mM sodium chloride, pH 7.2), was added to the activated silica gel. The suspension was agitated for 1 h at room temperature. The coupling efficiency was estimated by means of the Bradford protein assay of the initial solution and the eluate. Uncoupled active groups were blocked by incubation in 5 mL of buffer C (1 M monoethanolamine and 0.5 M sodium chloride, pH 8.0) with gentle agitation at room temperature for 1 h. After buffer C was discarded, three 5 mL portions of buffer D (0.1 M acetate buffer and 0.5 M sodium chloride, pH 4.0) and three 5 mL portions of buffer C were alternately injected into the silica gel. Finally, the silica gel was washed with 12 mL of PBS. Immunoaffinity columns with immobilized BBA-2187 scFv antibody were prepared by packing all of the prepared silica gel into 6 mL glass syringe barrels with Teflon frits. The columns were stored at 4 °C in the presence of 0.02% thimerosal until use.

Immunoaffinity Column Chromatography. All buffers and sample solutions were degassed before being loaded onto the columns. The immunoaffinity columns were conditioned with 5 mL of PBS, 5 mL of distilled water, 5 mL of 80% methanol, and 5 mL of distilled water to remove the matrix from the columns. The conditioned columns were loaded with distilled water or river water samples (50−200 mL) at a flow rate of about 5 mL min^{-1}, which had been filtered through a glass fiber filter (Whatman International Ltd., Maidstone, United Kingdom) and spiked with varied concentrations of BPA. After the columns were washed with 3 mL of 5% methanol, BPA was eluted with 3 mL of 80% methanol. The eluates were evaporated to dryness under reduced pressure at 35 °C, and the residue was dissolved in methanol (0.5 or 1 mL of methanol for HPLC; 2 or 4 mL of 10% methanol for ELISA). After use, the immunoaffinity columns were washed with 5 mL of distilled water followed by 5 mL of PBS and then stored as described previously until use.

SPE. Sample solutions were applied to Oasis HLB cartridge columns according to the manufacturer's instruction. Prior to use, the cartridges were conditioned with 5 mL of 10% methanol in methyl *tert*-butyl ether, 3 mL of 100% methanol, and 3 mL of distilled water. After the load of samples (adjusted to pH 3.0), the cartridges were washed with 3

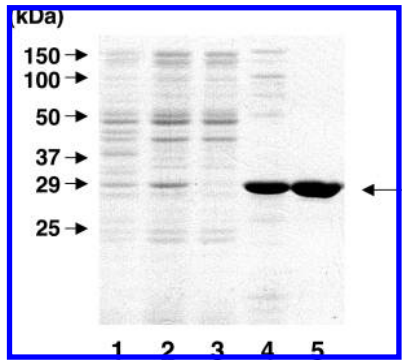

Figure 1. SDS-PAGE analysis of BBA-2187 scFv antibody after each of the purification steps. Lane 1, periplasmic fraction extracted from the recombinant *E. coli* cells expressing the BBA-2187 scFv gene; lane 2, fraction after an ammonium sulfate precipitation; lane 3, flow-through fraction from IMAC; lane 4, eluate from IMAC; and lane 5, eluate from anion-exchange chromatography. The gel was stained with Coomassie brilliant blue. The arrow indicates BBA-2187 scFv antibody.

mL of 5% methanol and then eluted with 6 mL of 10% methanol in methyl *tert*-butyl ether. The eluates were evaporated to dryness under reduced pressure at 35 °C.

Analysis of BPA by HPLC with Fluorescence Detection (HPLC-FLD). BPA in each of the samples was analyzed by HPLC-FLD (LaChrom series with the fluorescence detector L-7485, Hitachi High-Technologies Corp., Tokyo, Japan). A Cosmosil $5C_{18}$-AR-II column was employed for separation, and the mobile phase was composed of acetonitrile, water, and acetic acid (40:60:0.1, v/v/v) at a flow rate of 1 mL min^{-1}. The sample injection volume was 20 μL, and the BPA in the samples was detected by a fluorescence detector at 275 nm for excitation and at 300 nm for emission.

ic-ELISA. Microtiter plates (Nalge Nunc International, Rochester, NY) were each coated with 100 μL of BPA hapten-RSA conjugate (0.1 μg mL^{-1}) in PBS overnight at 4 °C and were then blocked with 250 μL of 20% Blocking One (Nacalai Tesque Co.) in PBS for 2 h at room temperature. After the plates were washed three times with PBS, BPA standard solution (50 μL $well^{-1}$) was added to each well; then, 50 μL of BBA-2187 scFv antibody (50 ng mL^{-1}) diluted in 4-fold concentrated PBS containing 10% Blocking One was added. The plates were incubated for 1 h at 25 °C. After the plates were washed three times with PBS, 100 μL $well^{-1}$ of the anti-HSV antibody (0.1 μg mL^{-1}, EMD Biosciences, Inc.) was added, and the plates were incubated for 30 min at 37 °C. After the plates were washed three times with PBS, 100 μL $well^{-1}$ of HRP-labeled goat antimouse IgG (diluted 2000-fold; Pierce Co., Rockford, IL) was added, and the mixture was incubated for 30 min at 37 °C. After the plates were washed three times with PBS, 100 μL $well^{-1}$ of the substrate solution (2 mg mL^{-1} of *o*-phenylenediamine and 0.03% hydrogen peroxide) in 100 mM citrate-phosphate buffer (32 mM citrate monohydrate and 68 mM disodium hydrogenphosphate 12−water, pH 5.0) was added, and the plates were incubated for 10 min at room temperature. The enzyme reaction was stopped with 50 μL of 4 N sulfuric acid, and the absorbance was measured at 492−630 nm in a microtiterplate reader (MTP-500, Corona Electric Co., Ibaraki, Japan). The eluates from the immunoaffinity columns were also analyzed with a BPA test kit (Horiba Biotechnology Co., Kyoto, Japan) with BBA-2187 scFv antibody, according to the manufacturer's instructions.

RESULTS

Preparation and Characterization of the BBA-2187 scFv Antibody. The recombinant *E. coli* carrying the gene encoding the BBA-2187 scFv antibody was cultured and then used for the preparation of a periplasmic fraction; the periplasmic fraction was subjected to ammonium sulfate precipitation, IMAC, and anion-exchange chromatography. The samples obtained by these purification steps were analyzed by SDS-PAGE (**Figure 1**).

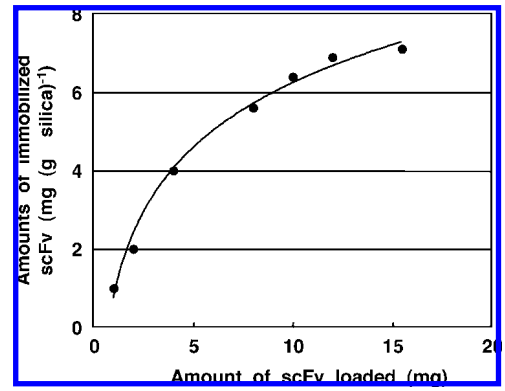

Figure 2. Immobilization of BBA-2187 scFv antibody on Wakogel C-300.

Purification of the scFv antibody (29 kDa) by IMAC was more efficient than ammonium sulfate precipitation, but some protein impurities remained in the fraction obtained after IMAC. Anion-exchange chromatography produced a single band for the BBA-2187 scFv antibody protein. Approximately 15 mg of BBA-2187 scFv antibody protein was obtained from a 5 L culture of the recombinant *E. coli* strain by these purification steps. The IC_{50} value of BPA toward the BBA-2187 scFv antibody is reported to be 0.32 ng mL^{-1} in ELISA, and this antibody shows weak cross-reactivity toward bisphenol E, bisphenol F, diethylstilbestrol, 4-octylphenol, and 4-(*tert*-octyl)phenol (*13*).

Immunoaffinity Column Chromatography of River Water Samples Contaminated with BPA. Immunoaffinity columns prepared with glass beads and with sepharose efficiently concentrated and purified BPA (data not shown) in river water samples, and the two types of columns showed nearly identical performance (data not shown).

For the immunoaffinity columns prepared with silica gel, the amount of scFv antibody bound to the silica gel increased dose dependently with the initial scFv antibody load (**Figure 2**). The amount of bound antibody was highest (7 mg [g silica]$^{-1}$) at a scFv load of 15 mg. The coupling efficiency was about 100% up to a scFv load of 4 mg scFv (g silica)$^{-1}$. Therefore, a scFv load of 4 mg scFv (g silica)$^{-1}$ was used for further studies. The other conditions for adsorption and elution of BPA and for the washing of the column were determined by comparison of the data obtained under various conditions (data not shown).

In the eluate obtained from immunoaffinity chromatography of a water sample from the Yodogawa River, BPA was detected at 5.9 min by HPLC-FLD, and the background signal was markedly lower than that observed for the eluate obtained from the Oasis HLB cartridge column (**Figure 3A**). The recovery rates of BPA from the Yodogawa sample were high for both columns, although the results obtained with the immunoaffinity and Oasis HLB cartridge columns slightly overestimated and underestimated the spiked concentrations of BPA, probably due to impurities in a silica gel of the immunoaffinity column (**Figure 3B**).

The immunoaffinity column retained BPA amounts ranging from 1.98 to 17.9 ng in water samples. The recovery of BPA was 89.0−109% (**Table 1**). Moreover, the different matrices of water samples from three different rivers did not affect the recovery rates of BPA from the column. Immunoaffinity chromatography indicated that the Yodogawa, Kamogawa, and Tamagawa rivers originally contained 26.4, 29.2, and 257 pg mL^{-1} of BPA, respectively. The Oasis HLB cartridge column also showed good recovery rates, both for distilled water and for the Yodogawa sample. The blank levels of both columns

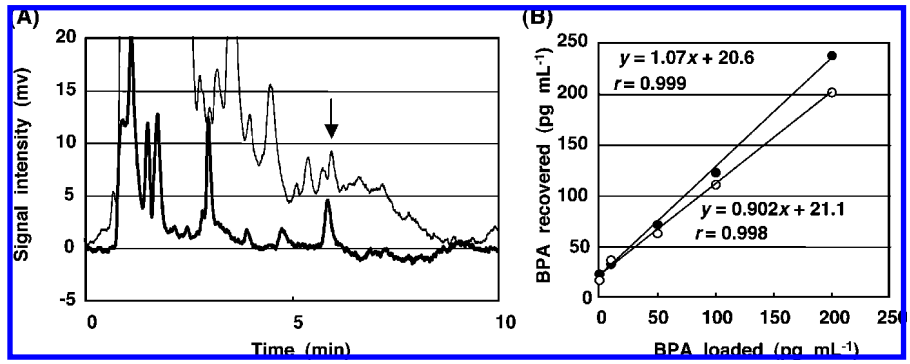

Figure 3. Purification of BPA from a sample of Yodogawa River water with the immunoaffinity and Oasis HLB cartridge columns. (**A**) HPLC chromatograms obtained with the immunoaffinity column (bold line) and the Oasis HLB cartridge column (thin line). Four hundred-fold concentrated samples of Yodogawa River water were applied. The arrow indicates BPA. (**B**) Correlation between recovered BPA and loaded BPA concentrations obtained with the immunoaffinity (closed circles) and Oasis HLB cartridge (open circles) columns.

Table 1. Recovery of BPA from River Water Samples by Immunoaffinity and Oasis HLB Cartridge Chromatographies

		loaded BPA		detected BPA			
column	water sample	spiked concn (pg mL^{-1})	loaded volume (mL)	detected concn (pg mL^{-1})[a]	recovery rate (%)	CV[b]	concn rate (fold)
immunoaffinity column	distilled water	0	200	0			1000
		10	200	9.89	98.9	4.49	1000
		100	100	95.1	95.1	5.23	100
	Yodogawa River	0	200	26.4		11.1	400
		10	200	35.3	89.0	6.01	400
		100	50	135	109	7.08	100
	Kamogawa River	0	100	29.2		6.58	200
		100	50	131	102	4.95	100
	Tamagawa River	0	50	257		4.20	100
		100	50	358	101	1.34	100
Oasis HLB column	distilled water	0	100	0			100
		100	100	113	113		100
	Yodogawa River	0	200	17.4			400
		100	50	112	94.6		100

[a] Detected by HPLC-FLD. [b] Coefficient of variation (n = 3 or 4).

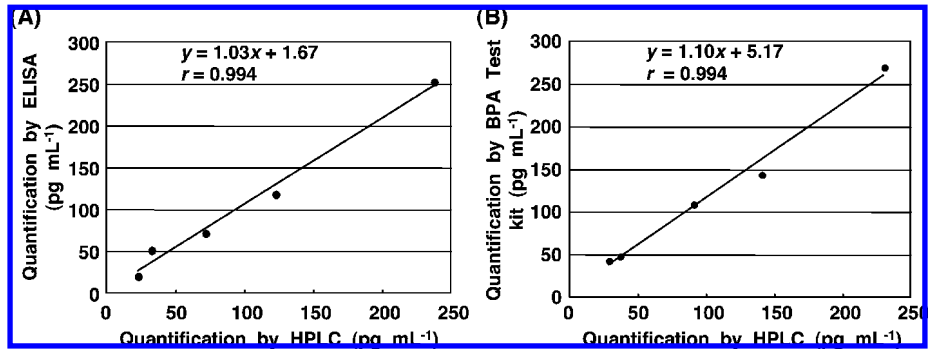

Figure 4. Correlation between (**A**) ELISA results and HPLC results and (**B**) BPA test kit results and HPLC results for samples of Yodogawa River water after immunoaffinity chromatography.

were 0. However, a 400-fold concentration of the Yodogawa samples caused a high background with the Oasis HLB cartridge column but not with the immunoaffinity column (**Figure 3A**).

We analyzed the eluates from the immunoaffinity column by means of ELISA using BBA-2187 monoclonal antibody and by means of a BPA test kit with BBA-2187 scFv antibody to investigate the influences of the column eluates on the ELISA results (**Figure 4**). The results for ELISA (r = 0.994) and the BPA test kit were highly correlated (r = 0.994) with the HPLC results. The immunoaffinity column was used more than 10 times without a decrease in BPA binding capacity under the conditions described in the Materials and Methods (data not shown).

DISCUSSION

Large-Scale Production of BBA-2187 scFv Antibody in Recombinant *E. coli*. For large-scale production of BBA-2187 scFv antibody, simple antibody purification steps are important for successful preparation of the immunoaffinity column. We used the pET-27b(+) vector and Rosetta (DE3) *E. coli* to produce large amounts of the scFv antibody in recombinant *E. coli*. The pET-27b(+) vector has a pelB signal sequence, which facilitates secretion of target proteins into the periplasmic space. Secretion into the periplasm has been used as a strategy for solubilization of proteins produced, because the bacterial periplasm is a compartment that allows efficient formation of

disulfide bonds, because of the oxidizing environment (*15*, *16*). Because scFv antibodies contain two disulfide bonds that are required for stable folding, we expected that periplasmic expression would be suitable for large-scale production of the scFv antibodies.

The expression plasmid was introduced into *E. coli* Rosetta (DE3), which was designed to enhance the expression of eukaryotic genes that contain codons rarely used in *E. coli*. This strain supplies tRNA for AGG, AGA, AUA, CUA, CCC, and GGA. Thus, Rosetta (DE3) provides an universal translation system that is not limited by the codon usage of *E. coli*.

Using these two approaches, we obtained 15 mg of purified BBA-2187 scFv antibody from 5 L of medium. The yield was markedly higher than the previously obtained yield of 10 mg of BBA-2187 scFv antibody employing pET-27b(+) and the BL21 (DE3) strain (data not shown). These results suggest that the scFv antibody gene codons are inappropriate for expression in recombinant *E. coli* using BL21 (DE3) and are consistent with the findings of Miller et al. (*17*). Moreover, the BBA-2187 scFv antibody is reported to be more sensitive than the BBA-2187 monoclonal antibody and have cross-reactivity similar to the original antibody (*13*).

Support Materials for High-Performance Immunoaffinity Column with BBA-2187 scFv Antibody. We chose silica gel as a support material, as it is rigid and allows chromatography to be performed at higher flow rates and higher pressures than other support materials. IgG antibody is commonly immobilized on silica gel with a large pore size (>300 Å diameter), such as Nucleosil (*18*−*20*). However, a large pore size and uniform particles are not required due to the small molecular size of scFv antibodies as compared to IgG. Therefore, we selected Wakogel C-300, which has small pores (70 Å) and is widely used for separation. Prevention of random orientation of the attached antibodies and steric hindrance of the each attached antibody, which reduces the number of active sites of the antibodies, should be considered for selection of an appropriate support material to achieve a high ability of the immunoaffinity columns (*21*).

Although approximately 4 mg of the scFv antibody was almost completely immobilized on 1 g of the modified silica gel in the present study, Berry and Pierce immobilized 3.2 mg of the Fv fragment on 1 g of the tresyl-activated silica gel Sorbsil C200, which has large pores (200 Å), with an 85% coupling efficiency (*22*). The surface areas of Wakogel C-300 and Sorbsil C200 are 450 and 299 $m^2\ g^{-1}$, respectively. This difference in surface area may have caused the difference between the binding capacities of the antibodies on the modified silica gels. Increased binding capacity may provide a high density of immobilized antibodies, resulting in high-performance concentration and purification. Therefore, it was suggested that high stability and rigid immobilization of scFv antibodies on the silica gel were important for high-performance concentration and purification of BPA.

Immunoaffinity Column Chromatography for Monitoring of BPA in River Water Samples. Three different water samples contaminated with ppt levels of BPA were tested on the immunoaffinity column. The results obtained are consistent with the results generated by environmental monitoring performed by the Ministry of the Environment of Japan. The efficiency of the immunoaffinity column for the detection of ppt levels of BPA was not affected by the sample matrices. The BPA concentrations estimated by ELISA and HPLC after use of the immunoaffinity column showed a high correlation, although the values obtained by means of ELISA slightly overestimated the spiked values. We believe that impurities in the methanol and the silica gels interfered with the results of ic-ELISA. In fact, the overestimation was reduced by washing of the silica gel with methanol (data not shown).

These results clearly show that the immunoaffinity column with immobilized BBA-2187 scFv antibody has the capacity for high-performance concentration and purification of BPA in various river water samples. Also, the combination of this column, ELISA, and HPLC was efficient for monitoring the ppt levels of BPA. Furthermore, silica gel-based immunoaffinity columns are markedly more efficient than columns prepared with glass beads or sepharose and have the advantage of being less expensive and disposable. However, problems remain with the large-scale production of the scFv antibody in recombinant *E. coli*, since the activity of the scFv antibody is often lower than the activity of the original monoclonal antibody due to protein aggregation, different codon usage, and instability resulting from truncation of the constant regions of IgG. Also, in this study, cross-reactivity of the BBA-2187 scFv antibody toward bisphenol E showed 6.5% as compared with BPA (*13*). Thus, cross-reactivity of the scFv antibody should be taken into account for specific purification.

SAFETY

This study was approved by the Committee for Safe Handling of Living Modified Organisms at Kobe University (Permission #14-10) and carried out according to the guidelines of the committee.

ABBREVIATIONS USED

BPA, bisphenol A; ELISA, enzyme-linked immunosorbent assay; FLD, fluorescence detection; HPLC, high-performance liquid chromatography; IMAC, immobilized metal affinity chromatography; ic, indirect competitive; scFv, single-chain variable fragment; SPE, solid-phase extraction.

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