Use of an immunoaffinity column for tetrachlorodibenzo-*p*-dioxin serum sample cleanup

Weilin L. Shelver*, Gerald L. Larsen, Janice K. Huwe

U.S. Department of Agriculture, Agriculture Research Service, Biosciences Research Laboratory, P.O. Box 5674, State University Station, Fargo, ND 58105-5674, USA

Abstract

Covalently linking 1-amino-3,7,8-trichlorodibenzo-*p*-dioxin with either keyhole limpet hemocyanin (KLH) or bovine serum albumin (BSA) provided antigens that generated antibodies in chickens. Competitive ELISA analysis demonstrated that the antibodies isolated from egg yolk (IgY) bound with 1,3,7,8-tetrachlorodibenzo-*p*-dioxin (1,3,7,8-TCDD). The antibodies were linked to CNBr-Sepharose to generate an immunoaffinity column. Radiolabeled 1,3,7,8-TCDD in a 0.05% Tween 20 solution was retained by the column and could be eluted by increasing the Tween 20 concentration. The binding efficiency for 10.7 ng per ml gel matrix ranged from 85 to 97%. Immunoaffinity columns generated by this method did not effectively bind ^{14}C-1,3,7,8-TCDD from serum samples. Diluting the serum 1:20 with 0.05% Tween 20 increased the binding efficiency. Alternately, ethanol–hexane extraction followed by solid phase extraction on a carbon column using a fat removal protocol also provided an appropriate preaffinity column cleanup for serum samples. After this preaffinity column cleanup, spiked serum samples applied to the immunoaffinity column showed binding efficiencies of over 90%. © 1998 Published by Elsevier Science B.V.

Keywords: Cleanup methods; Immunoaffinity column; Tetrachlorodibenzo-*p*-dioxin; Dioxins

1. Introduction

Polychlorinated dibenzo-*p*-dioxins (PCDDs) (Fig. 1) are toxic materials with one member, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), being one of the most toxic chemicals known to man [1]. Dioxins are byproducts in the production of a number of commercial chemicals, such as, herbicides, wood preservatives and chlorinated phenols. They may also be produced from commercial processes such as bleaching of paper pulp and by various incomplete
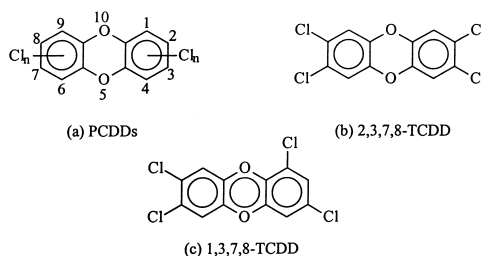

Fig. 1. (a) Structure and numbering system for PCDDs, $n=1$–4. (b) Structure of 2,3,7,8-TCDD. The most toxic congener of polychlorodibenzo-*p*-dioxins. (c) Structure of 1,3,7,8-TCDD. The congener used for this study.

---

*Corresponding author.

combustion processes such as incineration or forest fires. Because of these multiple origins and their slow degradation, dioxins are widely distributed in the environment [2].

Environmental dioxins accumulate in the food chain [3] as a result of their high lipophilicity and resistance to biochemical degradation. Currently acceptable daily doses of TCDD-equivalents range from 0.006 pg/kg body weight per day by the U.S. Environmental Protection Agency to 10 pg/kg body weight per day by the World Health Organization [4]. Major dioxin sources for human exposure are fish, meat and dairy products which are estimated to contribute 1–3 pg/kg body weight per day [5]. As a consequence most of the population in the United States, as well as other industrial nations, have detectable serum levels of 10 to 20 pg/g toxic equivalents of PCDDs [6].

The low concentration of multiple congeners of dioxins and the complex environmental matrices make the analysis of dioxins extremely time consuming and complex. The analyses include multiple cleanup steps combined with instrumentation capable of resolving the multiple congeners with high sensitivity such as high resolution gas chromatography–mass spectrometry (HRGC–MS) [7,8]. Although impressive detection limits have been obtained (low ppt to ppq levels), the long process of sample cleanup limits the number of samples which can be analyzed in a given time [9]. Evaluation of environmental issues frequently requires large numbers of analyses which may not be economically feasible using present methodology.

Previous attempts to decrease the cost of dioxin analysis included development of a radioimmunoassay (RIA) using a polyclonal antibody [10] and an enzyme linked immunosorbent assay (ELISA) using murine monoclonal antibodies [11,12]. These assays require fewer steps for sample cleanup and complement the HRGC–MS analysis. The ELISA or RIA techniques allow a higher throughput of sample analyses and can be used for preliminary screening for dioxins; however, the detection limits of these assays vary depending on the matrix involved [13]. The sensitivity of either the ELISA or RIA generally is two to three orders of magnitude less sensitive than HRGC–MS.

Utilizing the property of specific antigen recogni-

tion, immunoaffinity columns have proven useful in purifying small molecules from biological matrices; some specific examples are mycotoxins (aflatoxins [14], ochratoxin A [15]), corticosteroids [16] and beta-sympathomimetics [17]. This method should be particularly useful for complex biological matrices such as blood, milk, or urine. The advantages of immunoaffinity column purification for dioxins include the ability to selectively retain dioxin congeners, to decrease the amount of organic solvents needed for the cleanup (greater environmental friendliness), and to shorten sample cleanup time. After cleanup the sensitivity and specificity of HRGC–MS analysis would allow quantitation of the individual congeners.

In the present study, the feasibility of using an immunoaffinity column for cleanup of spiked ^{14}C-1,3,7,8-TCDD or ^{14}C-2,3,7,8-TCDD samples is described. The effects of various solvent systems, the steps necessary for increasing serum sample binding efficiency, and the cross-reactivity between dioxin congeners are examined.

2. Experimental

2.1. Materials

[14]C-1,3,7,8-TCDD (63.4 mCi/mmol) and ^{14}C-2,3,7,8-TCDD (63.5 mCi/mmol) were obtained from ChemSyn Science Laboratories (Lenexa, KS, USA). All other chemicals were obtained from Sigma–Aldrich (St. Louis, MO, USA) unless otherwise stated. Bovine serum was obtained from Sigma (St. Louis, MO, USA). Solvents of analytical grade or better were obtained from commercial vendors.

2.2. Synthesis of hapten and antigens

All dioxin compounds were considered toxic and were handled in a manner appropriate for chlorinated dibenzo-p-dioxins based on material safety data sheet information. The 1-amino-3,7,8-trichlorodibenzo-p-dioxin was synthesized by the method of Chae et al. [18] from 2,5-dichloro-1,3-dinitrobenzene and 4,5-dichlorocatechol monohydrate. An adipic acid moiety was chosen as the spacer group for linkage of the amino-substituted dioxin to protein. The acid

chloride of monomethyl adipate was prepared by a modification of the method of Taylor [19]. Monomethyl adipate was refluxed 4 h in thionyl chloride. Fractional distillation removed excess thionyl chloride and gave the product as a clear liquid (b.p. 105°C). Monomethyl adipoyl chloride and 1-amino-3,7,8-trichlorodibenzo-*p*-dioxin were reacted in dry pyridine at 0°C, and the purified adipamide methyl ester was hydrolyzed to the free acid under basic conditions as described by Albro et al [10]. All product structures were confirmed by NMR and/or GC–MS analysis.

Antigens were produced by linking the hapten with either bovine serum albumin (BSA) or keyhole limpet hemocyanin (KLH) in the presence of iso-butylchloroformate and tributylamine in 30% dioxane–0.2 *M* sodium borate (pH 8.7) [10]. Hapten to carrier protein binding ratios were determined by the method of Erlanger et al [20].

2.3. Antibody generation, purification and characterization

Chickens were housed individually in wire cages with food and water provided ad libitum. Four white Leghorn hens, age 6 months at the first immunization, were used for antibody production. Two of the chickens were immunized with antigen from BSA and two with antigen from KLH. Each chicken was injected intramuscularly in 2 sites at monthly intervals. Antigen (25 μg) was dissolved in 350 μl normal saline and mixed in a 1:1 ratio with complete Freund adjuvant for the first immunization. Subsequent monthly boosts employed incomplete Freund adjuvant instead of complete Freund adjuvant. Chicken eggs were collected each day and stored at 4°C until used. The chickens were euthanized at 12 months.

Antibodies were isolated from the egg yolk following the method of Keyler et al [21]. Egg yolks were washed with 10 m*M* phosphate buffer (pH 7.4), then mixed in a 1:1:1 ratio of yolk, buffer and 10.5% polyethylene glycol 8000 and the precipitate removed. The supernatant from this procedure was precipitated by adding half the yolk volume of 42% polyethylene glycol 8000. The precipitate was re-suspended with 25% ethanol, stored at −20°C for 1 h and centrifuged to remove lipids and polyethylene

glycol. Finally, the ethanol was removed by dialysis with 10 m*M* phosphate buffer (pH 7.4), and the resulting solution was stored at −20°C until used. The protein concentration was measured with a BIO-RAD protein assay kit (Bio-Rad Laboratories, Hercules, CA, USA) by the method of Bradford [22] using gamma globulin as the calibration standard. The purity of the antibody was measured by 10% SDS-PAGE under nonreducing conditions [23].

Antibody production was monitored with ELISA. Antibodies raised against the 1,3,7,8-TCDD-KLH conjugate were tested by coating the ELISA plate with 100 ng of the 1,3,7,8-TCDD-BSA conjugate. The plates were blocked with 1% fish gelatin before applying a series of serial dilution of antibody (from 800 to 1.6 ng/well). Goat antichicken IgG horse radish peroxidase 1:30 000 was used as the secondary antibody. The color developing reagent was *o*-phenylenediamine (40 μg/well). Color development was stopped at 30 min by adding 50 μl of 3 *M* HCl to the wells, and the absorbance was read at 490 nm. The specificity of the antibody was checked with a competitive inhibition ELISA. The procedure was the same as described for the ELISA except that 500 ng/well of primary antibody was added to various amounts of 1,3,7,8-TCDD as the competitor (0.8 to 50 ng/well). A murine monoclonal antibody specific for 2,3,7,8-TCDD was used as the positive control while polyclonal antibody specific for 2,2′,4,4′,5,5′-hexachlorophenyl was used as the negative control.

2.4. Generation of immunoaffinity column

The manufacturer's method for coupling a ligand to CNBr-activated Sepharose 4B (Pharmacia Biotech, Uppsala, Sweden) was followed. Briefly, 90 mg of antibody and 10 ml of activated CNBr-Sepharose 4B in 0.1 *M* NaHCO$_3$ (pH 8.3), 0.5 *M* NaCl were reacted at 4°C. The excess protein (BSA or antibody) was removed by washing with a large volume of coupling buffer. Excess active groups were blocked by 0.1 *M* Tris (pH 8), and washed alternately with 0.1 *M* Tris (pH 8), 0.5 *M* NaCl and 0.1 *M* acetate buffer (pH 4), 0.5 *M* NaCl. Protein content was measured for each wash-fraction to determine binding efficiency. The antibody generated from 1,3,7,8-TCDD-KLH was used for immuno-affinity column generation and for testing in this

study. The gel beads were packed in an open-end glass column. For the 1,3,7,8-TCDD studies, 10 ml columns (10.5×120 mm) were used. For the 2,3,7,8-TCDD study, 2 ml columns (7×50 mm) were used.

2.5. Application and elution of samples on immunoaffinity column

^{14}C-1,3,7,8-TCDD was used as a model compound for the development of the column and sample cleanup procedure in order to minimize handling of the extremely toxic 2,3,7,8-TCCD. ^{14}C-1,3,7,8-TCDD (650 or 100 ng) in acetone (1 μl) was dissolved in 10 ml of buffer [50 m*M* Tris (pH 7.5), 0.15 *M* sodium chloride, 0.05% Tween 20]. The sample was placed on the column, and the column was washed with 8–10 five ml fractions of buffer to remove any nonbound material. Bound sample was then eluted utilizing various concentrations of Tween 20 (0.2, 0.5, or 1%). The flow-rate was 0.3–0.5 ml/min, and all the column work was done at 4°C.

To determine if the components of the buffer affected the binding of the dioxin to the affinity column, ^{14}C-1,3,7,8-TCDD was applied to the immunoaffinity column in 10 ml of 0.05% Tween 20. The column was washed with 0.05% Tween 20, and dioxin was eluted by 0.5% Tween 20 as described in the previous paragraph.

Bovine serum (10 ml) spiked with ^{14}C-1,3,7,8-TCDD (100 ng) was applied directly or diluted 1:5 or 1:20 with 0.05% Tween before applying to the immunoaffinity column. Alternatively, spiked serum was extracted twice with ethanol–hexane (1:1, v/v) (1:2 ratio of serum to extractant). The organic layers were combined and applied to a 150 mg Carbograph column for solid phase extraction (Alltech Associates, Inc., Deerfield, IL, USA). The column was washed with hexane and dichloromethane before eluting the dioxin with toluene. The toluene was removed by evaporation. Ten ml of 0.05% Tween 20 were added, and the sample was sonicated for 30 min before applying to the immunoaffinity column. The immunoaffinity column was washed with 0.05% Tween 20 and eluted with 0.5% Tween 20 as described previously for the 1,3,7,8-TCDD controls.

The ability of the column to bind ^{14}C-2,3,7,8-TCDD was tested by applying 10 ng to a 2 ml column. The sample loading, washing and eluting procedures were the same as that used for 1,3,7,8-TCDD.

2.6. Postimmunoaffinity column cleanup and GC–MS analysis

The postimmunoaffinity column cleanup was similar to our previous report [24]. Briefly, the eluant (0.5% Tween 20) was extracted with ethanol–hexane (1:1, v/v). The hexane extract was passed through a glass column packed with 6 g acid silica [25] and 3 g of anhydrous sodium sulfate. The hexane was evaporated by a stream of nitrogen using 10 μl dodecane as a keeper solvent. The dodecane solution was analyzed by GC–MS: HP 5890 series II GC (Hewlett–Packard Co., San Fernando, CA, USA); VG Autospec mass spectrometer (Micromass, Inc., Beverly, MA, USA); 15 m DB-5MS column, 0.25 mm I.D., 0.25 μm film thickness (J&W Scientific, Folsom, CA, USA). The temperature program was: initial temperature 100°C for 2 min, increased to 310°C at 5°C/min, and maintained at 310°C for 4 min. The monitoring was performed using the ion at 322 to detect ^{14}C-1,3,7,8-TCDD.

3. Results

3.1. Antibody generation, purification and characterization

Antigens had 11 mol hapten/mol BSA (MW 66 kDa used for calculation), and 471 mol hapten/mol KLH (MW 3000 kDa used for calculation [26]). All of the immunized chickens had elevated antibody titers as determined by the ELISA after the first boost. Isolated IgY had a purity greater than 70% based on SDS-PAGE analysis. The average production of antibodies was about 40 mg/egg. Since we desired cross reactivity with a broad range of dioxin congeners, the antibody was not purified by a 1,3,7,8-TCDD-affinity column which would have enriched the antibody activity toward that particular congener.

In competitive ELISA analyses, 1,3,7,8-TCDD was bound to the primary antibody which made it unavailable to the coating antigen and decreased the

absorption when compared to blanks where the competition agent (1,3,7,8-TCDD) was absent. The competitive inhibition gave a concentration-related response toward the amount of 1,3,7,8-TCDD added to the well. Solubilization of the highly hydrophobic TCDDs for use in the analysis was difficult. Nonionic detergents improve the solubility of TCDD in an aqueous system [10,12]. In order to solubilize 1,3,7,8-TCDD for ELISA analyses, the chemical was sonicated 2 h in a buffer containing 50 mM Tris–HCl, 0.5% bovine serum albumin, and 0.1% Tween 20.

3.2. Generation of immunoaffinity column

The coupling efficiency for ligands to CNBr-Sepharose 4B was 86±22%. Out of five coupling experiments, four showed over 90% coupling efficiency; however, one particular batch had a coupling efficiency of <50%, which contributed to the large variation in binding efficiencies.

3.3. Immunoaffinity column for TCDDs cleanup

Preliminary experiments demonstrated that 1,3,7,8-TCDD bound to the immunoaffinity column and nonionic detergents such as Tween 20 sucessfully eluted the TCDDs. Solvent systems containing 30% acetone, 20% dioxane, or 5% acetonitrile were unable to elute the dioxin from the immunoaffinity column. Common strategies for affinity columns utilizing low pH (1% propionic acid) or high salt concentrations (3 M NaSCN) to elute off the target compound failed to elute the dioxin off the immunoaffinity column. A high concentration of acetone (95%) eluted the dioxin off the immunoaffinity column, however, produced large variability in recovery.

Fig. 2 shows the elution of ^{14}C-1,3,7,8-TCDD (in buffer) with different concentrations of Tween 20. Tween 20 concentrations of 0.5 or 1% provided good recoveries of ^{14}C-1,3,7,8-TCDD with practical elution volumes of 20 to 40 ml which were used in all subsequent studies. Elution with 0.2% Tween 20 solution was too slow to be practical.

Table 1 summarizes the analytical data from the immunoaffinity column methods development with 1,3,7,8-TCDD. Comparison of different loading
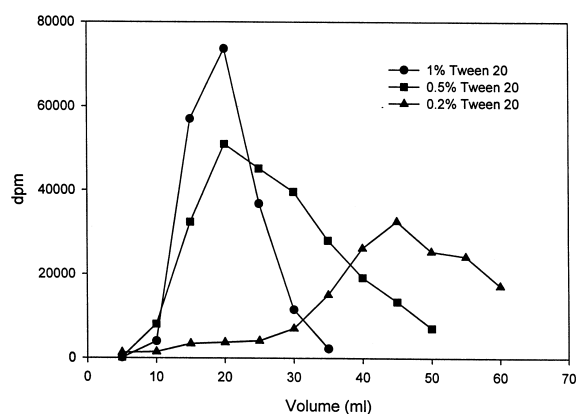

Fig. 2. Elution of ^{14}C-1,3,7,8-TCDD with different concentrations of Tween 20.

amounts, application matrices, and percentage of nonbound (in wash) versus bound sample (in eluent), as well as differences for negative control and specific immunoaffinity columns are presented. Sample loads of 10.7 ng/ml gel gave efficient column binding; however, loads of 64.5 ng/ml gel appeared to exceed the binding capacity and resulted in only 70% binding efficiency. The presence of buffer or saline in the application matrix did not affect column binding. A biological matrix such as bovine serum required extensive dilution or preaffinity column cleanup before binding efficiencies reached 90%.

To verify that binding to the column was due to specific antigen–antibody interactions, two negative control columns were constructed. The first contained BSA coupled to Sepharose and showed no binding of the ^{14}C-1,3,7,8-TCDD indicating 1,3,7,8-TCDD did not bind indiscriminately to the protein or the Sepharose support. The second column constructed of IgY from chickens prior to immunization further confirmed the specificity of our analytical column (Table 1).

The antibodies showed cross-reactivity with 2,3,7,8-TCDD. When 5 ng of ^{14}C-2,3,7,8-TCDD/ml gel was applied to the immunoaffinity column, 2,3,7,8-TCDD was retained and later eluted by 0.5% Tween 20. The recovery efficiencies were 51.8±6.5% (n=3). Subsequent elutions with higher concentrations (2 and 10%) of Tween 20 resulted in an additional 17% recovery. Analysis of the gel

Table 1
Analytical data demonstrating the application of the immunoaffinity column for purifying 1,3,7,8-TCDD

Load (ng/ml gel)	n	Application matrix	Wash (0.05% Tween 20)	Eluant (0.5% Tween 20)
a. Specific IgY–CNBr Sepharose column				
64.5	2	Tris/saline/0.05% Tween 20	26; 30	65.6; 72.1
10.7	2	Tris/saline/0.05% Tween 20	2; 2.2	86.9; 97.4
11.4±2	3	0.05% Tween 20	1.8; 0.8; 10.4	85; 87.8; 83.2
10.7	2	Serum	66.1; 51.9	19.4; 28.8
12.4	2	Serum 1:20	9.9; 15.2	75.8; 68.5
8.8±3	4	Serum after extraction and Carbograph cleanup	5.8±5.9	91.7±8.6
b. Negative control				
53.6[a]	3	Tris/saline/0.05% Tween 20	81; 82; 88.7	1.7; 0; 0
12.5[a]	3	0.05% Tween 20	93.7; 99.7; 90.5	2.7; 0.2; 6.6
64.5[b]	2	Tris/saline/0.05% Tween 20	95.4; 96	—[c]

[a] Nonspecific IgY–CNBr-Sepharose.
[b] BSA–CNBr-Sepharose.
[c] Not available.

beads by combustion showed only 4% of the ^{14}C adhering to the beads.

The immunoaffinity column was reasonably robust. The binding efficiency did not decrease even after repeated use. When stored at 4°C in 10 mM phosphate buffer pH 7.4 and 0.02% sodium azide, the column was stable for a minimum of 3 months.

3.4. Application of immunoaffinity column to serum analyses

Fig. 3 compares the elution profiles of a control sample of ^{14}C-1,3,7,8-TCDD (in buffer) and a sample of ^{14}C-1,3,7,8-TCDD in bovine serum. ^{14}C-1,3,7,8-TCDD (in buffer) bound to the column completely and was eluted with 0.5% Tween 20 (panel 1). No protein was detected in the eluate indicating that the antibody was not leaching from the column. When the spiked serum sample was applied to the immunoaffinity column, a significant amount of ^{14}C (>50%) was not bound and coeluted with a protein peak in the wash buffer (panel 2). An additional 25% was specifically bound and eluted with 0.5% Tween 20. Dilution of the serum 1:5 with loading buffer did not significantly increase the binding efficiency; however, when the serum was diluted 1:20 with loading buffer the binding efficiency improved to over 70% (Table 1). An alternative precolumn preparation for serum samples

involved partitioning with ethanol–hexane (1:1, v/v) followed by a Carbograph solid phase extraction to remove proteins and lipids prior to immunoaffinity
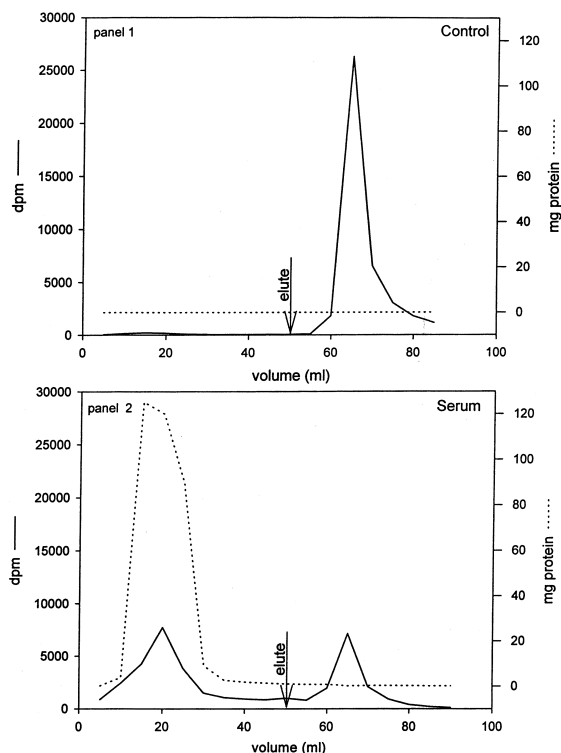

Fig. 3. Elution pattern of ^{14}C-1,3,7,8-TCDD (—) and proteins (- - -) for control (panel 1) and serum sample (panel 2).

chromatography. Both steps were found to be necessary to retain the immunoaffinity column binding efficiency. Recovery of ^{14}C from the two-step procedure was 83%. After organic extraction and Carbograph solid phase extraction the immunoaffinity column retained over 90% of the sample which subsequently could be eluted with a higher concentration of Tween 20 (0.5 or 1%) (Table 1).

3.5. Postimmunoaffinity column cleanup and GC–MS analysis

By using an ethanol–hexane extraction followed by passage through an acid silica column, the nonionic detergent was removed and samples were ready for GC–MS analysis. Total recovery from both steps was 70%.

4. Discussion

In the development phase of this project we prepared a hapten corresponding to 1,3,7,8-TCDD, a relatively nontoxic TCDD, to generate polyclonal antibodies. Although the antibodies were directed against the 1,3,7,8-TCDD, considerable cross-reactivity to the 2,3,7,8-TCDD was expected, because in coupling the hapten to the protein (BSA or KLH) the 3,7,8 positions are exposed, and these positions are identical in both congeners. Other groups have used this hapten to generate polyclonal antibodies from rabbit serum [10] and a murine monoclonal antibody [11] which have shown extensive cross-reactivity to 2,3,7,8-TCDD and other dioxin congeners. The chicken antibodies generated by our group also showed cross-reactivity between 1,3,7,8-TCDD and 2,3,7,8-TCDD and have proved to be a suitable source for generating an immunoaffinity column. Immunoaffinity columns of this type are expected to retain a mixture of congeners with closely related structures such as the dioxins and furans. The individual congener concentrations, necessary to evaluate total toxic equivalency, can then be determined by HRGC–MS.

Loading the sample onto the column with either buffer containing 0.05% Tween 20 or distilled water containing 0.05% Tween 20 gave similar results. By eliminating the buffer, the column cleaning and regeneration steps were simplified. A critical factor

in the sample loading step is controlling the solubility of the TCDD. If the TCDD is not soluble in the loading solvent, it precipitates on top of the column and is difficult to elute. In this study, 0.05% Tween 20 proved to be an effective solvent and gave good recoveries from the immunoaffinity column.

Owing to the physical properties of dioxins, the major interaction between dioxins and the antibodies would be in a region which contains a hydrophobic pocket instead of through polar interactions. Exploration of elution conditions provided confirmation for the hydrophobic nature of the dioxin–antibody interaction. Traditionally, analytes bind to affinity columns and can be eluted by increasing the salt concentration or decreasing pH. This was not the case for the dioxin affinity purification since dioxins have weak ionic interactions. Rather, the primary interaction may be π–π stacking or some other form of hydrophobic binding. High concentrations of organic solvent such as 95% acetone efficiently eluted the dioxin off the immunoaffinity column; however, the recovery was variable. Nonionic detergents were suitable for eluting the dioxins off the immunoaffinity column due to their hydrophobic nature.

The low recovery of ^{14}C-2,3,7,8-TCDD from the immunoaffinity column could be caused by several factors. The 2,3,7,8-TCDD could bind to the immunoaffinity column more strongly than 1,3,7,8-TCDD does, thus requiring higher concentrations of Tween 20 to elute it off the column. Another possibility is that 2,3,7,8-TCDD binds nonspecifically to the plastic and glass. Since the combustion of the gel beads showed only a 4% recovery of 2,3,7,8-TCDD, the 30% which was not accounted for in the washes and eluent could be binding to the glass column.

Since greater than 90% of the 1,3,7,8-TCDD was bound when the column was loaded with 10 ng samples using either buffered or aqueous solution, the low binding efficiency of spiked serum samples demonstrated a matrix effect which interfered with 1,3,7,8-TCDD binding to the antibody. Serum samples contain lipids and proteins, either of which could interfere with the binding efficiency of ^{14}C-1,3,7,8-TCDD. Bagnati et al. have reported that dilution of serum five- to twenty-fold with buffer increased binding efficiencies [27] to immunoaffinity columns. We found a minimal increase in the binding

efficiency when we diluted the serum sample fivefold (data not shown). However, when a extensive dilution (1:20) was made, the immunoaffinity column binding efficiency improved to about 70%. The increase in sample volume, however, increased loading time significantly. Alternatively, extracting the ^{14}C-1,3,7,8-TCDD with ethanol–hexane and using a solid phase extraction column to remove lipids gave excellent recoveries. The dioxin was subsequently retained and eluted from the immunoaffinity column with efficiencies comparable to applying the sample in aqueous solution.

5. Conclusion

This study uses antibodies which recognize dioxins as a purification tool to: (1) decrease the steps necessary for dioxin analysis; (2) shorten the length of time needed for analysis; (3) decrease the consumption of organic solvents used for sample cleanup; and (4) reduce the overall cost of dioxin analysis. The concept of using an immunoaffinity column to shorten the cleanup of samples contaminated with dioxin from aqueous systems has been clearly demonstrated to be viable. Work to improve and automate the process is underway.

References

[1] L.S. Birnbaum, Chemosphere 27 (1993) 469.
[2] S. Safe, Crit. Rev. Toxicol. 21 (1990) 51.
[3] G.F. Fries, D.J. Paustenbach, J. Toxicol. Environ. Health 29 (1990) 1.
[4] A. Schecter, J. Startin, C. Wright, M. Kelly, O. Rapke, A. Lis, M. Ball, J.R. Olson, Environ. Health Persp. 102 (1994) 962.
[5] J.R. Startin, in: A. Schecter (Ed.), Dioxins and Health, Plenum Press, 1994, New York, p. 115.
[6] A. Schecter, O. Rapke, P. Furst, J.J. Ryan, Organohalogen Comp. 33 (1997) 473.
[7] A.P.J.M. de Jong, A.K.D. Liem, R. Hoogerbrugge, J. Chromatogr. 643 (1993) 91.
[8] D.G. Patterson, J.S. Holler, C.R. Lapeza Jr., L.R. Alexander, D.F. Groce, R.C. O'Connor, S.J. Smith, J.A. Liddle, L. Needham, Anal. Chem. 58 (1986) 705.
[9] J.A. Van Rhijn, W.A. Traag, T.W. Kulik, L.G.M.Th. Tuinstra, J. Chromatogr. 595 (1992) 289.
[10] P.W. Albro, M.I. Luster, K. Chae, S.K. Chaudhary, G. Clark, L.D. Lawson, J.T. Corbert, J.D. McKinney, Toxicol. Appl. Pharmacol. 50 (1979) 137.
[11] L. Stanker, B. Watkins, N. Rogers, M. Vanderlaan, Toxicol. 45 (1987) 229.
[12] M. Vanderlaan, L.H. Stanker, B.E. Watkins, P. Petrovic, S. Gorbach, Environ. Toxicol. Chem. 7 (1988) 859.
[13] J. Sherry, Chemosphere 34 (1997) 1011.
[14] A. Kussak, B. Andersson, K. Andersson, J. Chromatogr. B 672 (1995) 253.
[15] B. Zimmerli, R. Dick, J. Chromatogr. B 666 (1995) 85.
[16] R. Bagnati, V. Ramazza, M. Zucchi, A. Simonella, F. Leone, A. Bellini, R. Fanelli, Anal. Biochem. 235 (1996) 119.
[17] H. Hooijerink, R. Schilt, E.O. Van Bennekom, F.A. Huf, J. Chromatogr. B 660 (1994) 303.
[18] K. Chae, L.K. Chao, J.D. McKinney, J. Agric. Food. Chem. 25 (1977) 1207.
[19] H.T. Taylor, J. Org. Chem. 0 (1958) 3922.
[20] B.F. Erlanger, F. Borek, S.M. Beiser, S. Lieberman, J. Biol. Chem. 228 (1957) 713.
[21] D.E. Keyler, D.J.W. Goon, W.L. Shelver, C.A. Ross, H.T. Nagasawa, J.V. St. Peter, P.R. Pentel, Biochem. Pharmacol. 48 (1994) 767.
[22] M. Bradford, Anal. Biochem. 72 (1976) 248.
[23] B. Perbal (Ed.), A Practical Guide to Molecular Cloning, John Wiley and Sons, Inc., New York, 1988, Ch. 3, p. 20.
[24] W.L. Shelver, J.K. Huwe, Organohalogen Comp. 31 (1997) 46.
[25] W.E. Turner, S.G. Isaacs, D.G. Patterson, Jr., L.L. Needham, Polychlorinated dioxins and dibenxofurans, in: C. Rappe, H.R. Buser, B. Dodet, I.K. O'Neill (Eds.), Environmental Carcinogens Methods of Analysis and Exposure Measurement, Vol. 11, IARC Scientific Publications No 108, 1991, Oxford, p. 346.
[26] K.R. Shuler, R.G. Dunham, P. Kanda, J. Immuno. Method. 156 (1992) 137.
[27] R. Bagnati, M.G. Castelli, L. Airoldi, A. Ubaldi, R. Fanelli, J. Chromatogr. 527 (1990) 267.