Covalent binding of dioxin metabolites to a 79 kDa protein isolated from bile of rats dosed with either 1,2,7,8-; 1,3,7,8-; or 1,4,7,8-tetrachlorodibenzo-*p*-dioxin.

C. Wiener and G.L. Larsen
USDA, ARS, Biosciences Research Laboratory, P.O.Box 5674-Univ. Sta., Fargo, ND 58105

Introduction

Dioxins are a group of halogenated aromatic hydrocarbons which are formed as unwanted by-products of a variety of thermal and chemical processes. Three non-toxic dioxin congeners, 1,2,7,8-; 1,3,7,8-; and 1,4,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), were selected for metabolism studies for several reasons: (1) the animals can ingest these compounds in their environment, (2) very little work has been conducted on them, and (3) the compounds can be dosed at a high mass level to increase the quantities of metabolites which can be isolated.

Materials and Methods

Individual studies were conducted on ^{14}C-labelled 1,2,7,8-TCDD, 1,3,7,8-TCDD, and 1,4,7,8-TCDD in bile-duct cannulated rats. ^{14}C-Labelled congeners were purchased from Chemsyn Scientific Laboratories, Lenexa, KS. Six male Sprague-Dawley rats were dosed orally for each experiment and bile was collected daily for three days after dosing. Bile samples were individually chromatographed on Sephadex G-75 column (4.5 x 90cm; G-75) and Sephacryl S-200 (2.2 x 85cm; S-200) as previously described [1]. Both G-75 and S-200 columns were equilibrated and run in 0.05 M potassium phosphate buffer (pH 7.2). The method used for sodium dodecylsulfate-polyacrylamide electrophoresis (SDS-PAGE ,13% polyacrylamide) was that of Maizel [2] which was run under reducing conditions. SDS-PAGE gels were stained with Coomassie blue. The molecular weight (MW) of the bile-protein/dioxin complex was determined using S-200 and SDS-PAGE. The following proteins were used to estimate the MW of the unknown protein by comparison with their elution volumes from S-200: bovine albumin (66,200), ovalbumin (43,000), chymotrypsinogen A (25,000), and ribonuclease A (13,700). The following standard globular proteins were used for estimation of MW by SDS-PAGE: trypsin inhibitor (21,500), carbonic anhydrase (31,000), ovalbumin (42,900), bovine albumin (66,200), and phosphorylase B (97,000). Isoelectric focusing was conducted using FMC IsoGel® Agarose IEF plates (pH 3-10, 0.6mm) and the following pI standards: cytochrome C, pI 10.2; myoglobin (whale), major pI 8.2 and minor pI 7.7; myoglobin (horse), major pI 7.4 and minor pI 7.0; carbonic anhydrase, pI 6.1; ß-lactoglobulin (A,B), A pI 5.4 and B pI 5.5; ovalbumin, pI 4.8; glucose oxidase, pI 4.2; and amyloglucosidase, pI 3.6. Radioactivity was extracted from the protein with organic solvents (hexane, toluene and ethyl acetate). Thin layer chromatography (TLC) of the extracted radioactivity used silica gel TLC plates developed with 50:50 v/v hexane:methylene chloride.

Results and Discussion

The bile-protein/dioxin complex for all three congeners eluted from the G-75 column between 500-750 ml (Figure 1A). This fraction from G-75 was subsequently applied to a S-200 column (Figure 1B). The bile-protein/dioxin complex eluted from the S-200 column between 200-300 ml for the three congeners. The association of radioactive 1,3,7,8-TCDD with a bile-protein is shown in Figure 1A and 1B by co-chromatography of the radioactivity with protein. Similar G-75 and S-200 elution profiles were observed in bile of rats dosed with either 1,2,7,8- or 1,4,7,8-TCDD (data not shown). SDS-PAGE of each of the bile-protein/dioxin complexes isolated from 0-24 h bile from rats dosed with 1,2,7,8-, 1,3,7,8- and 1,4,7,8-TCDD revealed a single major band of protein with apparent molecular weights of 81.5 kDa, 75.7 kDa, and 80.0 kDa, respectively (79.1 kDa average). The S-200 elution volume for the bile-protein/dioxin complex isolated from 1,2,7,8-TCDD dosed rats indicated an apparent weight of 104 kDa which, with the average 79 kDa MW from SDS-PAGE, indicates that the protein in this complex is a monomer of 79 kDa MW. S-200 was used to give a rough MW estimate of the native (intact subunits) protein. SDS-PAGE was used to obtain the MW of subunits and is also considered to be more accurate. Isoelectric focusing studies on this 79 kDa bile-protein/dioxin complex had a pI of 5.7. Attempts to sequence the protein were unsuccessful because of an apparent N-terminus blockage.

In the 1,2,7,8-TCDD study, 0.92% of the dose was bound to the 79 kDa protein in the 0-24 h bile (Table 1). There was no bile-protein/dioxin complex observed in the 24-48 h and 48-72 h bile. In the 0-24 h bile, ^{14}C associated with the 79 kDa protein was not extractable with organic solvents which indicated covalent binding to the protein. This protein fraction was subjected to acid hydrolysis and extracted with ethyl acetate. The organic fraction, containing most of the ^{14}C, was chromatographed using TLC which indicated that a metabolite of ^{14}C-1,2,7,8-TCDD was covalently bound to the 79 kDa protein.

In the 1,3,7,8-TCDD study, 0.22%, 1.15%, and 0.12% of the dose bound to the 79 kDa protein in the 0-24 h, 24-48 h, and 48-72 h bile fractions, respectively (Table 1). In the 0-24 h and 24-48 h bile, ^{14}C associated with the 79 kDa protein was covalently bound because ^{14}C was not extractable from the 79 kDa protein with organic solvents. Acid hydrolysis of the 79 kDa protein and TLC of the hydrolyzate is further evidence that a metabolite of ^{14}C-1,3,7,8-TCDD was covalently bound to the 79 kDa protein.

In the 0-24 h, 24-48 h, and 48-72 h bile from rats dosed with 1,4,7,8-TCDD, 3.43%, 0.008%, and 0.051% of the dose was bound to the protein, respectively (Table 1). In the 1,4,7,8-TCDD study 60% of the ^{14}C associated with the protein in the 0-24 h bile was extracted using organic solvents. The extractability indicated that part of the ^{14}C was bound non-covalently and that the remaining 40% appeared to be bound covalently to the protein. The extractable radioactivity co-chromatographed with authentic 1,4,7,8-TCDD on TLC indicated that 1,4,7,8-TCDD was non-covalently bound to the 79 kDa protein. The 40% that was not extractable was subjected to acid hydrolysis and was extracted into ethyl acetate. Sixty eight percent of the acid hydrolyzed ^{14}C co-chromatographed on TLC with authentic 1,4,7,8-TCDD. This apparent covalent binding of 1,4,7,8-TCDD may involve a metabolite of 1,4,7,8-TCDD covalently bound to the protein which upon acid hydrolysis reverted back to parent 1,4,7,8-TCDD as has been previously observed with fecal 1,4,7,8-TCDD metabolites [3] and arene oxides of phenanthrene and naphthalene [4]. TLC data on the remaining 33% of the hydrolyzable ^{14}C indicated it was a metabolite of 1,4,7,8-TCDD.

In conclusion, although the bile protein has not been identified, its physiological role may be altered when metabolites of the dioxin congeners are bound covalently to it. This ultimately may affect the health and production efficiency of the animal.

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References

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Table 1. Total ^{14}C bound to the 79 kDa bile protein in three dioxin congeners as a percentage of the total dose.

Bile	1278-TCDD (%)	1378-TCDD (%)	1478-TCDD (%)
0-24 hr	0.915	0.22	3.43
24-48 hr	0	1.15	0.008
48-72 hr	0	0.12	0.051

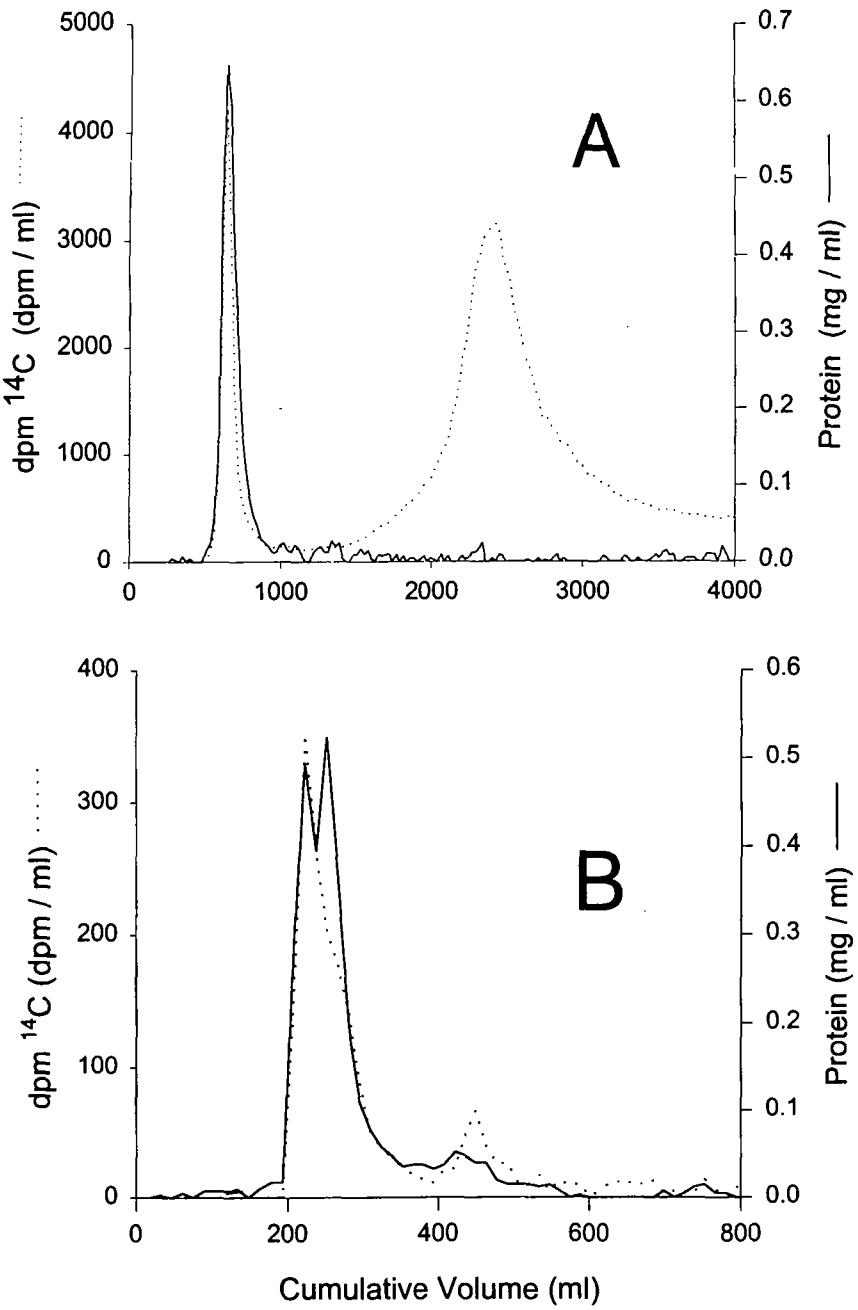

Figure 1. Elution patterns from (A) Sephadex G-75 and (B) Sephacryl S-200 in the partial purification of rat biliary protein associated with ^{14}C-1,3,7,8-tetrachlorodibenzo-p-dioxin.

Fractions were assayed for protein (——) and radioactivity (----).