0026-895X/89/050713-07$02.00/0

MOLECULAR PHARMACOLOGY, 35:713-719

Characterization of a Specific Binding Protein for 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin in Human Thymic Epithelial Cells

JON C. COOK[1] and WILLIAM F. GREENLEE

Department of Cellular and Molecular Toxicology, Chemical Industry Institute of Toxicology, Research Triangle Park, North Carolina 27709

Received August 24, 1988; Accepted February 22, 1989

SUMMARY

Specific toxic and biochemical responses elicited by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) in human thymic epithelial (TE) cells in culture are mediated by the TCDD receptor protein. Characterization of the physicochemical properties of the TCDD receptor in cytosol fractions from cultured human TE cells indicates that this protein is similar, but not identical, to the extensively studied receptor species present in mouse and rat liver. The human TCDD receptor sediments at 9.1 S on sucrose density gradients at 0°, undergoes a temperature-dependent conversion at 20° to a species sedimenting at 10.7 S, and partially dissociates in the presence of 0.4 M KCl, as judged by the appearance of a peak sedimenting at 5 S. Both the temperature- and salt-dependent changes in the observed physical properties of the human TCDD receptor are inhibited by sodium molybdate. Two molybdate-stabilized binding species can be resolved from TCDD specific binding isotherms to human TE cytosol. Under identical conditions, only a single TCDD specific binding component was detected in cytosol fractions from both mouse and rat liver. Mixing cytosol prepared from human TE cells with hepatic cytosol fractions from C57BL/6 mice revealed the presence of a heat-labile, trypsin-sensitive factor in human TE cells that inhibits TCDD specific binding to the murine hepatic receptor. Molybdate stabilized the mouse receptor against the actions of this human inhibitory factor. Molybdate also may stabilize the human TCDD receptor, as indicated by the 2- to 3-fold increase in total TCDD specific binding measured in human TE cytosol fractions in the presence of this metallo-oxyanion.

TCDD is the prototype for the halogenated aromatic compounds, a large group of environmental toxicants that includes the dibenzo-*p*-dioxins, dibenzofurans, biphenyls, and azo- and azoxybenzenes. Studies in various animal models and in human and animal cells in culture have established that the most characteristic pathologic lesions produced by TCDD and isosteric halogenated aromatic compounds are mediated by an intracellular receptor protein (to be designated in this report as the TCDD receptor) (reviewed in Refs. 1 and 2). The physicochemical properties of the TCDD receptor protein present in cytosol fractions from rodent liver and the murine hepatoma cell line Hepa 1c1c7 have been well characterized (3–5) and appear in general to be analogous to those previously reported for the glucocorticoid (6) and other steroid hormone receptors (reviewed in Ref. 7). Like its steroid counterpart, the rodent TCDD receptor protein possesses distinct ligand- and DNA-binding domains (8–10) and functions as a *trans*-acting effector of gene expression (11–13).

Previous investigations from this laboratory have focused on the development of *in vitro* models for study of the mechanisms of TCDD toxicity to skin and thymus. Results obtained from these model systems have demonstrated the involvement of the TCDD receptor in TCDD-induced epidermal hyperkeratinization (14) and suppression of TE-dependent thymocyte maturation (15). The latter response has been modelled using both mouse and human reconstituted thymus cell populations and results from direct actions of TCDD on TE target cells, mediated by the TCDD receptor protein (15, 16).

Relatively little is known about the human TCDD receptor. A specific binding protein for TCDD has been identified in fetal and adult lung samples (17, 18), placenta (19, 20), normal keratinocytes (14), squamous cell carcinoma lines (21, 22), and early passage cultures of human TE cells (15). In this report, the findings of an initial characterization of the physicochemical properties of the TCDD receptor from human TE cells are presented.

Experimental Procedures

Materials. [1,6-^{3}H]TCDD (36 Ci/mmol) was a generous gift from Dr. George W. Lucier (National Institute of Environmental Health Sciences) and was originally purchased from Chemsyn Science Laboratories (Lenexa, KS). The [^{3}H]TCDD was purified by high pressure

This work was presented in part at the Society of Toxicology 26th Annual Meeting, Washington, DC, March, 1987.

[1] Supported by a Chemical Industry Institute of Toxicology Postdoctoral Fellowship. Current address: Haskell Laboratory, E. I. du Pont de Nemours and Co., Newark, DE 19714.

ABBREVIATIONS: TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; BSA, bovine serum albumin- B6, C57BL/6; SD, Sprague-Dawley; TCDF, 2,3,7,8-tetrachlorodibenzofuran; TE, thymic epithelial; HEPES, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid.

liquid chromatography (23). [*methyl*-^{14}C]Methylated-BSA was bought from New England Nuclear (Boston, MA). Aprotonin, calf thymus and salmon testes DNA, chymostatin, diisopropyl fluorophosphate, iodoacetamide, leupeptin, pepstatin A, phenylmethylsulfonyl fluoride, and sodium molybdate were from Sigma Chemical Co. (St. Louis, MO). Trypsin (2.5%) and soybean trypsin inhibitor (7000 $N\alpha$-benzoyl-L-arginine ethyl ester units/mg) were purchased from GIBCO Laboratories (Grand Island, NY). All other chemicals were obtained from sources previously described (15).

Animals. Female B6 mice (4 weeks of age) and Crl:CDBR SD rats (~100 g) from Charles River Breeding Laboratories, Inc. (Kingston, NY) were quarantined on arrival for 2 weeks and were found to be free of pathogenic viruses by a standard virus titer screen (rat/mouse assessment profile; Microbiological Associates, Bethesda, MD). Animals were housed in polycarbonate shoe-box cages with filter tops at 72° ± 2°F and 50 ± 10% humidity with a 12-hr light/dark cycle. NIH-07 pelleted diet and tap water were available *ad libitum*.

Cell culture. Early passage cultures of human TE cells were established by methods described previously (15). Human thymus samples

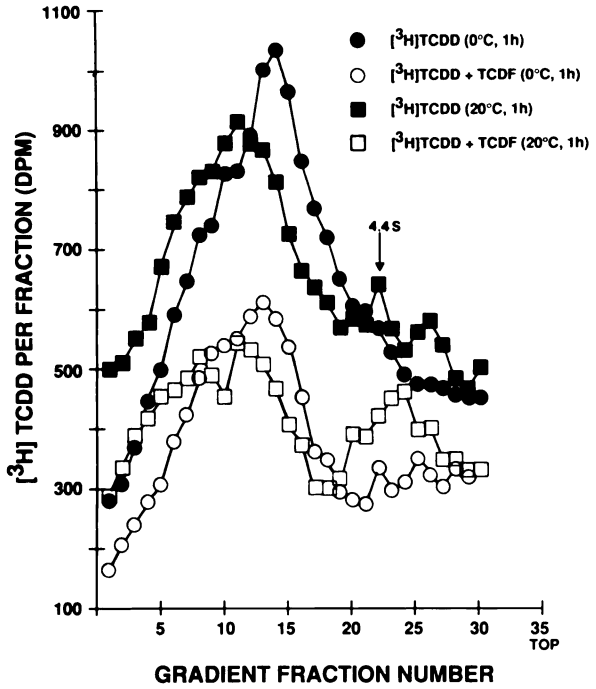

Fig. 1. Temperature-dependent shift in the sedimentation profile of [^{3}H]TCDD binding to human TE cytosol in HEDG buffer. [^{3}H]TCDD was incubated for 1 hr at either 0° (*circles*) or 20° (*squares*) with human TE cytosol in the presence or absence of 200-fold molar excess of unlabeled TCDF. The labeled cytosol fractions were sedimented through linear (10–30%) sucrose gradients as described in Experimental Procedures. *S* values for total TCDD binding peaks are 9 (●) and 10 (■).

TABLE 1

Summary of the molybdate stabilization of the temperature- and salt-dependent shifts in the sedimentation of the TCDD receptor from three human TE strains

Sucrose density gradient analysis of [^{3}H]TCDD binding to human TE cytosol fractions was performed as described in Experimental Procedures. Cytosol was incubated with ligand for 1 hr at the specified temperature. The human TE strains examined are designated HuTE-L, HuTE-M, and HuTE-N.

Conditions		Sedimentation Values ($S_{20,w}$)		
Incubation temperature	Buffer	HuTE-L[a]	HuTE-M[b]	HuTE-N[b]
0°	HEDG	9 ± 0.1	9 ± 0.2	9 ± 0.0
0°	HEDGM	9 ± 0.1	9 ± 0.1	9 ± 0.0
20°	HEDG	11 ± 0.2[c]	10 ± 0.0[c]	10 ± 0.1[c]
20°	HEDGM	9 ± 0.0	9 ± 0.2	9 ± 0.1
0°	HEDGK	9 ± 0.3	9 ± 0.3	9 ± 0.0
		5 ± 0.1[c]	5 ± 0.0[c]	5 ± 0.0[c]
0°	HEDGKM	9 ± 0.1	9 ± 0.1	9 ± 0.0

[a] Values are expressed as the mean ± standard error of three experiments.
[b] Values are expressed as the average ± range of two experiments.
[c] The value indicated was significantly different ($p < 0.01$) from the value obtained at 0° in HEDG buffer as assessed by analysis of variance (31).

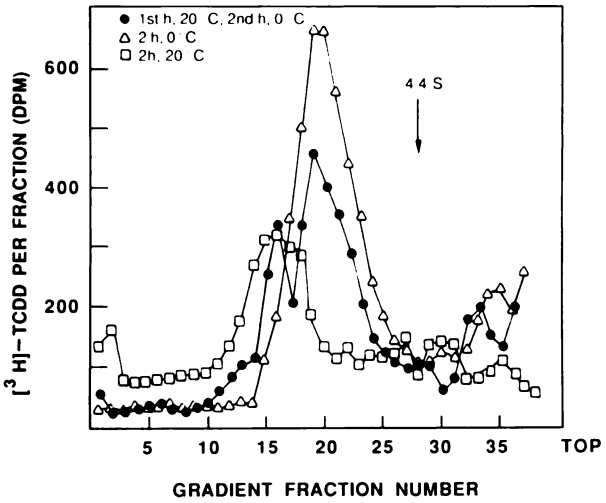

Fig. 2. Reversibility of the temperature-dependent shift in the sedimentation profile of [^{3}H]TCDD specific binding to human TE cytosol in HEDG buffer. Three incubation conditions were compared, 2 hr at 0°; 2 hr at 20°; and 1 hr at 20° followed by 1 hr at 0°. The profiles shown represent specific binding. *S* values are 9 (△, ●) and 10 (□, ●).

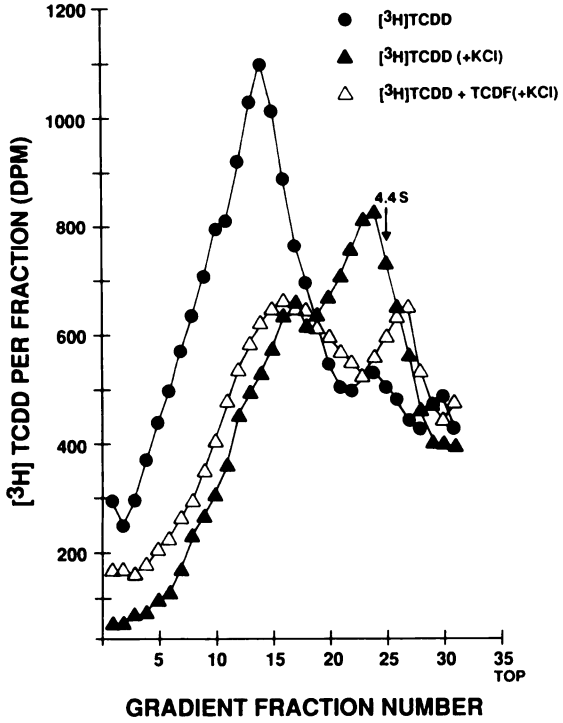

Fig. 3. Salt-dependent shift in the sedimentation profile of [^{3}H]TCDD binding to human TE cytosol. [^{3}H]TCDD was incubated for 1 hr at 0° with TE cytosol fractions in the presence (HEDGK buffer) or absence (HEDG buffer) of 0.4 M KCl. For the high salt samples, sucrose density gradients were prepared with buffers containing 0.4 M KCl. *S* values are 9 (●) and 5 (▲).

were from children (2 months to 16 years of age) undergoing corrective cardiac surgery.

Buffers. The following buffers were used: HEDG (25 mM HEPES, 1.5 mM EDTA, 1 mM dithiothreitol, 10% (v/v) glycerol, pH 7.6), HEDGK, (HEDG buffer with 0.4 M KCl), HEDGM, (HEDG buffer with 20 mM sodium molybdate), and HEDGKM, (HEDG buffer with 0.4 M KCl and 20 mM sodium molybdate).

Measurement of [^{3}H]TCDD specific binding. Cytosol fractions were prepared in HEDG buffer as described previously (15, 24). Cytosol fractions (0.5 ml; 4–5 mg of protein/ml) were incubated for 2 hr at 20° with [^{3}H]TCDD (0.1–5 nM) in the presence (nonspecific binding) or absence (total binding) of a 200-fold molar excess of TCDF. Total and nonspecific binding of [^{3}H]TCDD were quantitated by adsorption of the protein-bound ligand onto hydroxylapatite (25). Free TCDD was calculated by subtracting total binding from the total amount of radiolabeled compound added to each sample. Specific binding was calculated by subtracting nonspecific from total binding.

Sucrose density gradients. Qualitative changes in the properties of the TCDD protein binding species were monitored by centrifugation through linear (10 to 30%) sucrose density gradients in a Sorval® TV-865 vertical tube rotor, with 6-ml capacity ultracrimp tubes (26). Cytosol samples were incubated with 5 nM [^{3}H]TCDD, in the presence or absence of 500 nM TCDF for 1 hr at 0° or 20° as described above and the unbound radioligand was adsorbed onto dextran-coated charcoal (10 mg of charcoal: 1 mg of dextran) (15). At 0°, 95–100% of equilibrium binding was achieved by 1 hr. A 330-μl sample of the charcoal-treated sample was sedimented through the sucrose density gradient by centrifugation (370,000 × g_{av} for 2 hr at 2°) and 150-μl fractions were collected using a Hoefer FS101 gradient tube fractionator. The radioactivity in each fraction was quantitated in a Packard Tri-Carb (Model 460 CD) liquid scintillation counter. Sedimentation coefficients for the radioactive peaks were calculated as described previously (27), with ^{14}C-labeled BSA (4.4 S) as the standard. The [^{14}C]-labeled BSA marker was added to each sample and is indicated by an arrow in all figures. Alkali-soluble protein was measured by the method of Bradford (28) with BSA (fraction V) as the standard.

Protease inhibitors. Stock solutions of the following protease inhibitors were prepared and added to 100 ml of HEDG buffer to yield the final concentrations indicated in the legend to Fig. 5: phenylmethylsulfonyl fluoride (1.7 mg/ml, in ethanol); chymostatin (25 mg/ml, in dimethyl sulfoxide); and pepstatin A (25 mg/ml, in ethanol). The other inhibitors examined were added directly to the HEDG buffer. The HEDG buffer containing the protease inhibitors was used to prepare cytosol from human TE strain Q. Total and nonspecific TCDD binding was assayed by sucrose density gradient analysis as described above.

Results

Characterization of a TCDD specific binding protein in human TE cells. A trypsin-sensitive TCDD specific binding species sedimenting at approximately 9 S in sucrose density gradients at 0° is detectable in cytosol prepared from human TE cells (Fig. 1). The human TCDD receptor is converted to a species sedimenting at 10 S when incubated at 20° for 1 hr (Fig. 1). For each of the strains examined, the value obtained for the sedimentation coefficient (S) at 20° was significantly different ($p < 0.01$) from the value obtained at 0° (Table 1). The temperature-dependent conversion of the TCDD receptor to a faster sedimenting form was not observed in hepatic cytosol fractions from SD rats or B6 mice (data not shown). Conversion to the faster sedimenting form can be at least partially reversed if the incubation temperature is returned to 0° (Fig. 2).

When incubated in the presence of 0.4 M KCl for 1 hr at 0°, the human TCDD receptor appears to partially dissociate, as judged by the presence of a peak sedimenting at 5 S on sucrose density gradients (fig. 3; Table 1). Both the temperature- and salt-dependent changes in the sedimentation profile of the human TCDD receptor are prevented by the inclusion of 20 mM sodium molybdate in the incubation and analysis buffers (Fig. 4, Table 1).

In the presence of seven protease inhibitors with activity against 14 different proteases (including calcium-dependent thiol protease), both total and nonspecific TCDD binding was increased (Fig. 5); however, the calculated value for specific TCDD binding was approximately 25% lower than the value obtained in the absence of inhibitors (28 versus 39 fmol/mg of protein, respectively). These findings suggest that human TE cytosol does not contain common protease activity toward the TCDD receptor under the experimental conditions used in this study. The decrease in TCDD specific binding activity in the presence of the protease inhibitors ranged from 10 to 25% (this experiment) and may result from nonspecific interaction of the inhibitors with the TCDD receptor.

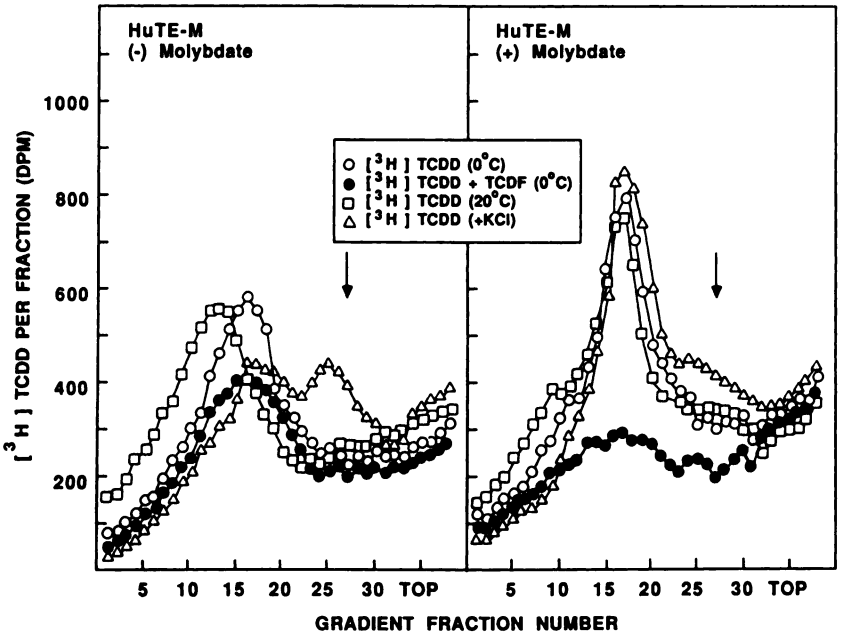

Fig. 4. Molybdate stabilization of the temperature- and salt-dependent shifts in the sedimentation profile of [^{3}H] TCDD binding to human TE cytosol. Cytosol fractions were incubated with [^{3}H]TCDD for 1 hr at either 0° or 20° (HEDG buffer) or for 1 hr at 0° in the presence 0.4 M KCl (HEDGK buffer). For each of the incubation conditions shown in the *right panel*, the buffers used contained 10 mM sodium molybdate. This same concentration of molybdate was included in the buffer used to prepare the sucrose density gradients (see Experimental Procedures). S values for the *left panel* are 9 (0°), 10 (20°), and 5 (0.4 M KCl). S values for the *right panel* are 9 (all peaks).

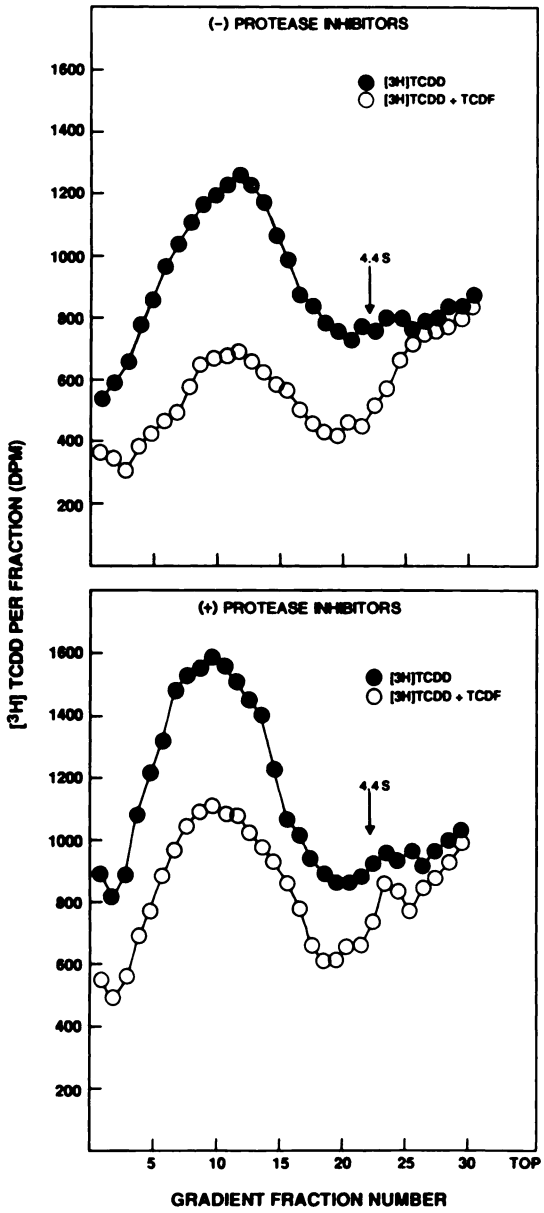

Fig. 5. Sucrose density gradient analysis of [^{3}H]TCDD binding to human TE cytosol in the presence of protease inhibitors. Cytosol fractions were prepared in HEDG buffer and were incubated with [^{3}H]TCDD in the absence (*upper*) or presence (*lower*) of seven protease inhibitors for 1 hr at 0°, as described in Experimental Procedures. The protease inhibitors added to the incubation buffer were aprotonin (0.1 mg/ml), chymostatin (0.1 mg/ml), diisopropyl fluorophosphate (0.1 mM), iodoacetamide (1.0 mM), leupeptin (0.1 mg/ml), pepstatin A (0.1 mg/ml), and phenylmethylsulfonyl fluoride (0.1 mM).

Evidence for molybdate-stabilized multiple binding species. The results of Scatchard analysis of the TCDD specific binding isotherms to cytosol fractions from three human TE strains, L, M, and N, are shown in Fig. 6. In the absence of sodium molybdate, the linearized binding data best fit a single component model. The calculated values for the dissociation constant (K_d) and total receptor content (n) for the three strains examined were similar (K_d, 0.17 to 0.38 nM; n, 20.4 to 35.7 fmol/mg of protein). Inclusion of 20 mM sodium molybdate in the incubation and analysis buffers resulted in the appearance of a second, lower affinity (K_d values ranging from 3.6 to 6.3 nM), component. In the presence of molybdate, the total receptor content was increased approximately 3-fold, with most of the measured binding distributed within the lower affinity component (ranging from 60%, strain L, to 90%, strain N). The multiple binding components resolvable from Scatchard analysis of TCDD binding in the presence of molybdate may represent distinct receptor proteins or different activation states of a single receptor species (see Discussion).

Evidence for an inhibitor of TCDD specific binding in human TE cytosol. The specific binding of [^{3}H]TCDD to hepatic cytosol from B6 mice was used to monitor for the potential existence of an endogenous TCDD receptor ligand or inhibitor in human TE cytosol. Mixing cytosol from B6 mouse liver with cytosol from human TE strain L cells resulted in a 66% decrease in the total binding expected for an additive response (i.e., B6 plus human TE-L) (Fig. 7). Preheating the human TE cytosol sample for 10 min at 57° before mixing with the mouse liver cytosol prevented the observed decrease in TCDD specific binding. Because the human TE cytosol is mixed with the B6 mouse liver cytosol before the addition of [^{3}H]TCDD, these observations suggest that the heat-sensitive factor in the human TE cytosol is inhibiting TCDD binding to specific sites in the B6 cytosol, rather than destroying binding sites occupied by TCDD. As shown in Fig. 8, trypsinization of the human cytosol also prevented much of the decrease (83% of an additive response for B6 and trypsin-treated human TE-M) in specific binding of [^{3}H]TCDD to the mouse cytosol in the mixed samples. The trypsin was neutralized by addition of soybean trypsin inhibitor to the human TE cytosol before addition to the B6 cytosol. Control experiments using B6 cytosol demonstrated that the trypsin was completely neutralized by the soybean trypsin inhibitor (data not shown). These observations suggested that the heat-labile inhibitory factor in the human cytosol was a protein. Molybdate stabilized the mouse hepatic TCDD receptor against the human inhibitory factor (Fig. 9). Molybdate also may stabilize the human TCDD receptor, as judged by the 2- to 3-fold increase in total [^{3}H]TCDD specific binding (Figs. 4 and 6) measured in the presence of this metallo-oxyanion.

Discussion

The data presented in this report indicate that the TCDD receptor species present in cytosol fractions from cultured human TE cells is similar, but not identical, to the extensively studied rodent hepatic TCDD receptor protein. The human TCDD receptor sediments at approximately 9 S on sucrose density gradients at 0° (Fig. 2), partially dissociates in the presence of 0.4 M KCl (Fig. 3), and can undergo a reversible temperature-dependent conversion (at 20°) to a species sedimenting at 10.7 S (Fig. 2). The TCDD receptor from rat liver readily dissociates in the presence of high salt; however, the receptor from mouse liver is relatively resistant (3). The biologic significance of the temperature-dependent conversion of the human TCDD receptor to a faster sedimenting form is unknown.

Molybdate inhibits the observed temperature- and salt-dependent changes in the physical properties of the human TCDD receptor (Fig. 4). Molybdate has been reported to stabilize the TCDD receptor protein present in human placenta (20) but only partially stabilizes the rodent receptor (4). In the present study it was observed that, in the absence of molybdate, only a single high affinity (K_d value approximately equal to 0.25 nM) binding component was resolvable from TCDD specific binding

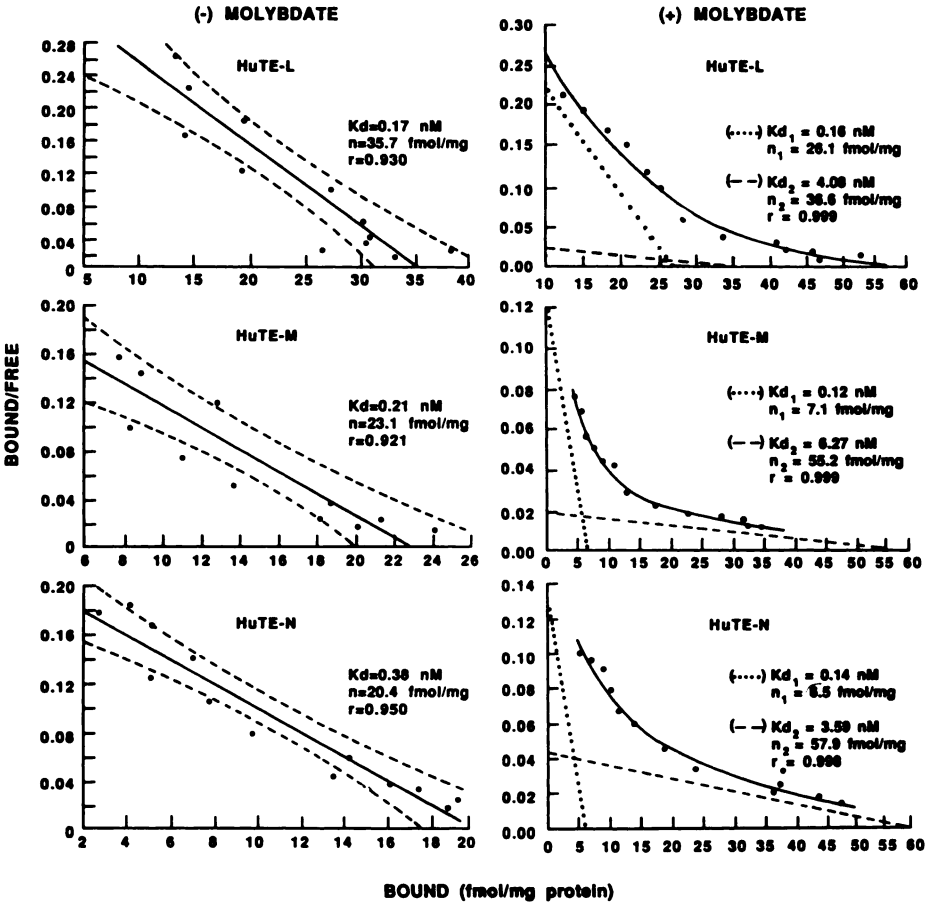

Fig. 6. Analysis of the [^{3}H]TCDD specific binding components detected in cytosol fractions from three human TE strains. [^{3}H]TCDD specific binding to TE cytosol was assayed by absorption of protein-bound ligand onto hydroxylapatite as described in Experimental Procedures. [^{3}H]TCDD binding isotherms (concentration range of [^{3}H]TCDD, 0.1 to 5 nM) to cytosol from each human TE strain were obtained in the presence or absence of 20 mM molybdate and were analyzed according to the method described by Scatchard (32). The dissociation constant (K_d), total receptor number (n), and correlation coefficient (r) are shown for each plot. The *dashed lines* in the *left panels* represent 95% confidence intervals for a single-component binding species from cytosol prepared in a HEDG buffer. The data shown in the *right panels* were from cytosol prepared in a HEDGM buffer and were fitted to a two-component model. The *dotted* and *dashed lines* represent the high and low affinity binding sites, respectively. Using an F test based on the extra sum of squares principle (33), the two-component model significantly ($p < 0.005$) improves the goodness of fit compared with the one-component model for each strain.

isotherms to human TE cytosol fractions; however, inclusion of molybdate in the binding buffer resulted in a 2- to 3-fold increase in receptor binding, associated with the presence of a second, lower affinity (K_d value approximately equal to 5 nM), binding species (Fig. 6). Under identical conditions, only a single binding species was detected in cytosol fractions from either rat or mouse liver (data not shown). The multiple TCDD binding species observed in the presence of molybdate may represent distinct receptor proteins. In this case molybdate could be stabilizing a labile species of the TCDD receptor. Alternatively, molybdate could stabilize different activation states of a single TCDD receptor protein. Activation of a single TCDD receptor species may result from action of a specific protein kinase modulating receptor phosphorylation. It has been observed that the total TCDD receptor binding measured in cytosol from a mouse hepatoma cell line is dependent on the cellular ATP concentration (29).

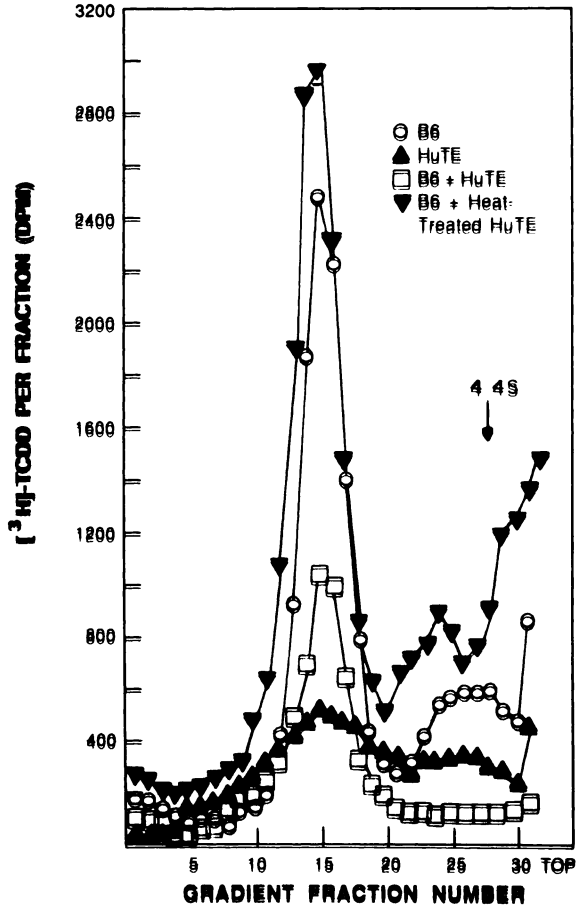

Fig. 7. Detection of a heat-sensitive inhibitor of [^{3}H]TCDD specific binding in human TE cytosol. [^{3}H]TCDD specific binding was determined by sucrose density gradient analysis (see Experimental Procedures) on the following samples: 250 μl of cytosol from B6 mouse liver (9.8 mg of protein/ml) plus 250 μl of HEDG buffer (○); 250 μl of cytosol from human TE (strain M) cells (4.2 mg protein/ml) plus 250 μl of HEDG buffer (▲); 250 μl of B6 mouse liver cytosol plus 250 μl of human TE cytosol (□) and 250 μl of B6 mouse liver cytosol plus 250 μl of human TE cytosol, which had been incubated at 57° for 10 min before mixing and assay of [^{3}H]TCDD binding (▼). All samples were prepared in HEDG buffer and were mixed before incubation with the radiolabeled ligand for 1 hr at 20°. The sedimentation profiles shown represent specific [^{3}H]TCDD binding.

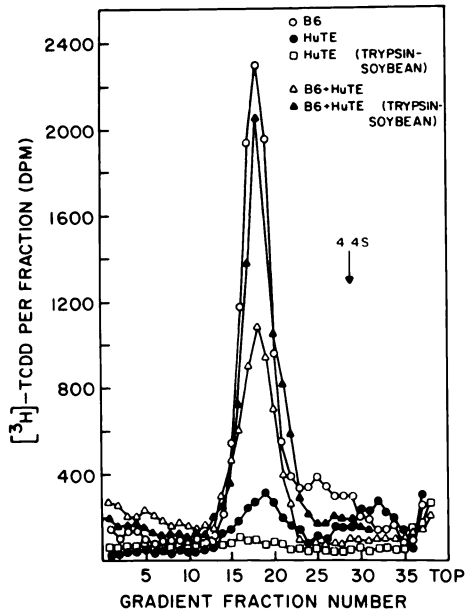

Fig. 8. Effect of trypsinization of human TE cytosol on [^{3}H]TCDD binding inhibitor activity. [^{3}H]TCDD specific binding was determined by sucrose density gradient analysis (see Experimental Procedures) on the following samples: 250 μl of cytosol from B6 mouse liver (7.0 mg of protein/ml) plus 250 μl of HEDG buffer (○); 250 μl of cytosol from human TE (strain M) cells (6.75 mg protein/ml) plus 250 μl of HEDG buffer (●); 250 μl of human TE cytosol, which was incubated for 20 min at 20° with trypsin (0.2 mg/ml cytosol), followed by a 10-min incubation with soybean trypsin inhibitor (1 mg/ml cytosol), plus 250 μl of HEDG buffer (□); 250 μl of B6 mouse liver cytosol plus 250 μl of human TE cytosol (△) and 250 μl of B6 mouse liver cytosol plus 250 μl of trypsin-treated human TE cytosol (▲). All samples were prepared in HEDG buffer and were mixed before incubation with the radiolabeled ligand for 1 hr at 20°. The sedimentation profiles shown represent specific [^{3}H]TCDD binding.

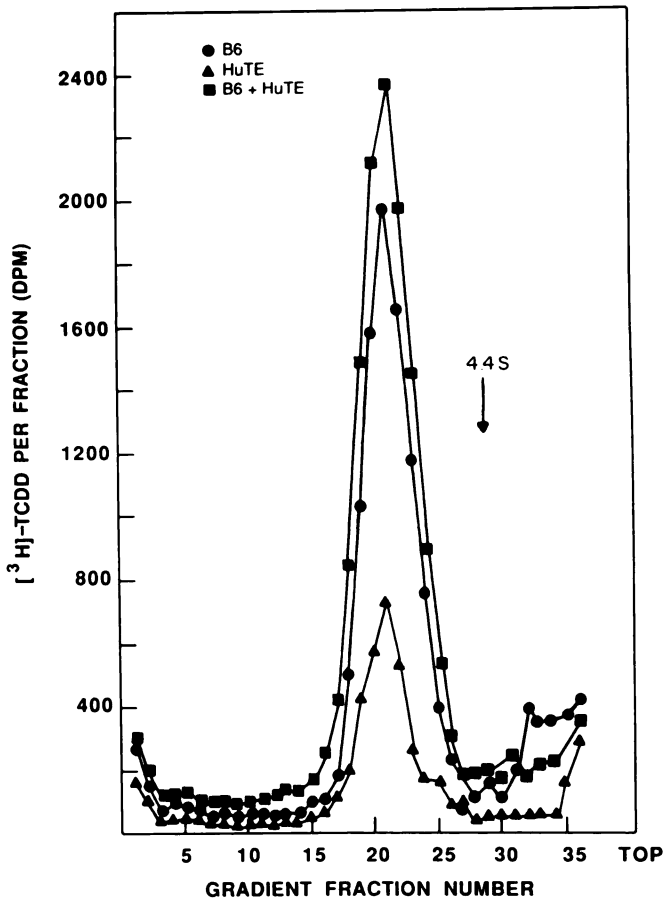

Fig. 9. Molybdate stabilization of [^{3}H]TCDD specific binding against human TE cytosolic inhibitor activity. [^{3}H]TCDD specific binding was determined by sucrose density gradient analysis (see Experimental Procedures). HEDGM buffer was used in the preparation of cytosol fractions and the linear sucrose density gradients. [^{3}H]TCDD specific binding was determined on the following samples: 250 μl of cytosol from B6 mouse liver (7.0 mg protein/ml) plus 250 μl buffer (●), 250 μl of cytosol from human TE (strain M) cells (6.0 mg protein/ml) plus 250 μl of buffer (▲) and, 250 μl of B6 mouse liver cytosol plus 250 μl of human TE cytosol (■). All samples were mixed before incubation with the radiolabeled ligand for 1 hr at 20°.

Mixing experiments with cytosol from mouse liver and human TE cells revealed the presence of a human cytosolic factor that inhibited TCDD binding to the mouse receptor (Figs. 7–9). This factor was shown to be heat labile and trypsin sensitive, suggesting that it is a protein. It does not appear to be a protease because no increase in nonspecific binding or appearance of slower (lower molecular weight) sedimenting species was observed in the sucrose density gradient profiles (Fig. 7) and addition of seven protease inhibitors had no effect on the receptor profiles (Fig. 5). The protease inhibitors examined included agents specific for the calcium-dependent thiol proteases previously reported to degrade the TCDD receptor (30). Molybdate was found to stabilize the mouse receptor against the human inhibitory factor (Fig. 9). Molybdate also may stabilize the human TCDD receptor against the actions of this inhibitory factor, as indicated by the 2- to 3-fold increase in TCDD specific binding (Figs. 4 and 6); however, TCDD specific binding is detectable in the absence of molybdate (Figs. 4 and 6), suggesting either that a TCDD receptor species exists that is resistant to inhibition or that the binding detected results from incomplete inhibition by the cytosolic factor.

Acknowledgments

We are indebted to Dr. Benson R. Wilcox (Chief of Thoracic Surgery at the School of Medicine, University of North Carolina) and his staff at the North Carolina Memorial Hospital for providing human thymus specimens and to Dr. George W. Lucier (National Institute of Environmental Health Sciences) for the generous gift of [^{3}H]TCDD. We also thank Drs. Thomas A. Gasiewicz, Allan B. Okey, and Alan Poland for generously providing preprints of work from their laboratories.

References

1. Poland, A., and J. Knutson. 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin and related halogenated aromatic hydrocarbons: examination of the mechanism of toxicity. *Annu. Rev. Pharmacol. Toxicol.* **22**:517–554 (1982).
2. Greenlee, W. F., R. Osborne, K. M. Dold, L. G. Hudson, M. J. Young, and W. A. Toscano, Jr. Altered regulation of epidermal cell proliferation and differentiation by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). *Rev. Biol. Toxicol.* **8**:1–35 (1987).
3. Denison, M. S., L. M. Vella, and A. B. Okey. Structure and function of the *Ah* receptor for 2,3,7,8-tetrachlorodibenzo-*p*-dioxin: species difference in molecular properties of the receptors from mouse and rat hepatic cytosols. *J. Biol. Chem.* **261**:3987–3995 (1986).
4. Denison, M. S., L. M. Vella, and A. B. Okey. Hepatic *Ah* receptor for 2,3,7,8-tetrachlorodibenzo-*p*-dioxin: partial stabilization by molybdate. *J. Biol. Chem.* **261**:10189–10195 (1986).
5. Cuthill, S., L. Poellinger, and J.-A. Gustafsson. The receptor for 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in the mouse hepatoma cell line Hepa 1c1c7: a comparison with the glucocorticoid receptor and the mouse and rat hepatic dioxin receptors. *J. Biol. Chem.* **262**:3477–3481 (1987).
6. Poellinger, L., and D. Gullberg. Characterization of the hydrophobic properties of the receptor for 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. *Mol. Pharmacol.* **27**:371–376 (1985).
7. Gustafsson, J.-A., J. Carlstedt-Duke, L. Poellinger, S. Okret, A.-C. Wilkstrom, M. Bronnegard, M. Gillner, Y. Dong, K. Fuxe, A. Cintra, A. Harfstrand, and L. Agnati. Biochemistry, molecular biology, and physiology of the glucocorticoid receptor. *Endocrine Rev.* **8**:185–234 (1987).
8. Carlstedt-Duke, J. M. B., U. B. Harnemo, B. Hogberg, and J.-A. Gustafsson. Interaction of the hepatic receptor protein for 2,3,7,8-tetrachlorodibenzo-*p*-dioxin with DNA. *Biochim. Biophys. Acta* **672**:131–141 (1981).

9. Wilhelmsson, A., A.-C. Wikstrom, and L. Poellinger, Polyanionic binding properties of the receptor for 2,3,7,8-tetrachlorodibenzo-*p*-dioxin: a comparison with the glucocorticoid receptor. *J. Biol. Chem.* **261**:13456–13463 (1986).
10. Hannah, R. R., J. Lund, L. Poellinger, M. Gillner, and J.-A. Gustafsson. Characterization of the DNA-binding properties of the receptor for 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. *Eur. J. Biochem.* **156**:237–242 (1986).
11. Jones, P. B. C., D. R. Galeazzi, J. M. Fisher, and J. P. Whitlock Jr. Control of cytochrome P_1-450 gene expression by dioxin. *Science (Wash. D. C.* **227**:1499–1502 (1985).
12. Jaiswal, A. K., F. J. Gonzalez, and D. W. Nebert. Comparison of human mouse P_1-450 upstream regulatory sequences in liver- and nonliver-derived cell lines. *Mol. Endocrinol.* **1**:312–320 (1987).
13. Sogawa, K., A. Fujisawa-Sehara, M. Yamane, and Y. Fujii-Kuriyama. Location of regulatory elements responsible for drug induction in the rat cytochrome P-450c gene. *Proc. Natl. Acad. Sci. USA* **83**:8044–8048 (1986).
14. Osborne, R., and W. F. Greenlee. 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) enhances terminal differentiation of cultured human epidermal cells. *Toxicol. Appl. Pharmacol.* **77**:434–443 (1985).
15. Cook, J. C., K. M. Dold, and W. F. Greenlee. An *in vitro* model for studying the toxicity of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin to human thymus. *Toxicol. Appl Pharmacol.* **89**:256–268 (1987).
16. Greenlee, W. F., and R. A. Neal. The *Ah* receptor: a biochemical and biologic perspective, in *The Receptors* (M. Conn, ed.) Vol. 2. Academic Press, New York, 89–129 (1985).
17. Roberts, E. A., N. H. Shear, A. B. Okey, and D. K. Manchester. The *Ah* receptor and dioxin toxicity: from rodent to human tissues. *Chemosphere* **14**:661–674 (1985).
18. Roberts, E. A., C. L. Golas, and A. B. Okey. *Ah* receptor mediating induction of aryl hydrocarbon hydroxylase: detection in human lung by binding of 2,3,7,8-[^{3}H]tetrachlorodibenzo-*p*-dioxin. *Cancer Res.* **46**:3739–3743 (1986).
19. Wong, T. K., T. Sloop, and G. W. Lucier. Nondetectable concentrations of human placental *Ah* receptors are associated with potent induction of microsomal benzo[*a*]pyrene hydroxylase in individuals exposed to polychlorinated biphenyls, quaterphenyls, and dibenzofurans. *Toxicol. Appl. Pharmacol.* **85**:60–68 (1986).
20. Manchester, D. K., S. K. Gordon, C. L. Golas, E. A. Roberts, and A. B. Okey. *Ah* receptor in human placenta: stabilization by molybdate and characterization of binding of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, 3-methylcholanthrene, and benzo[*a*]pyrene. *Cancer Res.* **47**:4861–4868 (1987).
21. Hudson, L. G., R. Shaikh, W. A. Toscano, and W. F. Greenlee. Induction of 7-ethoxycoumarin-*O*-deethylase activity in cultured human epithelial cells by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD): evidence for TCDD receptor. *Biochem. Biophys. Res. Commun.* **115**:611–617 (1983).
22. Harper, P. A., C. L. Golas, and A. B. Okey. Characterization of the *Ah* receptor and aryl hydrocarbon hydroxylase induction by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin and benz[*a*]anthracene in the human A431 squamous cell carcinoma line. *Cancer Res.* **48**:2388–2395 (1988).
23. Gasiewicz, T. A., and R. A. Neal. 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin tissue distribution, excretion, and effects on clinical chemical parameters in guinea pigs. *Toxicol. App. Pharmacol.* **68**:131–139 (1979).
24. Cook, J. C., and E. Hodgson. The induction of cytochrome P-450 by isosafrole and related methylenedioxyphenyl compounds. *Chem.-Biol. Interact.* **54**:299–315 (1985).
25. Gasiewicz, T. A., and R. A. Neal. The examination and quantitation of tissue cytosolic receptors for 2,3,7,8-tetrachlorodibenzo-*p*-dioxin using hydroxylapatite. *Anal. Biochem.* **124**:1–11 (1982).
26. Tsui, H. W., and A. B. Okey. Rapid vertical tube rotor gradient assay for binding of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin to the *Ah* receptor. *Can. J. Physiol. Pharmacol.* **59**:927–931 (1981).
27. Martin, R. G., and B. N. Ames. A method for determining the sedimentation behavior of enzymes: application to protein mixtures. *J. Biol. Chem.* **236**:1372–1379 (1961).
28. Bradford, M. M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **72**:248–254 (1976).
29. Gudas, J. M., and O. Hankinson. Reversible inactivation of the *Ah* receptor associated with changes in intracellular ATP levels. *J. Cell Physiol.* **128**:449–456 (1986).
30. Poland, A., and E. Glover. Ca^{2+}-dependent proteolysis of the *Ah* receptor. *Arch. Biochem. Biophys.* **261**:103–111 (1988).
31. Steel, R. G., and J. H. Torrie. *Principles and Procedures of Statistics: A Biometrical Approach.* McGraw-Hill, New York (1980).
32. Scatchard, G. The attractions of proteins for small molecules and ions. *Ann. N. Y. Acad. Sci.* **51**:660–672 (1949).
33. Munson, P. J., and D. Rodbard. LIGAND: a versatile computerized approach for characterization of ligand-binding systems. *Anal. Biochem.* **107**:220–239 (1980).

Send reprint requests to: Dr. William F. Greenlee, Department of Cellular and Molecular Toxicology, Chemical Industry of Toxicology, P. O. Box 12137, Research Triangle Park, NC 27709.