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Characterization of the Hydrophobic Properties of the Receptor for 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin

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SUMMARY

The hydrophobic character of the receptor for 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) has been estimated by its tendency to adsorb to hydrophobically interacting matrices. The receptor is adsorbed to uncharged pentyl-Sepharose but not to butyl-Sepharose at 1 M NaCl. It is also adsorbed to phenyl-Sepharose or Cibacron blue-Sepharose at lower ionic strengths (0–0.15 M NaCl). Elution of adsorbed receptor could not be achieved under mild conditions (decreasing salt concentration, increasing glycerol concentration). A concentration of 0.2% (w/v) of the detergent 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate (CHAPS) was required to desorb the receptor from pentyl-Sepharose with an approximate yield of 11–14% of the specific [^{3}H]TCDD-binding activity. The CHAPS-treated receptor exhibited the same physicochemical characteristics as that in crude cytosol (4–5 S, Stokes radius ~60 Å). Furthermore, the effects of detergents other than CHAPS on hydrodynamic parameters and on [^{3}H]TCDD binding to the receptor were studied. In conclusion the TCDD receptor showed more pronounced hydrophobic properties than those reported for steroid hormone receptors.

INTRODUCTION

Several lines of evidence indicate that the biological responses produced by TCDD[1] and its congeners are mediated by their binding to an intracellular, soluble receptor protein (reviewed in Ref. 1). One interesting property of these compounds is their potency to induce an isozyme of cytochrome P-450 (usually designated cytochrome P-448 in the rat) associated with aryl hydrocarbon hydroxylase activity (2). A "two-step" model for the control of the cytochrome P-448 gene by the TCDD receptor has been postulated: subsequent to ligand-binding, the receptor undergoes a poorly understood process which results in the accumulation of the inducer-receptor complex within the cell nucleus (1), followed by the accumulation of enzyme-specific mRNA (3, 4).

Both this model for the mechanism of induction and the physicochemical properties of the TCDD receptor are strikingly similar to properties reported for mammalian steroid hormone receptors (reviewed in Refs. 5 and 6). Thus, as the steroid hormone receptors, the TCDD receptor sediments at around 4–5 S in hypertonic buffers (7–9), has a Stokes radius around 60 Å (3, 8, 10) and an apparent molecular weight around 100,000 (8), adsorbs to hydroxylapatite, and interacts with polyanions (e.g., DNA-cellulose and heparin-Sepharose) (8, 10). Competition experiments, however, have not indicated any affinity of steroid hormones for the TCDD receptor (11). Likewise, TCDD-receptor ligands lack affinity for several steroid receptors tested (8). Furthermore, one monoclonal antibody against the rat liver glucocorticoid receptor does not cross-react with the TCDD receptor (8).

Resolution of the molecular characteristics of the TCDD receptor and those of other soluble receptors is, however, a key step in relating structure to function for these regulatory proteins. Hence, experiments were performed to elucidate the hydrophobic (12) characteristics of the TCDD receptor and compare these with known properties of steroid hormone receptors.

MATERIALS AND METHODS

Chemicals. [1,6-^{3}H]TCDD (28 Ci/mmol) was a generous gift from Dr. A. Poland (Madison, WI). Unlabeled TCDF was kindly supplied by Dr. C. Rappe (Umeå, Sweden). [1-^{14}C]Glucose (45 Ci/mol) was from New England Nuclear Corp. (Boston, MA). "Single peak" pig insulin was purchased from Kabi Vitrum (Stockholm, Sweden). Polyethylene sorbitan monooleate (Tween 80), Triton X-100, 2-hydroxyethylmercaptan, activated charcoal (Norit A), cytochrome *c* (equine heart), hemoglobin (bovine), albumin (bovine serum fraction V), rabbit IgG, and catalase (bovine liver) were purchased from Sigma Chemical Co. (St. Louis, MO). A Shaltiel (13) series of alkyl-agarose (agarose-C$_n$, n = 2, 4, 6, 8, 10) was purchased from Miles-Yeda (Rehovot, Israel). Sephacryl S-300, Sephadex G-25, phenyl-Sepharose, blue Sepharose,

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[1] The abbreviations used are: TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; TCDF, 2,3,7,8-tetrachorodibenzofuran; CHAPS, 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate; CMC, critical micelle concentration; R_s, Stokes radius.

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blue dextran 2000, and dextran T-70 were from Pharmacia Fine Chemicals (Uppsala, Sweden). Uncharged butyl- and pentyl-Sepharose (14) were generously supplied by Dr. S. Påhlman (Uppsala, Sweden). CHAPS was purchased from Pierce Chemical Co. (Rockford, IL). Berol 185 (a condensation product of *n*-alcohols of the chain length C-10, C-12, C-14, and ethylene oxide) was from Berol Scandinavia (Stenungsund, Sweden). All other chemicals were analytical grade products from either Sigma or Merck AG (Darmstadt, F. R. G.).

Animals. Male Sprague-Dawley rats weighing 180–240 g were used throughout this study.

Buffers. The following buffer was routinely used: 20 mM potassium phosphate, 1 mM ethylenediaminetetraacetic acid, 2 mM 2-hydroxyethylmercaptan, 10% (w/v) glycerol, pH 7.2. Where indicated, the glycerol was omitted or increased to 50% (w/v), and, in some (indicated) cases, salt or detergents were added to the buffer.

Preparation of cytosol. Rat liver cytosol was prepared as described (8). The cytosol was quickly frozen in aliquots and stored at −70°. It has been reported that no losses of the receptor-binding capacity of [^{3}H]TCDD can be detected during storage up to 9 months (3).

Ammonium sulfate precipitation. Receptor was precipitated by the dropwise addition of a saturated buffer solution of $(NH_4)_2SO_4$ to yield a final concentration of approximately 55% saturation. After 30 min, the suspension was centrifuged at 25,000 × g_{av} for 10 min. The pellet was resuspended in buffer not containing glycerol before application to hydrophobic matrices.

Hydrophobic interaction chromatography. Unlabeled crude cytosol or $(NH_4)_2SO_4$-precipitated receptor were chromatographed on 2- to 3-ml hydrophobic matrices packed into 1.5 × 6 cm glass columns. Samples were adsorbed to the resins in the standard buffer containing 0–5% (w/v) glycerol at various ionic strengths. The columns were then washed with 3–4 column volumes of the application buffer and with buffers containing no salt, 20–50% (w/v) glycerol or various concentrations of detergents at a flow rate of approximately 8–11 ml/cm^2/hr. Pooled fractions obtained by the washing steps were then chromatographed on Sephadex G-25 equilibrated in the standard buffer to remove the washing buffer and assayed for receptor content as described below. The degree of substitution of the uncharged alkyl-agarose resins was estimated from the volume of glycidyl ether used for the gel synthesis (15).

In vitro labeling of receptor with [^{3}H]TCDD. [^{3}H]TCDD was added either to crude cytosol or to pooled fractions obtained from hydrophobic interaction chromatography. In both cases, samples were incubated for 2 hr at 0–4° with approximately 3 nM [^{3}H]TCDD in the absence or presence of a 200-fold molar excess of unlabeled TCDF dissolved in *p*-dioxan [final concentration, ≤1% (v/v)]. After incubation, unbound ligand was removed by treatment with dextran-coated charcoal (3.8 mg of charcoal and 0.38 mg of dextran T-70/ml of sample) as previously described (8). The dextran-charcoal-treated material was then analyzed for specific binding of [^{3}H]TCDD by the following procedures.

Sedimentation analysis and gel permeation chromatography. These analyses were carried out as described before (8) using linear 10–40% (w/v) sucrose gradients prepared in buffer containing or lacking 0.4 M KCl and siliconized columns of Sephacryl S-300 (1 × 115 cm), respectively.

General methods. Protein concentrations were determined by the method of Lowry *et al.* (16) with bovine serum albumin as standard. Radioactivity was measured in Scintillator 299 from Packard (Downers Grove, IL) in an LKB-Wallac 1216 Rackbeta II (Stockholm, Sweden) scintillation spectrometer with an average counting efficiency of 40%.

Safety precautions. Since TCDD and TCDF are extremely toxic compounds (1), their use necessitates special handling and disposal procedures as outlined by Poland *et al.* (11).

RESULTS

Earlier experiments have shown that free (unbound) [^{3}H]TCDD is adsorbed to the gel matrix when [^{3}H]TCDD-labeled liver cytosol is analyzed by gel permeation chromatography (8, 10). Therefore, we chose to apply the TCDD receptor to a series of hydrophobic matrices in a nonliganded state. Subsequent to chromatography, pooled fractions were labeled with radioligand and assayed for receptor binding.

In initial experiments, unlabeled liver cytosol was applied in 1 M NaCl to a Shaltiel series of alkyl derivatives attached to agarose by the cyanogen bromide method (13, 16a) (agarose-C_n, n = 2, 4, 6, 8, 10). Specific binding of [^{3}H]TCDD was only detectable in the flow-through fraction obtained from passage of cytosol over ethyl- and butyl-agarose as assayed on sucrose gradients. CNBr-activated agarose alone did not retard the receptor (not shown). Since CNBr-activated agarose gels have been shown to exhibit both electrostatic and hydrophobic interactions with proteins (16b), we chose butyl- and pentyl-Sepharose gels synthesized without introducing charged groups (14) for the further characterization of the receptor.

As noted above with the Shaltiel butyl-agarose, the TCDD receptor was not retained on uncharged butyl-Sepharose (60–70 mmol of substituent/mol of galactose) after application in 1 M NaCl (Fig. 1A). As in crude cytosol (7–9), the receptor in the flow-through fraction sedimented around 8–10 S under low salt buffer conditions (Fig. 1A), whereas it sedimented around 4–5 S in high salt buffers (not shown). Under identical experimental conditions, sucrose gradient analysis did not reveal any specific binding of [^{3}H]TCDD in the flow-through fraction of pentyl-Sepharose of a similar degree of substitution as above (Fig. 1B). If the degree of sub-

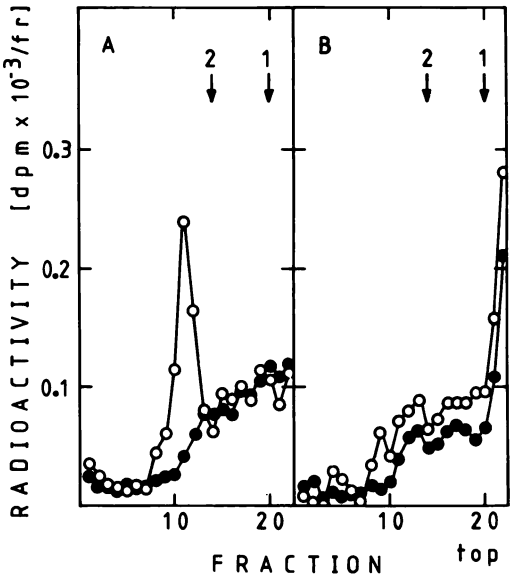

FIG. 1. *Sedimentation analysis of [^{3}H]TCDD-binding components in the flow-through fractions of uncharged butyl-(A) and pentyl (B)-Sepharose*

Liver cytosol (2 ml, ~37 mg of protein) was chromatographed on butyl- and pentyl-Sepharose in buffer containing 1 M NaCl and no glycerol. Protein-containing fractions in the flow-through were pooled, labeled with 3 nM [^{3}H]TCDD in the presence (●) or absence (○) of a 200-fold molar excess of unlabeled TCDF and analyzed for specific [^{3}H]TCDD binding by low salt linear 10–40% (w/v) sucrose gradient centrifugation as described under Materials and Methods. Sedimentation markers were: *1*, cytochrome *c* (1.7 S); and *2*, IgG (6.6 S).

stitution of the pentyl-Sepharose was lowered to approximately 30–40 mmol of substituent/mol of galactose, the receptor was still completely retained (not shown). For the following characterization of the receptor, alkyl-agarose resins of the higher degree of substitution were chosen.

The influence of the ionic strength on the interaction between the TCDD receptor and uncharged pentyl-Sepharose was studied (Fig. 2). Unlabeled cytosol was applied to the gel in buffer containing 5% (w/v) glycerol and 0, 0.5, 0.75, or 1.0 M NaCl, respectively. Comparable amounts of specific [^{3}H]TCDD-binding could be detected in the flow-through fractions if no salt (not shown) or 0.5 M NaCl (Fig. 2A) had been present in the application buffer, whereas less receptor was recovered in 0.75 M NaCl (Fig. 2B). The presence of 5% (w/v) glycerol did not disturb the adsorption of receptor in 1 M NaCl noted above (Fig. 2C). This was also the case if $(NH_4)_2SO_4$-precipitated receptor was applied to the resin at a similar ionic strength (data not shown). It was not possible to desorb the retained receptor by either decreasing the ionic strength or increasing the glycerol concentration in the washing buffer, two commonly used mild procedures for desorbing proteins from hydrophobically interacting matrices (20). After these two washes, only 2–3% of the initially applied amount of protein was still adsorbed. No gross changes in sedimentation behavior of the receptor could be detected on sucrose gradients after treatment with 1–2 M NaCl or $(NH_4)_2SO_4$ at 55% saturation (not shown).

At "low" ionic strength (0–0.15 M NaCl), the receptor was firmly adsorbed to phenyl-Sepharose and blue Sepharose. Also in this case, it was not possible to desorb the receptor by the mild conditions described above. Furthermore, we could not elute the receptor from blue Sepharose by increasing the ionic strength (0.15–1.0 M NaCl) (data not shown), a procedure known to desorb the vitamin D receptor from blue dextran Sepharose (17, 21).

The interaction of the TCDD receptor with pentyl-Sepharose and its interruption by detergents was studied. The choice of detergent was based on the ability of the detergent to preserve (i) the molecular parameters and (ii) the ligand-binding characteristics of the receptor found in crude cytosol. In line with this, the effect of three different classes of detergents on the sedimentation characteristics of the TCDD receptor was tested (Fig. 3). Only the zwitterionic detergent CHAPS preserved the sedimentation of the receptor at 8–10 S under low ionic strength conditions (Fig. 3B), whereas treatment of cytosol with sodium deoxycholate revealed a broad peak of specific [^{3}H]TCDD binding at around 11–12 S (Fig. 3B). The same effect was noted for the nonionic detergents Triton X-100 (not shown) and Tween 80.[2] Table 1 summarizes the effect of various detergents on the recovery of specific [^{3}H]TCDD-binding from detergent-treated cytosols as determined by sucrose gradient analysis, regardless of the shape and S value of the specific binding

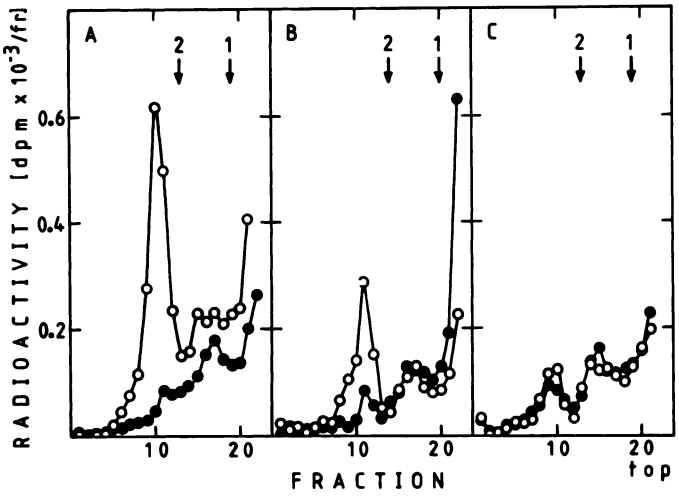

FIG. 2. *Adsorption of the TCDD receptor to pentyl-Sepharose in 0.5 (A), 0.75 (B), or 1.0 M (C) NaCl*

Liver cytosol (2 ml, ~43 mg of protein) was applied to pentyl-Sepharose in buffer containing 0.5, 0.75, or 1 M NaCl and 5% (w/v) glycerol and chromatographed as described under Materials and Methods. Protein-containing flow-through fractions were pooled, labeled with 3 nM [^{3}H]TCDD in the presence (●) or absence (○) of 600 nM unlabeled TCDF, and assayed for specific [^{3}H]TCDD binding on low salt 10–40% (w/v) sucrose gradients as described under Materials and Methods. The same sedimentation markers as in Fig. 1 were used.

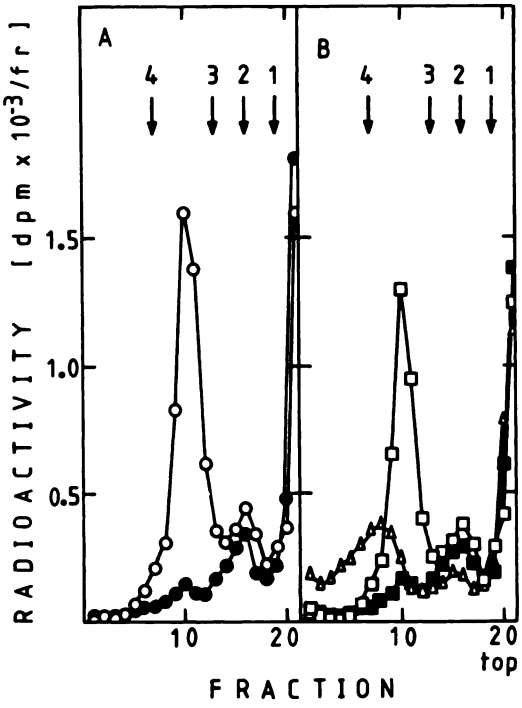

FIG. 3. *Effect of detergents on the sedimentation profile of [^{3}H] TCDD-labeled cytosol*

Liver cytosol (20–24 mg of protein/ml) was (A) left in buffer alone (○) or (B) treated with 0.2% (w/v) CHAPS (□) or 0.2% (w/v) sodium deoxycholate (△) for 1 hr and subsequently labeled with [^{3}H]TCDD and assayed on low salt 10–40% (w/v) sucrose gradients as described under Materials and Methods. Parallel incubations of receptor in the presence of a 200-fold molar excess of unlabeled TCDF were also analyzed (●, ■). The sedimentation markers were: *1*, cytochrome *c* (1.7 S); *2*, bovine serum albumin (4.4 S); *3*, IgG (6.6 S); and *4*, catalase (11.3 S).

[2] L. Poellinger, J. Lund, E. Dahlberg, and J.-Å. Gustafsson, manuscript in preparation.

TABLE 1

Effect of detergent treatment on specific binding of [^{3}H]TCDD in liver cytosol

Liver cytosol (12 mg of protein/ml) was incubated with various concentrations of the detergents or with buffer alone before labeling with 3 nM [^{3}H]TCDD in the presence or absence of a 200-fold molar excess of unlabeled TCDF. Specific binding was assayed on 10–40% (w/v) sucrose gradients as described under Materials and Methods. The effect of detergents is expressed as the percentage of specific binding in the control remaining after treatment. Each value represents the mean of duplicate determinations on sucrose gradients.

Detergent	Concentration	Specific binding
	%	% control
None	0	100
Sodium deoxycholate (w/v)	0.1	67.0
	0.2	31.2
CHAPS (w/v)	0.1	63.3
	0.2	66.5
	0.4	45.1
Berol 185 (v/v)	0.2	63.5
	0.4	35.4

peak. Treatment of cytosol with sodium deoxycholate, CHAPS, or the nonionic detergent Berol 185 at concentrations below CMCs reduced the specific binding of [^{3}H]TCDD to 63–67% of the control value. At concentrations close to the CMC values for the detergents used, only 31–45% of the control specific binding was recovered.

When unlabeled cytosol was chromatographed on pentyl-Sepharose in the presence of 1 M NaCl, approximately 95 ± 2% ($n = 7$)[3] of the applied amount of protein and no significant amounts (<5 fmol/mg of protein) of the total receptor concentration (cf. above) were recovered in the flow-through fraction. Addition of 0.2% (w/v) CHAPS resulted in a further recovery of 2–3% of the applied amount of protein (Fig. 4A). In order to avoid adsorption of receptor at this low protein concentration (~0.15–0.25 mg/ml) to the charcoal during receptor analysis, we included insulin in the fractions (final concentration, ~0.9–1.0 mg of insulin/ml) before labeling with [^{3}H]TCDD. The binding of [^{3}H]TCDD to insulin was less than 5% of the total [^{3}H]TCDD concentration, as determined by charcoal-dextran adsorption (data not shown). Analysis for [^{3}H]TCDD binding in the "insulin-stabilized" CHAPS eluate revealed receptor binding in the 8–10 S region in the absence of NaCl (Fig. 4B) or in the 4–5 S region in the presence of 0.4 M KCl (not shown). Furthermore, gel permeation chromatography of this material resulted in the total inclusion of specifically bound [^{3}H]TCDD ($R_s \cong 59 \pm 3$ Å, $n = 3$)[3] (Fig. 5). The recovery of receptor in the CHAPS elution step was 12 ± 2% ($n = 3$) of the applied receptor concentration, and the purification was 9- to 12-fold over that in crude cytosol, as estimated by sedimentation analysis. No significant further gain in purification was achieved if $(NH_4)_2SO_4$ precipitation preceded the hydrophobic interaction chromatography.

Using both the S and R_s values obtained for the CHAPS-eluted receptor, an M_r ~100,000–110,000 may

[3] Values are expressed as mean ± standard deviation of number of determinations shown in parentheses.

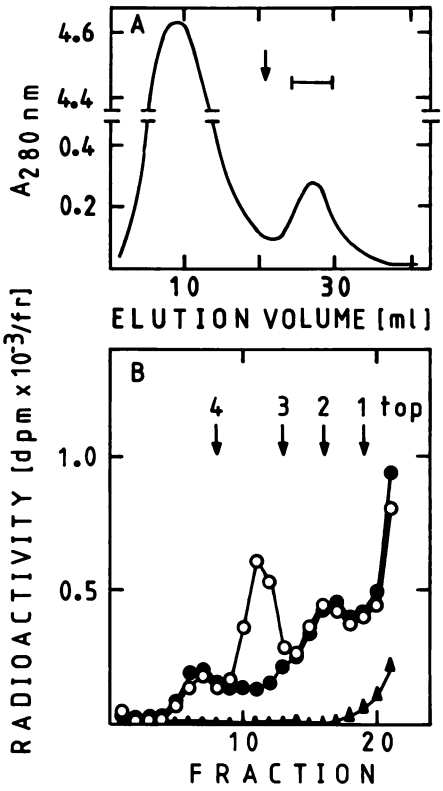

FIG. 4. *Elution of the TCDD receptor from pentyl-Sepharose by 0.2% (w/v) CHAPS*

A, liver cytosol (2 ml, 46 mg of protein, ~33 fmol of receptor/mg of protein) was applied to pentyl-Sepharose in 1 M NaCl, whereafter the column was washed with 20 ml of buffer containing 1 M NaCl and 20 ml of buffer containing 0.2% (w/v) CHAPS. The change in washing buffer is indicated by an arrow. The CHAPS-eluted material was fractioned in 1-ml portions into tubes containing ~1 mg of insulin/100 µl of buffer, pooled (pool indicated by a bar), and labeled with 3 nM [^{3}H]TCDD in the presence (●) or absence (○) of a 200-fold molar excess of unlabeled TCDF as described under Materials and Methods. B, labeled material was assayed on low salt 10–40% (w/v) sucrose gradients for specific binding of [^{3}H]TCDD as described under Materials and Methods. Binding of [^{3}H]TCDD to insulin alone (1 mg/ml) was assayed on separate gradients (▲). The same sedimentation markers were used as in Fig. 3.

be estimated according to Siegel and Monty (22): $M_r = 424 \cdot S R_s$ (assuming a partial specific volume of 0.725 cm^3/g and a solvation factor of 0.2 g of solvent/g of solute). All the determined physical properties of the receptor eluted with CHAPS were in agreement with those reported for the receptor in crude cytosol (8).

DISCUSSION

Previous studies have indicated striking similarities in physicochemical properties between mammalian steroid hormone receptors and the TCDD receptor (3, 8, 9). The present study demonstrates the presence of hydrophobic regions on the rat liver TCDD receptor by its interaction with alkyl agaroses. A chain length of C ≧ 5 appears to be sufficiently hydrophobic to retain the receptor on the resin in 1 M NaCl. The potential importance of nonpolar sites within or on soluble receptors and other steroid-binding molecules has been indicated by Wolff *et al.* (18) who showed that most of the binding energy in these

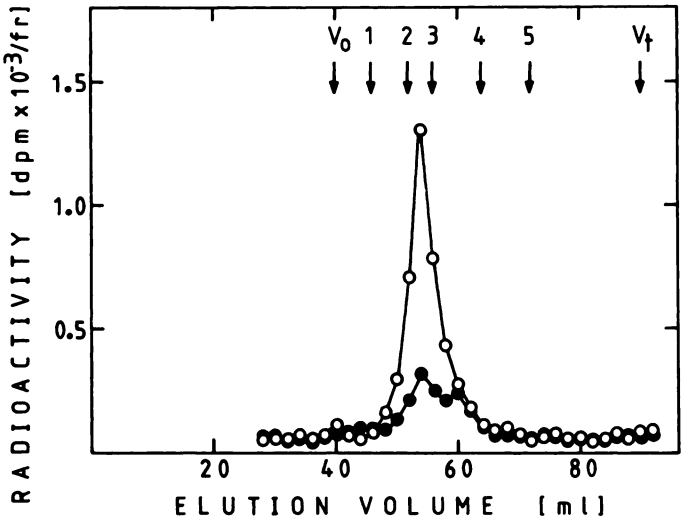

FIG. 5. *Gel permeation chromatography of the TCDD receptor eluted from pentyl-Sepharose by CHAPS*

Liver cytosol (2 ml, 46 mg of protein) was chromatographed on pentyl-Sepharose as in Fig. 4. Material eluted with 0.2% (w/v) CHAPS was labeled with 3 nM [^{3}H]TCDD in the presence (●) or absence (○) of 600 nM unlabeled TCDF. Aliquots (1 ml) were chromatographed on Sephacryl S-300 (1.0 × 115 cm) as described under Materials and Methods. Elution was carried out with buffer containing 0.02% (w/v) sodium azide and 0.15 M NaCl at a flow rate of approximately 5 ml/cm^2/hr. Fractions (2 ml) were collected and assayed for radioactivity. The columns were calibrated with blue dextran 2000 (V_0), [^{14}C]glucose (V_t), and the following standard proteins: *1*, thyroglobulin (86.1 Å); *2*, ferritin (61.5 Å); *3*, catalase (51.3 Å); bovine serum albumin (35.9 Å); and *5*, cytochrome *c* (17.9 Å).

systems results from hydrophobic interactions. It has also been reported that estradiol-binding to the estrogen receptor and the gross structure of the receptor protein are affected by chaotropic anions of the Hoffmeister series (19, 23), thus indicating the importance of water structure around a hydrophobic binding site or around the whole receptor molecule. Therefore, we chose to apply the TCDD receptor to hydrophobic matrices in the presence of ions of a mild chaotropic character (NaCl or $(NH_4)_2SO_4$), though the effect of chaotropic ions on the adsorption of proteins to hydrophobic matrices has been shown to be proportional to their chaotropicity (24).

Hydrophobic interactions have been reported for the rabbit uterine progesterone receptor (25). This receptor was retained in 0.6 M NaCl on octyl-decyl-agarose and effectively desorbed by the mild conditions described above (25). Bruchowsky *et al.* (26) have described similar results for the rat prostatic nuclear androgen receptor. The resins used (26), however, terminate in amino groups, thus superimposing ion-exchange properties upon the hydrophobic character of the resins. Both non-liganded and liganded steroid receptor forms adsorbed to and eluted from the alkyl-agaroses in a similar manner (25, 26), indicating separated, distinct sites on the receptor for the hydrophobic interaction with the matrix and ligand-binding.

When the TCDD receptor was eluted from pentyl-Sepharose by 0.2% (w/v) CHAPS, a 9–12-fold purification and a low yield of receptor were achieved. This purification yield might not only reflect the efficiency of CHAPS in desorbing the receptor from the resin, but also difficulties in labeling the partially purified receptor with ligand. Such difficulties have been described after chromatography of unlabeled steroid hormone receptors (27) and have been observed during labeling of heparin-Sepharose eluates with [^{3}H]TCDD (8). It is possible that these differences might be even more pronounced in the presence of a detergent.

Phenyl-agarose has been used for the partial purification of a specific binding protein for 3-methylcholanthrene ($M_r \cong$ 30,000–50,000) (28). It appears, however, as if 3-methylcholanthrene and TCDD bind to the same receptor protein (8, 9).[2] In view of the difficulties in desorbing the TCDD receptor from phenyl-Sepharose and the differences in M_r of the two proteins, the protein isolated by Tierney *et al.* (28) might represent nonreceptor material very similar to a binding species extensively characterized by Zytkowicz (29). In this study, Sephadex LH-20 chromatography was used to separate bound and free ligand in cytosol fractionated by $(NH_4)_2SO_4$ precipitation. It is conceivable that the TCDD receptor is adsorbed to this resin in the presence of $(NH_4)_2SO_4$, thus removing the receptor from the sample analyzed for specific binding sites for polycyclic aromatic hydrocarbons.

Although a phenyl group generally displays a hydrophobicity intermediate between the straight chain alkyls *n*-butyl and *n*-pentyl (15), respectively, the TCDD receptor interacted approximately as firmly with both phenyl- and pentyl-Sepharose. This might be due to factors other than the hydrophobicity of the attached groups only, e.g., the degree of substitution or more specific interactions (π-π interactions) with possible phenyl and tyrosyl groups of the protein.

The binding of proteins to Cibacron blue dyes has been shown to involve both electrostatic and hydrophobic interactions (30). In the case of steroid hormone receptors, an electrostatic nature in the steroid receptor-Cibacron blue interaction, possibly involving a polynucleotide-binding site, has been suggested by several authors (17, 21, 31, 32). The firm binding of the TCDD receptor to blue Sepharose might have been due to the more pronounced hydrophobic properties of the TCDD receptor, though also the TCDD receptor has been shown to interact with polyanions such as DNA (10) and heparin (8).

Though the hydrophobic properties of the TCDD receptor may be exploited to obtain a moderate purification of the receptor, hydrophobic interaction chromatography might be valuable as a step before affinity chromatography of the receptor with the aim to remove proteins interacting nonspecifically with the affinity ligand. Affinity chromatography has proven to be a powerful technique in the purification of the glucocorticoid receptor (33).

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