Carcinogen-binding Proteins in the Rat Ventral Prostate: Specific and Nonspecific High-Affinity Binding Sites for Benzo(a)pyrene, 3-Methylcholanthrene, and 2,3,7,8-Tetrachlorodibenzo-p-dioxin

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ABSTRACT

The polychlorinated dibenzodioxin [3H]-2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and the carcinogens [3H]benzo(a)pyrene and [3H]-3-methylcholanthrene bound to saturable binding sites in cytosol from the rat ventral prostate. Analysis of equilibrium binding parameters in diluted cytosol preparations indicated an apparent Kd of ~2 nM and a binding capacity of approximately 1 nmol/mg cytosolic protein, corresponding to ~5% of the total protein content. However, gel permeation chromatography analysis as well as velocity sedimentation analysis on sucrose gradients of [3H]TCDD-labeled rat prostatic cytosol indicated binding of [3H]TCDD to two discrete species. These analyses indicated a sedimentation coefficient of 3.6-3.8S, a Stokes radius of 25-28 Å, and a calculated relative molecular weight of 42,000-45,000 for the most abundant binding species. The other binding species sedimented at 4-5S under high ionic strength conditions and at 8-10S under low ionic strength conditions and had a Stokes radius of approximately 60 Å, a relative molecular weight of ~100,000, and an estimated concentration of 5-20 fmol/mg cytosolic protein. Binding of [3H]TCDD to this species was displaceable by a 200-fold m excess of 2,3,7,8-tetrachlorodibenzo-p-dioxin. Therefore, this species was tentatively identified as the TCDD receptor. The polychlorinated dibenzodioxin [3H]TCDD were found to be similar to the characteristics of a protein previously purified from the rat ventral prostate, prostatic secretory protein, which binds androgens as well as estramustine, a nitrogen mustard derivative of estradiol. The binding of estramustine to diluted prostatic cytosol was shown to be competitively inhibited by 2,3,7,8-tetrachlorodibenzo-p-dioxin. Moreover, purified prostatic secretory protein bound [3H]TCDD, 3Hbenzo(a)pyrene, as well as [3H]-3-methylcholanthrene. It is suggested that binding to this protein is responsible for the high-binding capacity of carcinogens in cytosol from the rat ventral prostate.

INTRODUCTION

As a chemical etiology of prostatic cancer recently has been suggested (1, 2), several studies have addressed the issue whether the prostatic gland possesses enzymes necessary for the metabolic activation of chemical carcinogens. In the rat ventral prostate of untreated rats, it has been shown that the cytochrome P-450-dependent microsomal enzyme activity AHH3 as well as immunodetectable amounts of the major β-naphthoflavone-inducible isozyme of cytochrome P-450, cytochrome P-450c (the form mainly responsible for AHH-activity) are very low. Following treatment with β-naphthoflavone or TCDD, an approximately 500-fold induction of cytochrome P-450c is detectable by both immunochemical techniques and AHH activity determinations (3-7). Moreover, this induction in the prostate has been shown to be accompanied by an increased capability to form mutagenic metabolites from the promutagens 2-aminofluorene and B(a)P as determined by the Ames' Salmonella/microsome assay (6).

A substantial amount of evidence indicates that the induction of cytochrome P-450c in several tissues is mediated by binding of the inducing agent to an intracellular, soluble receptor protein, which in a poorly understood process leads to an increased affinity of the ligand-receptor complex for target sites within the cell nucleus (8). TCDD is one of the most potent agonists known today for both the enzyme-induction response (9) and receptor binding (10). Other agonists include such well-known carcinogens as 3-MC and B(a)P (10). The TCDD receptor has been demonstrated in several extrahepatic tissues in both rats and mice (11, 12). Surprisingly, no TCDD receptor has been reported for the rat ventral prostate, possibly indicating a nonreceptor mediated induction process of cytochrome P-450c in this tissue. However, a very high degree of nonspecific (nonreceptor) binding of [3H]TCDD to an unidentified component in undiluted cytosol from the rat prostate has been reported (11, 12). It has also been reported that [3H]B(a)P readily interacts with components in rat prostatic cytosol (13). In this paper, we characterize the binding of various radioligands ([3H]TCDD, [3H]-3-MC, and [3H]B(a)P) to ventral prostatic cytosol and fractions thereof.

MATERIALS AND METHODS

Chemicals. [1,6-3H]TCDD (28 Ci/mmol) was a generous gift from Dr. A. Poland (Madison, WI). [G-3H]-3-MC (27 Ci/mmol) and [G-3H]B(a)P (25 Ci/mmol) were from the Radiochemical Centre, Amersham, England. [1-14C]-Glucose (8 Ci/mol) was from New England Nuclear Corp. (Boston, MA). Unlabeled TCDD was kindly supplied by Dr. C. Rappe (Umeå, Sweden). Estramustine and [2,4,6,7-3H]estramustine (107 Ci/mmol) were generously provided by Leo Pharmaceuticals (Helsingborg, Sweden). Activated charcoal (Norit A), cytochrome c (equine heart), albumin (bovine serum fraction V), rabbit IgG, and catalase (bovine liver) were purchased.

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from Sigma Chemical Co. (St. Louis, MO). Sephacryl S-300, Sephadex G-25 and G-100 fine, DEAE-Sephrose, heparin-Sepharose, blue dextran 2000, and dextran T-70 were from Pharmacia Fine Chemicals (Uppsala, Sweden). All other chemicals were analytical grade products from either Sigma or Merck AG (Darmstadt, Federal Republic of Germany).

Animals. Male Sprague-Dawley rats weighing 200-300 g were used throughout this study.

Buffer. The following buffer was routinely used: 20 mM potassium phosphate-1 mM EDTA-2 mM 2-hydroxyethylmercaptan-10% (w/v) glycerol, pH 7.2.

Preparation of Cytosol. Rats were killed by cervical dislocation and the two ventral prostate lobes were immediately excised and removed onto ice. All successive work was carried out at 0-4°C unless indicated differently. The prostates were finely minced with a pair of scissors in approximately 5-10 vol of buffer and thereafter homogenized in 2 vol of buffer in a teflon/glass Potter-Elvehjem homogenizer. The homogenate was then centrifuged at 140,000 x g for 45 min. The supernatant, hereafter termed cytosol, was removed and care was taken to avoid the floating lipid layer.

Purification of PSP. The mincing procedure of the prostatic gland resulted in release of secretory fluid from the ductules and lumina (14) which subsequently together with the cytosol was used for purification of PSP by anion-exchange and gel permeation chromatography as described by Heyns et al. (15). Purified PSP was quickly frozen in aliquots and stored at -70°C. In some cases, PSP was delipidated by precipitation in acetone (16, 17) before assessment of ligand binding.

In Vitro Labeling Conditions. For determination of TCDD receptor binding, crude cytosol or various chromatographic fractions of cytosol were labeled with 5-5 nm [3H]TCDD at the indicated protein concentrations for 2-18 h at 0-4°C, usually in the absence or presence of a 200-fold excess of unlabeled TCDF. TCDF was used instead of unlabeled TCDD to determine specific binding in view of its greater solubility in water and similar affinity for the receptor (10). Bound and free ligand were usually separated by DCC adsorption (1.9% (w/v) charcoal and 0.19% (w/v) dextran T-70, final concentrations) as previously described (18). In some cases, a hydroxylapatite-based assay for receptor binding of TCDD (19) was used to separate free as well as specifically and nonspecifically bound [3H]TCDD. PSP was labeled with the indicated concentrations of radioligand at 37°C for 30 min, whereafter DCC adsorption was performed at 0-4°C (0.1%-1% charcoal, final concentrations, as indicated). All receptor or PSP ligands were dissolved and added to the incubations in dioxane, the concentration of which never exceeded 1% (v/v).

Velocity Sedimentation Analysis. Labeled samples (200 μl) were layered onto linear 5-20% sucrose gradients in buffer (containing 0.4 M KCl in indicated cases), followed by centrifugation in an SW 60 Ti rotor in a Beckman L8-M centrifuge at 260,000-320,000 x g for 14-18 h to a preset cumulative centrifugal effect (α2) of 1.7 x 1012 rad2/s for TCDD receptor analysis on 10-40% (w/v) sucrose gradients (17) and 2.15 x 1011 rad2/s for PSP-binding analysis on 5-20% (w/v) sucrose gradients. Fractions (200 μl/fraction) were collected from the bottom of the centrifuge tubes by gravity flow. Cytochrome c (1.7S), bovine serum albumin (4.5S), γ-globulin (6.6S), and catalase (11.3S) were 4C-labeled (20) and used as sedimentation markers. Sedimentation velocities of the receptor peaks were calculated according to Martin and Ames (21).

Gel Permeation Chromatography. Analytical gel permeation chromatography on 1-x 115-cm columns of Sephacryl S-300 was performed at a flow rate of 5 ml/cm2/h as previously described (18). The columns were equilibrated in buffer containing 0.15 M NaCl and 0.02% (w/v) sodium azide. Sephadex G-100 was equilibrated in buffer containing 0.02% (w/v) sodium azide and packed into a siliconized column (1.5 x 87 cm). The flow rate was 14 ml/cm2/h and the sample volumes were approximately 1% of the total column volume. The columns were calibrated with the following standard proteins: thyroglobulin (86.1 Å); ferritin (61.5 Å); catalase (51.3 Å); bovine serum albumin (35.9 Å); and cytochrome c (17.9 Å).

Calculation of Hydrodynamic Properties. Relative molecular weights, and frictional ratios due to shape, f/f0, were calculated from the Stokes radius, Rn, and sedimentation coefficient as described by Siegel and Monty (22), assuming a partial specific volume of 0.725 cm3/g and a solvation factor of 0.2 g/g of solute (18). Axial ratios, a/b, for hydrodynamically equivalent prolate ellipsoids were derived from the values listed by Schachman (23). S and Rn values for the protein standards were obtained from the literature (24, 25).

Heparin-Sepharose Chromatography. Heparin-Sepharose was equilibrated in buffer and packed into a siliconized column (2.6 x 8 cm). Nonlabeled cytosol was applied to the column which was then washed with 2-3 column vol of buffer, whereafter retained material was eluted with 1 column vol of buffer containing 0.5 M NaCl at a flow rate of 4 ml/cm2/h.

Saturation Studies. Saturation studies were performed in siliconized glass tubes at cytosolic protein concentrations of approximately 1 μg/ml buffer (26, 27) containing 0.1% (w/v) gelatin (26). Gelatin was included in the incubation mixture in order to avoid adsorption of ligand or protein to the wall of the incubation tube. Under these conditions, up to 10 nm [3H]TCDD and approximately 25 nm [3H]BaP and [3H]-3,4,5-MC, respectively, could be dissolved. Diluted cytosol or purified PSP were incubated in 0.5-ml aliquots with varying concentrations of ligand at 37°C for 30 min. The tubes were removed onto ice, and unbound ligand was adsorbed by the addition of 0.5 ml of an ice-cold DCC suspension (1.5% (w/v) charcoal-0.05% (w/v) dextran T-70-0.1% (w/v) gelatin in buffer). The incubations were agitated and left on ice for 10 min. The charcoal was then pelleted by centrifugation at 1500 x g for 10 min, whereafter the supernatant was assayed for radioactivity. To determine the total radioactivity added, aliquots (generally, 20 μl) of the incubations were removed shortly before the addition of the DCC. Binding data were analyzed by Scatchard plots (28) or double-reciprocal plots using linear regression analysis to determine apparent equilibrium parameters of binding.

General Methods. Protein concentrations were determined using the method of Lowry et al. (29), with bovine serum albumin as the standard. Radioactivity was measured in a Packard 299 from Packard (Downers Grove, IL) in an LKB-Wallac Rackbeta II scintillation spectrometer (Stockholm, Sweden) with an average counting efficiency of 40%.

Safety Precautions. Since TCDD and TCDF are extremely toxic compounds (8), their use necessitated special handling and disposal procedures as outlined by Poland et al. (10).

RESULTS

By means of gel permeation chromatography of [3H]TCDD-labeled crude rat ventral prostatic cytosol on Sephacryl S-300 it was possible to distinguish between two discrete [3H]TCDD-binding species which both were included into the gel (Fig. 1). The first peak of radioactivity with an Rn of approximately 60 Å was nearly completely abolished by labeling of the crude cytosol in the presence of a 200-fold excess of TCDF. The 60-Å binding entity was, however, partly obscured by high levels of a major [3H]TCDD-binding species eluting in the 25- to 28-Å region of the column. Under these experimental conditions, binding of [3H]TCDD to this entity was nondisplicable (nonspecific) and disturbed the further characterization of the 60-Å [3H]TCDD-binding species. It was therefore investigated whether heparin-Sepharose chromatography of nonlabeled cytosol could remove any interfering nonspecific binding. It has previously been established that the TCDD receptor readily interacts with heparin-Sepharose both in the presence or absence of ligand (18). It was possible to label the material eluted from heparin-Sepharose with...
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Fig. 1. Gel permeation chromatography of rat prostatic cytosol labeled with [3H]TCDD. Prostatic cytosol (10 mg protein/ml) was incubated with 5 nm [3H]TCDD for 2 h at 0-4°C in the absence (o) or presence (•) of a 200-fold M excess of unlabeled TCDF. Bound and free ligand were separated by adsorption to heparin-Sepharose column and then washed with 2-3 column vol of buffer. Retained material was eluted with 1 column vol of buffer containing 0.5 M NaCl and desalted on Sephadex PD-10 columns. The eluates were incubated with 3 nm [3H]TCDD for 2 h at 0-4°C, in the absence (o) or presence (•) of a 200-fold excess of unlabeled TCDF. Bound and free ligand were separated by DCC adsorption [final concentration of charcoal, 1.9% (w/v)]. A sample volume of 1 ml was then chromatographed as described under "Materials and Methods." Fractions (2.0 ml) were collected and assayed for radioactivity. The columns were calibrated with blue dextran 2000 (V0), [14C]glucose (V), and the following standard eluates: 1, thyroglobulin (86.1 S); 2, bovine serum albumin (4.4 S); 3, IgG (6.6 S), and 4, catalase (11.3 S).

Fig. 2. Velocity sedimentation analysis of [3H]TCDD-labeled heparin-Sepharose eluates. Nontagged prostatic cytosol (5 ml; 50 mg protein) was applied to a heparin-Sepharose column and then washed with 2-3 column vol of buffer. Retained material was eluted with 1 column vol of buffer containing 0.5 M NaCl and desalted on Sephadex PD-10 columns. The eluates were incubated with 3 nm [3H]TCDD for 2 h at 0-4°C, in the absence (o) or presence (•) of a 200-fold excess of unlabeled TCDF. Bound and free ligand were separated by DCC adsorption [final concentration of charcoal, 1.9% (w/v)]. Aliquots (200 ¡l) were then layered onto 10-40% (w/v) sucrose gradients prepared in buffer (A) or in buffer containing 0.4 M KCl (B). The gradients were centrifuged and fractions were collected as described under "Materials and Methods." Sedimentation markers were: 1, cytochrome c (1.7 S); 2, bovine serum albumin (4.4 S); 3, IgG (6.6 S); 4, catalase (11.3 S); and 5, cytochrome c (17.9 S).

Table 1
Comparison of physicochemical properties of the major [3H]TCDD-binding species in rat prostatic cytosol and PSP

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Major [3H]TCDD-binding species</th>
<th>PSP</th>
</tr>
</thead>
<tbody>
<tr>
<td>S (S-1)</td>
<td>3.6-3.8</td>
<td>3.7a</td>
</tr>
<tr>
<td>Mw</td>
<td>40,000-45,000</td>
<td>38,000b-46,000c</td>
</tr>
<tr>
<td>R0 (A)</td>
<td>25-28</td>
<td>29c</td>
</tr>
<tr>
<td>l/f0</td>
<td>1.1-1.2</td>
<td>1.3c</td>
</tr>
<tr>
<td>Axial ratio</td>
<td>2-5</td>
<td>5-6d</td>
</tr>
<tr>
<td>Isoelectric point</td>
<td>4.7-5.0f</td>
<td>4.6-4.9f</td>
</tr>
</tbody>
</table>

a Heynse and De Moor (17).
b Calculated sum of the molecular weight of the different PSP subunits (39).
c Forsgren et al. (37).
d Calculated values from the sedimentation coefficient (17) and molecular weight (37) (cf. "Materials and Methods").
e Carlstedt-Duke (11).
f Heyns et al. (36).
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ical characteristics of a major secretory protein in the rat ventral prostate, often referred to as PSP (17, 34). In view of these data we examined the binding of various TCDD receptor ligands to crude cytosol as well as to purified PSP.

When crude prostatic cytosol or purified PSP was labeled with saturating concentrations of [3H]TCDD at 37°C, a rapid association of ligand could be observed, as determined by DCC adsorption. After 30 min, maximal association was reached and represented approximately 85% of the total radioactivity. Therefore, charcoal-resistant binding slowly declined, so that after 24 h approximately 10% of the maximal binding remained. At 0°C, the association rate was slow; first after 24 h, a plateau was reached both in crude cytosol and incubations using pure preparations of PSP. However, in both cases this binding represented only approximately 30–40% of the maximal binding observed at 37°C (data not shown). Analysis of the purified PSP preparations on 5–20% (w/v) sucrose gradients revealed a single symmetrical peak of radioactivity in the same region of the gradients (3.5–4S) (Fig. 3A) as the major [3H]TCDD-binding entity in crude cytosol (cf. above). The radioactivity recovered under this peak accounted for approximately 78% of the total radioactivity. It was also possible to label purified PSP with [3H]-3-MC or [3H]B(a)P, as determined by velocity sedimentation (Fig. 3B). In the case of [3H]-3-MC and [3H]B(a)P, 65 and 46% of the total radioactivity were recovered, respectively, on the sucrose gradients.

Saturation of binding of [3H]TCDD and [3H]estramustine to the major binding species was obtained only when cytosol was diluted to a protein concentration of approximately 1 µg/ml in the presence of 0.1% (w/v) gelatin. This binding was further evaluated by Scatchard analysis (28). Typical Scatchard plots (r > 0.9) were obtained for the binding of both compounds. This is in agreement with a single class of binding sites with uniform affinity for the tested ligand. Typical plots are shown in Fig. 4. Both [3H]TCDD and [3H]estramustine bound to the same maximal number of sites (~1 nmol/mg cytosolic protein; ±5% of the total cytosolic protein content), suggesting binding of the two ligands to the same site in cytosol. The apparent Kd in this particular experiment was 4.1 nM for [3H]estramustine and 1.9 nM for [3H]TCDD.

To further investigate if halogenated PAH interacted with the same class of binding sites in diluted prostatic cytosol as did [3H]estramustine, binding of various concentrations of [3H]estramustine was determined in the absence or presence of 5 or 10 nm TCDF, respectively. TCDF was chosen as a structural analog of TCDD because of its more pronounced solubility in water (10). The binding data obtained were analyzed according to Lineweaver and Burk (35) using linear regression analysis. Points, mean of duplicate determinations.

Fig. 4. Representative Scatchard plots comparing binding of [3H]estramustine and [3H]TCDD to rat ventral prostatic cytosol. Cytosol (≈1.3 µg protein/ml) was incubated in the presence of 1% (w/v) gelatin at 37°C for 30 min with increasing concentrations of [3H]estramustine, 0–10 nm (C) or [3H]TCDD, 0–5 nm (C). The incubations were terminated by the addition of 0.5 ml of an ice-cold DCC suspension yielding a final concentration of charcoal of 1% (w/v). Subsequent to DCC adsorption (see "Materials and Methods"), free ligand was calculated by subtracting bound ligand from the total concentration of ligand present in the incubation. From these data, Scatchard plots (25) were constructed by least squares linear regression analysis.

Fig. 5. Competitive binding of TCDF to [3H]estramustine-binding sites in prostatic cytosol. Cytosol (≈1.3 µg protein/ml buffer containing 0.1% gelatin) was incubated at 37°C for 30 min with 0.5–1.0 nM [3H]estramustine in the absence (C) or presence of 5 (•) or 10 (D) nm unlabeled TCDF. Specific binding was determined by DCC adsorption (final concentration of charcoal, 1% (w/v)) as described under "Materials and Methods," and the obtained data were plotted according to Lineweaver and Burk (35) using linear regression analysis. Points, mean of duplicate determinations.

total cytosolic protein content), suggesting binding of the two ligands to the same site in cytosol. The apparent Kd of this particular experiment was 4.1 nM for [3H]estramustine and 1.9 nM for [3H]TCDD.

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Fig. 3. Velocity sedimentation analysis of purified PSP labeled with [3H]TCDD (A) or [3H]-3-MC (C) and [3H]B(a)P (B). Purified PSP (≈1 mg protein/ml) was incubated with 5 nm [3H]TCDD, 5 nm [3H]-3-MC, or 5 nm [3H]B(a)P at 37°C for 30 min. Bound and free ligand were separated by DCC adsorption (final concentration of charcoal, 1% (w/v)). Aliquots (200 µl) were then layered onto 5–20% (w/v) sucrose gradients prepared in buffer. The gradients were centrifuged and fractions were collected as described under "Materials and Methods." Sedimentation markers were: 1, myoglobin (2.0S); 2, ovalbumin (3.5S); and 3, bovine serum albumin (4.4S).

Fig. 5. Competitive binding of TCDF to [3H]estramustine-binding sites in prostatic cytosol. Cytosol (≈1.3 µg protein/ml buffer containing 0.1% gelatin) was incubated at 37°C for 30 min with 0.5–1.0 nM [3H]estramustine in the absence (C) or presence of 5 (•) or 10 (D) nm unlabeled TCDF. Specific binding was determined by DCC adsorption (final concentration of charcoal, 1% (w/v)) as described under "Materials and Methods," and the obtained data were plotted according to Lineweaver and Burk (35) using linear regression analysis. Points, mean of duplicate determinations.

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Titration of diluted prostatic cytosol with [3H]-3-MC or [3H]-B(a)P revealed nonlinear, upward concave curves when apparent equilibrium binding data were analyzed according to Scatchard (28) (Fig. 6) indicating at least two classes of binding sites. Interestingly, in addition to low-affinity binding, two straight lines could be extrapolated from the concave graphs (r = 0.90 and 0.92, respectively) indicating the presence of a high-affinity binding entity with a Kd of approximately 2 nm for [3H]-3-MC and [3H] B(a)P, respectively. In addition, these lines seem to have an intercept with the abscissa in the Scatchard plots similar to the values obtained for [3H]TCDD and [3H]estradiol (cf. Fig. 4).

DISCUSSION

In the present investigation, equilibrium binding studies indicate the presence of a major, saturable, high-affinity binding site for [3H]TCDD in diluted cytosol from the rat ventral prostate at a concentration of about 1 nmol/mg of total cytosolic protein. In contrast to this observation, hydrodynamic characterization by gel permeation chromatography and sucrose gradient sedimentation analysis indicates that [3H]TCDD binding to rat prostatic cytosol is composed of at least two discrete binding species. The major fraction of bound [3H]TCDD was associated with a symmetrical molecule of 3.6-3.8S, an Rs of 25-28 A, and a molecular weight of approximately 40,000-45,000. These physicochemical characteristics agree well with the properties of PSP, a protein purified by several laboratories from the rat ventral prostate. (36-38). PSP is a tetrameric glycoprotein with an isoelectric point of about 4.6-4.9 (36) consisting of two subunits, one containing the two different polypeptides termed CI (Mr ~ 10,200) and C3 (Mr ~ 8,600) and the other containing C2 (Mr ~ 10,500) and C3 (34, 39). The carbohydrate content of PSP has been estimated to be 3.2% (36). The most prominent functional characteristic of PSP appears to be steroid binding (17, 36). Androgens induce PSP synthesis (40, 41) and bind to PSP with low affinity (Kd ~ 1 uM) (17, 34). Moreover, it is known that estradiol mustard derivatives which are used in treating prostatic carcinoma (42) bind to PSP with high affinity (Kd ~ 10-30 nm) (26, 27, 37). The biological function of PSP is presently not known.

Thus far, it has been shown that the C2 polypeptide (cf. above) can bind steroids (36, 38) and that the CI and C2 chains exhibit amino acid sequence homology with each other (34) and with rabbit uteroglobin (39), whereas the C3 chain exhibits amino acid homology with human α2-macroglobulin (43). In the present study, [3H]TCDD bound to a saturable binding species in crude cytosol with high affinity (Kd ~ 2 nm). Several lines of evidence support the contention that this binding species in fact represents PSP: (a) both [3H]estradiol and [3H]TCDD interacted with the same maximal number of binding sites in crude cytosol as determined by Scatchard plot analysis; (b) double-reciprocal plot analysis according to Lineweaver and Burk (35) showed that TCDF, a structural analogue of TCDD, competitively inhibited estradiol binding to crude cytosol; (c) the physicochemical properties of the major [3H]TCDD-binding entity in crude cytosol closely resemble those reported for PSP (cf. above); and (d) [3H] TCDD as well as [3H]B(a)P and [3H]-3-MC readily reacted with purified PSP as assayed by velocity sedimentation analysis.

Extrapolation of equilibrium binding data calculated according to Scatchard (28) indicated that both [3H]B(a)P and [3H]-3-MC bound to a similar saturable binding species in diluted cytosol as [3H]TCDD and possibly with a similar affinity (Kd ~ 2 nm). It has previously been shown that [3H]B(a)P and [3H]-3-MC interact with a 4S, high-capacity, high-affinity carcinogen-binding protein in rat liver cytosol with very similar affinities (Kd = 2.5 and 2.8 nm, respectively) (44, 45). A similar protein binding [3H]B(a)P with a Kd of 1.8 nm has recently been described in a mouse embryo cell line (46).

The mechanism of induction of cytochrome P-450c in the rat ventral prostate is intriguing. There exists an increasing amount of data on the induction of AHH activity and cytochrome P-450c in the rat ventral prostate (3-7). However, no TCDD receptor has been observed in this tissue in the rat (11, 12), whereas the receptor has been detected in mouse prostatic cytosol (12) suggesting a nonclassical nonreceptor mediated induction process of cytochrome P-450c in the rat prostate. Interestingly, analytical gel permeation chromatography of [3H]TCDD-labeled crude cytosol also indicated binding of [3H]TCDD to a discrete component in cytosol with an Rs of ~60 A. Moreover, prechromatography of unlabeled cytosol on heparin-Sepharose revealed the presence of a specific [3H]TCDD binding species which sedimented at 8-10S under low-ionic strength conditions and 4-5S under high-ionic strength conditions and which was hardly distinguishable in crude cytosol. This salt dependent sedimentation shift is characteristic of the TCDD receptor in several tissues (18, 30) and of steroid hormone receptors in general (47). Furthermore, the physicochemical characteristics of this binding species, i.e., sedimentation coefficients, Rs, and molecular weight are identical to those observed in rat liver cytosol for the TCDD receptor (18, 33). In view of these data, we believe that this particular binding species represents the TCDD receptor and that its detection in crude cytosol is severely hampered by the high abundance of high-affinity [3H]TCDD-binding to PSP. In spite of the abundance of the major [3H]TCDD-binding species, analysis of crude cytosol by gel permeation chromatography indicates the presence of the receptor. Several explanations are conceivable for this observation, e.g. (a) a subsaturating concentration of [3H]TCDD with regard to binding to the major binding

P. Söderkvist, L. Poellinger, and J.-Å. Gustafsson, unpublished results.

Fig. 6. Scatchard analysis of the binding of [3H]B(a)P and [3H]-3-MC to prostatic cytosol. Cytosol (1.3 μg protein/ml buffer containing 0.1% gelatin) was incubated at 37°C for 30 min with increasing concentrations (0-20 nm) of [3H]B(a)P (L) and [3H]-3-MC (C) before binding was assayed as described under "Materials and Methods" and in the legend to Fig. 4. Points, mean of duplicate determinations. The broken lines were extrapolated from the graphs by linear regression analysis (r = 0.90-0.92) to obtain tentative Ks for a possible high-affinity component.
species and (b) differences in affinities of the TCDD receptor and the major binding species, respectively. For instance, the rat hepatic TCDD receptor has a $K_d$ for $[^3H]TCDD$ of 0.2–0.8 nm (19). Moreover, no information is available at the present on the dissociation rates of $[^3H]TCDD$ from either the major binding species or the TCDD receptor. Gel permeation chromatography analysis on Sephacryl S-300 was carried out during approximately 24 h. Therefore, the loss of bound ligand due to dissociation and association to the gel matrix might be considerable.

The pronounced abundance of PSP in prostatic epithelial cells (520% of the total protein content in cytosol) and in seminal fluid (17) confers a potentially high binding capacity for chemical carcinogens like B(a)P and 3-MC as well as for toxic substances such as chlorinated dibenzodioxins and dibenzofurans and might cause the accumulation of these and possibly related substances in the prostate. High-capacity binding of the chlorinated hydrocarbon insecticides dieldrin and $\alpha\,\beta$-dichlorodiphenytrichloroethane to a 3.5S androgen-binding protein in cytosol from the rat ventral prostate has previously been described (48). The properties of this protein are similar to those of PSP (cf. Table 1). In vivo studies have shown that $\alpha\,\beta$-dichlorodiphenyltrichloroethane accumulates in the mouse prostate (49). A 3–4S carcinogen- and TCDD-binding protein has been observed in mouse prostate cytosol, albeit at lower concentrations as compared to in rat prostatic cytosol (12). Furthermore, in vivo uptake and secretion of 3-MC by the rat and dog prostates has been demonstrated (50). Thus, it is conceivable that PSP is of relevance for the tissue-specific accumulation and secretion of structurally related PAH.

It is interesting that a secretory protein exhibiting amino acid homology with PSP, uteroglobin (39) (cf. above), also binds steroids with a similarly low affinity as compared to PSP ($K_d \equiv 1$ nm) and polychlorinated biphenyl congeners such as 4,4′,4′′-bis((methylsulfonyl)-2,2′,5,5′-tetrachlorobiphenyl with high affinity ($K_d = 2.5–15$ nm) (51). The physiological roles of either PSP or uteroglobin or the toxicological implications of the PAH-protein interaction are presently not understood. Furthermore, it is not known if high-capacity binding of PAH by e.g. PSP is of importance for the mechanism of induction of cytochrome P-450c. However, it has been suggested that carcinogen-binding molecules in rat liver may participate in the microsomal metabolism of PAH as intracellular transport proteins (52, 53). Unfortunately, these molecules have not yet been identified or purified. It is conceivable that PSP might be functionally similar to such a molecule in rat liver. Therefore, the rat ventral prostate might present an attractive model for further studies of the mechanism of induction of cytochrome P-450c and the microsomal metabolism of PAH.

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