Biochemical Pharmacology, Vol. 43, No. 5, pp. 965–970, 1992.
Printed in Great Britain.

0006-2952/92 $5.00 + 0.00

SPECIFIC BINDING OF POLYCHLORINATED BIPHENYLS TO RAT LIVER CYTOSOL PROTEIN

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(*Received* 13 *August* 1991; *accepted* 2 *December* 1991)

Abstract—Specific binding of polychlorinated biphenyls (PCBs) to rat liver cytosol protein has been detected using the ^{3}H-labeled PCB probe 2,2',4,4',5,5'-hexachlorobiphenyl (6-CB). Binding of 6-CB to cytosol protein is displaced by its non-radioactive congener, is of high affinity ($K_d \approx 3$ nM) and is saturable (maximal binding capacity $B_{max} \approx 600$ pmol/mg protein). 6-CB binding is not found in liver cytosol of animals fed a PCB-supplemented diet (500 ppm PCB for 5 days). Binding is also *in vitro* inhibited by high concentrations of triglyceride. PCB congeners such as 3,3',4,4',5-pentachlorobiphenyl as well as the thyroid hormones 3,5,3',5'-tetraiodothyronine and 3,5,3'-triiodothyronine (the latter hormone with an order of magnitude lower affinity) compete for the PCB binding site. On the other hand, a number of biochemically important compounds including the PCB core compound biphenyl and the hormone ligands dexamethasone and estradiol, as well as 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, do not compete for the 6-CB binding site. The data provide the first evidence of specific binding of unmetabolized PCB congeners to distinct binding sites in rat liver cytosol.

The world-wide and intensive use of polychlorinated biphenyls (PCBs†) has brought about the presence of low levels of these pollutants in many kinds of human and animal tissues. Even minute amounts of such persistent chemicals can cause toxic effects in resting and in proliferating cells [1, 2]. A particularly serious role of PCB has been in the field of carcinogenesis [3], specifically in the tumor-promoting activity in rodents [4–6].

Studies on inducible receptor proteins have shown that PCBs are able to inhibit binding of TCDD to its cytosolic receptor, i.e. the *Ah* receptor [7, 8]. In particular, the higher chlorinated PCB congeners compete with TCDD for the high-affinity binding site of the *Ah* receptor [9]. Subsequently, quantitative structure–activity relationships for PCB binding were determined [10, 11].

Moreover, structure-dependent high-affinity binding of PCB congeners to the human thyroxine transport protein were derived from *in vitro* binding tests to human prealbumin [12], with the various PCB congeners having vastly differing affinities. The authors later reported on a similar thyroxine-specific binding protein in rat liver nuclear extract with an estimated molecular mass of 45 kDa [13]. A series of polychlorinated hydroxybiphenyls were shown to compete with estradiol for the mouse uterine estrogen receptor [14], yet the PCB ligand with the greatest affinity had still a 42-fold weaker binding affinity than the natural hormone.

These results were derived from competition experiments with radioactive ligands and non-radioactive PCB congeners. An alternative approach would involve a direct search for specific PCB binding proteins using ^{3}H- or ^{125}I-labeled PCB probes.

This has been done by investigating PCB binding to blood plasma proteins. ^{3}H-Labeled 3,3',4,4'-tetrachlorobiphenyl was shown to associate with transthyretin [15]. However, binding was traced to an unidentified metabolite and binding parameters were not reported.

In rat and mouse lung cytosol a cytoplasmic binding protein for the PCB metabolite 4,4'-bis(methylsulfonyl) - 2,2',5,5' - tetrachlorobiphenyl has been characterized [16]. Using a ^{3}H-labeled probe, specific saturable binding with high affinity could be demonstrated. PCB congeners with an all-chlorine substitution such as 2,2',4,4',5,5'-hexachlorobiphenyl were weak competitors (IC_{50} = 3 μM). The binding protein had an apparent molecular mass of 13 kDa [17]. Binding activity was highest in the lung (Clara cells), lower in other tissues and undetectable in the liver [17].

Recently, the iodinated tetrachlorobiphenyl [4,4'-^{125}I^2]diiodo-2,2',5,5'-tetrachlorobiphenyl was synthesized and used to directly investigate binding of this "hexahalobiphenyl" to the murine hepatic *Ah* receptor [18]. The compound was able to displace competitively TCDD from the *Ah* receptor. Additional specific binding to other cytosolic proteins was not observed.

We synthesized 6-CB to search directly for a PCB binding protein in rat liver cytosol. We present evidence for the specific binding of 6-CB to a soluble protein and report on some ligand binding properties.

MATERIALS AND METHODS

The chlorinated hydrocarbons 2,2',4,4',5,5'-hexachlorobiphenyl, 3,3',4,4',5-pentachlorobiphenyl and TCDD were obtained from Promochem (Wesel). The commercial PCB mixture Clophen A-50 was kindly provided by Dr Wrabetz (Bayer AG,

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† Abbreviations: 6-CB, ^{3}H-labeled 2,2',4,4',5,5'-hexachlorobiphenyl; PCB, polychlorinated biphenyl; HAP, hydroxyapatite; TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; thyroxine, 3,5,3',5'-tetraiodothyronine.

Leverkusen). The synthesis of 6-CB is described below. Biphenyl was obtained from Riedel-de Haën (Seelze). Triolein, dexamethasone, the thyroid hormones, and 17β-estradiol were purchased from Sigma (Taufkirchen). TLC plates (Silicagel 60 and 60 F_{254}) were from Merck (Darmstadt). HAP was obtained from Bio-Rad (München), as was the assay kit to quantitate protein. The reagents for polyacrylamide gel electrophoresis were from Pharmacia LKB (Freiburg). All other reagents were of analytical grade; all suppliers are in the F.R.G.

Synthesis of 6-CB

Synthesis of 2,2',4,4',5,5'-hexachloro-3,3'-diiodobiphenyl. The substance was synthesized according to published methods [19–21]. The start compound was 2,2',5,5'-tetrachlorobenzidine (2.6 g; TCI Chemicals, Tokyo, Japan). Diazotation and chlorine substitution yielded 2,2',4,4',5,5'-hexachlorobiphenyl (1.6 g; m.p. 101°). Treatment with nitric acid and subsequent reduction yielded 3,3'-diamino-2,2',4,4',5,5'-hexachlorobiphenyl (0.3 g; m.p. 190°). Iodine substitution of the diazonium salt of this compound (200 mg) yielded 2,2',4,4'-5,5'-hexachloro-3,3'-diiodobiphenyl (145 mg). The product was purified by preparative TLC; the R_f (*n*-hexane) = 0.60. Analytical data: m.p. 175°; mass spectrometry: m/z = 610 (100%), 575 (20%, M^+-Cl), 562 (10%, M^+-CHCl), 540 (5%, M^+-2Cl), 518 (5%, M^+-CCl2), 483 (2%, M^+-I), 448 (30%, M^+-ClJ), 356 (10%, M^+-2J); analysis for $C_{12}H_2Cl_6J_2$ (in %): C 23.7 (theoretical: 23.3); H 0.3 (0.3); Cl 34.8 (35.4); J 41.4 (41.0).

Tritium exchange. Radioactive labeling of the hexachlorodiiodobiphenyl was carried out by Amersham Buchler (Amersham, U.K.) via the iodine–tritium exchange reaction. The crude product was purified by TLC (*n*-hexane; R_f = 0.65) and was controlled for chemical and radiochemical purity. The compound was shown to be indistinguishable from 2,2',4,4',5,5'-hexachlorobiphenyl by TLC in several systems with different mobile phases, by High Performance TLC, by reversed phase HPLC and by mass spectrometry. The final specific radioactivity was calculated as being 0.8 TBq/mmol (22 Ci/mmol).

Treatment of animals

Sprague–Dawley rats (6–8 weeks old, without reference to sex) were housed in the GSF Central Animal Station. They were fed a standard lab. diet; access to food and water was *ad lib.* If required, groups of two animals were fed a standard lab. diet supplemented with 0, 0.5, 5, 50, and 500 ppm of PCB (Clophen A-50, mean chlorine content 54%) for 1 and 5 days, respectively [22].

Preparation of liver cytosol

The animals were anesthesized with ether. Livers were removed, minced, rinsed and homogenized in ice-cold KP-buffer (10 mM potassium phosphate, 5 mM $MgCl_2$, 1 mM EDTA, 1 mM dithiothreitol, 0.24 M sucrose; pH 7.5). The homogenates (approximately 4 mL/g tissue) were centrifuged at 800 *g* (10 min) to remove nuclei and cellular debris. After aspiration of the top lipid layer, the suspension was centrifuged at 105,000 *g* (60 min). Portions (2 mL) of the resultant cytosol supernatant (with an average content of 25 mg protein/mL) were stored at −80° until use. Protein was determined according to Bradford's method [23] using the "Bio-Rad" protein assay.

PCB analysis

The PCB content of cytosol samples (1–9 mL of the supernatant) was analysed by Dr J. Schmitzer (Institut für Ökologische Chemie, GSF, München, F.R.G.), according to a standardized extraction and clean-up procedure for environmental samples of chlorinated hydrocarbons [24, 25].

Ammonium sulfate fractionation

A saturated solution of $(NH_4)_2SO_4$ was slowly added at 4° to stirred samples of liver cytosol (containing typically 350 mg of protein) to a final salt concentration of 48%. After 45 min of continuous stirring, the solution was centrifuged (10,000 *g*, 20 min). The supernatant was dialysed overnight against 10 mM potassium phosphate (pH 7.5) and concentrated by ultrafiltration to a volume of about 5 mL. The yield was typically 160 mg of protein.

HAP chromatography

Cytosol samples (typically containing 8–9 mg of protein in 1.6 mL of KP-buffer) were incubated with 400,000 cpm = 10 ng (≈8 nM) of 6-CB in the absence and presence of 5 μg of the unlabeled congener. After overnight incubation at 4°, the samples were applied on HAP columns (4-mL bed volume), previously equilibrated with 10 mM of potassium phosphate buffer, pH 7.5). Proteins were eluted by means of a linear potassium phosphate gradient (10–500 mM potassium phosphate). Ten fractions/hr were collected; fraction volume was 3.5 mL. Fractions were analysed for protein and ^3H-radioactivity; development of the gradient was monitored by conductivity measurement. The two columns were operated in parallel in order to precisely localize regions of specific 6-CB binding.

Binding analysis

Routine binding analysis was carried out with a miniature technique adapted from the above protocol: 40,000 cpm = 1 ng of 6-CB were dissolved in ethanol and placed into silicon-coated glass test tubes; other ingredients (for example competing chemicals for binding) were added at this stage. After evaporation of the solvent by the gentle air stream of a hood, 0.6 mg of protein in 0.25 mL of KP-buffer were added. Non-specific binding was determined in parallel assays in the presence of a 500-fold excess of the unlabeled congener. After incubation at 4° overnight, a 50-μL sample was withdrawn to estimate the actual concentration of 6-CB. The remaining portions (0.2 mL) were loaded on small HAP columns (0.25 mL bed volume) which had been prepared in short Pasteur pipettes and equilibrated with 10 mM of KP-buffer. The columns were washed with 2 × 0.4 mL of 15 mM KP-buffer, binding activity was then recovered by one-step elution with 0.8 mL of 70 mM KP-buffer.

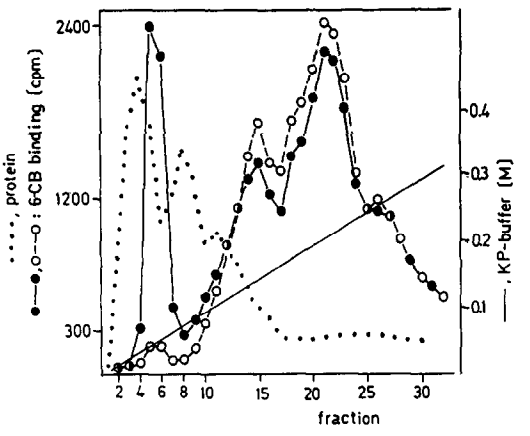

Fig. 1. Specific binding of 6-CB to rat liver cytosol protein. Rat liver cytosol (8 mg of protein in 1.6 mL of KP-buffer) was incubated with 400,000 cpm of 6-CB in the absence (●—●) and presence (○---○) of a 500-fold molar excess of non-radioactive 2,2',4,4',5,5'-hexachlorobiphenyl. Proteins were separated on two in-parallel operating HAP columns. The distribution profiles of protein (• • • •) and of 6-CB radioactivity (●——●, ○----○) are shown after elution with a linear (10–500 mM potassium phosphate) salt gradient (——).

RESULTS AND DISCUSSION

6-CB binding to cytosol protein

We used 6-CB to probe rat liver cytosol for specific high-affinity binding. Indeed, incubation and protein separation on a HAP column revealed the presence of displaceable 6-CB binding activity (Fig. 1). ^{3}H-Radioactivity is eluted by a linear salt gradient at 50–60 mM potassium phosphate. Binding is confined to a narrow range of cytosol proteins within the tailing edge of the first protein peak and is displaced in the presence of a 500-fold excess of unlabeled hexachlorobiphenyl. For comparison we labeled cytosol proteins with [^{3}H]TCDD under identical conditions and analysed TCDD binding. The *Ah* (TCDD-) receptor was eluted from the HAP column at 150–170 mM phosphate, in accordance with published results [26]. Thus, in addition to the *Ah* receptor there is a distinct cytosolic binding protein for PCB.

Effect of PCB feeding

We analysed also 6-CB binding to liver cytosol protein from animals which had been fed a PCB-supplemented diet (0.5–500 ppm of PCB). The initial portions of the 6-CB binding profiles after HAP chromatography are shown in Fig. 2. As shown in the upper panels of Fig. 2, a feeding period of 1 day was too short a time to influence specific 6-CB binding activity. However, after 5 days of feeding, the binding activity was decreased after feeding 50 ppm of PCB, and abolished after feeding 500 ppm of PCB as part of the diet (Fig. 2, lower panels).

This effect could be accounted for either by a lack of binding protein or by saturated binding sites. The former hypothesis appears unlikely, since the protein composition of all cytosols was identical, as judged from the identical protein staining pattern after SDS–PAGE. Support for the alternative hypothesis comes from analysis of the PCB content of liver cytosol extracts. Uptake of PCB was virtually linear with the PCB content in the diet, reaching concentrations of 25 μM (Table 1). This is a level sufficiently high to saturate any specific PCB binding sites in cytosol (see below). However, attempts to restore 6-CB binding by removing the PCB with dextran-coated charcoal were of only limited success: at best, 25% of the original binding activity was restored (data not shown). Thus, additional factors must contribute to 6-CB binding inhibition. For example, a diet highly supplemented with PCB is known to lead to pathological changes in rat liver ("fatty liver"): the liver cytosol of the animals is enriched with fat, the greater part in the form of droplets of triglyceride [27–29]. To test this hypothesis we carried out an *in vitro* binding experiment in the presence of lipid. As seen in Table 2, 6-CB binding to cytosol protein from untreated rats is inhibited by triolein in a concentration-dependent way.

The results suggest that 6-CB binding to cytosol protein from PCB-fed animals is for the most part blocked by PCB-induced lipid accumulation rather than by competition with PCB congeners supplied in the diet.

Competition assays

A number of compounds including aromatic organochlorine compounds and steroid hormone derivatives was assayed in binding competition experiments. The results are shown in Fig. 3. Clearly, non-radioactive 2,2',4,4',5,5'-hexachlorobiphenyl replaces effectively its radioactive congener 6-CB from the common binding site (Fig. 3, panel A), as do PCB congeners with chlorine substitution in other ring positions (such as 3,3',4,4',5-pentachlorobiphenyl). The unsubstituted parent compound biphenyl as well as the glucocorticoid derivative dexamethasone, the steroid hormone 17β-estradiol and the dioxine TCDD do not compete (Fig. 3, panel B and unpublished results). The lack of TCDD competition gives additional evidence of the different nature of the PCB binding protein and the *Ah* receptor. On the other hand, the thyroid hormones thyroxine and 3,5,3'-triiodothyronine do compete with 6-CB, thyroxine being about one order of magnitude more efficient than 3,5,3'-triiodothyronine (Fig. 3, panel B).

Some properties distinguish the PCB binding protein from the thyroxine transport protein (prealbumin, transthyretin) found in serum and liver. Our experiments show that the PCB binding protein has a charge different to that of transthyretin (as revealed by HAP ion exchange chromatography), has a lower molecular mass (about 40 kDa, not yet published), is not found in rat serum (unpublished observations) and—most importantly—has a reversed affinity for the thyroid hormones, affinity being an order of magnitude higher for thyroxine than for 3,5,3'-triiodothyronine.

Estimation of binding parameters

It is notoriously difficult to determine the saturation

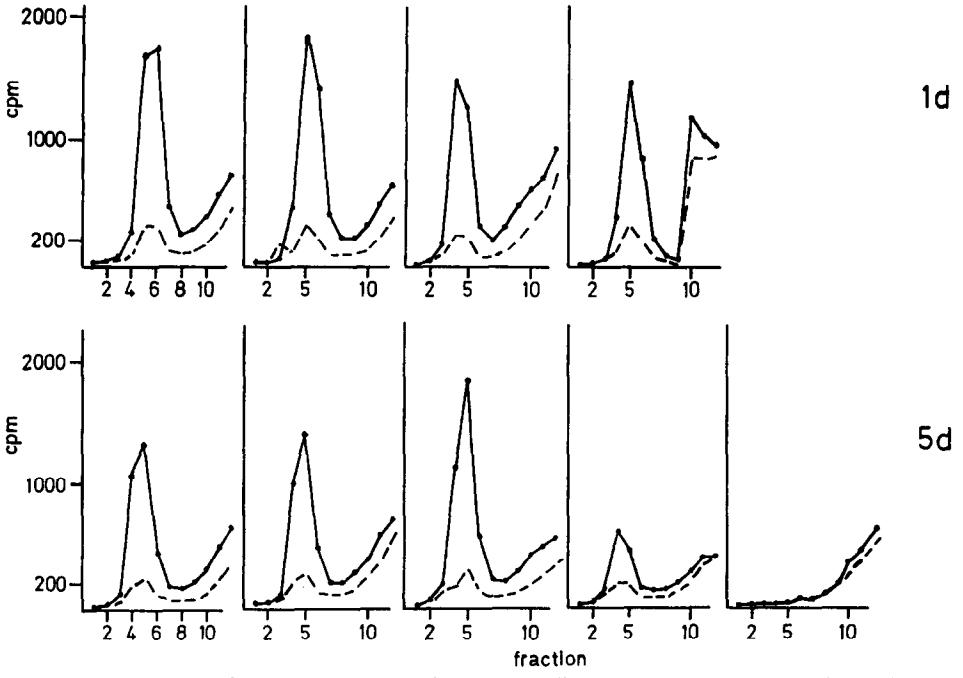

Fig. 2. Influence of PCB feeding on specific 6-CB binding. Rats were fed a standard lab. diet supplemented with 0, 0.5, 5, 50 and 500 ppm of PCB. Liver cytosol was prepared after 1 day (upper panels) and 5 days (lower panels). Specific 6-CB binding was determined as described in the legend to Fig. 1. The panels show the initial part only of the radioactivity elution profiles obtained in the absence (——) and presence (----) of the non-radioactive congener (cf. Fig. 1), as arranged from left to right for 0, 0.5, 5, 50 and 500 ppm of PCB in the diet.

Table 1. PCB content of the liver cytosol of rats having been fed a PCB-supplemented diet

PCB supplementation (μg PCB/g diet)	PCB content (μg PCB/g liver)	PCB ratio (liver:diet)
0.5	0.005	0.01
5	0.15	0.03
50	1	0.02
500	10	0.02

Animals were fed a PCB-supplemented diet for 5 days as described (see Materials and Methods). Samples (1–9 mL of cytosol supernatant) were extracted with *n*-hexane/acetone = 2/1, and the extracts filtered over short Florisil columns (0.5 mL of bed volume). Overall recovery—as checked by [^{3}H]hexachlorobiphenyl tracer—was 25%. The extracts were subjected further to a standardized clean-up procedure [24, 25] and identified by gas chromatography (Dr J. Schmitzer, Institut für Ökologische Chemie, GSF).

Table 2. Inhibition of 6-CB binding by triglyceride

Triolein (mM)	6-CB binding (pg/mg protein)
0	49
0.04	40
0.2	14
1	11

Samples of 0.6 mg of cytosol protein from untreated rats were incubated with 50,000 cpm (≈ 8 nM) of 6-CB in the presence of varying amounts of triolein (see Materials and Methods). Analysis was by means of the miniature binding technique.

binding of extremely lipophilic ligands to soluble proteins. We succeeded in getting a good estimate of 6-CB specific binding parameters by using the protein fraction of hepatic cytosol obtained after partial purification by ammonium sulfate treatment and ion exchange chromatography on HAP. Binding was analysed by means of the miniature HAP binding technique. The results of an equilibrium binding study are shown in Fig. 4. Displaceable high-affinity binding, determined as the difference between total 6-CB binding minus non-specific binding in the presence of a 500-fold excess of non-radioactive congener, approaches saturation (Fig. 4, upper panel). Replotting the data according to Scatchard's method yields the equilibrium dissociation constant $K_d \approx 3$ nM and the total number of binding sites $B_{max} \approx 600$ pg/mg protein = 2 pmol/mg protein (Fig. 4, lower panel). These parameters indicate high-affinity protein–ligand interaction.

We had noted previously rapid distribution of 6-CB into the cell nucleus of human monolayer cells [30]. Association with DNA was demonstrated by the formation of photo-induced 6-CB/DNA

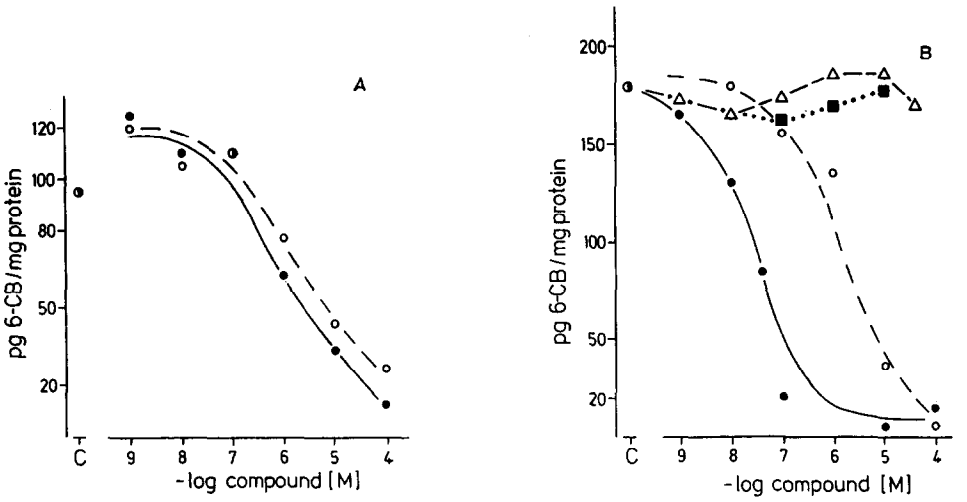

Fig. 3. Competitive binding assays. Cytosol was incubated with 6-CB in the presence of serial dilutions of a number of competitors. Binding analysis was by means of the miniature binding technique (see Materials and Methods). Panel A: 2,2',4,4',5,5'-hexachlorobiphenyl (●——●) 3,3',4,4',5-pentachlorobiphenyl (O----O). Panel B: thyroxine (●——●); 3,5,3'-triiodothyronine (O----O); biphenyl (■ • • • • ■); dexamethasone (△----△).

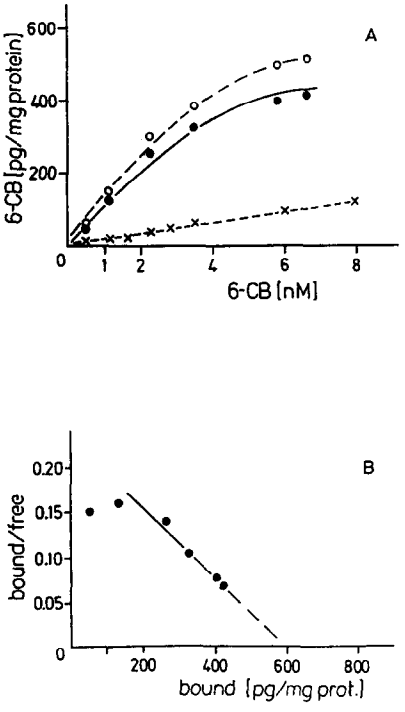

Fig. 4. Saturation binding assay. The 6-CB binding protein was partially purified by treatment with ammonium sulfate (48% saturation) and HAP chromatography. Samples (0.5 mg of protein in 0.25 mL) were incubated at 4° overnight with increasing concentrations of 6-CB. Binding activity was analysed by means of the miniature binding technique. Total 6-CB binding (O----O); non-specific binding (i.e. 6-CB bound in the presence of a 500-fold excess of non-radioactive congener) (×----×); specific 6-CB binding (●——●). Panel A: Plot of equilibrium saturation binding; panel B: Scatchard plot of specific binding.

nucleotide adducts [30]. The rapid contact with DNA following uptake suggested the possibility of a soluble cellular carrier protein being involved in the intracellular transport of PCB. However, in contrast to its presence in the cytosol, the 6-CB binding protein is not found in nuclear extracts (K. Buff and E. Schmitz, unpublished observations). Thus, a role in mediating nuclear binding (similar to that of a hormone binding protein) seems unlikely for the cytosol PCB binding protein. We are currently purifying the protein as a prerequisite to further investigation of its structure and biological function.

Acknowledgements—We acknowledge the contributions by Dr H.-S. Jenke (GSF, Abteilung für Zellchemie; animal feeding protocol), as well as by Drs E. Clausen and J. Schmitzer (GSF, Institut für Ökologische Chemie; mass spectrometry of 6-CB and PCB analysis).

REFERENCES

1. Kimbrough RD, Human health effects of polychlorinated biphenyls (PCBs) and polybrominated biphenyls (PBBs). *Annu Rev Pharmacol Toxicol* **27**: 87–11, 1987.
2. Safe S, Polychlorinated biphenyls (PCBs), dibenzo-*p*-dioxins (PCDDs), dibenzofurans (PCDFs), and related compounds: environmental and mechanistic considerations which support the development of Toxic Equivalent Factors (TEFs). *Crit Rev Toxicol* **21**: 51–88, 1990.
3. Kimbrough RD, Squire RA, Linder RE, Strandberg JD, Montali RJ and Burse VW, Induction of liver tumors in Sherman strain female rats by polychlorinated biphenyl Aroclor 1260. *J Natl Cancer Inst* **55**: 1453–1459, 1975.
4. Kimura NT, Kanematsu T and Baba T, Polychlorinated biphenyl(s) as a promotor in experimental hepatocarcinogenesis in rats. *Z Krebsforschg* **87**: 257–266, 1976.

5. Oesterle, D and Deml E, Dose-dependent promoting effect of polychlorinated biphenyls on enzyme-altered islands in livers of adult and weanling rats. *Carcinogenesis* **5**: 351–355, 1984.
6. Silberhorn EM, Glowert HP and Robertson LW, Carcinogenicity of polyhalogenated biphenyls: PCBs and PBBs. *Crit Rev Toxicol* **20**: 439–496, 1990.
7. Poland A, Glover E and Kende AS, Stereospecific, high affinity binding of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin by hepatic cytosol. *J Biol Chem* **251**: 4936–4946, 1976.
8. Okey AB, Bondy GP, Mason ME, Kahl GF, Eisen HJ, Guenthner TM and Nebert DW, Regulatory gene product of the *Ah* locus. Characterization of the cytosolic inducer–receptor complex and evidence for its nuclear translocation. *J Biol Chem* **254**: 11636–11648, 1979.
9. Bandiera S, Safe S and Okey AB, Binding of polychlorinated biphenyls classified as either phenobarbitone-, 3-methylcholanthrene- or mixed-type inducers to cytosolic *Ah* receptor. *Chem Biol Interact* **39**: 259–277, 1982.
10. Safe S, Bandiera S, Sawyer T, Robertson L, Safe L, Parkinson A, Thomas PE, Ryan DE, Reik LM, Levin W, Denomme MA and Fujita T, PCBs: structure–function relationships and mechanisms of action. *Environ Health Perspect* **60**, 47–56, 1985.
11. McKinney JD, Chae K, McConnell EE and Birnbaum LS, Structure–induction versus structure–toxicity relationships for polychlorinated biphenyls and related aromatic hydrocarbons. *Environ Health Perspect* **60**: 57–68, 1985.
12. Rickenbacher U, McKinney JD, Oatley SJ and Blake CCF, Structurally specific binding of halogenated biphenyls to thyroxine transport protein. *J Med Chem* **29**: 641–648, 1986.
13. McKinney J, Fannin R, Jordan S, Chae K, Rickenbacher U and Pedersen L, Polychlorinated biphenyls and related compound interactions with specific binding sites for thyroxine in rat liver nuclear extracts. *J Med Chem* **30**: 79–86, 1987.
14. Korach KS, Sarver P, Chae K, McLachlan JA and McKinney JD, Estrogen receptor-binding activity of polychlorinated hydroxybiphenyls: conformationally restricted structural probes. *Mol Pharmacol* **33**: 120–126, 1988.
15. Brouwer A and van den Berg KJ, Binding of a metabolite of 3,4,3′,4′-tetrachlorobiphenyl to transthyretin reduces serum vitamin A transport by inhibiting the formation of the protein complex carrying both retinol and thyroxine. *Toxicol Appl Pharmacol* **85**: 301–312, 1986.
16. Lund J, Brandt I, Poellinger L, Bergman A, Klasson-Wehler E and Gustafsson J-A, Target cells for the polychlorinated biphenyl metabolite 4,4′-bis(methylsulfonyl)-2,2′,5,5′-tetrachlorobiphenyl. Characterization of high affinity binding in rat and mouse lung cytosol. *Mol Pharmacol* **27**: 314–323, 1985.
17. Lund J, Nordlung L and Gustafsson J-A, Partial purification of a binding protein for polychlorinated biphenyls from rat lung cytosol: physicochemical and immunochemical characterization. *Biochemistry* **27**: 7895–7901, 1988.
18. Biegel L, Davis HD, Rosengren R, Safe L and Safe S, 2,2′,4,4′,5,5′-Hexachlorobiphenyl as a 2,3,7,8-tetrachlorodibenzo-*p*-dioxin antagonist in C57BL/6J mice. *Toxicol Appl Pharmacol* **97**: 561–571, 1989.
19. Safe S and Hutzinger O, The mass spectra of polychlorinated biphenyls. *J Chem Soc Perkin Transact* **I**: 686–691, 1972.
20. Lucas HJ and Kennedy ER, Iodobenzene. *Org Synth Coll* **II**: 351–352, 1943.
21. Hutzinger O, Pothier C and Safe S, Synthesis of chlorobiphenyls labeled with Deuterium and chlorine-36. *J Assoc Off Anal Chem* **55**: 753–75620, 1972.
22. Jenke H.-S, Löwel M and Berndt J, Modes of action and combination effects of polychlorinated biphenyls and hexachlorocyclohexane on the regulation of rat liver 3-hydroxy-3-methylglutaryl coenzyme A reductase. *Chem Biol Interact* **65**: 175–186, 1988.
23. Bradford MM, 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.
24. Rohleder H, Staudacher H and Sümmermann W, Hochdruck-Flüssig-Chromatographie zur Abtrennung lipophiler chlororganischer Umweltchemikalien von Triglyceriden in der Spurenanalyse. *Fresenius Z Anal Chem* **279**: 152–153, 1976.
25. Oxynos K, Schmitzer H, Gebefügi I, Dürbeck HW and Ouzounis K, Vorkommen von persistenten Organochlorverbindungen in verschiedenen Kläranlagen (Beiträge zur Umweltprobenbank). *VDI Bericht* **745**: 441–449, 1989.
26. Poellinger L, Lund J, Gillner M, Hansson L-A and Gustafsson J-A, Physicochemical characterization of specific and nonspecific polyaromatic hydrocarbon binders in rat and mouse liver cytosol. *J Biol Chem* **258**: 13535–13542, 1983.
27. Jonsson Jr. HT, Walker Jr. AM, Greene WB, Hughson MD and Hennigar GR, Effects of prolonged exposure to dietary DDT and PCB on rat liver morphology. *Arch Environ Contam Tox* **10**: 171–183, 1981.
28. Baumann M, Deml E, Schäffer E and Greim H, Effects of polychlorinated biphenyls at low dose levels in rats. *Arch Environ Contam Tox* **12**: 509–515, 1983.
29. Saito M, Polychlorinated biphenyls-induced lipid peroxidation as measured by thiobarbituric acid-reactive substances in liver subcellular fractions of rats. *Biochim Biophys Acta* **1046**: 301–308, 1990.
30. Buff K, Wegenke M and Bründl A, Photo-induced formation of DNA adducts of 2,2′,4,4′,5,5′-hexachlorobiphenyl in cultured human cells. *Biochem Pharmacol* **38**: 22773–2779, 1989.