0026-895X/94/061022-06$03.00/0

MOLECULAR PHARMACOLOGY, 46:1022-1027, 1994.

ACCELERATED COMMUNICATION

Purification to Homogeneity of the Heteromeric DNA-Binding Form of the Aryl Hydrocarbon Receptor from Rat Liver

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Received July 15, 1994; Accepted September 14, 1994

SUMMARY

The aryl hydrocarbon receptor (AhR) is a transcriptional enhancer that is activated by the binding of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and related toxic xenobiotics, as well as some naturally occurring compounds. Ligand binding inititates 1) dissociation of the ligand-bound monomeric AhR from the ligand-unoccupied multimeric complex and 2) biochemical and/or conformational changes that enable association of the ligand-bound monomer with other proteins. This heteromeric complex has high affinity for specific elements [dioxin-responsive elements (DREs)] in the regulatory regions of a number of structural genes, the induction and/or repression of which may be a mechanism of toxicity of TCDD. We have developed a relatively simple and rapid procedure that enables purification to homogeneity of a TCDD-bound receptor complex. The final step of purification is based on binding to an oligonucleotide containing the specific DRE sequence that is found in the upstream region of the *CYP1A1* structural gene. The purified complex retains *in vitro* DRE-binding function. Silver staining and Western blot analyses demonstrate that the complex consists of the AhR ligand-binding monomer of ~104 kDa, plus two proteins (94 and 96 kDa) that are recognized by antibodies prepared against the AhR nuclear translocator protein. Previous attempts to purify a DRE-binding form of the AhR were unsuccessful because of dissociation of the complex during chromatography; this is the first report of an isolated functional complex. The purified preparation will be valuable in further studies of receptor regulation and function.

The AhR is a member of a broad class of transcription factors containing a basic helix-loop-helix motif, which functions in protein-protein dimerization and sequence-specific DNA binding (1). The transcriptional activity of this protein has been shown to be regulated by its high affinity interaction with a number of xenobiotics, including the toxic and carcinogenic TCDD and related halogenated aromatic hydrocarbons (reviewed in Ref. 2). Although the exact mechanisms whereby the AhR mediates the extreme toxicity of these compounds are not clearly understood, it has been postulated that the inappropriate induction and/or repression of genes regulated by the AhR may result in an altered differentiation state in a variety of cell types. Indeed, morphological and biochemical features that are characteristic of altered cellular differentiation, rather than cell death and necrosis, appear to be the most common consequences of exposure of mammals to these toxicants (3). It is also not known whether, how, or when the activity of the AhR may be regulated in the absence of these xenobiotics.

The ligand-unoccupied AhR exists as a multimeric complex containing the 90-kDa heat shock protein and possibly other proteins (4, 5). Ligand binding to the AhR initiates a poorly understood sequence of events that ultimately result in a transcriptionally active receptor. These events include dissociation of the 90-kDa heat shock protein (6) and a conformational and/or biochemical alteration of the AhR, as evidenced by increased receptor thermostability (7, 8), decreased dissociation of bound ligand (6), and acquisition of the ability to dimerize with an additional protein (9, 10). The latter event confers recognition of *cis*-acting elements (DREs) located in regulatory regions of particular structural genes such as *CYP1A1* (11, 12), the glutathione-*S*-transferase gene (13), and the NAD(P)H:quinoline reductase gene (14).

A transcriptionally active AhR form in mouse hepatoma cells exists as a heterodimer with a protein, termed Arnt, that has sequence homology with the AhR, including a basic helix-loop-helix motif (15, 16). Although the data clearly indicate that Arnt is a functional dimerization partner with the AhR, it is possible that multiple Arnt-like proteins exist that enable the AhR to regulate a diversity of genes. Such regulatory diversification has been demonstrated for a variety of transcription

This work was supported in part by National Institutes of Health Grant ES02515 and Center Grant ES01247.

ABBREVIATIONS: AhR, aryl hydrocarbon receptor(s); N_3IBr_2DD, 2-azido-3-iodo-7,8-dibromodibenzo-*p*-dioxin; Arnt, aryl hydrocarbon receptor nuclear translocator; CYP1A1, cytochrome P4501A1; DRE, dioxin-responsive element; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; SDS, sodium dodecyl sulfate; PAGE, polyacrylamide gel electrophoresis; TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; BSA, bovine serum albumin.

factors (1, 17, 18). Recent investigations using guinea pig and mouse AhR have also provided evidence for at least two heteromeric DNA-binding forms of the receptor (19, 20). Whether these other proteins associated with the AhR are analogous or identical to Arnt remains to be determined. Nevertheless, these data suggest the existence of multiple heteromeric AhR-containing complexes, the relative abundance of which may be species and/or tissue dependent.

To further advance our understanding of the proteins, factors, and conditions necessary for the AhR to act as a transcription factor, we have explored approaches for the purification of a functional, i.e., DRE-binding, form of the AhR. Although purification of the murine AhR has been achieved under denaturing conditions (21), previous attempts to purify a functional AhR form have not been successful. Partial purification of ligand-bound receptor has recently been achieved by us[1] and others (22), but the complex lost the ability to bind to the DRE under *in vitro* conditions. This was apparently due to dissociation of the heteromeric complex during chromatographic procedures (22). In the studies reported here, we have purified to homogeneity a TCDD·AhR complex from rat liver cytosol. This complex retained DRE-binding function and was found to contain only AhR monomer and Arnt.

Experimental Procedures

Chemicals. [^{3}H]TCDD (40 Ci/mmol) was obtained from Chemsyn Science Laboratories (Lenexa, KS), unlabeled TCDD was from Cambridge Isotopes (Cambridge, MA), and [^{125}I]N_3IBr_2DD (2176 Ci/mmol) was a gift from Dr. A. Poland (University of Wisconsin, Madison, WI). [α-^{32}P]ATP (3000 Ci/mmol) was from NEN Research Products (Boston, MA). Calf thymus DNA, cyanogen bromide-activated Sepharose CL-4B, and α-lactalbumin were purchased from Sigma Chemical Co. (St. Louis, MO), and avidin-agarose was purchased from Pierce Chemicals (Rockford, IL). Protein concentrations in purified samples were measured with the Bio-Rad dye reagent using BSA as standard; otherwise, the method of Waddell (23) was used.

Buffers. HEDG buffer consisted of 25 mM HEPES, 1.5 mM EDTA, 1 mM dithiothreitol, and 10% (v/v) glycerol, pH adjusted to 7.6 at room temperature.

Animals and tissue preparation. Male Sprague-Dawley rats (250–300 g; Charles River, Wilmington, MA) were housed with a 12-hr light-dark cycle and were allowed food and water *ad libitum*. Livers were perfused with HEDG plus 1.15% KCl, and cytosol was prepared as described previously (24), with the addition of the protease inhibitors phenylmethylsulfonyl fluoride (0.3 mM), aprotinin (4 μg/ml), and leupeptin (25 μM). Cytosol was adjusted to approximately 15 mg/ml protein and was used immediately. For transformation of the AhR to a DNA-binding state, cytosol was incubated with 4–10 nM TCDD (including [^{3}H]TCDD tracer, in *p*-dioxane; 5 μl/ml) overnight (approximately 16 hr) at 4° (24, 25).

Synthetic oligodeoxyribonucleotides. The complementary oligodeoxyribonucleotides 5′-GATCCGGCTCTTCTCACGCAACTCCGAGCTCA-3′ (noncoding strand) and 5′-GATCTGAGCTCGGAGTTGCGTGAGAAGAGCCG-3′ (coding strand) were synthesized and ^{32}P-labeled at the 5′ ends using T4 polynucleotide kinase. The annealed oligonucleotide contains a single core recognition sequence (underlined) for the DNA-binding form of the AhR (11). An analogous oligonucleotide was prepared that contained 5-methylcytosine in both strands within the core recognition sequence, 5′-CAm^{5}CGCAA-3′ and 5′-TTGm^{5}CGTG-3′ (Operon Technologies, Alameda CA).

DNA-Sepharose chromatography. DNA-Sepharose was prepared from calf thymus DNA and Sepharose CL-4B as described (24). To the [^{3}H]TCDD-labeled, transformed cytosol was added a saturated ammonium sulfate solution to a final concentration of 33%, as described previously (10). Precipitated protein was pelleted at 10,000 × *g*, dissolved in HEDG buffer, and adjusted to a conductivity equivalent to approximately 0.07 M NaCl. The solution was again centrifuged briefly at 10,000 × *g* before being loaded, at 4°, onto a DNA-Sepharose column (1 × 34 cm) that had been equilibrated with HEDG plus 0.05 M NaCl. The column was washed with HEDG plus 0.14 M NaCl to remove low affinity DNA-binding proteins, and then transformed AhR was eluted with HEDG plus 0.35 M NaCl. Aliquots of fractions were analyzed for [^{3}H]TCDD by scintillation counting, to determine the position of the receptor. These fractions were generally supplemented with carrier protein (BSA or α-lactalbumin, 1 mg/ml), pooled, and frozen (−80°) until further processing. Before use, these fractions were adjusted to 0.07–0.08 M NaCl by dilution and/or desalting (PD10 column; Pharmacia-LKB). The α-lactalbumin obtained commercially was found to contain a number of contaminating proteins with molecular masses similar to that of the AhR, as determined by SDS-PAGE; therefore, it was purifed by G-75 Sephadex chromatography before use.

Avidin-agarose chromatography. The partially purified AhR obtained by DNA-Sepharose chromatography was further purified based on its specific binding to the DRE. The method used was a modification of the procedure described by Hagenbuchle and Wellauer (26). The noncoding strand (see above), biotinylated at the 5′ end, was purchased from Research Genetics (Huntsville, AL) and annealed to the coding strand. AhR-containing fractions from DNA-Sepharose chromatography were precleared of avidin-binding proteins by batch incubation with avidin-agarose (125 μl), centrifuged and filtered to remove the gel, and then incubated with biotin-DRE plus herring sperm DNA (1 μg/ml), EDTA (5 mM), and aprotinin (5 μg/ml) for 50 min at room temperature. An 8–10-fold excess of biotinylated DRE was used, based on the AhR present in each preparation (determined with [^{3}H]TCDD). After incubation, the sample was loaded (10 ml/hr, at 4°) onto a column packed with 1 ml of avidin-agarose. After washing at 20 ml/hr with HEDG plus 0.12 M NaCl, AhR was eluted with a NaCl gradient containing α-lactalbumin (0.5 mg/ml). Peak fractions were diluted to decrease the salt concentration and were then concentrated in Microsep centrifugal concentrators (Filtron Technology, Northborough, MA).

Gel retardation assay. Cytosolic protein (60–90 μg) or aliquots of the fractions from DNA-Sepharose chromatography were incubated for 20 min at room temperature with 25,000–35,000 cpm of ^{32}P-labeled DRE, at a final concentration of 0.08 M NaCl, after a 15-min equilibration with 200 or 100 ng of herring sperm DNA, respectively. Purified AhR was incubated with ^{32}P-labeled DRE without herring sperm DNA, at 0.1 M NaCl, and with 20–40 μg of BSA. In our hands, these modified conditions optimized binding to the DRE. These mixtures were then analyzed by nondenaturing gel electrophoresis and autoradiography as described previously (11).

Immunoblotting. Proteins separated by SDS-PAGE (6.8% resolving gel) were transferred to Immobilon-P membranes (Millipore, Bedford, MA) using a Semi-Phor apparatus (Hoefer, San Francisco, CA), and the membranes were blocked with 3% nonfat dry milk in 50 mM Tris, pH 7.5, 150 mM NaCl, 0.2% Tween 20. Duplicate halves of the membrane were blotted with either affinity-purified anti-AhR [prepared by Multiple Peptide Systems (San Diego, CA) against the amino-terminal peptide (27)] or anti-Arnt (kindly provided by A. Poland). Bands were visualized by means of the enhanced chemiluminescence method (Amersham).

Results and Discussion

Purification procedure. Rat hepatic cytosol contains approximately 100 fmol of AhR/mg of protein (28). Given a molecular mass of 106 kDa for the AhR in rats (29), there are 10.6 ng of AhR protein/mg of cytosolic protein. If the further assumption is made that Arnt (approximately 90 kDa) (15) is the only protein necessary to interact with the AhR to confer

[1] T. A. Gasiewicz and C. J. Elferink, unpublished observations.

DNA binding, then there are about 19.6 ng of active AhR·Arnt complex/mg of cytosolic protein. Thus, an enrichment of approximately 50,000-fold is necessary for purification of the TCDD·AhR·Arnt complex to homogeneity. In the present studies, [^{3}H]TCDD-labeled AhR contained in 90–150 ml of rat hepatic cytosol (15 mg/ml protein) was routinely partially purified by ammonium sulfate precipitation and calf thymus DNA-Sepharose chromatography (see Experimental Procedures), which we have previously reported to yield a DRE-binding form of the AhR (10). Additional methods were sought to further purify the receptor under conditions that were selective yet mild enough to retain DRE-binding activity.

Immunoaffinity purification of the AhR was attempted using a polyclonal anti-AhR antibody directed against the amino-terminal region (27). With this antibody covalently linked to Protein A-agarose (S & S, Keene, NH), [^{3}H]TCDD-labeled receptor could not be eluted with either 0.1 M glycine, pH 2.5, or 0.1 M triethylamine, pH 11, but was successfully eluted with 3–3.5 M $MgCl_2$. The eluted receptor was, however, not consistently able to bind to the DRE in the gel retardation assay, even after desalting procedures (data not shown). However, DRE binding could be reconstituted by the addition of C57BL/6-Ah^{dd} mouse hepatic cytosol, which contains defective AhR (data not shown). This suggested that, whereas ligand-bound AhR could be eluted, other factors necessary for DRE-binding had dissociated.

We additionally attempted recognition site-specific affinity chromatography similar to that described previously (30, 31), using a DRE-containing oligonucleotide coupled to Sepharose. Using a variety of binding and elution conditions, we were unable to achieve recovery of a receptor complex that maintained DRE binding.[2,3] These results are similar to those recently published by Mason *et al.* (22) and presumably indicate dissociation of the complex during chromatography. It is possible that, due to the nature of the covalent linkage of the oligonucleotide to Sepharose, a less than optimal AhR-Arnt interaction with the DRE is obtained. Therefore, we used an alternate method in which a biotinylated DRE-containing oligonucleotide was incubated with the partially purified transformed AhR before loading onto an avidin-agarose column (see Experimental Procedures). [^{3}H]TCDD-labeled AhR eluted from this column at approximately 0.3–0.35 M NaCl, using a linear gradient (Fig. 1). ^{3}H present in these fractions accounted for 40–50% of the total radioactivity eluted. From the relatively small amounts of cytosol routinely used, these purified fractions contained approximately 15% of the protein-bound [^{3}H]TCDD measured in crude cytosol using the hydroxyapatite assay (32) (Table 1); the protein concentration (assayed in preparations in which elution gradient buffer was not supplemented with α-lactalbumin) was below the limit of detection of our assay (~0.5 μg/ml). This represents a >43,000-fold purification over cytosol. Rechromatography of the flow-through fractions, with or without reincubation with biotin-DRE, resulted in <10% binding to the avidin-agarose (data not shown). This verifies that the capacity of the column was not exceeded by our conditions and that the [^{3}H]TCDD-labeled receptor in the flow-through fraction represents complexes that either never had or have lost high affinity DRE-binding capability.

[2] T. A. Gasiewicz and C. J. Elferink, unpublished observations.
[3] E. C. Henry and T. A. Gasiewicz, unpublished observations.

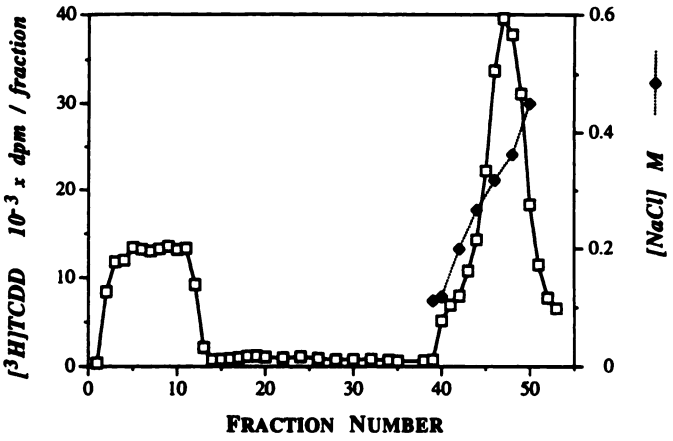

Fig. 1. Purification of AhR by avidin-agarose chromatography. Peak fractions of transformed [^{3}H]TCDD·AhR partially purified by DNA-Sepharose chromatography were incubated with biotin-DRE oligonucleotide, as described in Experimental Procedures, and then loaded onto an avidin-agarose column. After washing with 0.12 M NaCl, receptor was eluted with a NaCl gradient as shown. Fractions of 1 ml were collected throughout, and aliquots were removed for counting of ^{3}H (□) and measurement of NaCl concentrations by conductivity (♦).

TABLE 1

Purification of the DRE-binding form of the AhR

Data are averages from the two experiments in which avidin-agarose elution buffer contained no carrier protein.

Fraction	Protein	AhR recovery	Purification[a]
	%	%	*fold*
Cytosol	100	100	1
Ammonium sulfate	6	68	12
DNA-Sepharose	0.4	31	76
Avidin-agarose	≪0.03	15	>43,000[b]

[a] Recovery was estimated by quantifying by scintillation counting the amount of [^{3}H]TCDD obtained from each procedure. Estimates of recoveries and fold purification were calculated relative to crude hepatic cytosol.
[b] Calculations were based on a concentration of protein of <0.5 μg/ml, which is the limit of detection of the assay used.

Characterization of the purified receptor. Gel retardation analysis of the ^{3}H-labeled fractions from the avidin-agarose column, using the [^{32}P]DRE oligonucleotide, showed the formation of a protein-DNA complex (Fig. 2, *lane 6*) having the same mobility as that in fractions eluting from the calf thymus DNA-Sepharose column (Fig. 2, *lane 4*). The presence of a protein carrier, either α-lactalbumin or BSA, was found to be essential for maximum DRE binding of this purified receptor. Sequence specificity of DNA binding was demonstrated by competition with the [^{32}P]DRE by unlabeled DRE oligonucleotide (Fig. 2, *lanes 8* and *9*) and lack of competition by a methylated DRE (Fig. 2, *lane 7*). The slightly lower mobility of the DRE-protein complex in crude cytosol (Fig. 2, *lane 2*), compared with the more purified samples, was observed consistently and presumably reflects the presence of other proteins in the crude cytosol that may interact with the DRE·AhR complex to retard its migration.

In control experiments, vehicle (*p*-dioxane)-treated cytosol (equal volume and protein concentration as matched TCDD-treated cytosol) was carried through the ammonium sulfate precipitation, DNA-Sepharose, and biotin-DRE-avidin-agarose procedures, using columns not exposed to TCDD. Gel retardation analysis of the fractions eluting from the avidin-agarose column at the same NaCl concentration as TCDD·AhR showed

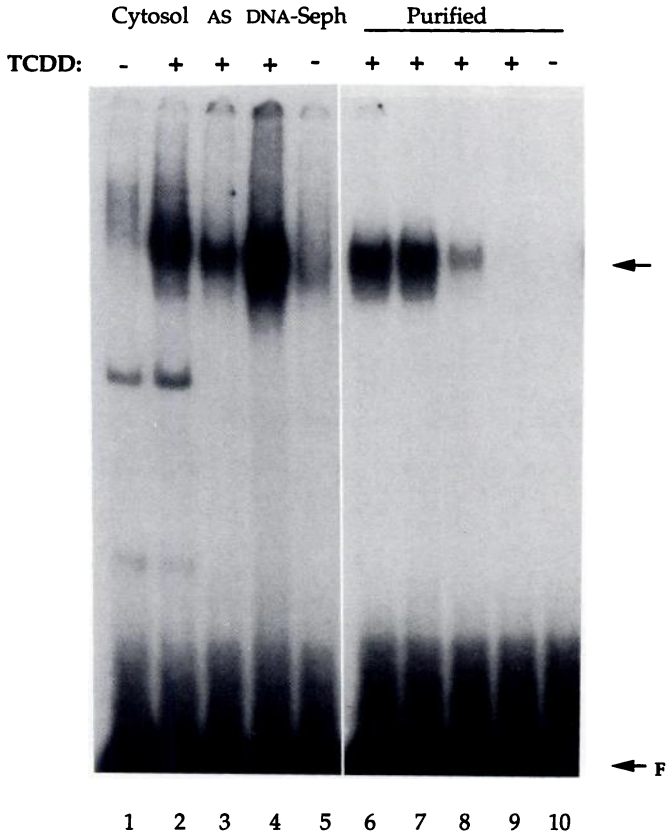

Fig. 2. Gel retardation analysis of AhR through stages of purification. *Lanes 1-6* and *10*, DRE-binding ability was assayed in crude cytosol before (*lane 1*) and after (*lane 2*) incubation with [^{3}H]TCDD, in the redissolved ammonium sulfate (*AS*) pellet of [^{3}H]TCDD-treated cytosol (*lane 3*), in peak [^{3}H]TCDD fractions eluted from DNA-Sepharose (*DNA-Seph*) (*lane 4*) and equivalent fractions from control cytosol (no TCDD) (*lane 5*), and in avidin-agarose eluates from [^{3}H]TCDD-treated (*lane 6*) and control (*lane 10*) samples. *Lanes 7-9*, 10 ng of methylated DRE (*lane 7*) or 10 ng (*lane 8*) or 100 ng (*lane 9*) of unlabeled DRE were added to the incubation of purified [^{3}H]TCDD·AhR with [^{32}P]DRE. *Arrow F*, free [^{32}P]DRE; *upper arrow*, TCDD-dependent protein-DRE complex.

no protein-DNA complex (Fig. 2, *lane 10*), indicating that the DRE complex (Fig. 2, *lane 6*) was TCDD dependent. Faint protein-DNA complexes were routinely observed in the control crude cytosol and eluates from the DNA-Sepharose columns (Fig. 2, *lanes 1* and *5*).

[^{3}H]TCDD·AhR-containing fractions from the avidin-agarose column were further analyzed by Western blotting and silver staining after separation by SDS-PAGE. Three predominant silver-stained bands, having molecular masses of approximately 104 ± 1.4, 96 ± 0.6, and 94 ± 0.6 kDa (mean ± standard deviation, six experiments), were consistently observed (Fig. 3, *lane 1*). The [^{125}I]N_3IBr_2DD photoaffinity-labeled AhR had the same molecular mass as the 104-kDa band (data not shown). Western blot analysis (Fig. 3, *lane 9*) also confirmed the 104-kDa band to be AhR. Both the 96- and 94-kDa bands were recognized by anti-Arnt antibody (Fig. 3, *lane 5*). Both of these proteins from rat liver are larger than the 87 kDa calculated for Arnt originally cloned from human cells (15), indicating interspecies variation in the molecular mass of Arnt, as also observed for AhR (29).

In control samples (without TCDD throughout the purification procedure), a silver-stained band with the same mobility as AhR (104 kDa) was consistently observed (Fig. 3, *lane 2*) but this protein was not recognized by our anti-AhR antibody (Fig. 3, *lane 10*). It is possible, however, that the silver-stained AhR band identified in TCDD-treated, purified samples (Fig. 3, *lane 1*) also contains the comigrating band observed in control preparations (Fig. 3, *lane 2*). Neither of the smaller proteins (94 and 96 kDa) observed in TCDD-labeled, purified fractions was detected in control fractions with silver stain or anti-Arnt (Fig. 3, *lanes 2* and *6*). A prominent, higher molecular mass band (108 ± 1.8 kDa) was consistently visible in purified control samples (Fig. 3, *lane 2*) and was not detected in the purified TCDD·AhR complex (Fig. 3, *lane 1*). Several additional, more faintly silver-stained bands were also visible in this particular purified control and were apparently absent in TCDD-treated samples (Fig. 3, *lane 1*). In the DNA-Sepharose fractions of control cytosol, both AhR and Arnt were recognized by our antibodies (Fig. 3, *lanes 4* and *8*), consistent with the detectable gel shift signal (Fig. 2, *lane 5*). Additionally, a second band of higher molecular mass that cross-reacted with anti-AhR was observed in control DNA-Sepharose fractions (Fig. 3, *lane 8*) but not in those fractions from TCDD-treated cytosol (Fig. 3, *lane 7*) or in purified samples (Fig. 3, *lanes 9* and *10*). Taken together, these results suggest that cytosol contains a small amount of receptor complex that can bind to calf thymus DNA-Sepharose but that TCDD is necessary for the specific DRE binding in the final step of purification. The two proteins (104 and 108 kDa) observed in purified control samples (Fig. 3, *lane 2*) apparently bind to the biotin-DRE-containing oligonucleotide during the incubation before avidin-agarose chromatography but cannot bind DRE in the gel retardation assay. The fact that these proteins are not co-purified from TCDD-treated cytosol is consistent with the possibility that they may bind loosely to the DRE oligonucleotide at sites that are inaccessible when the TCDD·AhR·Arnt complex is bound to the core DRE sequence. We have not attempted further characterization of these proteins from the untreated cytosols.

It is not known whether the 94- and 96-kDa proteins represent two isoforms of Arnt or two different Arnt-like dimerization partners of AhR that share an antigenic determinant. It is possible that the smaller one is a proteolytic product of the larger, although we consider it unlikely, because 1) both always appear at fairly equal intensities (on Western blots and with silver stain), 2) protease inhibitors were present during purification, 3) the molecular masses of the proteins are so close, and 4) no proteolysis of the AhR was apparent. Without further analysis, such as sequencing, the relationship between these two proteins cannot be determined at present. We presume that the 94- and 96-kDa proteins interact separately with the ligand-bound AhR to form DRE-binding complexes, which is consistent with the calculated molecular mass of 150–180 kDa determined by hydrodynamic analysis of transformed AhR in crude hepatic cytosol and nuclear AhR from mouse hepatoma cells (25, 33). If these proteins represent isoforms of Arnt, it will be of interest to determine whether they have differential roles in mediating the gene-specific alterations elicited by TCDD and related xenobiotic AhR ligands. It is clear from the silver-stained gels, however, that the DRE-binding complex, as purified, consists of only AhR and Arnt, with no additional high molecular mass factors; if additional components are involved, they must be small.

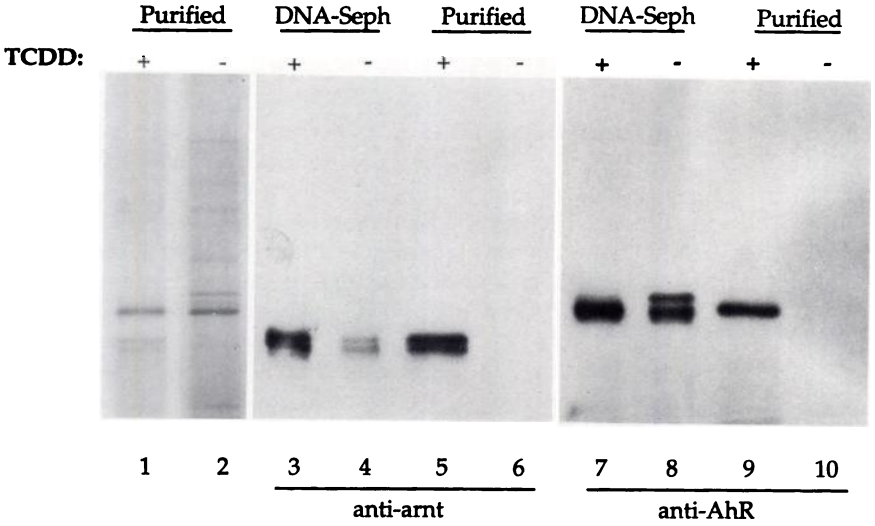

Fig. 3. Silver staining and Western blot analysis of partially purified and purified AhR. Samples from DNA-Sepharose (*DNA-Seph*) and avidin-agarose chromatography were mixed with loading buffer and separated by SDS-PAGE. *Lanes 1* and *2*, the gel was fixed and silver stained. *Lanes 3-10*, proteins were transferred to Immobilon membrane and duplicate halves of the membrane were blotted with anti-Arnt (*lanes 3-6*) or anti-AhR (*lanes 7-10*), followed by incubation with secondary antibody and detection using the enhanced chemiluminescence method (see Experimental Procedures). *Numbers at right*, molecular masses of markers (in kDa).

In summary, using a relatively simple and rapid purification procedure we have obtained a TCDD·AhR·Arnt complex that retains DRE-binding activity *in vitro* and is purified essentially to homogeneity, as judged by the lack of other bands on silver-stained gels and the >43,000-fold purification. The purified complex consists of the ligand-binding AhR monomer of ~104 kDa plus a doublet of proteins, of 96 and 94 kDa, that are both recognized by an anti-Arnt polyclonal antibody. This preparation will be useful for further analysis of molecular events involved in the transcriptional control of AhR-responsive genes. It should be pointed out that our purification protocol is based on selective binding of the AhR complex to a particular DRE sequence (upstream of *CYP1A1*); therefore, if AhR does dimerize with proteins other than Arnt to form complexes that have different DNA-binding specificities, these would be excluded during this purification.

Acknowledgments

We thank Dr. Alan Poland for the kind gifts of anti-Arnt antibody and [^{125}I]N_3IBr_2DD, as well as Matthew Mahon for his many helpful suggestions.

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