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CHARACTERIZATION OF THE ARYL HYDROCARBON RECEPTOR IN THE HUMAN C-4II CERVICAL SQUAMOUS CARCINOMA CELL LINE

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Abstract—Treatment of C-4II human cervical squamous carcinoma cells with 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) gave a concentration-dependent increase in ethoxyresorufin *O*-deethylase (EROD) activity. The EC_{50} for this response was approximately 1 nM and the maximum induced activity was 27 pmol/min/mg protein. The molecular properties of the cytosolic and nuclear aryl hydrocarbon (Ah) receptor complex were determined by velocity sedimentation analysis, photoaffinity labeling, gel retardation using a consensus dioxin responsive element (DRE), and DNA-Sepharose, DRE-Sepharose and Sephacryl S-300 gel permeation column chromatography. The apparent molecular masses of the cytosolic and nuclear photoaffinity-labeled Ah receptor complexes were 110 kDa and differed from the corresponding values obtained for the Ah receptor from other animal species. In contrast, most of the other molecular properties of the Ah receptor were not significantly different from those previously reported for other species. The relative Ah-responsiveness of the C-4II cells was assessed by determining the ratio of the induced EROD activity/nuclear Ah receptor levels for a submaximal inducing dose of [^{3}H]TCDD. The induced activity/binding ratio for the human C-4II cells was 0.77 and was at least one order of magnitude lower than the corresponding value for the Ah-responsive rat hepatoma H-4-II E cells.

The aryl hydrocarbon (Ah†) receptor protein has been widely identified in mammalian tissues [1]. Although endogenous ligands for this receptor have not been determined, the Ah receptor binds with high affinity to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and related toxic halogenated hydrocarbons, polynuclear aromatic hydrocarbons and other structurally diverse ligands [2, 3]. TCDD elicits a broad spectrum of toxic and biochemical responses and there is good evidence which supports a role for the Ah receptor in mediating these effects [3–5]. The induction of cytochrome P4501A1 (*CYP1A1*) gene expression by TCDD has been investigated thoroughly and the results indicate that the liganded nuclear Ah receptor complex acts as a transcriptional enhancer and interacts with specific *cis*-acting genomic sequences, namely dioxin-responsive elements (DREs), in the 5′-flanking region of the *CYP1A1* gene [5–7]. Unlike the steroid hormone receptors, the nuclear Ah receptor complex appears to be a heterodimer [8, 9].

The Ah receptor protein has been identified in several human tissues and cell lines, including placenta, lungs, tonsils, thymic epithelial cells, lymphoblastoid cells and in several transformed cell lines [10–17]. Research in this laboratory has focused on characterizing the Ah receptor in transformed human cell lines which can be used as models for investigating the molecular mechanisms of TCDD-induced gene transcription and the role of the Ah receptor in modulating cellular growth and differentiation [17, 18]. This study describes the characterization of the Ah receptor in C-4II human cervical squamous carcinoma cells which were originally established from an invasive squamous carcinoma of the human uterine cervix [19].

MATERIALS AND METHODS

Chemicals and biochemicals. TCDD (37 Ci/mmol) was prepared by chlorination of [1,6-$^3H_2$]dibenzo-*p*-dioxin and purified by high pressure liquid chromatography to greater than 95% purity. Unlabeled TCDD, 2,3,7,8-tetrachlorodibenzofuran (TCDF), 7-amino-2,3-dibromodibenzo-*p*-dioxin and 7-iodo-2,3-dibromodibenzo-*p*-dioxin (IDBDD) are routinely prepared in this laboratory (>98% pure by gas chromatographic analysis). [^{125}I]Sodium iodide (1800 Ci/mmol) was obtained from the Amersham Corp. Hydroxylapatite was purchased from Bio-Rad Laboratories (La Jolla, CA) ^{14}C-Labeled bovine serum albumin and catalase (prepared in this laboratory) were used as external standards for determination of sedimentation coefficients (*S* values). [γ-^{32}P]ATP (3000 Ci/mmol) was obtained from New England Nuclear (Boston, MA). All other chemicals and biochemicals used in these studies were the highest quality available from

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† Abbreviations: Ah, aryl hydrocarbon; DMSO, dimethyl sulfoxide; DRE, dioxin-responsive element; EROD, ethoxyresorufin *O*-deethylase; IDBDD, 7-iodo-2,3-dibromodibenzo-*p*-dioxin; SDS–PAGE, sodium dodecyl sulfate–polyacrylamide gel electrophoresis; TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; and TCDF, 2,3,7,8-tetrachlorodibenzofuran.

commercial sources. [^{125}I]DBDD was synthesized directly from 7-amino-2,3-dibromodibenzo-*p*-dioxin as described [18].

Cell growth and formation of nuclear receptor complexes with [^{3}H]*TCDD or* [^{125}I]*DBDD.* The C-4II human cell line was obtained from the American Type Culture Collection (Rockville, MD). Cells were grown in minimum essential medium and supplemented with 5% fetal bovine serum plus 10 mL antibiotic–antimycotic solution (Sigma), 1 μM sodium pyruvate, 1 g glucose, and 6 μg insulin per liter. Cells were grown in 150-cm^2 culture flasks in an air:carbon dioxide (95:5) atmosphere at 37°. After reaching confluency, the cultures were trypsinized, washed once with used culture medium, and resuspended in this medium in 25-cm^2 flasks at a concentration of 3×10^6 cells/mL (final volume 10 mL). Radioligands, [^{3}H]TCDD (5 nM) and [^{125}I]-DBDD (0.4 nM), in dimethyl sulfoxide (DMSO) were added to the cell culture flasks so that the final concentration of DMSO in the culture medium was 0.5%. Nuclear extract baselines were obtained by cotreatment with a 200-fold excess of TCDF. The flasks were incubated by gentle shaking for 2 hr at 37°. After incubation, the suspended cells were dcanted into 50-mL centrifuge tubes and centrifuged at 1000 *g*. This and all subsequent procedures were performed at 4°.

Preparation of nuclear extract and cytosol. Harvested cells were washed twice in 30 mL of HEGD buffer (25 mM HEPES, 1.5 mM EDTA, 1 mM dithiothreitol, and 10% glycerol, pH 7.6). The washed cell pellet was resuspended in 1 mL of HED buffer (HEGD buffer without the glycerol) and incubated for 10 min. After incubation, the suspended cells were transferred to a 2-mL homogenizing tube with an additional 1 mL HEGD buffer added, and homogenized using a Teflon pestle/drill apparatus. The homogenate was transferred to a centrifuge tube, 10 mL of HEGD buffer was added, and the mixture was centrifuged at 4000 *g* for 10 min. The resulting pellet was washed twice with 30 mL of HEGD buffer. The pellet was then resuspended in 3 mL of HEGD buffer containing 0.5 M potassium chloride (pH 8.5) and allowed to stand at 4° for 1 hr. Nuclei prepared by this method were found to be intact and appeared to be greater than 90% free of extranuclear cellular component contamination, as determined by microscopic examination. The cytosolic fraction was obtained from the initial centrifugation of the cellular homogenate using HEGDM buffer (HEGD buffer plus 20 μM sodium molybdate).

Sucrose density gradient analysis. Unbound and loosely bound [^{3}H]TCDD was removed by adding dextran-coated charcoal (1 mL of 0.01:0.001% charcoal:dextran per mL of nuclear extract pelleted from HEG buffer) to the nuclear extracts. The dextran-coated charcoal was resuspended using a Vortex mixer, and the sample was incubated for 10 min at 4° before the dextran-coated charcoal was removed by centrifugation at 4000 *g* for 10 min at 4°. Aliquots (300 μL) of sample were layered on linear sucrose gradients (5% to 25%) prepared in HEG buffer containing 0.4 M potassium chloride. Gradients were centrifuged at 4° for 2.5 hr at 404,000 *g*. After centrifugation, 30 fractions were collected from each gradient, and radioactivity in each fraction was determined by liquid scintillation counting. Analysis of the cytosolic receptor was similar to that described above except that the high salt was omitted from the sucrose gradients.

In vitro *photoaffinity labeling studies* [18]. Nuclear extracts (1 mL, 0.5 mg protein/mL) containing the radioligand-bound Ah receptor complex were placed in quartz ampoules (dimensions: 0.6 (i.d.) × 24 cm]. The samples were positioned 1.5 to 2.0 cm from the light source and irradiated for 5 min. The light source was a 450-W medium pressure Hanovia mercury arc lamp filtered by a pyrex water-cooled (4°) immersion well. The pyrex immersion well effectively filtered the light source so that the samples were irradiated at wavelengths of > 300 nm. The irradiated samples were removed after appropriate times and stored at 4° until processed.

Quantitation of photocovalent adduct formation. Aliquots of the irradiated nuclear extract (50 μL) were placed on individual 2-cm Whatman GF/A glass-fiber filters and air-dried at room temperature. The filters were placed into 40 mL of ice-cold 10% (w/v) trichloroacetic acid for 30 min at 4°, removed, and placed into 40 mL of ice-cold methanol for 30 min at 4°. The last step was repeated, and the filters were removed from methanol and allowed to dry at 20°. The radioactivity of the dried filters was determined by liquid scintillation counting.

Separation of covalently-labeled receptor by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE). The irradiated nuclear extract (1 mL, 0.5 mg protein/mL) was treated with 10% (w/v) cold trichloroacetic acid (final concentration 7%) overnight at 4° and centrifuged at 4000 *g* for 10 min. The protein pellet was washed three times with cold methanol to remove residual trichloroacetic acid. The dried samples were dissolved in 100 μL of distilled water plus 2 μL sodium hydroxide (2 N); then 30 μL 5 × Laemmli's sample buffer was added. The samples were incubated for 5 min at 100°, loaded onto a 7.5% polyacrylamide slab gel (14 × 9 × 0.15 cm), and electrophoresed for 5 hr at 25 mA at 18°, utilizing the same upper and lower buffer (25 mM Tris base, 190 mM glycine and 3.5 mM SDS, pH 8.3). After electrophoresis, the gel was fixed in a solution containing 7% (v/v) glacial acetic acid and 50% (v/v) methanol. The fixed gel was transferred to the filter paper and dried under vacuum at 80°. The dried gel was autoradiographed by loading onto X-AR Omat X-ray film and stored at −80° and developed after 1–2 weeks.

Ethoxyresofurin O-deethylase (EROD) activity. EROD was basically assayed as described by Pohl and Fouts [20] with some modifications. Trypsinized cells were plated into 25-cm^2 tissue culture flasks (10^5 cells/mL), allowed to grow until approximately 60% confluency, and treated with the appropriate concentration of TCDD for 24 hr. Cells were harvested by manual scraping from the culture flasks, centrifuged at 1000 *g* for 5 min (4°), and resuspended in 100 μL Tris–sucrose buffer (38 mM Tris–HCl, 0.2 M sucrose, pH 8.0). Aliquots of the cells suspension (50 μL) were incubated with 1.15 mL cofactor solution (containing 1 mg bovine serum

albumin, 0.7 mg NADH, 0.7 mg NADPH, and 1.5 mg $MgSO_4$ in 0.1 M HEPES buffer, pH 7.5) in a 37° water bath for 2 min. The reaction was started by adding 50 μL ethoxyresorufin solution (1 mg/40 mL) for a 15-min incubation and stopped by adding 2.5 mL methanol. Samples were centrifuged at 1000 *g* for 10 min. The supernatant was used for fluorescence measurement at an excitation wavelength of 550 nm, and an emission wavelength of 585 nm.

Gel permeation chromatography of the Ah receptor complex on sephacryl S-300. Sephacryl S-300 gel was equilibrated with HEGD buffer containing 0.4 M KCl and packed into a siliconized glass column (1.5 × 100 cm). The column was calibrated with standard proteins (Rs values): thyroglobulin (8.5 nm), apoferritin (6.1 nm), catalase (5.2 nm), alcohol dehydrogenase (4.7 nm), bovine serum albumin (3.6 nm), ovalbumin (2.7 nm) and cytochrome *c*(1.7 nm). Blue dextran was used as an indicator of the void volume. Two milliliters of nuclear extract (treated with dextran-coated charcoal) was applied to the column, which was eluted by gravity at the rate of 8 mL/hr; 1 mL per fraction was collected and radioactivity was determined by scintillation counting.

Chromatography of nuclear Ah receptor complexes on DNA Sepharose. The preparation of the DNA-Sepharose column in this laboratory was carried out as previously described [17]. Two milliliters of nuclear extract was diluted to 10 mL with HEGD buffer and applied to the DNA-Sepharose column and eluted with 40 mL of HEGD buffer to remove unbound complexes. The column was subsequently eluted with a 100 mL linear gradient consisting of 0 to 0.8 M sodium chloride in HEGD buffer. The elution rate was 10 mL/hr and 1.5-mL fractions were collected. Alqiuots from each fraction were measured for radioactivity and salt concentrations as determined by conductivity measurements. The column was reused after further elution with 5 vol. of 0.8 M sodium chloride in HEGD buffer and then equilibrated in HEGD buffer (pH 7.6).

Chromatography of the nuclear Ah receptor complexes on DRE Sepharose. Complementary strands of the synthetic oligonucleotide containing the sequence 5′-GATCTGGCTCTTCTCACG - CAACTCCG-3′ were synthesized, purified by polyacrylamide gel electrophoresis and annealed as described [21]. The oligonucleotide was labeled at the 5′ end using T4-polynucleotide kinase and [γ-^{32}P]ATP [22], and the DNA oligomers were covalently attached to CNBr-activated Sepharose 4B by a slight modification of the method reported by Kadonaga and Tjian [23]. The DNA (100 μL in water) was added to the swollen gel and the reaction slurry shaken at 20° for 16 hr with 0.1 M *N*-ethyl - *N*′ - (3 - dimethylaminopropyl)carbodiimide hydrochloride. The DRE-Sepharose coupling product was then washed and packed as previously described [17]. The columns were equilibrated with HEGD buffer (pH 7.6) containing 40 mM EDTA. The procedure of analysis and elution of the nuclear Ah receptor complex on the DRE-Sepharose column was the same as described above for the DNA-Sepharose column.

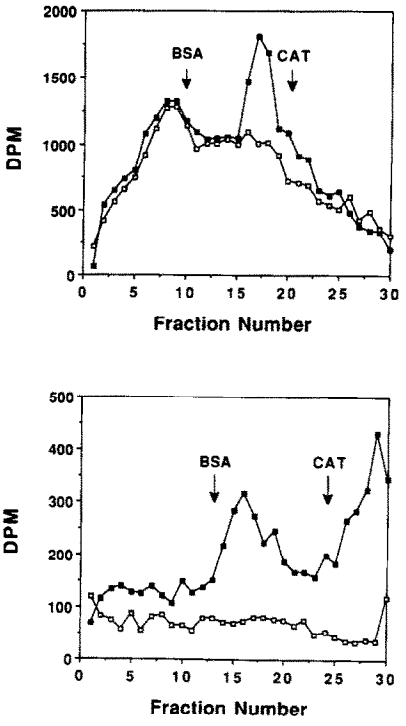

Fig. 1. Sucrose density gradient elution profiles of the cytosolic (top) and nuclear Ah receptor complexes from the human cervical C-4II carcinoma cell line. The cells were treated with 5 nM [^{3}H]TCDD (■) or 5 nM [^{3}H]TCDD plus 1 μM unlabeled TCDF (□) for 2 hr; the cytosolic and nuclear fractions were isolated and analyzed by velocity sedimentation on sucrose gradients as described in Materials and Methods. The position of the specifically-bound Ah receptor complex was determined by using ^{14}C-labeled catalase (CAT) and bovine serum albumin (BSA) as internal standards [17, 18].

Gel retardation analysis. Complementary strands of the synthetic ^{32}P-labeled DRE was also used for the gel retardation assay [17, 21]. Samples (5 μL) of nuclear extract (2 mg protein/mL) from the control (DMSO) and TCDD-treated cells were incubated in HEGD buffer with 200 ng poly(dI-C) for 15 min at 20° to bind nonspecific DNA-binding proteins. Following the addition of ^{32}P-labeled-DRE, the mixture was incubated for a further 15 min at 20°, loaded onto a 4% polyacrylamide gel (acrylamide : bisacrylamide, 30 : 0.8) and run at 110 V in 0.09 M Tris borate and 0.002 M EDTA, pH 8.0. Gels were dried and protein-DNA binding was visualized by autoradiography.

RESULTS

The nuclear and cytosolic Ah receptor complexes were obtained from cell suspensions that were incubated in the presence of the radioligands for 2 hr. The procedure has been used previously in studies with rodent and human cell lines [18] since relatively high intracellular yields of both cytosolic and nuclear receptor complexes are obtained. Similar

Table 1. Molecular properties of the Ah receptor complexes from human C-4II cervical squamous carcinoma cells by sucrose density gradient analysis and Sephacryl S-300 gel permeation chromatography*

Parameter	Nuclear Ah receptor	Cytosolic Ah receptor
Concentration (fmol/mg protein)	26 ± 0.6	74 ± 8.4
Sedimentation coefficients ($S_{20,w}$)	6.5 ± 0.4	8.9 ± 0.3
Stokes radii (nm)	6.8 ± 0.20	7.5 ± 0.09
Relative molecular mass (M_r)	182,030 ± 5,460	275,475 ± 3,379
Frictional ratio (f/f_o) shape	1.8 ± 0.05	1.7 ± 0.01
Axial ratio (a/b) prolate ellipsoid	16.0 ± 0.48	14.4 ± 0.15

* Results are expressed as means ± SD for at least three separate determinations; the techniques used to determine these data are outlined in Materials and Methods.

results (but lower overall yields) were obtained when the attached cells were incubated with the radioligands. In addition, the suspended cells retained their Ah-responsiveness for up to 24 hr (unpublished observations). The results in Fig. 1 illustrate the sucrose density gradient elution profile of the cytosolic and nuclear Ah receptor complexes from C-4II cells. The specifically-bound nuclear (6.5 *S*) and cytosolic (8.9 *S*) peaks were observed; and additional specifically-bound peak (fractions 25–30) of unknown origin was also detected (Fig. 1, bottom). The overall cellular concentrations of the specifically-bound Ah receptor complexes were determined by incubating the cells with 5 nM [^{3}H]-TCDD for 2 hr followed by isolation of the cytosolic and nuclear fractions. Velocity sedimentation analysis of these fractions showed that the levels were 26 ± 0.6 (nuclear) and 74 ± 8.4 (cytosolic) fmol/mg protein. Both the cytosolic and nuclear Ah receptor complexes from the C-4II cells were chromatographed using Sephacryl S-300 gel permeation chromatography and the results from the chromatographic and velocity sedimentation analysis were used to determine some of the molecular properties of the Ah receptor complexes (Table 1). The sedimentation coefficients, Stokes radii, relative molecular masses (M_r), frictional (f/f_o) and axial ratios for the nuclear and cytosolic Ah receptor complexes were 6.5 ± 0.4 and 8.9 ± 0.3 *S*; 6.8 ± 0.20 and 7.5 ± 0.09 nm; 182,030 ± 5,460 and 275,475 ± 3,379; M_r 1.8 ± 0.05 and 1.7 ± 0.01 f/f_o and 16.0 ± 0.48 and 14.4 ± 0.15 a/b respectively.

The apparent molecular masses of the nuclear and Ah receptor binding protein from the C-4II cells were also determined by photoaffinity labeling using [^{125}I]DBDD as the photolabile high affinity ligand. After photolysis the photoaffinity-labeled complexes were analyzed by denaturing SDS–PAGE, and the results (Fig. 2) illustrate that the apparent molecular mass of the nuclear and cytosolic Ah receptor protein was 110 kDa.

The results in Fig. 3 summarize elution patterns for the nuclear Ah receptor complex from DNA-Sepharose and DRE-Sepharose columns. Two specifically-bound peaks were eluted from the DNA-Sepharose columns at salt concentrations of 0.31 and 0.40 M; salt concentrations of 0.37 and 0.43 M

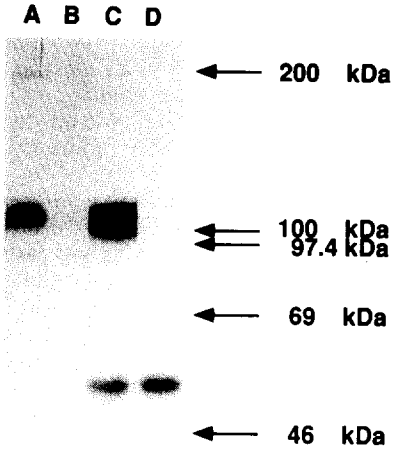

Fig. 2. SDS–PAGE separation of photoaffinity labeled nuclear (lane A) and cytosolic (lane C) Ah receptor from C-4II cells. The cells were treated with [^{125}I]DBDD and nuclear and cytosolic fractions were photolyzed, separated by SDS–PAGE, and visualized by autoradiography as described [18]. Lanes B and D are derived from cells treated with [^{125}I]DBDD and a 200-fold excess of TCDF. The molecular weight markers used in this study were myosin (200 kDa), phosphorylase *b* (97 and 100 kDa), bovine serum albumin (69 kDa) and ovalbumin (46 kDa).

were required for elution of the two peaks from the DRE-Sepharose column. In addition, the binding of the nuclear Ah receptor complex to a synthetic oligonucleotide DRE was also determined using a gel retardation assay. Incubation of nuclear extracts from C-4II cells treated with 10 nM TCDD and ^{32}P-labeled DRE gave a retarded band in the gel shift assay (see Fig. 4), whereas this band was reduced significantly in intensity using nuclear extracts from untreated cells. The Ah-responsiveness of these cells was determined by the concentration-dependent induction of EROD activity by TCDD. The results (Fig. 5) clearly demonstrate that the EROD activity in solvent (DMSO)-treated cells was non-detectable; the maximum induced enzyme activity was 27 pmol/

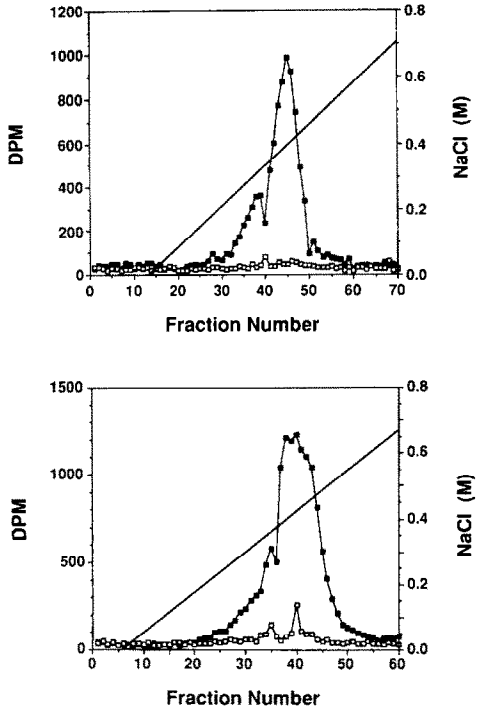

Fig. 3. DNA-Sepharose (top) and DRE-Sepharose chromatography of the nuclear Ah receptor complex from C-4II cells. Nuclear extracts from cells treated with 5 nM [^{3}H]TCDD (■, total binding) and 5 nM [^{3}H]TCDD plus 1 μM TCDD (□, nonspecific binding) were eluted from the columns using salt gradients as described in Materials and Methods.

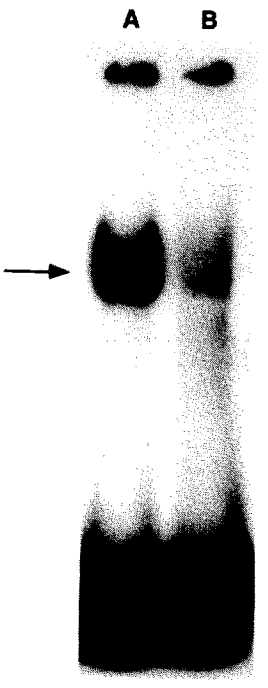

Fig. 4. Gel retardation of the DRE and nuclear extracts from C-4II cells treated with 5 nM TCDD (lane A) or DMSO (solvent) (lane B). The nuclear extracts were incubated with ^{32}P-labeled DRE, separated by gel electrophoresis, and visualized by autoradiography as described in Materials and Methods. The relative mobility of the retarded DRE–Ah receptor complex from C-4II cells was similar to results previously reported for transformed human Hep G2 cells [18].

min/mg protein with an EC_{50} value of approximately 1 nM for this induction response.

DISCUSSION

The biochemical and toxic potency of TCDD in laboratory animals or mammalian cells in culture is highly variable and dependent on the sex, age, species and genetic background of target cells or organs [2–5]. For example, TCDD causes a dose-dependent increase in hepatocellular carcinomas in female but not male Sprague–Dawley rats [24]; TCDD causes chloracne in some animal species (e.g. hairless mice, rabbits, humans and monkeys) but not others (most strains of mice, rats and guinea pibs). The reason for the interspecies- and strain-dependent differences in TCDD-responsiveness is unknown.

The induction of *CYP1A1* gene expression is a multistep process which includes the following sequence of events [5–7]: (i) initial binding of TCDD to the unoccupied cytosolic Ah receptor; (ii) transformation of the liganded cytosolic receptor complex into a form with increased DNA binding properties; (iii) cytosol-nuclear translocation of the transformed complex; and (iv) interaction of this complex with specific *cis*-acting genomic binding sites (DREs). The Ah-responsiveness of a specific tissue may also be dependent on the number of DREs in the 5′-flanking region of the *CYP1A1* gene and the presence of other factors which may inhibit or increase gene transcription [25, 26]. The results of several studies have suggested that in some target cells, the molecular properties of the Ah receptor may be indicative of intraspecies Ah-responsiveness. For example, the decreased Ah-responsiveness of mutant cells derived from the wild-type mouse Hepa 1c1c7 cell line has been associated with either decreased Ah receptor levels or a defect in the formation of the transformed cytosolic Ah receptor complex [27–29] which is the presursor for the transcriptionally active nuclear heterodimer.

Poland and coworkers have examined some of the inter- and intraspecies differences in the molecular properties of the Ah receptor using photoaffinity labeling and receptor binding assays [30–32] and have demonstrated that at least four allelic forms of the murine Ah receptor could be distinguished by their thermolability and apparent molecular masses [30]. The interstrain differences in the affinities of TCDD for the Ah receptor were not readily determined due to the different thermolabilities of the Ah receptor. Moreover, the thermolability of the Ah receptor coupled with high levels of non-specific binding can give K_D values (determined by traditional Scatchard analysis) which are inaccurate and do not reflect the true ligand–receptor affinities

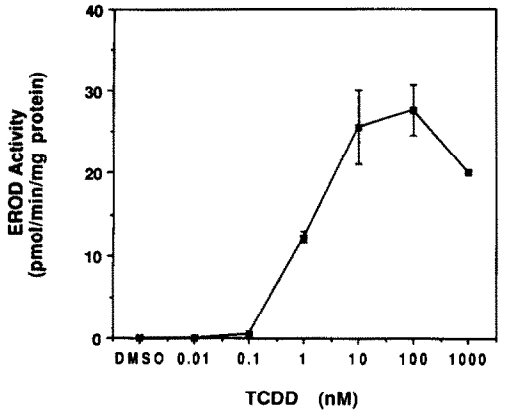

Fig. 5. Concentration-dependent induction of EROD activity in C-4II cells by TCDD. The results are expressed as means ± SD for at least three determinations at each concentration. The constitutive EROD activity in this cell line was not detectable.

[33, 34]. Not surprisingly, the apparent K_D values for ligand–Ah receptor binding in human lymphoblastoid cells [14], human placenta [10] and human tonsils [12] were also highly variable (4.6 to 5.8 nM, 5 to 8 nM and 3 to 12 nM, respectively). However, it is conceivable, that some of the other molecular properties of the Ah receptor may be useful as indicators of Ah-responsiveness.

This study reports the characteristics of the Ah receptor and the Ah-responsiveness of the C-4II human cervical carcinoma cell line [19] and the results will form the basis for developing approaches to assess the possible differences in Ah-responsiveness of human cell lines to toxic halogenated and polynuclear aromatic hydrocarbons.

The results in Table 1 and Fig. 1 summarize several properties of both the cytosolic and nuclear Ah receptor complexes. The sedimentation coefficients for the nuclear and cytosolic Ah receptor complex (i.e. 6.5 and 8.9 *S*, respectively) were similar to the values obtained using comparable experimental conditions for the Ah receptor from other human cell lines, rodent cell lines and rodent liver [1]. Moreover, the Stokes radii, relative molecular masses, and frictional and axial ratios for the nuclear and cytosolic Ah receptor from the human C-4II cells and from rat and mouse liver cytosol were also similar [35, 36]. For example, Denison and coworkers [35] have reported that the sedimentation coefficients, Stokes radii, relative molecular mass, and frictional and axial ratios for the cytosolic Ah receptor from rat and mouse liver cytosol were 8.8 to 9.4 *S*; 7.0 to 7.1 nm; 257,000 to 277,000 M_r 1.7 f/f and 12 to 12.4 a/b, respectively. The only major difference between these data and the results for the human C-4II cells was the higher axial ratio value (14.4) for the human cells. However, in previous studies in this laboratory [36], the axial ratio values obtained for rat and mouse cytosol (13.0 and 14.2, respectively) were similar to the data obtained in this study. Thus, the molecular properties of the C-4II human Ah receptor which are summarized in Table 1 do not distinguish significantly between the human and rodent Ah receptor complexes.

Photoaffinity labeling of the Ah receptor with the high affinity ligand, [^{125}I]DBDD, followed by denaturing SDS–PAGE gave a specifically-labeled 110 kDa band for both the cytosolic and nuclear receptor. These data suggest that there is a common nuclear and cytosolic ligand binding subunit and comparable results have been reported for the Ah receptor from the Hepa 1c1c9 mouse hepatoma cell line [37]. The apparent molecular mass of the photoaffinity-labeled human Ah receptor observed in this study (110 kDa) is similar to results from previous studies from this laboratory on the human Hep G2 cell Ah receptor [18] but somewhat different from results previously reported in HeLa cells (106 kDa) [32]; however, the variation in human forms of the Ah receptor binding protein and the significance of this variability require further study using identical assay and photoaffinity labeling conditions.

The properties of the nuclear Ah receptor from the C-4II cells were also investigated by chromatography in DNA-Sepharose and DRE-Sepharose columns (Fig. 3) and by the gel retardation assay (Fig. 4). The specifically-bound nuclear Ah receptor was eluted as a major and minor peak from both columns at salt concentrations between 0.31 and 0.43 M and these properties were similar to those reported for other human and rodent receptor preparations [17]. The results of the gel shift assay (Fig. 4) with nuclear extracts from cells treated with 10 nM TCDD gave a [^{32}P]DRE–protein complex which exhibited a mobility that was indistinguishable from those observed with nuclear receptor complexes from transformed rodent cell lines [18]. Thus, the results from this study indicate that the molecular properties of the Ah receptor complex from the human C-4II cells and from other species and cell lines are similar with the exception of the apparent molecular masses of the Ah receptor ligand binding subunit as determined from photoaffinity labeling studies [31–33].

The results summarized in Fig. 5 illustrate that TCDD elicits a concentration-dependent induction of EROD activity with an EC_{50} value of approximately 1 nM and a maximum induced activity of approximately 27 pmol/min/mg protein. These values suggest that the C-4II cells are significantly less responsive to TCDD than H-4-II E cells which have been investigated previously in this laboratory [38]. For example, in the rodent cell line maximal induction of EROD activity was observed at a concentration of 1 nM with an EC_{50} value of 0.084 nM [38]. Moreover, at a concentration of 1 nM, the TCDD-induced EROD activity was 286 ± 11.4 pmol/min/mg protein in the H-4-II E cell line. A more direct comparison of the Ah responsiveness of the two cell lines can be assessed by comparing the ratio of the induced EROD activity/fmol receptor mg^{-1} (induced activity/binding ratio) at submaximal or near-maximal inducing doses of TCDD. In the

human C-4II cells treated with 5 nM [^{3}H]TCDD, the estimated EROD activity was 20 pmol/min/mg protein (from Fig. 5) and the corresponding level of nuclear Ah receptor was 26 fmol/mg protein. Thus, the induced activity/binding ratio was 0.77. In contrast, the ratio in rat hepatoma H-4-II E cells treated with 1 nM [^{3}H]TCDD was 14.3 which was approximately 20-fold higher than in the human cell line. The induced activity/binding ratio is a measure of the activity of the nuclear Ah receptor as an inducer of P4501A1 activity, and provides a quantitative measure of the Ah-responsiveness of different cell lines. Another approach would be to determine the induced activity/binding ratios for several cell lines at a specific concentration of TCDD (e.g. 1 nM) which results in submaximal induction activity. Current studies in this laboratory are investigating a variety of approaches which can be used to delineate possible differences in the Ah-responsiveness of diverse human cell lines to TCDD and related compounds.

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