MOLECULAR PROPERTIES OF THE HUMAN Ah RECEPTOR: INTRASPECIES VARIABILITY

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ABSTRACT
The Ah-responsiveness (CYP1A1 induction by TCDD) of the following cell lines was investigated, A431 (epidermoid carcinoma), C-4II (cervical carcinoma) Hep G2 (hepatoblastoma) LS180 (colon adenocarcinoma) MCF-7 (breast adenocarcinoma), MDA-MB-231 (breast adenocarcinoma) and T-47D (breast ductal carcinoma). TCDD caused a more than 35-fold induction of CYP1A1 mRNA levels in T-47D human breast cancer cells, whereas no significant induction was observed in MDA-MB-231 breast cancer cells. There was no correlation between TCDD-responsiveness and levels of nuclear Ah receptor. Likewise, there was no correlation between the affinity of the TCDD-Ah receptor complex for binding to its dioxin responsive elements (DREs) and transactivation. The heterogeneity in molecular weight (Mr) of the Ah receptor complex among the cell lines was significant. In T-47D cells, the apparent Mr values for the Ah receptor complex was 206-kDa, whereas in the MDA-MB-231 cell line, the Mr value was 175-kDa. Photoaffinity labeling showed that the Ah receptor ligand-binding subunit is a 110 kDa protein in all 7 cell lines. Thus, the diversity observed in Mr values was possible related to the Arnt protein which interacts with the Ah receptor to form the nuclear heterdimeric complex. Monoclonal antibodies to the Arnt protein bound with the nuclear Ah receptor complexes from both MDA-MB-231 and T-47D cells and the results suggest that the Arnt protein may be truncated in MDA-MB-231 cells. It is possible that the deleted region of the Arnt gene plays an important role in the transcriptional enhancer activity of the Ah receptor complex.

INTRODUCTION
No endogenous ligand for the aryl hydrocarbon (Ah) receptor has yet been found, but exogenous compounds such as 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) bind to the receptor with high affinity and TCDD has been widely used to delineate the cellular and molecular mechanisms underlying Ah receptor-mediated responses[1]. The cytosolic Ah receptor in rodents is a 250-300 kDa heteromeric complex consisting of the Ah receptor ligand binding subunit and possibly 2 heat shock protein 90 subunits[2]. Upon binding of ligand, the heat shock proteins dissociate and a transformed Ah

receptor-Arnt heterodimer is formed[3]. The 180-200 kDa complex accumulates in the nucleus, where it binds to specific DNA sequences, dioxin responsive elements (DRE's)[4], thus activating gene transcription. The most thoroughly characterized gene induced by the Ah receptor, the *CYP1A1* gene, has been used in this study as an indicator of Ah-responsiveness. The molecular properties of 7 human cell lines originating from different tissues have been investigated in order to determine if there is any correlation between the molecular properties of the Ah receptor and Ah-responsiveness.

MATERIALS AND METHODS

Cell growth and formation of nuclear receptor complexes with [3H]TCDD or [125I]DBDD: The A431 (epidermoid carcinoma), C-4II (cervical carcinoma), Hep G2 (hepatoblastoma), LS180 (colon adenocarcinoma), MCF-7 (breast adeno-carcinoma), MDA-MB-231 (breast adenocarcinoma) and T-47D (breast ductal carcinoma) human cells lines were obtained from the American Type Cell Culture collection. 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 treated with [3H]TCDD (5 nM) or [125I]-DBDD (0.4 nM) in dimethyl sulfoxide (DMSO) and incubated for 2 hr at 37°C. Nuclear extract baselines were obtained by cotreatment with 200-fold excess of TCDF. Nuclear extracts were obtained as described[5].

Sucrose density gradient analysis of nuclear extracts: Aliquots (300 μl) of sample were layered onto linear sucrose gradients (5-25%) and centrifuged at 4°C for 2.5 hr at 404,000 g. Thirty fractions were collected from each gradient. Radioactivity in each fraction was determined by liquid scintillation counting. [14C]-Labeled bovine serum albumin (4.4 S), alcohol dehydrogenase (7.4 S) and catalase (11.3 S) were used as markers. For immunoassay, monoclonal anti-Arnt protein antibody was incubated with nuclear extract at the protein ratio 1:200 at 4°C overnight and analyzed by gradient as described above.

In vitro photoaffinity labeling studies: Nuclear extracts containing the radioligand-bound Ah receptor complex were irradiated at wavelengths of > 300 nm for 5 min. The light source was a 450-W medium pressure mercury arc Hanovia lamp filtered by Pyrex water-cooled (4°C) immersion well. The quantitation and separation of the covalently labeled receptor were carried out as described[6].

Gel permeation chromatography: Sephacryl S-300 gel was equilibrated with HEGD buffer containing 0.4 M KCl and packed into siliconized glass column (1.5 cm x 100 cm). Two ml of nuclear extract was applied to the column, and eluted by gravity at the rate of 8 ml/hr. Fractions were collected and radioactivity was determined by scintillation counting.

Gel retardation analysis: Complementary DRE oligonucleotide containing the sequence 5′-GATCTGGCTCTTCTCACGCAACTCCG-3′ was synthesized, purified by polyacrylamide gel electrophoresis, annealed and endlabeled using T4-polynucleotide kinase and [γ–32P]ATP. DNA binding was measured using a gel retardation assay[4], 10 μg nuclear extract from the control (DMSO) and TCDD treated cells were incubated with 1μg poly d(I-C). Reaction mixtures were separated by a 5% polyacrylamide gel. Protein-DNA binding was visualized by autoradiography. The

DRE-Ah receptor complex was quantitated using Betascope 603 Blot Analyzer.
Northern Blot analysis: The plasmid containing the 1.2 kb CYP1A1 cDNA was a gift from Dr. Alan Anderson (laval University, Quebec City, Canada). RNA was extracted using the acidic guanidinium thiocyanate extraction procedure followed by a 1.2% agarose gel electrophoresis and transfer to a nitrocellulose membrane as described[7]. The membrane was prehybridized for 18-24 hr at 65°C and hybridized for 24 hr with addition of [32P]-labeled probe (106 cpm/ml). The blot was quantitated by using Betascope 603 Blot Analyzer and visualized by autoradiography. The CYP1A1 mRNA was standardized against b-tubulin mRNA.

RESULTS AND DISCUSSION

The molecular properties of the Ah receptor from several human cell lines with variable Ah-responsiveness have been analyzed. The Ah-responsiveness in the cell lines: A431 (epidermoid carcinoma), C-4II (cervical carcinoma), Hep G2 (hepatoblastoma), LS180 (colon adenocarcinoma) MCF-7 (breast adenocarcinoma), MDA-MB-231 (breast adenocarcinoma) and T-47D (breast ductal carcinoma) was evaluated by determining the induction of *CYP1A1* gene expression by TCDD. Table 1 summarizes the level of CYP1A1 mRNA as determined by Northern blot analysis.

Table 1. TCDD-inducibility of an Ah receptor-DRE retarded band and CYP1A1 mRNA levels in human cell lines.

	AhR-DRE Complex*			CYP1A1 mRNA**		
Cells	Control	TCDD	TCDD/Control	Control	TCDD	TCDD/Control
MDA	30±2.7	79±5.2[a]	2.6	0.04±0.01	0.06±0.02[b]	1.5
A431	79±7.9	115±12[a]	1.5	0.16±0.01	1.22±0.45[a]	7.6
Hep G2	30±1.6	114±2.3[a]	3.8	0.11±0.2	2.10±0.29[a]	19.1
MCF-7	13±0.5	56±1.8[a]	4.3	0.08±0.01	0.66±0.01[a]	8.3
T-47D	60±10	124±9[a]	2.1	0.13±0.07	4.60±0.49[a]	35.4
C-4II	16±1.6	37±2.7[a]	2.3	0.16±0.02	1.20±0.14[a]	7.5
LS180	26±2.7	43±4.3[a]	1.7	0.10±0.01	1.02±0.33[a]	10.2

a; Significantly higher ($p<0.01$) than observed for the control cells.
b; *No significant induction.*
*; CPM, determined by Betascope 603 Blot Analyzer.
**; Relative units over ß-tubulin.

There was a broad range of TCDD responsiveness ranging from 35-fold induction in T-47D cells to no induction in the MDA-MB-231 cell line. Velocity sedimentation analysis of nuclear extracts were performed, and the results show that there was no correlation between levels of the Ah receptor and Ah-responsiveness (Table 2). Another variable that might influence Ah-responsiveness is the binding affinity of the nuclear TCDD-AhR-complex with DRE's, a prerequisite for transcriptional activation. Gel mobility shift assays showed that the nuclear complex in all 7 cell lines bound to a DRE, and the binding was TCDD-dependent. The ratio of TCDD/DMSO binding of the Ah receptor-DRE interaction showed no correlation with CYP1A1 mRNA inducibility by TCDD (Table 1). The molecular weight of the Ah receptor complex was determined by gel permeation chromatography and velocity sedimentation analysis. As shown in Table 2, there was a significant variability in the Mr values for the Ah

receptor complex among the cell lines. For example, the calculated Mr for T47-D and MDA-MB-231 cells was 205.8 ±0.5 and 174.9±6.4, respectively.

Table 2. Molecular Parameters of the Nuclear Ah Receptor-[3H]TCDD Complex In Seven Human Cell Lines Analyzed by Velocity Sedimentation and Gel Permeation Chromatography

Cell Lines	Level[a] (fmol/mg protein)	S (20,W)[a]	MW (kD)[b]
MDA	83.5±3.5	6.40±0.01	174.9±6.4
A431	27.7±1.2	6.57±0.29	186.3±4.9
HepG2	42.4±2.1	6.90±0.10	199.1±3.0**d
MCF-7	24.5±3.0	7.23±0.29	221.2±5.8**
T-47D	167.1±3.4	6.90±0.01	191.3±5.1*
LS180	29.9±1.0	6.73±0.29	195.7±4.8**

a; determined by sedimentation analysis.
b; determined by sedimentation analysis and gel permeation chromatography.
c; * indicates $P < 0.05$; ** indicates $P < 0.01$.
d; Mean±SD for at least 3 determinations.

This variability was not due to differences in the ligand-binding subunit of the Ah receptor complex since photoaffinity labeling showed that it is a well-conserved 110-kDA protein in all human cancer cell lines (data not shown). The variation in molecular weight must then be due to other proteins associated to the Ah receptor complex. Using antibodies to the Arnt protein, velocity sedimentation showed that Arnt is present both in MDA-MB-231 and in T-47D cells. In MDA cells, the Ah receptor complex before and after addition of Arnt antibody sedimented at 6.40±0.01 S and 7.40±0.28 S, respectively, whereas in T-47D, the complex sedimented at 6.90±0.01 S and 8.51±0.28 S. The data suggest that changes in the Arnt protein may play a crucial role in intercellular Ah-responsiveness, and research on the possible structural differences in the Arnt gene are currenly being investigated.
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