Effects of Proteolysis on the Nuclear Aryl Hydrocarbon (Ah) Receptor from Human and Mouse Cancer Cell Lines

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ABSTRACT

The differences in the molecular properties of the nuclear aryl hydrocarbon (Ah) receptor from human Hep G2 and mouse Hepa 1c1c cells were investigated by time- dependent partial proteolysis with chymotrypsin or trypsin followed by column chromatographic and velocity sedimentation analysis. The sedimentation coefficients, Stokes radii and apparent molecular weights of the untreated human and mouse Ah receptor complexes were similar. The major differences in the trypsin- and chymotrypsin-treated human and mouse nuclear Ah receptor complex was associated with a low molecular weight fragment (25.7-29.7-kDa) observed after treatment with trypsin. The time- and concentration-dependent effects of trypsin and chymotrypsin on the proteolytic digest map of the human and mouse Ah receptor were determined using receptor preparations which were photoaffinity labeled with [^{125}I]7-iodo-2,3-dibromodibenzo-*p*-dioxin (I-DBDD) as the radioligand, separated by SDS-PAGE followed by autoradiography. The human Ah receptor was significantly more resistant to proteolysis by trypsin or chymotrypsin than the mouse Ah receptor; however, the major photolabeled proteolytic products from both cell lines were observed at Mr 48- and 45-kDa. This suggests that there are common structural features in their ligand binding sites.

INTRODUCTION

The aryl hydrocarbon (Ah) receptor binds with high affinity to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) , 3-methylcholanthrene (MC) and structurally-related aromatic and halogenated aromatic hydrocarbons[1-2]. The receptor has been identified in several different organs and tissues of mammalian species and cells in culture[3]. Although the mechanism of Ah receptor-mediated responses resembles that of the steroid and thyroid hormone receptors, there are important differences in their structural properties and organization. For example, most nuclear steroid hormone receptor complexes function as homodimers whereas the Ah receptor complex appears to be a heterodimer[4]. There is also a remarkable inter- and intraspecies heterogeneity in the apparent Mr values for the Ah receptor binding protein[5-6]. This study was designed to further probe the heterogeneity and domain organization of the Ah receptor by comparing the properties of the proteolysis products of the nuclear Ah receptor from mouse Hepa 1c1c7 and human Hep G2 cells.

METHODS

Cell growth and Preparation of nuclear extract Cells were grown as described[7]. After reaching confluency, the cultures were harvested. Radioligands, [^{3}H]-TCDD (5 nM) and [^{125}I]-DBDD (0.4 nM) in DMSO were added to the suspended cell in culture flasks and incubated by gentle shaking for 1 hr at 37° C. Nuclear extract baselines were obtained by

cotreatment with 200-fold excess of 2,3,7,8-TCDF. Nuclear extracts were prepared as described[7].

Limited proteolytic digestion Freshly prepared nuclear extracts were incubated with several concentrations of proteases at 20° C for various times. The reactions were stopped by chilling on ice and adding a 20-fold excess protease inhibitor.

Sucrose density gradient analysis, *in vitro* photoaffinity labeling, gel permeation chromatography, DNA-Sepharose chromatography and DRE-Sepharose chromatography were carried out as previously described[8].

Separation of covalently labeled receptor and degraded fragments by SDS-PAGE The irradiated nuclear extracts (1 ml, 0.5 mg protein/ml) were digested as described above. Samples were treated with 10% (w/v) cold trichloroacetic acid (final concentration 7%) overnight at 4° C. The protein pellet was washed 3 times with cold methanol. The dried samples were dissolved in Laemmli's sample buffer and separated on a 10% polyacrylamide slab gel for 5 hr at 25 mA at 18° C. After electrophoresis, the gel was fixed and dried under vacuum at 80° C. The dried gel was autoradiographed by loading onto X-AR Omat X-ray film and stored at -80° C and developed after 1-2 weeks.

RESULTS

The nuclear extracts from mouse Hepa 1c1c7 and human Hep G2 cells were obtained after treating the cells with 5 nM [^{3}H]-TCDD for 2 hr followed by extraction with high salt and digested as described. The results from velocity sedimentation and gel permeation chromatographic analysis were summarized in Table 1. Incubation with chymotrypsin for 60 min gave similar sedimentation coefficients (5.3 and 4.9 S), Stoke radii (4.9 and 4.8 nm) and apparent Mr values (107- and 99-kDa) for the Hepa 1c1c7 and Hep G2 cells respectively. In contrast, trypsin treatment followed by gel permeation chromatography gave two peaks for Hepa 1c1c7 and one peak in Hep G2 cells. The protease-digested nuclear extracts were also chromatographed on DNA- and DRE-Sepharose columns. The concentrations of salt required to elute the specifically-bound peaks derived from Hepa 1c1c7 and Hep G2 cells for the DNA-Sepharose and DRE-Sepharose columns were 0.35 and 0.34 M, and 0.33 and 0.31 M, respectively. Similar results were obtained after incubation with chymotrypsin and trypsin The time- and concentration-dependent effects of chymotrypsin on the Hepa 1c1c7 and Hep G2 nuclear Ah receptor complexes were also determined by photoaffinity labeling followed by SDS-PAGE separation of the photoaffinity labeled products which were visualized by autoradiography (Figure 1). The results show that photoaffinity labeling of the native nuclear Ah receptor from Hepa 1c1c7 and Hep G2 cells gave a major radiolabeled band at 95- and 110-kDa respectively, which corresponded to the Mr values previously reported for the Ah receptor ligand-binding protein. Incubation of Hep G2 Ah receptor complex for up to 90 min with chymotrypsin (1 μg/mg protein) resulted in minimal degradation of photoaffinity labeled 110-kDa Ah receptor protein (Figure 1). In contrast, the Hepa 1c1c7 nuclear Ah receptor was partially degraded by low concentration of chymotrypsin (1 μg/mg protein) to give at least two relatively high molecular weight products with apparent Mr values of 71 and 48 k-Da. A repeat of the proteolysis using a higher concentration of chymotrypsin (5 μg/mg protein) resulted in the rapid breakdown of the nuclear Ah receptor from both cell lines. The dominant photolabeled degradation products were observed at Mr 48- and 45-kDa for both receptor preparations. For trypsinization, both the Hepa 1c1c7 and Hep G2 were relatively resistant to hydrolysis at a low concentration (1 μg/mg protein) but showed a rapid time-dependent breakdown using 5 μg/mg protein. Again, a major proteolysis product from both the human and mouse Ah receptor was a 41-kDa band.

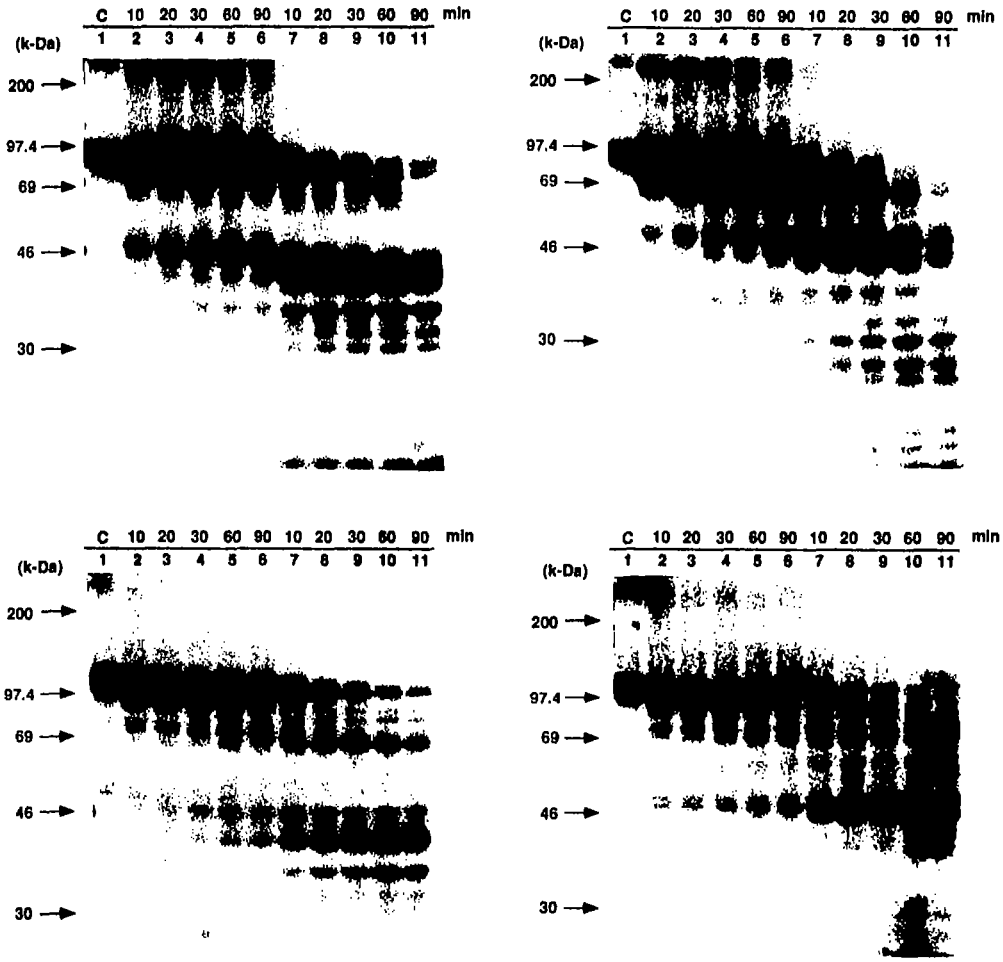

Figure 1. Autoradiographs of denaturing SDS-PAGE separation of photoaffinity-labeled nuclear Ah receptor from Hepa 1c1c7 (top) and Hep G2 (bottom), cells treated with trypsin (left) and with chymotrypsin (right) at 20° C. The sample in lane 1 was not treated with enzyme and represents the photoaffinity-labeled nuclear Ah receptor; samples in lanes 2 through 6 were treated with enzyme (1 μg/mg protein) for 10, 20, 30, 60 and 90 min, respectively. Samples in lanes 7 through 11 were treated with enzyme (5 μg/mg protein) for 10, 20, 30, 60 and 90 min, respectively. [^{125}I]-DBDD was used as the photoaffinity label for these experiments.

DISCUSSION

In this study, the effects of trypsin- and chymotrypsin-induced proteolysis of the nuclear Ah receptor complex from mouse Hepa 1c1c7 and human Hep G2 cells are compared in order to delineate species-dependent structural differences. Under non-denaturing conditions, treatment with chymotrypsin showed that the molecular properties of the Ah receptor from both cell lines were comparable. The major differences between the molecular properties of

Table 1. Molecular properties of the nuclear Ah receptor complex from the Hepa 1c1c7 and Hep G2 cells after treatment with trypsin or chymotrypsin[a]

Parameters	Cells	Native	Chymotrypsin (5 μg/mg protein) 60 min	Trypsin (5 μg/mg protein) 60 min	
				Frag. 1	Frag. 2
$S_{20,W}$[b]	Hepa-1	6.4±0.1	5.3±0.2	4.7±0.2	3.1±0.2
	Hep G2	6.0±0.1	4.9±0.2	4.7±0.6	
Rs (nm)	Hepa-1	6.9±0.1	4.9±0.1	4.4±0.1	2.0±0.1
	Hep G2	7.0±0.1	4.8±0.2	4.4±0.2	
M.W.(k-Da)[c]	Hepa-1	181.4±0.3	107.1±1.3	84.3±1.7	25.7±0.7
	Hep G2	173.1±2.6	98.5±3.0	86.2±3.6	

a; Mean±SD for at least 3 determination.
b; determined by sedimentation analysis.
c; determined by sedimentation analysis and gel permeation chromatography.

the human versus mouse nuclear Ah receptor complex using non-denaturing condition were observed after treatment with trypsin (5 μg/mg protein) for 10 or 60 min in which a low molecular weight minor peak was formed only with the mouse Hepa 1c1c7 nuclear Ah receptor complex. The time- and concentration-dependent effects of trypsin and chymotrypsin on the proteolytic digest map of the human and mouse Ah receptor was also determined. The results illustrate several major differences between the mouse and human Ah receptor (Figure 1). For example: (a) the Hep G2 Ah receptor was relatively resistant to chymotrypsin (1 ug/mg protein) whereas under comparable condition the Hepa 1c1c7 Ah receptor degraded to give two bands at Mr 48- and 71-kDa; (b) the overall pattern of bands formed using a higher concentration of chymotrypsin (5 μg/mg protein) was also different for the mouse and human Ah receptor; and (c) after treatment with trypsin, the overall pattern of photolabeled bands was also different for the human and mouse Ah receptor. Despite these differences, the results also showed that the major photolabeled fragments which formed after treatment of the mouse or human nuclear Ah receptor complex with trypsin (41-kDa) or chymotrypsin (45- and 48-kDa) were indistinguishable by SDS-PAGE analysis. These data from studies on the proteolysis of the photolabeled Ah receptor suggest that there are some common features in the structure of the ligand binding site of the Ah receptor from the mouse and human cell lines. (Supported by the National Institutes of Health, ES-03554)

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