COMPARTIVE BIOCHEMICAL ANALYSIS OF THE MECHANISM OF Ah AND STEROID HORMONE RECEPTOR ACTION

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ABSTRACT:

The Ah receptor (AhR) appears to mediate many, if not all, of the biological and toxic effects of halogenated aromatic hydrocarbons (HAHs). Biochemical and genetic studies have indicated that HAH:AhR complexes induce cytochrome P450IA1 activity in a manner analogous to that described for steroid hormones and their receptors. Although comparison of the physiochemical and biochemical properties of AhR and hormone receptors (HR) reveal some similarities, several striking differences were also observed. The most significant differences were related to variation in receptor-DNA interactions and the number of gene products involved in receptor function. These observations are suggestive of a different molecular mechanism of action. Thus the AhR system appears to represent a class of receptors similar to, yet distinctly different from those described for steroid hormone receptors.

COMPARISON OF Ah RECEPTOR- AND STEROID HORMONE RECEPTOR-RESPONSIVE SYSTEMS

The Ah receptor (AhR) is a soluble intracellular protein that binds a variety of halogenated and nonhalogenated aromatic hydrocarbons, such as 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD, dioxin), saturably and with high affinity (1,2). Many, if not all, of the morphological, biochemical, teratogenic and immunological effects of TCDD may be mediated by the AhR (3,4). The mechanism of induction of cytochrome P450IA1, by TCDD, is similar to induction of specific responsive genes by steroid hormone receptors (SHRs) (5,6). TCDD binds to the AhR and, following transformation of the AhR to its DNA-binding form, TCDD:AhR complexes accumulate within the nucleus. The high affinity binding of transformed TCDD:AhR complexes to specific DNA sequences (dioxin responsive enhancers (DREs)) located 5'-ward of the cytochrome P450IA1 gene results in enhanced transcription of the gene (6-9). The similarity in mechanism of action suggests that the AhR may be a member of the steroid and thyroid hormone receptor superfamily (10).

Comparison of the biochemical properties of the AhR and its specific DNA recognition sequence with those from SHRs responsive systems reveal several similarities although striking differnces are observed (Table 1). Several observations are suggestive of distinct differences in the molecular mechanism of AhR and SHR action:

1) Although the cytosolic form of these receptor groups are similar in size and shape, transformed (nuclear) AhR is significantly larger than nuclear SHRs. These results suggest that transformed AhR are composed of at least two distinct subunits, the exact identity of which is currently unknown.

Table 1. Comparison of AhR and Hormone Receptor Systems.

Characteristic	AhR/DRE	SHR/HRE
Ah Receptor		
1. Sedimentation Coefficient		
Low Salt (0.1M)	8-10S[17,18]	8-10S[19]
High Salt (0.4M)	4-6S	3.5-4.5S
2. Molecular Weight		
A. *Cytosolic:*		
Low Salt (0.1M)	250-300K[17,18]	250-310K[19]
High Salt (0.4M)	100-130K	90-100K
B. Nuclear:	~176K[20]	100-130K[21]
C. Ligand Binding Subunit (SDS PAGE)	95-124K[22]	~100K[23]
3. HSP90 Bound to Receptor	Yes[24]	Yes[25]
4. p59 Bound to Receptor	No[26]	Yes[27]
5. Molybdate Stabilization	Partial[28]	Yes[29]
6. Reactive SH Groups	Yes[30]	Yes[29]
7. Complimentation Groups	Three+[15,16]	One[31,32]
8. Phosphorylation Required for Ligand Binding	No[33]	Yes/No[34]
9. DNA Binding Inhibited by Metal Chelators	No[35]	Yes[36]
DNA Recognition Site		
1. Sequence Motif	Single Site[9]	Dyad Symmetry[11-13]
2. Numbers of Receptors Bound per recognition motif	One[14]	Two[11-13]

2) SHR DNA recognition sequences exhibit at least partial dyad symmetry and appear to bind receptor homodimers (11-13). In contrast, the AhR DNA concensus sequence has no obvious dyad symmetry and studies using a radioiodinated TCDD congener indicates that TCDD:AhR complexes bind to its specific DNA recognition site in a one to one stoichiometric ratio (9,14).

3) All of the steroid hormone receptor genes isolated to date, encode all of the functional determinants for hormone binding, nuclear localization, DNA binding and transcriptional enhancement in one protein or complimentation group (for review see 10). In contrast, the function of the AhR appears to involve at least

three genetically distinct complimentation groups (15,16). One of these affects the ability of the protein to bind TCDD, another the ability of the TCDD:AhR to bind to DNA (16), and, while the function of the remaining one is currently not known, it appears to affect both the ability of the AhR to bind both ligand and DNA (15). These different complimentation groups may represent distinct proteins which modify the function of the AhR protein (which, in this instance, would contain all of the functional determinants) or represent separate AhR subunits (such as distinct ligand binding- and DNA-binding subunits).

Taken together, these observations imply that transformed (nuclear) AhR is heteroprotein complex which may contain distinct ligand- and DNA-binding subunits. The identity and determination of whether one or both subunits directly interact with the DRE must await purification of the AhR and generation of probes against these proteins. Thus, the AhR system appears to represent a class of receptors similar to yet distinctly different from those described for steroid hormone receptors.

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