THE AH RECEPTOR AND ITS LIGANDS: A COMPARATIVE PERSPECTIVE

Mark E. Hahn, Sibel I. Karchner, and Diana G. Franks

Woods Hole Oceanographic Institution, Woods Hole, MA, U.S.A.

Introduction

The aryl hydrocarbon receptor (Ah receptor or AHR) was initially discovered by virtue of its ability to bind planar aromatic molecules such as halogenated dioxins, halogenated biphenyls, and polynuclear aromatic hydrocarbons, leading to the induction of cytochrome P4501A1 (CYP1A1)[1]. Consistent with this, the AHR has a well-known role in regulating the adaptive response to xenobiotics. However, the AHR may have physiological functions in addition to the regulation of xenobiotic-metabolizing enzymes[2]. Indeed, the existence of an endogenous regulator (endogenous ligand?) of the AHR was suggested soon after its discovery[3]. Understanding both types of AHR functions requires information on the ligand specificity of this protein. In pursuing this objective, it is necessary to consider the diversity of AHR proteins that exists both among species (AHR orthologs in different evolutionary lineages) and within species (AHR paralogs within a single lineage). Qualitative and quantitative differences in ligand specificity can occur among all these AHRs; the patterns of such differences will inform our understanding of AHR functions.

Differences in AHR number among species

A single, high-affinity AHR exists in all mammals examined to date[4, 5]. In contrast, the AHR displays considerable structural and functional diversity in non-mammalian species[5]. Recent findings reveal that the *AHR* gene has undergone duplication and diversification in chordates, resulting in at least four gene subfamilies within the *AHR* gene family: AHR1, AHR2, AHR3, and AHR repressor (AHRR)[6]. Additional duplications of AHR1 and AHR2 have occurred in some fish lineages, such that a single fish species may contain up to five AHR forms (in addition to an AHRR). For example, the recently assembled genome of the pufferfish *Fugu rubripes*[7] contains multiple genes related to the killifish (*Fundulus heteroclitus*) AHR1 and AHR2 that we identified earlier[8, 9]. Two of the predicted *Fugu* AHR proteins are AHR1-like, while three are AHR2-like. Multiple AHR2 isoforms have been identified also in salmonid fishes[10, 11]. In addition, a new AHR form (AHR3) is found in some elasmobranch fishes (R.R.Merson & M.E.Hahn, manuscript in preparation). The unusual diversity among fish AHRs may affect our ability to extrapolate between rodent and aquatic models.

Differences in AHR ligand-binding properties among species

Another possible factor affecting extrapolation among species is variation in the structure-activity relationships for AHR binding and toxicity. The set of compounds known to bind mammalian AHRs is large and structurally diverse [12, 13]. In light of this, we have sought to determine whether fish and mammalian AHRs differ in their ability to bind various AHR ligands. Initially, we evaluated several batch ligand-binding assays that have been used successfully with rodent AHRs: the hydroxylapatite adsorption [14], protamine sulfate precipitation [15], and filter-binding assays [16]. Despite extensive efforts and modifications, measurement of specific binding of [^{3}H]2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) to *in*

vitro-expressed fish AHRs was not reliably accomplished using these assays, in contrast to results obtained with the *in vitro*-expressed mouse AHR, which was run as a positive control in each case. Interestingly, the *in vitro*-expressed human AHR also could not be studied using these methods ([17] and unpublished), in agreement with results of others [18].

Because the batch AHR assays were not effective with fish and human AHRs, we used the more labor-intensive sucrose density gradient competitive binding assay[19] to analyze the structure-binding relationships of fish and mammalian AHRs. Using *in vitro* translated mammalian AHR(1) and fish AHR1 and AHR2 forms, we found that the binding of [^{3}H]TCDD could be displaced by planar halogenated compounds (2,3,7,8-tetrachlorodibenzofuran, 3,3',4,4'-tetrachlorobiphenyl), non-halogenated aromatic hydrocarbon (benzo[a]pyrene), a flavonoid ([]-naphthoflavone), an indole (indigo), and linear tetrapyrroles (bilirubin, biliverdin). Although there were some relatively small quantitative differences among receptors in the degree of competition, the overall structure-binding relationships for this set of compounds appears to be similar among fish and mammalian AHRs.

There are, however, some interesting differences in the ligand-binding activities between mammalian AHRs and some fish AHRs, for some ligands. For example, fish are relatively less sensitive than mammals to AHR-dependent activation of transcription by mono-*ortho* PCBs such as PCB-105 (2,3,3',4,4'-pentachlorobiphenyl)[20, 21]. Our results with *in vitro*-expressed proteins and fish cells[22] show that this difference is due not to differences in the relative affinity of AHR binding but rather to differences in intrinsic efficacy for activation of the AHR. Another example of an intriguing difference between mammals and fish can be found in the zebrafish (*Danio rerio*). This species possesses both an AHR1 and an AHR2, but zebrafish AHR1—the ortholog of the mammalian AHR—has lost the ability to bind both halogenated and non-halogenated AHR ligands[23].

The identification of AHR homologs in invertebrates (*Caenorhabditis elegans*[8, 24], *Drosophila melanogaster* [25], and *M. arenaria* [26]) provided an opportunity to explore the conservation of AHR ligand-binding characteristics over nearly a billion years of evolution. We measured the specific binding of [^{3}H]TCDD to *in vitro*-expressed AHRs from these species in comparison to the *in vitro*-expressed human AHR. A clear peak of specific binding was obtained with the human AHR, but there was no evidence of specific [^{3}H]TCDD binding with any of the invertebrate AHRs[26]. Similar results were obtained with the nonhalogenated AHR agonist [^{3}H][]-naphthoflavone (BNF). Thus, the lack of specific binding to the prototypical AHR ligands TCDD and BNF appears to be a property shared by all known invertebrate AHR homologues, distinguishing them from most vertebrate AHRs[24, 26]. Moreover, these results suggest that the adaptive role of the AHR as a regulator of CYP1A and other xenobiotic metabolizing enzymes may have been a chordate or vertebrate innovation[5]. Thus, sensitivity to the developmental toxicity of dioxins and related chemicals may have had its origin in the evolution of the dioxin-binding capacity of the AHR in the chordate lineage. Focused studies in early chordates such as the sea squirt *Ciona* [27] and the lamprey *Petromyzon* [8] will be needed to test this hypothesis. If the ability to bind dioxins and related compounds first evolved in chordates, invertebrate AHRs may prove valuable in identifying physiological AHR ligands, if they exist.

Endogenous compounds and marine natural products as AHR ligands

Several natural chemicals have been identified as ligands for the AHR (reviewed elsewhere[13]). These include endogenous animal metabolites as well as natural products synthesized by plants or microbes. A recently identified endogenous AHR ligand, 2-(1'H-indole-3'-carbonyl)-thiazole-4-carboxylic acid methyl ester (ITE), was isolated from mammalian lung[28]. Although its physiological role is still uncertain, ITE exhibits high-affinity binding to mammalian AHR and to both fish AHR1 and AHR2, showing that the ability of AHRs to bind this compound is conserved in vertebrates[28].

Several natural products found in marine or freshwater environments have been identified as AHR ligands. These include halogenated compounds such as the halogenated dimethyl bipyrroles[29] and several brominated indoles (M.Hahn, M.Denison, D. Franks, & T.Higa, manuscript in preparation) as well as non-halogenated chemicals such as brevetoxin[30] and retene[31]. Given the enormous variety and structural diversity of halogenated and non-halogenated marine natural products[32, 33], other AHR ligands are certain to be found among them. It will be interesting to determine whether in aquatic organisms the AHR might play some role in chemical ecology, as we speculated earlier[34].

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