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BINDING SPECIFICITY OF DIVERSE AHR LIGANDS INTERPRETED BY MOLECULAR MODELING

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Introduction

The Aryl hydrocarbon Receptor (AhR) is a ligand-dependent basic helix-loop-helix-Per-Arnt-Sim (bHLH-PAS) containing transcription factor that responds to exogenous and endogenous chemicals with the induction of gene expression and production of diverse biological and toxic effects. The mechanism is initiated by ligand binding to the cytosolic AhR, which is present in a multiprotein complex including hsp90. Among the AhR domains involved in its functional activities, the PAS-B is responsible for ligand binding and also involved in hsp90 interaction [1] [2].

While the best-characterized high affinity ligands for the AhR include a variety of toxic halogenated aromatic hydrocarbons (HAHs), polycyclic aromatic hydrocarbons (PAHs), and PAH-like chemicals, other natural, endogenous and synthetic AhR ligands with diverse structure and physico-chemical characteristics ("non-classical" ligands) have also been identified [3]. Analysis of the specific binding interactions of these ligands within the AhR ligand binding domain (LBD) would allow detailed analysis of the key molecular events regulating the mechanisms of ligand-dependent and ligand-specific AhR activation. Even if no experimental structure for the AhR has yet been determined, new information has become available on experimental structures of AhR homologous proteins. Based on this information, we previously developed theoretical models of the AhR LBD and validated them by mutagenesis and functional analysis. These studies highlighted the most important residues for the 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD)
binding [4]. In addition, a specifically developed molecular docking protocol [5] applied to these models allowed us to correctly classify a large set of HAHs and PAHs as high or low affinity AhR ligands [unpublished results].

Aim of our current studies is to combine molecular modeling methods with experimental approaches to gain insights into the binding specificity of AhR ligands with different structures and properties [6]. This requires computational approaches able to properly take into account the receptor conformational changes associated with binding to such diverse molecules.

Materials and methods

Homology Modeling : 10 homology models of the PAS-B domain of the mouse AhR (gi|7304873) were developed, each one based on a HIF-2α template structure (PDB id: 3F1N, 3F1O, 3F1P, 3H7W, 3H82, 4GHI, 4GS9, 4XT2, 4ZP4, 4ZQD). Models were produced by MODELLER [7]. All the ligands and solvent molecules in the HIF-2α internal cavity were maintained during the modeling steps. The quality of the final models was assessed by analyzing the DOPE score profile, and by using the PROCHECK program and the ProSa web server.

Molecular Docking: The modeled structures were refined by energy minimization with MacroModel [8], maintaining the crystallographic molecules inside the cavity. Flexible ligand docking was performed using Glide XP [9]. The obtained poses were refined and their binding free energy ($_{\Delta}G_{bind}$) was obtained by the MM-GBSA (Molecular Mechanics Generalized Born/Surface Area) approach implemented in Prime [10]. The final pose of each ligand was selected on the basis of the lowest $_{\Delta}G_{bind}$.

Results and discussion

Binding of a set of AhR agonists (Figure 1) was computationally studied. They include "classical" and "non-classical" ligands [3]. To describe their binding specificity we used a molecular docking approach planned to take into account flexibility and plasticity of the receptor binding cavity. This was achieved by considering an ensemble of different receptor conformations (ensemble docking) obtained by homology modeling from 10 templates.

An extended set of mutagenesis studies on binding of these chemicals [6] was used to confirm the calculated binding geometries.

The results provided insight into the molecular determinants of the different binding modes associated to the diverse ligands.
For example, the binding geometries obtained for TCDD, 3-Methylcholanthrene (3MC) and Leflunomide are shown in Figure 2. The comparison suggests that the main interactions with the sidechains in the binding cavity are different for the three ligands. The steric hindrance of the 3MC prevents it from reaching the most internal region of the cavity that is instead available for the TCDD and that contains some residues experimentally confirmed as essential for TCDD binding [5]. However most of the stabilizing interactions are shared by these two planar and aromatic "classical" ligands. In contrast, the AhR is predicted to accommodate the smaller and more flexible ligand Leflunomide in a smaller region near to the entrance of the cavity. Here its binding is stabilized by a reduced number of interactions with the internal sidechains, some of which involving polar residues. The observed differences in binding geometries may explain the lower binding affinity observed for Leflunomide with respect to the classical ligands [11].
In conclusion, the proposed new modeling approach based on docking to an ensemble of modeled receptor conformations appears to provide a useful approach in which to investigate and understand at the molecular level differences in the binding of diverse ligands to the AhR LBD.

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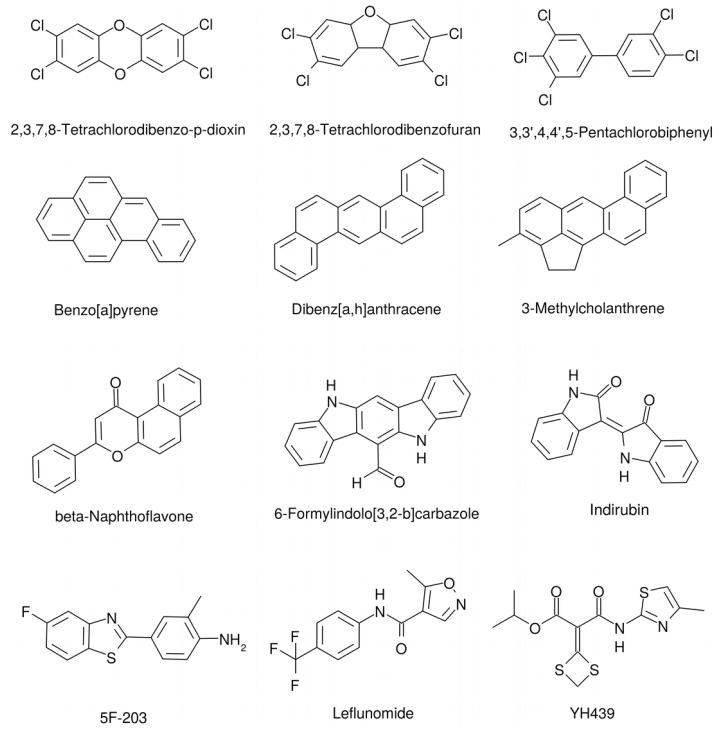

Figure 1. AhR ligands studied.

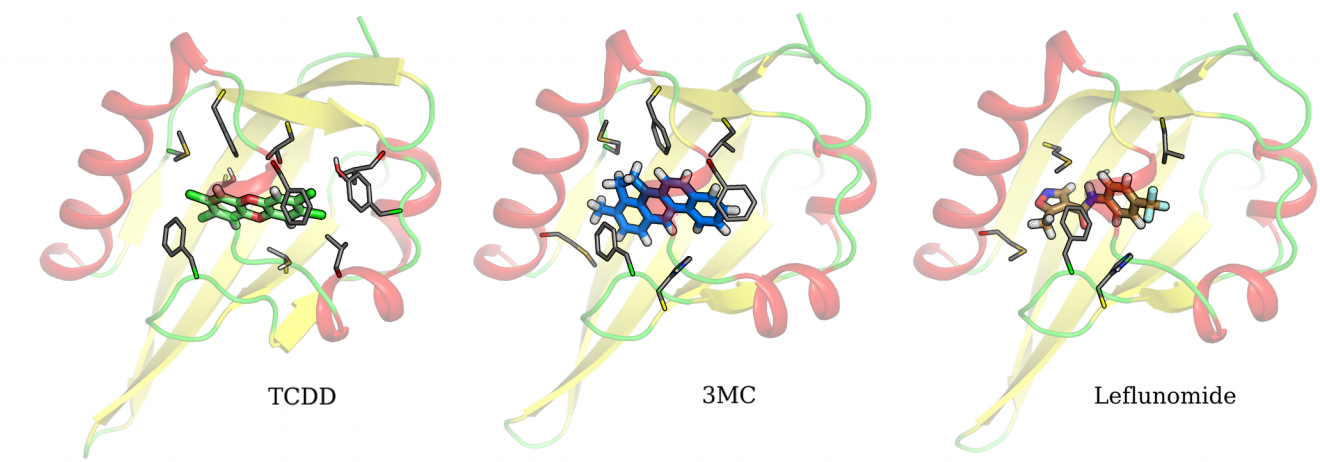

Figure 2. AhR docking poses. Models are depicted with their secondary structures and the ligands with different colors. Residues that mostly contribute to the binding pose stabilization (determined by ΔG_{bind} analysis) are shown as sticks.