# NEW INSIGHTS INTO THE STRUCTURE OF THE mAhR LIGAND BINDING DOMAIN

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## Introduction

Rationalization of the structural basis for ligand binding and activation of the Aryl hydrocarbon Receptor (AhR) requires detailed information about its three-dimensional structure. However, no X-ray or NMR-determined structures of the liganded or unliganded protein are available to date.

For these reasons we developed a theoretical model for the Ligand Binding Domain (LBD) of the mouse AhR by homology modelling techniques.[1] Modelling was based on the crystal structures of homologous proteins belonging to the Per-Arnt-Sim (PAS) superfamily that were available: the bacterial photoactive yellow protein, PYP, the human potassium channel, HERG, and the heme binding domain of the bacterial $O_2$ sensing FixL protein. These are evolutionary rather distant PAS domains which show different functionalities and different cofactor-binding properties. However, they reveal a highly conserved structural framework. Among the three reference structures, our analysis suggested FixL as the best template for modelling.

The proposed model has constituted an initial framework for analysing the AhR ligand-binding mechanism.[1,2] What clearly emerged is that a good steric complementarity exists between the modelled binding cavity and the molecular structures of PCDDs and closely related halogenated aromatic hydrocarbons. Some preliminary hypotheses have been advanced on the residues that may be involved in binding of these ligands within the cavity. However, many of the recently identified AhR ligands[2,3] have structures that are not easily accommodated into the modelled cavity. This evidence suggests that additional key residues could lie at the entrance of the proposed binding pocket.

To analyse all these hypotheses in greater detail, further refinement and testing of the model, based on experimental evidences, is required. In particular, site directed mutagenesis coupled with ligand binding experiments may allow validation of the key residues proposed to be involved in ligand binding. These studies are in progress. In addition, the X-ray structure of the LOV2 domain from the phototropin segment of the photoreceptor PHY3 has been recently published.[4] This domain, that shares homology with the PAS superfamily, may add information useful to complete the structural framework on which the first model was based. New models of the AhR LBD, derived on the basis of these new experimental data by homology modelling techniques, have been developed and compared with the previous model.

## Methods and Materials

The structural alignment of template proteins was derived from FSSP[5] database. On the basis of previous data and considerations[1] the multiple alignment of the thirteen known sequences of AhRs against the templates was driven by the secondary structure prediction on the LBD (residues 230-421).[6] The prediction was obtained with the JPRED[7] web system. Secondary elements predicted with high confidence were directly aligned. For a small region with lower confidence on structural prediction, a

comparison with the sequence alignment of mAhR, HERG and PHY3 confirmed and supported the structural choices. This sequence alignment was built with ClustalW.[8]

The alignments were visualised and edited with Seaview[9] interactive display program.

The 3D modelling and loop search was performed with the aid of SWISS-MODEL Comparative Protein Modelling Server and the Swiss-PdbViewer.[10] Side-chain conformations were optimised with SCWRL 2.9.[11]

Resulting models were submitted to the Biotech Validation Suite for Protein Structures and a further refinement procedure was carried out on the basis of validation results.[12] 3D visualisation and figures were produced with VMD[13] molecular visualization program.

**Results and Discussion**

The PHY3 LOV structure confirms the observation that PAS domains show highly conserved structural characteristics despite the low level of sequence homology (below 20 % identity in all the pairwise comparisons of the four known structures except for HERG and PHY3 which are 30 % identical). The general fold retained by the four structures includes a helix-turn-helix element, a five-stranded b-sheet and a long central a-helix, called helical connector.

The different functionalities of these domains are reflected by their different cofactor-binding properties: the 4-hydroxycinnamic acid is covalently bound in PYP, the iron protophorphyrin is coordinated to an histidine residue in FixL, FMN noncovalently bounds the PHY3 LOV domain. HERG does not contain any cofactor. Also the shape and the dimension of the binding cavities reflect the different binding properties of these domains. See, as an example, the superposition of the FixL and PHY3 PAS domains in Figure 1. The different positions of the helical connector with respect to the b scaffold are associated to different inclinations of the cofactor molecules, that point to the opposite sides of the helix.

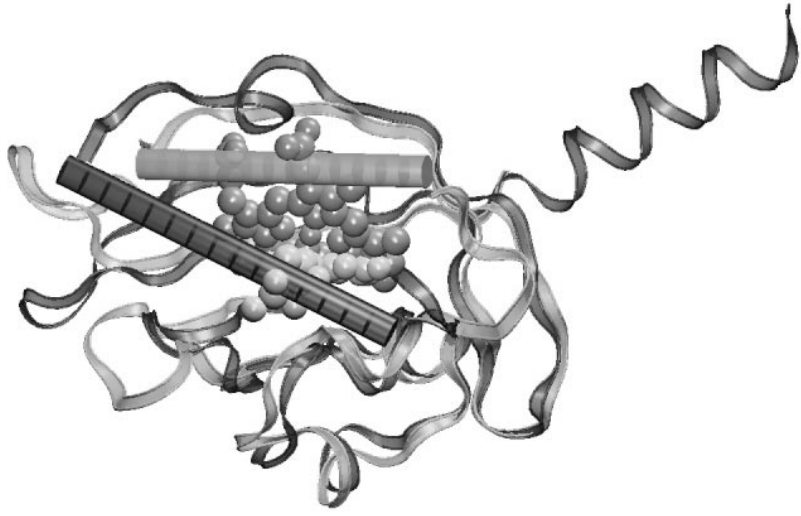

**Figure 1.** Superposition of the FixL (blue) and the PHY3 (green) PAS domain structures with cofactors. The helical connectors are represented as cylinders.

In this framework, the choice of the template structure is a crucial step in modelling the mAhR LBD and in deriving information about binding.

Two models were built on the basis of a multiple alignment of all the four reference structures, by selecting the PHY3 and HERG PAS domains as templates. The former looks particularly suitable as it noncovalently bounds a cofactor, the flavin mononucleotide (FMN) molecule, that exibits stereo-electronic properties very similar to PCDDs. The latter shows a remarkable structural similarity with the phototropin LOV domain. The new models of mAhR LBD (for residue 277-380 and 276-383, respectively) along with the previous one, based on the FixL template structure, may give complementary information about the LBD structure and the key elements for binding.

An open question from the previous model was the relative position of the helical connector with respect to the b scaffold and the consequent shape of the binding cavity. Both the new models suggest a different entrance to the binding pocket, located on the opposite side of the helical connector, due to a closer distance of this helix from the b-sheet. A similar cavity shape is conserved in all the models.

Three deeply buried residues, already identified in the previous modelled cavity, are conserved in the same fashion also in the new models. R282, A375 and Q377 define a molecular environment with steric and electronic features complementary to the TCDD: the Ala residue, with its short side-chain, leave enough room for ligand accomodation, while the Arg and Gln could favourably interact with the dichlorobenzene moiety.

The analysis of residues involved in the interaction of the PHY3 LOV domain with its cofactor shows a polar region on the pyrimidine side of the isoalloxazine ring and an hydrophobic pocket around the dimethylbenzene moiety. In all the models of mAhR the same polar region is reproduced and the essential residues (Q377 and Q358) are conserved.

The comparison of all the homology models of the LBD confirms some of the preliminary hypotheses and suggests new directions for investigating both the ligand approach and the specific interactions involved in ligand binding.

The overall fold and dimension of the binding cavity are consistent in all the models. The central role of the helical connector in receiving and transducing the ligand signal seems conserved in the mAhR as in the other PAS domains. The hypothesis of an essential polar pocket within the cavity finds more support with these additional structural proposals. A new question arising from the new models is the relative localisation of the cavity entrance with respect to the sides of the helical connector.

**Acknowledgments**

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**References**

1. Procopio M., Lahm A., Tramontano A., Bonati L. and Pitea D. (2002) Eur. J. Biochem. 269, 13.
2. Denison M.S., Pandini A., Nagy S.R., Baldwin E.P. and Bonati L., *submitted.*
3. Denison M.S., Heath-Pagliuso S. (1998) Bull. Environ. Contam. Toxicol. 61, 557.
4. Crosson S. and Moffat K. (2001) Proc. Natl. Acad. Sci. USA. 98, 2995.
5. Holm L. and Sander C. (1996) Science 273, 595.
6. Coumailleau P., Poellinger L., Gustafsson J.-A. and Whitelaw M.L (1993) J. Biol. Chem. 270, 25291.
7. Cuff J.A., Clamp M.E., Siddiqui A.S., Finlay M. and Barton G.J. (1998) Bioinformatics 14, 892.
8. Higgins D., Thompson J., Gibson T.Thompson J.D., Higgins D.G., Gibson T.J. (1994) Nucleic Acids Res. 22, 4673.
9. Galtier N., Gouy M. and Gaultier C. (1996) Comp. Appl. Biosc. 12, 543.

10. Guex N. and Peitsch M.C. (1997) Electrophoresis 18, 2714.
11. Bower M.,Cohen F.E. and Dunbrack R.L. (1997) J. Mol. Biol. 267, 1268.
12. http://biotech.ebi.ac.uk:8400.
13. Humphrey W., Dalke A. and Schulten K. (1996) J. Mol. Graph. 14, 33.