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STRUCTURE NOTE

Crystal structure of the ligand-binding domain of the retinoid X receptor from the ascidian polyandrocarpa misakiensis

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Key words: X-ray crystallography; nuclear receptor; apo-RXR LBD; tetramer; AF-2 helix.

INTRODUCTION

Nuclear receptors (NRs) form an important superfamily of transcription factors in metazoans. They regulate various biological processes, either by activating or repressing expression of target genes[1] such as morphogenesis, differentiation, and homeostasis.

Members of the NR superfamily share a common functional and structural organization. Their modular structure includes a variable N-terminal activation domain and two functionally independent and conserved domains in the central and C-terminal positions. The central DNA-binding domain allows them to associate with specific DNA response elements whereas the carboxy-terminal ligand-binding domain (LBD) is required for the binding of ligand molecules and for the transmission of the ligand signals at the transcriptional level. Ligands are usually small lipophilic molecules, such as steroid and thyroid hormones, retinoic acid and fatty acids, which specifically bind to the structurally conserved ligand-binding pocket in the LBD. In addition to its role in ligand binding, the LBD (i) interacts with transcriptional cofactors (corepressors and coactivators), (ii) is involved in receptor homodimerization, heterodimerization, or oligomerization, and (iii) contains the ligand-dependent transactivation function AF-2.[2]

All LBD structures display a fold organized into a three-layered α-helical sandwich that forms the hydrophobic ligand binding-pocket. Comparative analysis of LBDs led to the mousetrap model of the ligand-dependent conformational switch.[3] Upon ligand binding, there is a major repositioning of the "transactivation helix" H12 (AF-2 helix) that closes the ligand-binding pocket and induces the released of the corepressor. This H12 motion generates a surface where coactivator proteins can now bind.

The retinoid X receptor (RXR) is one of the most highly conserved members of the steroid/retinoid family

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of nuclear hormone receptors. It has been identified in species ranging from sponges to mammals.[1,4] RXRs from mammals have been studied most extensively, and it has been shown that they forms homodimers and tetramers and can regulate target gene expression when bound with 9-*cis* retinoic acid. RXR also forms heterodimers with numerous other nuclear receptors. These include the retinoic acid receptors, thyroid hormone receptors, vitamin D3 receptor, peroxisome proliferator activated receptors as well as several orphan receptors such as the liver X receptors, pregnane X receptor, and constitutively-activated receptor.[5] Because of its ability to interact with many other nuclear receptors, RXR is thus involved in multiple signaling pathways, such as cell growth, differentiation, homeostasis, metabolism, and development.[6]

To date, most of the RXR LBD crystal structures have been obtained using mammal proteins containing a ligand, only a few having been crystallized without any ligand. Here we present the crystal structure of the ligand-binding domain of the retinoid X receptor from the budding ascidian *Polyandrocarpa misakiensis*[7] crystallized without ligand. The comparison of this structure with previously determined one is of evolutionary interest.

Table I
Crystallographic Data and Refinement Statistics

X-ray source	ESRF/FIP BM30A beamline
Wavelength Å	0.979
Resolution (Å)	48.2–2.9 (2.975–2.9)[a]
Total reflections	143,184
Unique reflections	27,210
Redundancy	5.3
Completeness (%)	99.5 (98.6)[a]
R_{sym}[b] (last shell) (%)	7.1 (40.9)[a]
I/σ	18.5 (4.39)[a]
Space group	$P2_12_12_1$; a = 82.3 Å, b = 96.1 Å, c = 151.7 Å
R_{cryst}[c] (%)	26.7 (34.9)[a]
R_{free}[d] (%)	32.0 (38.1)[a]
R.m.s.d bonds (Å)	0.007
R.m.s.d angles (°)	1.066
Nonhydrogen protein atoms	6257
Water molecules	175
Average B factor (Å^2)	40.2

[a]Last shell.
[b]$R_{sym} = \Sigma_{hkl} \Sigma_i |I_{hkl,i} - \langle I_{hkl,i}\rangle| / \Sigma_{hkl} \Sigma_i |I_{hkl,i}|$ where $\langle I_{hkl,i}\rangle$ is the average intensity of the multiple hkl, observations for symmetry-related reflections.
[c]$R_{cryst} = \Sigma_{hkl} |F_{obs} - F_{calc}| / \Sigma_{hkl} |F_{obs}|$, where F_{obs} and F_{calc} are observed and calculated structure factors.
[d]R_{free} same definition as for R_{cryst}, but includes only 10% of data excluded from refinement.

METHODS

The *Polyandrocarpa misakensis* RXR (PmRXR) full cDNA gene (accession number AB030318) was used. The DNA region encoding the LBD (residues N103-V337) was amplified using *Pfu* polymerase and the oligonucleotides PmR-A2: 5′ GGTGAGGCTAGCAACCCTAAT GACG; *Nhe*I site underlined) and PmR-B1: 5′ GCT GGATCCTCAGTCAGAGCGCTG; *Bam*HI site underlined), and cloned in pET28a using the *Nhe*I and *Bam*HI restriction sites. The PmRXR LBD, fused to an N-terminal His_6-tag, was expressed and purified as previously described.[8] PmRXR His_6-LBD was crystallized by the hanging drop vapor diffusion method at 20°C using 17% PEG 4K, 0.1*M* PIPES pH 7.0, 15 m*M* NaCl, 1 m*M* *n*-dodecyl β-D-Maltoside and a protein concentration of 7 mg/mL. After transfer to a cryo-protecting solution containing crystallization reservoir solution with 20% ethylene glycol added to it, crystals were flash-cooled in liquid nitrogen. Native data sets were collected on the FIP-BM30A beamline at ESRF (Grenoble, France) using crystals grown in absence of ligand and coactivator peptide. The diffraction data were processed using XDS.[9] Crystals belong to the $P2_12_12_1$ space group with unit cell parameters a = 82.3 Å, b = 96.1 Å, c = 151.7 Å. The structure was solved by molecular replacement using Molrep[10] and the RXR moiety of the mouse RXR/RAR complex (Protein Data Bank (PDB) entry 1XDK) as a search model. Model building was made using O[11] and Coot[12] and refinements were carried out with CNS[13] and Refmac5.[14] The stereochemistry of the structure was checked with the program Whatcheck.[15] A total of 81.9% of the residues in PmRXR are in the most favored regions of the Ramachandran plot, 15.2% in the additional allowed regions and 3% in the generously allowed regions. Data and refinement statistics are summarized in Table I. The coordinates and the structure factors have been deposited in the Protein Data Bank, Research Collaboratory for Structural Bioinformatics, Rutgers University, New Brunswick, NJ (http://www.rcsb.org/) (PDB entry: 2Q60). Figures were generated with *PyMOL* (http://pymol.sourceforge.net).

RESULTS AND DISCUSSION

Polyandrocarpa misakiensis RXR LBD displays ~80% amino acid sequence identity with RXR LBD of vertebrates and all the residues reported to interact with the ligand 9-*cis* retinoic acid (9cRA) are conserved. However, in spite of many trials, we could not obtain crystals containing 9cRA even when they were grown in the presence of ligand and coactivator peptide.

The final model of the PmRXR crystal structure refined at 2.9 Å resolution comprises 796 amino acid residues and 175 water molecules. The asymmetric unit contains four RXR molecules organized as a tetramer and has a solvent content of 53%. In the four monomers, the last 15 C-terminal residues and the connecting loop between helix 1 and helix 3 (residues 120 to 142) are not ordered, and due to the lack of matching electron density, they were not included in the model. Two additional poorly-defined electron density peaks, probably

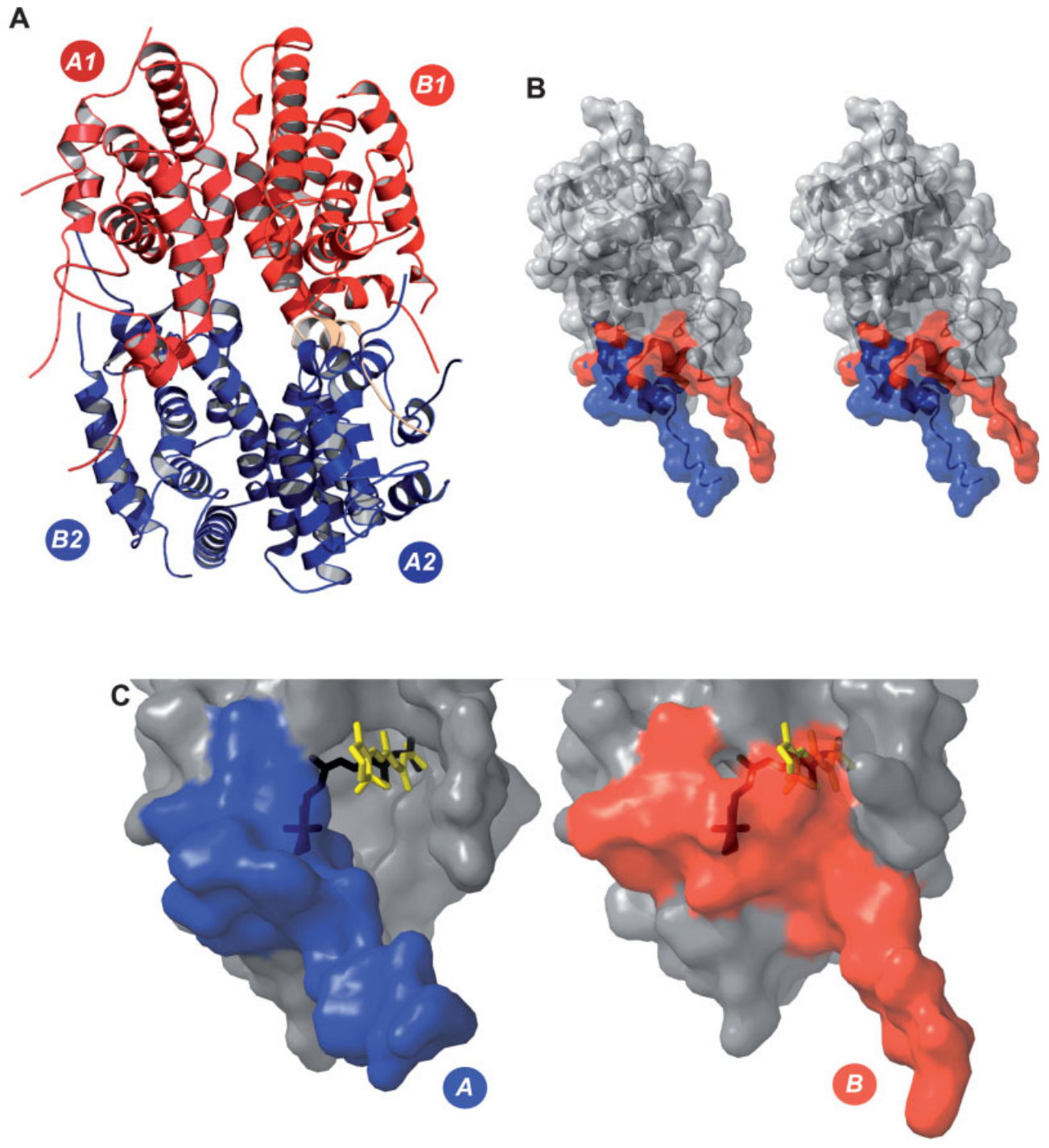

Figure 1

Structure of the Pm RXR LBD tetramer. (**A**) Overall ribbon representation of the tetramer consisting of a "bottom to bottom" dimer of dimers. The two red monomers (A1, B1) form one canonical dimer and the two blue ones, its symmetric (A2, B2). Overlap of monomer A H11/H12 onto monomer B is represented in beige. (**B**) Stereo view of the superposition of monomer A and B showing the two different H11/H12 conformations. (**C**) Close view of the ligand binding pocket of monomer A and B. Ligand molecules found in other RXR structures have been added in Pm RXR. The 9-*cis* retinoic acid from BgRXR (PDB code 1XIU) is depicted in black and the non-activating *trans* retinoic acid from hRXR (PBD code 1G5Y) in yellow.

corresponding to partially ordered detergent molecules, were found stuck in between the two dimers forming the tetramer. No model was fitted into these densities or included in the refinement procedure. Data set and refinement statistics are summarized in Table I.

The four PmRXR LBD monomers fold into the well-described three-layered α-helical sandwich typical of NR LBDs[16] with the hydrophobic ligand-binding pocket embedded inside the sandwich. Because there is no ligand in the hydrophobic pocket, the AF2 domain adopts an apo conformation[17] and protrudes from the main body of the LBD [Fig. 1(A)]. The unliganded LBD tetramer structure consists of a "bottom to bottom" dimer of dimers with the latter adopting the canonical butterfly shape.[18] In addition to the region that forms the canonical dimer interface (mainly residues from helix H10), three distinct regions in each monomer contribute to the apo-tetramer interface. These are the H3/H3 and H11/H11 interfaces, and H12 of each monomer, which protrudes from the body of the LBD towards the coactivator-binding site of an adjacent monomer within the symmetric dimer.

The tetrameric organization of the *P. misakiensis* unliganded LBD structure presented here is very similar (RMSD = 1.3 Å) to the human RXRα LBD apo-tetramer previously described by Gampe *et al.*[19] despite of the phylogenetic distance between the two organisms. According to these authors, this quaternary organization corresponds to a transcriptionally inactive auto-repressed complex because H12 physically prevents binding of coactivators to the LBD. However, following ligand binding, H12 will reposition, which may induce either the reorganization into holo-tetramers[8] or the dissociation into holo-dimers.[18]

Careful analysis of the PmRXR LBD tetramer reveals that the two monomers forming one canonical dimer do not have identical conformations. Superposition of monomers A and B [Fig. 1(B)] shows that both forms are very similar up to the C-terminal end of Helix 10 and that Helix 11 and 12 (starting from residue Glu 309) are in completely different positions. Monomer B is identical to the monomer found in the human RXRα apo LBD structure (PDB code: 1LBD) described by Bourguet *et al.*[17] In monomer A there is a displacement of H11 that moves away from the helical sandwich inducing an average shift of H12 of about 10 Å. This reorganization is necessary to allow the docking of H12 in the H3/H5 hydrophobic cleft region (coactivator binding site) in the adjacent dimer. As a consequence, it induces an opening of the ligand-binding pocket of monomer A, which leaves enough space for the ligand to slide inside [Fig. 1(C)]. This could be a way not only to keep the repressed RXR form "competent" for activation but also of "pre-selecting" the cognate ligand (through its carboxylate end). Then the H11 and H12 rearrangement stabilized by binding of the coactivator protein, will allow effective binding and subsequent trapping of the ligand inside the LBD. In one of the human apo-tetramer crystals, Gampe *et al.*[19] observed an unusual binding of two molecules of *trans* retinoic acid (tRA) resulting from the isomerization of the added *cis* retinoic acid. According to the authors, this binding has no particular significance or function. We think instead that it could reflect a snapshot of the first step of the ligand recognition and/or binding awaiting the fixation of the coactivator to be completed. Because this second event could not or did not happen, the 9cRA isomerized into tRA during the course of crystallization.

The crystal structure we present here is the second describing an unliganded RXR LBD organized as a tetramer. Gampe *et al.*[19] made the first description of this type of quaternary organization for the human RXRα and suggested that apo-tetramers correspond to a self-repressed storage form involved in the receptor regulation. The formation of tetramers in solution and the functional role of mammalian RXR tetramers in gene regulation have been reported (Ref. [1] and references therein). In the present work, we show that urochordates, the closest relatives of vertebrates,[20] have the ability to form apo-tetramers and strengthens the hypothesis of Gampe *et al.*[19] Because the ascidian homologue of RXR can also be organized as a tetramer in absence of ligand, it seems that apo-tetramer formation reflects a real physiological function that could be conserved in the whole chordate phylum.

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