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A ligand-entry surface of the nuclear receptor superfamily consists of the helix H3 of the ligand-binding domain

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ABSTRACT

We successfully simulated receptor-ligand complex holo-form formation using the human retinoid X receptor-α ligand-binding domain (LBD) and its natural ligand, 9-*cis* retinoic acid. The success of this simulation was strongly dependent on the findings for an initial structure between the apo-LBD and the ligand as well as the discovery of the driving forces underlying the ligand-trapping and subsequent ligand-induction processes. Here, we would like to propose the "helix H3 three-point initial-binding hypothesis," which was instrumental in simulating the nuclear receptor (NR) superfamily. Using this hypothesis, we also succeeded in simulating holo-form formation of the human retinoic acid receptor-γ LBD and its natural ligand, all-*trans* retinoic acid. It is hoped that this hypothesis will facilitate novel understanding of both the ligand-trapping mechanism and the simultaneous C-terminal folding process in NR LBDs, as well as provide a new approach to drug design using a structure-based perspective.

1. Introduction

The nuclear receptor (NR) is a transcription factor that controls many crucial biological events, such as morphogenesis and homeostasis. NRs constitute a superfamily and are widespread among eukaryotes. Five or six functionally separable domains are characteristic of the NR modular structure [1]. The most highly conserved NR domain throughout evolution is the DNA-binding domain (DBD), which recognizes a consensus DNA sequence in the promoter region of target genes. A moderately conserved ligand-binding domain (LBD) serves as a ligand-dependent molecular switch and is the basis of the ligand-dependent activation function-2 (AF-2) of transcription [2,3].

For the last two decades, structural information for NRs has been rapidly accumulating from X-ray crystallography and nuclear magnetic resonance (NMR) experiments; over 700 three-dimensional (3D) LBD structures and more than 70 DBD 3D structures have been reported. All crystallographically solved 3D structures of LBDs have revealed the presence of essentially only one common fold: a three-layered helical sandwich fold consisting of twelve helices (helices H1–H12) and small, antiparallel β-sheets (sheets S1 and S2) (Fig. 1B) [2,3]. Structural changes important for the signal transduction mechanism of NRs have been revealed by the 3D structures of the unliganded and liganded LBDs solved for the human retinoid

Abbreviations: hAR, human androgen receptor; hCAR, human constitutive androstane receptor; hCOUP-TFI, human chicken ovalbumin upstream promoter-transcription factor-I; hCOUP-TFII, human chicken ovalbumin upstream promoter-transcription factor-II; ssDAF-12, *Strongyloides stercoralis* DAF-12; hDAX-1, human dosage-sensitive sex-reversal adrenal hypoplasia congenital region on the X chromosome gene 1; dmDHR38, *Drosophila melanogaster drosophila* hormone receptor 38; hEAR2, human v-erbA related protein-2; hvEcR, Heliothis virescens ecdysteroid receptor; hERα, human estrogen receptor-α; mERRγ, mouse estrogen-related receptor-γ; rFXR, rat farnesoid X-activated receptor; hGR, human glucocorticoid receptor; hHMR, human testicular receptor 3; hHNF4α, human hepatocyte nuclear factor 4-α; mLRH-1, mouse liver receptor homolog-1; hLXRα, human liver X-receptor-α; hMR, human mineralocorticoid receptor; hNGFI-B, human nerve growth factor-induced B; hNOR, human neuron-derived orphan receptor; hNURR1, human nuclear receptor related 1; hPNR, human photoreceptor cell-specific nuclear receptor; hPPARγ, human peroxisome proliferator-activated receptor-γ; hPR, human progesterone receptor; hPXR, human pregnane-X receptor; hRARγ, human retinoic acid receptor-γ; rRORβ, rat retinoic acid-related orphan receptor-β; hRXRα, human retinoid-X receptor-α; hSF-1, human steroidogenic factor 1; hTRβ, human thyroid hormone receptor-β; hTR2, human testicular receptor 2; hTR4, human testicular receptor 4; dmUSP, *Drosophila melanogaster* ultraspiracle protein; rVDR, rat vitamin D receptor.

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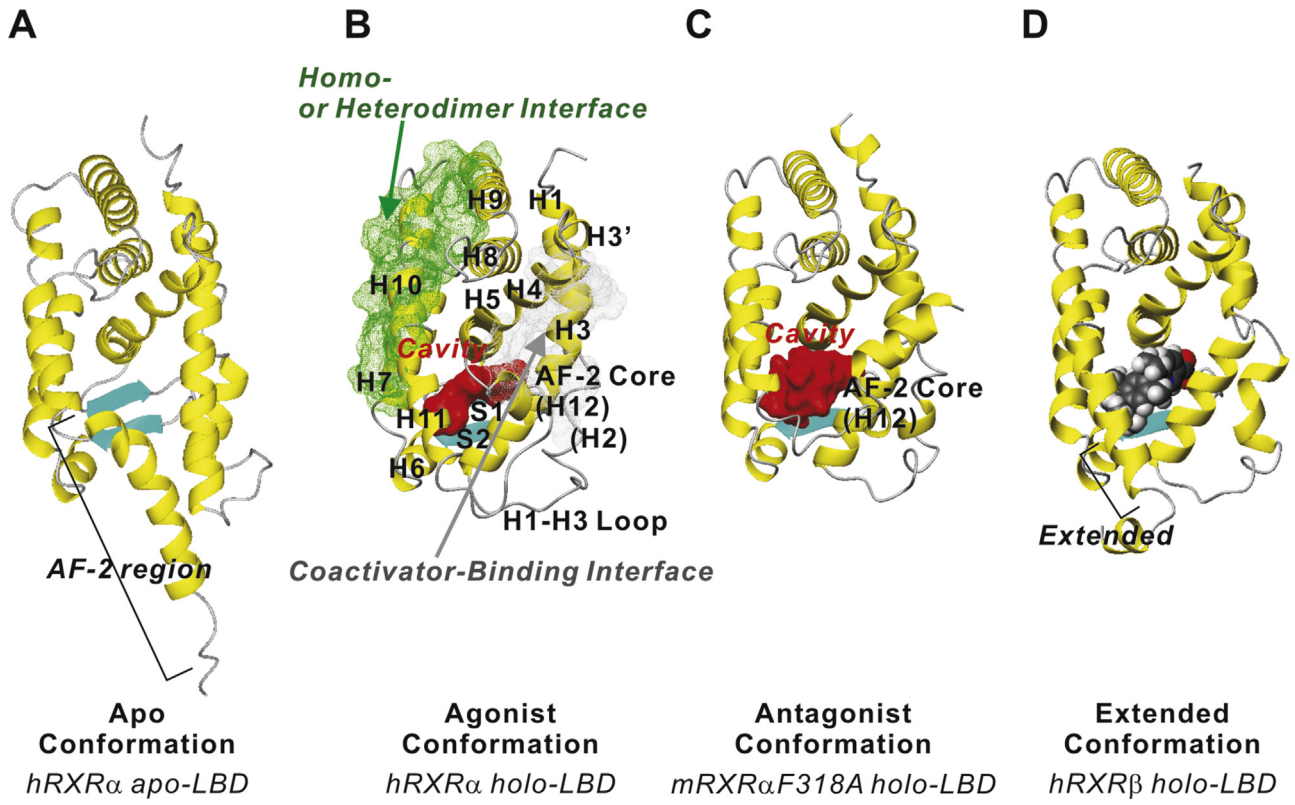

Fig. 1. Canonical structures of NR LBDs. (A) hRXRα apo-LBD (1LBD [2]) in the apo conformation. (B) hRXRα holo-LBD (1FM9 [4]) in the agonist conformation. (C) mRXRαF318A holo-LBD (1DKF [5]) in the antagonist conformation. (D) hRXRβ holo-LBD (1H9U [6]) in the α-helical, extended conformation.

X receptor-α (hRXRα) [2–4,7]. AF-2 architectures, located in C-terminal regions (helices H11–H12), significantly differed from each other in these two structures (Fig. 1A and B). In the liganded structure, the holo-LBD generated a hydrophobic cavity, and the liganded hydrophobic core of the LBD was stabilized by the covering amphipathic AF-2 architecture [3,4,7]. However, in the unliganded apo-LBD structure, the AF-2 architecture adopted the so-called apo conformation and was buried in the potential cavity [2]. Based on these observations, a mouse-trap mechanism was proposed for NRs [3]. To date, many structures for non-crystallographical homo- and heterodimers have been obtained. These structures have revealed either the agonist or antagonist conformation of the AF-2 architecture in the presence or absence of cofactor fragments (i.e., a coactivator or corepressor; Fig. 1B and C) [4,5,8–20]. In the case of the agonist complex, the AF-2 architecture adopted the agonist conformation, and the resulting AF-2 core (helix H12) simultaneously participated in the cofactor-binding site, thus creating a coactivator-binding site. In contrast, in the case of the antagonist complex, the AF-2 architecture adopted the so-called antagonist conformation, in which the AF-2 core interacted with its own cofactor-binding site composed of the helices H3, H3′, and H4 (Fig. 1B); this conformation of the AF-2 architecture prevents interactions with the cofactor's LXXLL motif (where X can be any amino acid), which is known to be the consensus sequence [9]. Agonist binding to the NR LBDs is associated with recruitment of coactivators, e.g., nuclear receptor coactivators (NCoAs) such as steroid receptor coactivator-1 (SRC-1) [17,20], glucocorticoid receptor-interacting protein 1 (GRIP1) [7], and transcriptional mediators/intermediary factor 2 (TIF2) [14,16,19], and corepressors such as nuclear receptor corepressors (NCoRs) and silencing mediator of retinoid and thyroid hormone receptors (SMRT) [12]. Moreover, several LBD-dimer interfaces have been confirmed to date (Fig. 1B) [2,4,5,8–10,13–18,20,21]. The results of previous studies have suggested that the AF-2 region is in a dynamic state, and that the formation of a receptor-ligand complex strongly biases this equilibrium toward either an agonist or antagonist conformation. Thus, NR LBDs control the formation of the cofactor-binding site and are thereby able to transmit the signal to the basal transcription machinery through the cofactor-binding interface.

We have recently conducted attempts to reveal the conserved local motifs involved in the common overall fold of the LBDs using structural and sequence alignment analyses of a large number of NR LBDs [22]. These local motifs were formed by conserved amino acids at particular positions, i.e., signal amino acids, in the class-dependent and -independent manner. In the previous study, we concluded that the signal amino acids construct an individual local motif and their assemblies determine the common overall fold of the NR LBD. Of these local motifs, three local motifs, i.e., AF-2 fixed motifs, were involved in the agonist conformation of the AF-2 region of the LBD. Receptor-agonist interactions increased the stability of these AF-2 fixed motifs in the agonist conformation. In contrast, perturbation of the AF-2 fixed motifs by a ligand or another protein molecule led the AF-2 architecture to adopt an antagonist conformation. In that study, we noted that the ligand-binding interface in holo-LBD mainly consisted of the residues of helix H3.

In the present paper, we propose a "helix H3 three-point initial-binding hypothesis," which facilitated the findings of the initial structure for the holo-form formation between an apo-LBD and a ligand as well as the discovery of the driving forces underlying the ligand-trapping mechanism of the NRs. On the basis of this hypothesis, we succeeded in simulating receptor-ligand complex formation, using the hRXRα LBD and the 9-*cis* retinoic acid (9cRA; Fig. 2) as well as the human retinoic acid receptor-γ (hRARγ) LBD and the all-*trans* retinoic acid (ATRA; Fig. 2).

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