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Minireview

Ligand–protein interactions in nuclear receptors of hormones

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Abstract Nuclear hormone receptors are transcription factors regulated by lipophilic ligands. These hormones bind to their nuclear receptor targets using an induced fit mechanism that triggers a large conformational change and generates the proper surface for the binding of protein coactivators. The molecular details of the various steps of this activation process or its inhibition by antagonists are now understood for several nuclear receptors.

Key words: Nuclear receptor; Ligand binding domain; Transcriptional regulation; Ligand–protein interaction; Selectivity; Molecular recognition

1. Introduction

Lipophilic hormones including retinoids, steroids, vitamin D_3, thyroxine and eicosanoids are potent regulators of development, cell division and differentiation, organ physiology, metabolism and homeostasis. The pleiotropic effects of these hormones are mediated through specific intracellular proteins belonging to the nuclear receptor (NR) superfamily [1], whose principal target is in the nucleus. These ligand-inducible transcription factors are represented throughout the animal kingdom in vertebrates, arthropods and nematodes. All the members of this superfamily share a common functional and structural architecture consisting of six domains [2] with a variable A/B amino-terminal region, a highly conserved C domain (~70–80 amino acids) responsible for specific DNA binding of the receptor to its target DNA sequence and weak dimerization, a flexible linker D region and the moderately conserved carboxy-terminal E domain (~250 amino acids) named the ligand binding domain (LBD) responsible for hormone binding and strong dimerization [3]. The two distinct autonomous activation transactivation functions AF-1 and AF-2 are carried by the A/B and E regions, respectively. In contrast to the constitutively active function AF-1, AF-2 is a ligand-dependent function which is critical for the regulation of transcription [4]. Physiologically, NRs can be active molecular entities in monomeric, homodimeric or heterodimeric association states [5,6].

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Abbreviations: AT-RA, all-*trans* retinoic acid; 9C-RA, 9-*cis* retinoic acid; NR, nuclear receptor; RAR, retinoic acid receptor; LBD, ligand binding domain

2. Ligand-induced conformational changes

2.1. Three major conformational states of NR LBDs

The crystal structures of several LBDs of different nuclear receptors have been solved: RXR (retinoid X receptor) [7,8], RAR (retinoic acid receptor) [9,10]; TR (thyroid receptor) [11], PPAR (peroxisome proliferator-activated receptor) [12–14]; ER (estrogen receptor) [15–17], PR (progesterone receptor) [18,19], and VDR (vitamin D_3 receptor) [20]. The two recently reported structures of NR LBD heterodimers in the case of RXR/RAR [21] and RXR/PPAR [22] make it possible to establish the structural basis of allosteric interactions between subunits within such NR LBD heterodimers. Taken together, all these structures confirmed the existence of a common fold [23] encompassing 12 α-helices (numbered H1–H12) and a β-turn, arranged as a three-layered antiparallel α-helical 'sandwich' (Fig. 1). These structures also point to the fact that major structural changes occur upon ligand binding [3,24,25]. The existence of a large conformational difference between an 'open' *apo*-form and a compact 'closed' *holo*-form was first illustrated by the comparison between the two X-ray structures of *apo*-RXRα [7] and *holo*-RARγ bound to all-*trans* retinoic acid (AT-RA) [9]. Furthermore, comparison of agonist- versus antagonist-bound LBDs structures revealed the existence of two distinct conformations for the C-terminal transactivation helix H12 positioned in two conserved hydrophobic grooves (therefore called agonist and antagonist grooves) displayed at the NR LBD surfaces (Fig. 1). These crystallographic studies strongly support the concept of the uniqueness of the *holo*-agonist conformation among all NR LBDs versus more variable conformations of the other functional states.

2.2. The coregulator binding sites

Several proteins interacting with NRs have been identified. These include some general transcription factors such as TFIIB and some TAFs (TATA-box binding protein-associated factors). The transcription regulation involves the recruitment of coactivators and corepressors and hormonal-dependent remodelling of chromatin via a subtle balance in the acetylation/deacetylation level of the histone components of nucleosomes triggered by acetylation or deacetylation enzymatic machineries [26–28]. Some corepressors have been identified that interact with *apo*-receptors and are released upon ligand/agonist binding [29,30]. For coactivators, the so-called TIFs (transcriptionally intermediary factors) act as transducers between the NR AF-2s and the basal transcriptional machinery; they interact with NR LBDs in a ligand/agonist- and AF-2 integrity-dependent manner. From the available

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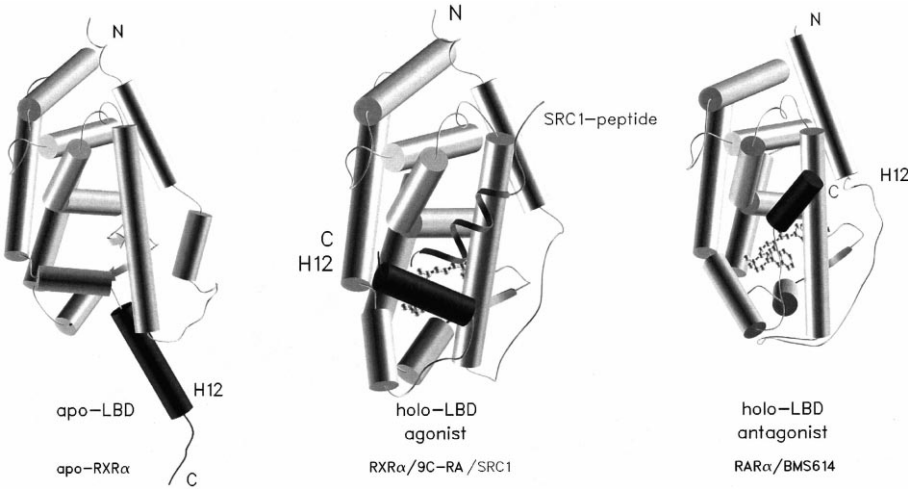


Fig. 1. The three conformational states of the nuclear receptor LBDs known so far: *apo*-form (left), *holo*-agonist (middle) and *holo*-antagonist (right) states, represented by *apo*-RXRα [7], RXRα/9C-RA [8] (model with SRC1 coactivator peptide from PPAR [13]) and RARα/BMS614 [21] (subunit of the RXRα heterodimer). Note the different positions of helix H12 (colored in black). In the antagonist position, H12 occupies precisely the position of the coactivator – the molecular basis of nuclear receptor antagonism.

crystal structures, it is inferred that NR LBDs are signal-responsive regulatory modules adopting distinct conformations as *apo*-receptors, *holo*-agonist-bound or *holo*-antagonist-bound species (Fig. 1) supporting or precluding interactions with proteins such as chaperones, coactivators [31–33] or corepressors [34–36]. The NR interacting domains of coactivators and corepressors have been analyzed and consensus sequences of type LXXLL (L: leucine, X: any amino acid) have been identified. Interestingly, LXXLL-like motifs are also present in the amphipathic helix H12 of NRs [13,16,31,33] and in the NR interacting domains (ID1, 2) of corepressors [34–36]. The crystal structures of three agonist complexes of NR LBDs, namely ERα [16], TRβ [37] and PPARγ [13] bound to LXXLL peptides, revealed that such peptides adopt a helical conformation and occupy the so-called antagonist groove, whereas the agonist groove is occupied by helix H12. The crystal structure of the RXR/RAR heterodimer bound to oleic acid and the RAR selective antagonist BMS614 also make it possible to visualize both LBDs in their antagonistic conformations [21] (see Fig. 1). This structural evidence indicates (i) the possibility of a competition mechanism in the occupancy of either agonist and antagonist surfaces by either corepressor or coactivator NR interacting domains (H12 in the 'antagonist' conformation mimics the coactivator NR box peptide (Fig. 1)), and (ii) the high degree of dynamic and conformational variability of the transactivation helix H12, a critical substructure in signal transduction by NRs. The ligand-induced transconformation of H12 together with the associated structural changes affecting mainly helices H3, H6 and H11 are crucial for the generation of transcriptionally active receptors; it generates a subset of surfaces that allow subsequent specific binding of coactivators and disrupt the interaction surfaces with corepressors.

2.3. Ligand binding mechanism: the RXR model

The existence of a conformational change between an 'open' *apo*-form and a compact 'closed' *holo*-form has been recently highlighted by the direct comparison between the two X-ray structures of *apo*-RXRα [7] and *holo*-RXRα bound to 9-*cis* retinoic acid (9C-RA) [8] showing the same receptor in its two extreme conformational states (Fig. 2). A similar conformation has been observed in the heterodimer RXR/PPAR bound to 9C-RA and rosiglitazone, respectively [22]. The *apo*-form exhibits an additional helix (H2) in the region connecting helix H1 and helix H3. In the *holo*-form, this segment unfolds and the flexible loop region sticks to the protein. This different conformation of loop H1–H3 probably illustrates the dynamics of this region that may act as a molecular spring accompanying the movement of helix H3 which undergoes a very large conformational change. Among the numerous NR LBD crystal structures solved, it has also been re-

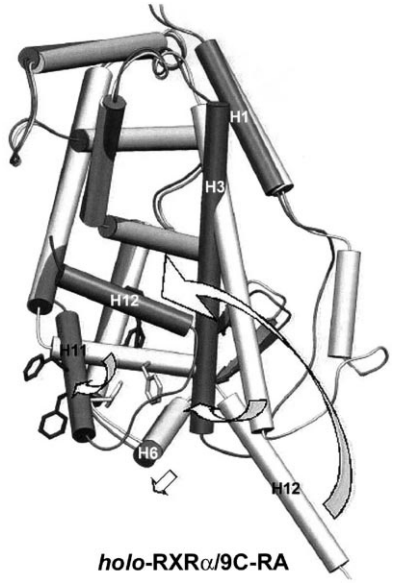


Fig. 2. Ligand-induced conformational changes in RXRα LBD. Superposition of *apo*-RXRα (in light gray) and *holo*-RXRα/9C-RA (in dark gray) LBD structures. The arrows emphasize the main structural changes affecting helices H3, H6, H11 and transactivation helix H12. For the sake of clarity the ligand has not been drawn. In the *apo*-form solvent-exposed phenylalanine residues are colored in black whereas the buried phenylalanine is drawn in light gray; in the *holo*-form the respective solvent accessibilities of these residues are inverted.

ported that this H1–H3 connecting region is often poorly ordered and shows little if any secondary structure. The N-terminal part of H3 rotates by about 90° around its helical axis and packs against the ligand binding pocket. This H3 movement, which is permitted by the displacement of helix H11 from its *apo*-position, is concomitant with the binding of the ligand in an induced fit mechanism. The conformational change brings essential residues into contact with the ligand, seals the binding pocket and generates the proper H12 binding surface.

While helix H11 is kinked in the RXRα *apo*-LBD thereby filling the unoccupied binding pocket, in the RXRα *holo*-LBD it adopts a regular α-helical conformation in the continuity of H10. In the absence of the ligand, H11 stabilizes the *apo*-form by filling the pocket with several hydrophobic residues (Leu-441, Phe-437 and Phe-438) while two other hydrophobic side chains from the same helix (Leu-436 and Phe-439) are exposed to the solvent. In the *holo*-form, H11 (displaced by helix H3) moves away and rotates by 180° around its own axis, generating a proper ligand binding site and helping the repositioning of helix H12. In the RXRα *holo*-LBD, 9C-RA occupies the binding pocket and the side chains of Leu-441, Phe-437 and Phe-438 are exposed to the solvent, while the side chains of Leu-436 and Phe-439 are internalized and form the lower part of the binding pocket. In conclusion, H11 exposes one face toward the solvent in the *apo*-state (presumably the corepressor interacting interface) and the other face in the *holo*-state. These movements of helix H11 emphasize the role of solvation versus desolvation processes in the structural transition. Such entropic effects are expected to be general to other NR LBDs.

The most striking conformational change affects helix H12 (Fig. 2) which is completely repositioned upon ligand binding: in the *apo*-form, H12 protrudes from the protein core and is exposed to the solvent, whereas in the *holo*-form it rotates and folds back toward the ligand binding pocket, thus inducing the compaction of the LBD. As a result, some key residues of the AF-2 AD core become exposed to the solvent and are available for interaction with coactivators mediating transcriptional activation. In summary, ligand binding, acting first through the rearrangement of helix H3, induces the repositioning of helix H12 by expelling helix H11.

3. Specificity of ligand recognition

NR ligands generally exhibit strong affinities for their targets (dissociation constants are in the nanomolar to micromolar range) which is associated with a high specificity. Ligand specificity is crucial from the point of view of both cellular transcription regulation and therapeutic applications. Considering the chemical similarity of natural ligands as estradiol and progesterone, or AT-RA and 9C-RA – the biologically active metabolites of vitamin A (Fig. 3A,B) – Nature has generated an astonishing potential for discriminating between these pairs. In the following, we discuss both examples. According to the phylogenetic analysis of their sequences, RXR, RAR, ER and PR belong to different subgroups of nuclear receptors. RAR and RXR LBDs have only modest sequence identity (30%), which is only slightly higher than that observed between the ER and PR LBDs (23%), whereas ER and RXR LBDs share 27% of their sequence [23]. Therefore, it is intriguing that estradiol and progesterone bind to distinct receptors, whereas 9C-RA binds to both RXR and RAR.

3.1. Estradiol versus progesterone

The crystal structures of estradiol bound to ER and progesterone bound to PR [15,18,19] illustrate how receptor-specific residues provide selective interactions with the ligand (Fig. 3A): the hydroxyl groups of estradiol act as hydrogen bond donors, whereas the ketone groups of progesterone act as hydrogen bond acceptors. Accordingly, in the vicinity of the 3-hydroxyl/3-keto groups, a key residue is converted from a hydrogen bond acceptor (Glu-353 of ERα) into a hydrogen bond acceptor (Gln-725 in PR) which optimizes the hydrogen bond pattern with one/two water molecules and the arginine and phenylalanine residues (Arg-394/Phe-404 in ERα, Arg-766/Phe-778 in PR). The arginine residue is conserved in the steroid receptor family, as well as in RAR (Arg-278) and RXR (Arg-316) where it is hydrogen-bonded to the carboxylate group of the ligand (Fig. 3C).

3.2. Ligand adaptability: AT-RA versus 9C-RA

Whereas RXR exclusively binds 9C-RA, RAR binds both AT-RA and 9C-RA stereoisomers [38,39]. The comparison of the crystal structures of AT-RA and 9C-RA bound to RARγ [9,10] addresses the question of recognition of different ligands by the same environment, i.e. the same receptor. Although the isomers of retinoic acid appear to have different shapes in the free state, the ligands turned out to be very similar once bound (Fig. 3B), as their intrinsic flexibility allows them to fit into a structurally unique ligand binding pocket [10]. The conformational adaptation to the cavity leads to an identical protein conformation. In particular, the agonist position of helix H12 is conserved (Fig. 1) and accounts for the similar binding and activation properties of AT-RA and 9C-RA.

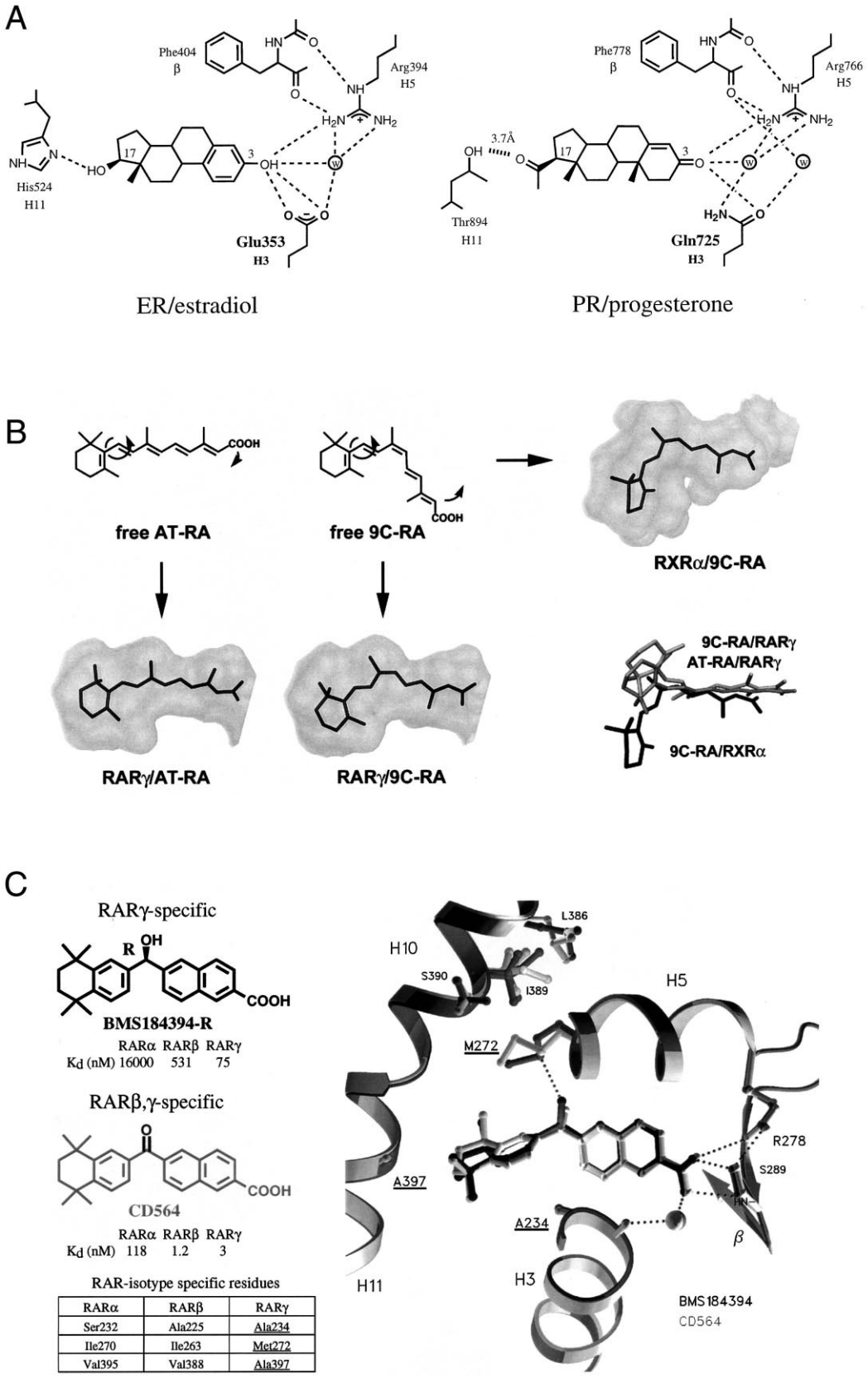
3.3. 9C-RA bound to RXR or RAR

The flexibility of 9C-RA allows it to adopt different conformations in RAR and RXR (Fig. 3B), as revealed by the crystal structures of the respective LBD complexes [8,10]. In RXR, 9C-RA exhibits a pronounced bend, whereas in RAR its shape is closer to that observed for AT-RA bound by RAR. The geometry of the RXRα ligand binding pocket selects the 9-*cis* isomer but excludes the all-*trans* isomer due to its flexure limit [10]. The lower affinity of RXRα for 9C-RA ($K_d = 2$ nM) compared to RARγ ($K_d = 0.2$–0.8 nM for AT-RA and 9C-RA) [40,41] could be explained by a smaller number of hydrophobic contacts which reflects the lower occupancy of the cavity in RXRα compared to that of RARγ.

Due to a different orientation and location of the β-ionone binding sites in RAR and RXR, the β-ionone ring of 9C-RA exhibits a rotation of about 90° (Fig. 3B). Therefore, in RXR it points to the bottom of the LBD (away from helix H12), whereas in RAR it makes hydrophobic contacts with helix H12. The ligand shift towards the center of the RXR cavity is accompanied by a shift of the side chain of the conserved Arg-316 (Arg-278 in RARγ, Fig. 3C) in order to maintain its interaction with the carboxylate group of 9C-RA.

3.4. NR isotypes as distinct pharmaceutical targets

The existence of receptor isotypes (e.g. ERα and β, LBD sequence identity 59%; RARα, β and γ, LBD sequence identity $>80\%$) raises the question of specific ligand recognition



achieved with ligand binding cavities that are highly conserved within a receptor subfamily. Moreover, specificity for a given isotype is of considerable importance for therapeutic applications. ER is a target for chemotherapeutic drugs against certain reproductive cancers, in particular breast cancer. Ligands like the agonist estradiol, the partial antagonist raloxifen or the phytoestrogen genistein (an ERβ-selective partial agonist) exhibit different pharmacological profiles with respect to the

Fig. 3. A: Schematic representation of the hydrogen bond networks between estradiol and ER (left), and between progesterone and PR (right). Note that Glu-353 of ERα is replaced by Gln-725 in order to match the hydrogen bond requirements [19]. B: Ligand adaptability of AT-RA and 9C-RA to either RAR (left and middle) or RXR (right) providing the structural basis of retinoic acid isomer specificity. Solvent-accessible surface delimiting the ligand binding pockets in RARγ and RXRα are depicted as white transparent envelopes with their respective ligands as observed in the crystal structures of the RARγ/AT-RA [9], RARγ/9C-RA [10] and RXRα/9C-RA [8] complexes. Right corner below: position of the ligands as obtained from a superposition of the protein–ligand complexes. C: The chemical similarity of the RARγ-selective BMS184394 and the RARβ,γ-selective CD564 makes it possible to attribute their different selectivity to their respective hydroxyl and keto groups. The superposition of the RARγ LBD complexes [49] reveals that BMS184394 forms a hydrogen bond with the sulfur atom of the RARγ-specific Met-272. Loss of this interaction (CD564) induces a conformational change of the Met-272 side chain, which goes with a rearrangement of 'second layer residues'.
←

ERα, β isotypes [42] as illustrated by the crystal structures of the ERα and ERβ LBDs bound to several of these ligands [15,17,39]. Two residues in the ERα, β pockets that are not conserved are presumably responsible for ER isotype selectivity.

Retinoids are involved in the treatment of various skin diseases and cancers, in particular breast cancer and acute promyelocytic leukemia [43–45]. Since the RARα, β and γ isotypes [46] correspond to distinct pharmacological targets, retinoids selective for the individual isotypes are required. Sequence alignment of the RAR isotypes shows that all but three residues in the ligand binding pocket are conserved [9,10,23] (Fig. 3C), and their crucial role for isotype selectivity has been demonstrated by site-directed mutagenesis [47].

3.5. RAR isotype selectivity

Several crystal structures of complexes of the hRARγ LBD bound to chemically closely related agonist retinoids have been reported recently [48,49]. They illustrate how synthetic retinoids achieve isotype selectivity, whereas the natural ligands behave as panagonists (Fig. 3C): for example, the RARγ-selective BMS184394 carries a hydroxyl group in the bridge connecting the two aromatic rings, whereas the RARβ,γ-selective CD564 has a keto moiety at that position (Fig. 3C). The hydroxyl group provides an additional hydrogen bond with the sulfur atom of the RARγ-specific Met-272 that is replaced by isoleucine residues in RARα and β. In contrast, the keto group of CD564 disrupts the hydrogen bond observed in the BMS184394 complex as illustrated by the different conformation of the Met-272 side chain, resulting in a shift from RARγ to RARβ,γ selectivity (Fig. 3C). In the same way, the isoleucine residues in RARα and β that correspond to Met-272 allow CD564 to bind but disrupt the hydrogen bond with RARγ-selective ligands. The molecular basis for RARγ selectivity appears to be unique, since the hydrogen bond with Met-272 is a common feature of all crystal structures of complexes with RARγ-selective ligands solved so far [48,49]. It is important to note that the correct orientation of the hydroxyl group is a prerequisite for RARγ selectivity and affinity since RAR turned out to exhibit a strong enantiomer selectivity. Inactive enantiomers may bind at high ligand concentrations but adopt energetically unfavorable conformations and exhibit close contacts with residues in the pocket [48]. In contrast to the just discussed introduction of specific ligand–protein interactions, RARα discrimination is probably based on steric contact between the ligand (CD564 for example) with the RARα-specific serine residue that replaces Ala-234 (RARγ, Fig. 3C), whereas panagonists (including AT-RA and 9C-RA) are smaller in this region and more flexible which allows accommodation to any of the RAR isotypes [49]. This is also suggested by the orientation of the serine side chain observed in an RARα LBD ligand complex [21], and corroborated by the small size of RARα-selective retinoids.

4. Concluding remarks

Over the last years joined efforts from different disciplines have provided insight into the regulation of gene activity by nuclear receptors. Numerous ligands have been synthesized for therapeutic applications, with the aim of being more isotype-specific and with fewer side effects for therapeutic applications. However, until recently their chemical structure and activation properties could not be correlated with the structure of their target. The increasing number of crystallographic data on ligand complexes may contribute to the structure-based drug design of new, in particular isotype-selective ligands. For example, the high resolution crystal structures of specific complexes revealed that flexible 'second layer residues' represent a second level of adaptation mediating different conformations of residues in close contact with the ligand (e.g. Met-272 in RARγ, see Fig. 3C). In the RXR pocket all residues are conserved whereas the second layer residues are not [8]. Considering combined adaptations of first and second layer residues may be the key for the design of RXR isotype-selective retinoids that are missing up to now.

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