MINIREVIEW

Small Molecule Modulation of Nuclear Receptor Conformational Dynamics: Implications for Function and Drug Discovery

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ABSTRACT

Nuclear receptors are targets for a wide range of ligands, both natural and synthetic, that regulate their activity and provide a means to pharmacologically modulate the receptor. Recent emphasis in the nuclear receptor field has focused on selective nuclear receptor modulators, which can display graded transcriptional responses and tissue selective pharmacological responses that deviate from the prototypical agonist or antagonist. Understanding the molecular mechanism of action of these selective modulators will provide significant insight toward the development of the next generation of modulators. Although most nuclear receptor structural studies have primarily focused on obtaining ligand-receptor cocrystal structures, recent studies implicate an important role for protein dynamics in the mechanism of action of nuclear receptor ligands. Here we review nuclear receptor studies reporting how ligands modulate the conformational dynamics of the nuclear receptor ligand-binding domain (LBD). A particular emphasis is placed on protein NMR and hydrogen/deuterium exchange (HDX) techniques and how they provide complementary information that, when combined with crystallography, provide detailed insight into the function of nuclear receptors.

Introduction

Nuclear receptors are modular domain transcription factors that regulate the expression of genes controlling a wide range of physiologic processes. Nuclear receptors are generally considered ligand-regulated transcription factors, although only about one-half of the 48 members in the human nuclear receptor superfamily have identified physiologic ligands. These ligand-regulated receptors have been successful targets for drugs treating a variety of human diseases. Primary examples include estrogen receptor (ER), the target for tamoxifen in breast cancer therapy; glucocorticoid receptor (GR), the target for dexamethasone and prednisolone as anti-inflammatory therapies; and peroxisome proliferator-activated receptors (PPARs) such as PPARγ, which is the target for rosiglitazone in type 2 diabetes therapy. Nuclear receptors share a conserved modular domain structure (Fig. 1A), including a N-terminal activation function-1 (AF-1) region and central DNA-binding domain (DBD). However, the primary target for drug discovery is the C-terminal ligand-binding domain (LBD), which contains the activation function-2 (AF-2) surface that serves as a binding site for coregulator proteins. The nuclear receptor LBD is the physiologic binding site for natural ligands such as 17β-estradiol (ER), cortisol (GR), and 1α,25-dihydroxyvitamin D3 (vitamin D receptor; VDR). In the absence of ligand, parts of the receptor LBD are conformationally mobile, or dynamic. Ligand binding stabilizes the receptor LBD conformation, which facilitates interactions with coregulator proteins that remodel chromatin, which controls polymerase binding and the expression of target genes (Fig. 1B).

Nuclear receptors can generally be divided into two classes, transcriptional activators and repressors. The accepted mechanism of action for nuclear receptor transcriptional activators (Fig. 1C) dictates that an agonist ligand binds to the LBD and increases the recruitment of coactivator proteins, which in turn increases the transcription of target genes...
genes. In the classic sense, an antagonist would block the binding of the agonist to the LBD and prevent the agonist from inducing a conformational change in the receptor. However, many antagonists described for nuclear receptors display inverse agonist activity for receptors with significant basal or constitutive transcriptional activity, where binding of the ligand increases recruitment of corepressor proteins and actively represses transcription. The mechanism of action of nuclear receptor ligands is complex, because the same ligand can have different tissue-, cell-, and promoter-specific action, often depending on the expression levels of coregulator proteins, and also display graded receptor recruitment of corepressor proteins and actively represses transcription. The mechanism of action of nuclear receptor ligands is complex, because the same ligand can have different tissue-, cell-, and promoter-specific action, often depending on the expression levels of coregulator proteins, and also display graded receptor recruitment of corepressor proteins and actively represses transcription. The mechanism of action of nuclear receptor ligands is complex, because the same ligand can have different tissue-, cell-, and promoter-specific action, often depending on the expression levels of coregulator proteins, and also display graded receptor recruitment of corepressor proteins and actively represses transcription.

Ligand-Receptor Crystal Structures and the Helix 12 Structure-Function Model

Many advances in our understanding of nuclear receptor function have come from structural biology efforts focused on the receptor LBD. The most common method of choice for these endeavors has been X-ray crystallography. Crystal structures of ligand-receptor complexes provide an atomic “snapshot” into the molecular mechanism of action of the receptor. Hundreds of crystal structures of nuclear receptor LBDs have been reported, culminating in a helix 12 structure-function model (Fig. 2) describing the molecular basis of ligand-modulated agonism (the “on” or transcriptionally active conformation) and antagonism (the “off” or transcriptionally repressed conformation). The LBD adopts a three-layered α-helical sandwich fold, consisting of 12 α-helices with a ligand-binding pocket (Pike, 2006). The hydrophobic AF-2 surface (helix 3/4/5/12 interface) provides a binding site for

Fig. 1. Overall structure of nuclear receptors and mechanism of action. (A) the conserved domain architecture of nuclear receptors consists of an N-terminal intrinsically disordered region called the activation function-1 (AF-1) domain, followed by a central DNA-binding domain (DBD) and a C-terminal ligand-binding domain (LBD) that contains the activation function-2 (AF-2) region. To illustrate the quaternary structure, the intact PPARγ/RXRα (yellow and pink, respectively) complex is shown bound to DNA, ligands, and coregulator peptides (green); PDB: 3DZY. (B) nuclear receptors bind to specific DNA response elements, recruit coregulator proteins, which remodel chromatin and controls polymerase binding, all of which controls the expression of specific target genes. (C) ligands that bind to the nuclear receptor LBDs elicit a variety of pharmacological responses, including activation (agonists), inactivation (antagonists or non-agonists), and, for receptors that are constitutively active, ligands can downregulate the constitutive response (inverse agonists).
coactivator proteins with an LXXLL recognition motif (Savkur and Burris, 2004). In the apo or unliganded form, helix 12 is generally thought to be extended away from the LBD, as is the case observed in the apo RXRα LBD crystal structure (Gampe et al., 2000). However, in the case of apo PPARγ (as described below), helix 12 does not adopt a single conformation but rather adopts multiple conformations in solution (Johnson et al., 2000; Hughes et al., 2012). Furthermore, as described below for ERs, helix 12 appears to be stabilized to the same degree in apo or liganded forms (Dai et al., 2008, 2009). It has been observed generally that agonist ligands, helix 12 docks to the core of the LBD and covers the bound agonists in the ligand-binding pocket. This conformation forms the AF-2 coregulator surface that allows for binding of coactivator proteins containing a LXXLL motif—an “active conformation.” However, upon binding an antagonist ligand, the AF-2 surface is blocked, which inhibits coactivator binding and facilitates corepressor binding—an “inactive conformation.” There are two general classes of antagonists: passive and active. Passive antagonists induce a helix 12 conformation that blocks the AF-2 surface. Active antagonists contain structural moieties that physically block the AF-2 surface, and for these ligands helix 12 can be found in a variety of positions or is not observed in the crystal structure because of structural disorder. Although this model generally explains the activity of agonist and antagonist ligands, it has not provided detailed insight into the mechanism of action of other classes of ligands, including graded/partial agonists, or the observation that the same ligand can have agonist activity in one tissue or cell type and antagonist activity in another.

**Ligand Modulation of NR Conformational Dynamics**

Although the helix 12 structure-function model (Fig. 2) derived from nuclear receptor LBD crystal structures provides a convenient model to correlate ligand activity via structure-function relationships, this model has not satisfactorily explained the mechanism of action for some types of ligands. The switching between “on” (agonist) and “off” (antagonist) conformations in crystal structures is generally referred to as structural plasticity of helix 12, although in some cases helix 12 “dynamics” are used to describe this model. Although crystal structures do not readily report on protein dynamics, there is a growing consensus that protein dynamics do indeed impact the function of nuclear receptors. A primary example of this has been illustrated with PPARγ. Unlike the structures of apo RXRα LBD, for example, which display helix 12 in an extended conformation away from the

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**Fig. 2.** The helix 12 structure-function model is a popular structure-function model in the nuclear receptor field. This model has been derived from ligand-receptor LBD cocrystal structures and correlates the position of helix 12 in the LBD to the functional response of the bound ligand. In the unliganded form, helix 12 is dissociated from the core of the LBD. For some proteins, such as the RXRs, this outward-facing position facilitates tetramer formation. For others, such as PPARγ, helix 12 is conformationally mobile and likely adopts multiple conformations. Upon binding an agonist ligand, helix 12 docks to the core of the LBD and covers the bound agonists in the ligand-binding pocket. This conformation forms the AF-2 coregulator surface that allows for binding of coactivator proteins containing a LXXLL motif—an “active conformation.” However, upon binding an antagonist ligand, the AF-2 surface is blocked, which inhibits coactivator binding and facilitates corepressor binding—an “inactive conformation.” There are two general classes of antagonists: passive and active. Passive antagonists induce a helix 12 conformation that blocks the AF-2 surface. Active antagonists contain structural moieties that physically block the AF-2 surface, and for these ligands helix 12 can be found in a variety of positions or is not observed in the crystal structure because of structural disorder. Although this model generally explains the activity of agonist and antagonist ligands, it has not provided detailed insight into the mechanism of action of other classes of ligands, including graded/partial agonists, or the observation that the same ligand can have agonist activity in one tissue or cell type and antagonist activity in another.
LBD (Bourguet et al., 1995), crystal structures of the PPARγ LBD in the apo form show the “active” conformation (Fig. 3A) (Nolte et al., 1998; Uppenberg et al., 1998). Namely, in the apo PPARγ LBD structures, helix 12 caps the ligand-binding pocket in the same manner as that seen when PPARγ LBD was co-crystallized with the PPARγ full agonist rosiglitazone (Nolte et al., 1998). Although the full PPARγ agonist ligand rosilitazone makes a number of contacts in the ligand-binding pocket, which were postulated to stabilize a conformation suitable to bind coactivator proteins, the apo versus rosilitazone-bound structures have not provided a conclusive mechanism for ligand-dependent PPARγ function. It is noteworthy that most crystallization conditions are in fact nonphysiologic and often use extreme measures to facilitate protein crystal formation, including extreme buffer pH, salt concentrations, and large amounts of dehydrating or precipitating agents. Thus the crystalized structural conformation could be expected to differ from a structure obtained under physiologic or more native-like conditions. On the other hand, subsequently we describe data from other structural techniques suitable to probe the structure and dynamics of the LBD in solution, under what could be considered more physiologic or more native-like conditions. Through a combination of NMR techniques suitable to probe the structure and dynamics of the LBD, we will provide mechanistic insight into the activity of PPARγ ligands. The helix 12 structure-function model has not adequately explained the mechanism of action of PPARγ ligands. The missing resonances localize to the ligand-binding pocket and activation function-2 (AF-2) coregulator interaction surface, which includes helix 12. Missing NMR resonances manifest due to a motion that occurs on a specific time scale, on the order of microseconds-to-milliseconds ($10^{-3}$-$10^{-6}$s), which is generally called “intermediate chemical exchange.” In contrast to apo LBD, the binding of a full or strong agonist, such as rosiglitazone, fully stabilizes the PPARγ LBD conformation, allowing the observation of nearly all NMR resonances in the full agonist PPARγ LBD (Johnson et al., 2000; Hughes et al., 2012). This binding event stabilizes the receptor NMR resonances observed to be in intermediate chemical exchange in the apo LBD. Before to these NMR experiments, the nuclear receptor field suggested that the LBD exists in discrete conformational states depending on the specific ligand bound to the receptor, and the ligand-binding event shifts the conformation from one state to another. However, the NMR data support a different model, one by which the apo LBD samples an ensemble of multiple conformations, and ligand binding stabilizes a subset of these conformations (Johnson et al., 2000). Similar observations have been made for PPARα (Cronet et al., 2001) and RXRα (Lu et al., 2006).

![Fig. 3. Ligand-induced differences in conformational dynamics provide a better model for understanding the mechanism of action of nuclear receptor ligands. The helix 12 structure-function model has not adequately explain the mechanism of action of PPARγ ligands, in particular those displaying graded activity. (A) Crystal structures of the PPARγ LBD all show the same conformation with helix 12 capping the ligand-binding pocket, whether crystallized in the apo form (yellow) or bound to ligand—such as the full agonist (rosiglitazone; green), near full agonist (MRL20; pink), or weak partial agonist (MRL24; blue). (B) Techniques useful in the study of protein conformational dynamics provide mechanistic insight into the activity of PPARγ with graded activity. In the apo form, the PPARγ LBD is conformationally dynamic. In this state, backbone NMR resonances are not observed for residues comprising the ligand-binding pocket, helix 11 and the AF-2 surface, which contain helix 12. Furthermore, these regions show dramatic and rapid exchange in HDX experiments. Full agonists, such as rosiglitazone, provide robust stabilization of the LBD in both NMR (more assigned NMR resonances) and HDX data (robust protection from HDX). Agonists with graded activity, such as MRL20 and MRL24, display gradations in their ability to stabilize the LBD, in particular on helix 3, 11, and 12—all in a manner that correlates with the graded response of the ligand. Namely, stronger agonists afford more stabilization compared with weaker agonists.](image-url)
Hydrogen/deuterium exchange (HDX) studies coupled to mass spectrometry have revealed a similar dynamic phenotype for the LBD upon binding ligands. The HDX experiment involves subjecting protein to D_2O for different times, allowing solvent accessible amide hydrogens to exchange for deuterium. When coupled to mass spectrometry, the protein sample is quenched at specific time points and digested, and the degree of HDX is quantitatively assayed in specific peptides. The number of amide deuteriums is a direct measure of solvent accessibility and conformational dynamics via changes in hydrogen bonding patterns (Hoofnagle et al., 2003), making this experiment sensitive to the conformation of the protein. This experiment is often performed as a differential analysis, where the exchange kinetics of the apo LBD are compared with the ligand-bound LBD. Apo PPAR_γ LBD exhibits considerable HDX in similar regions observed in intermediate conformational exchange by NMR, namely regions making up the ligand-binding pocket and the AF-2/helix 12 surface (Hamuro et al., 2006; Bruning et al., 2007; Hughes et al., 2012). Full PPAR_γ agonists, such as rosiglitazone or GW1929, display robust protection from HDX in these regions, suggesting these ligands change the conformation of the protein relative to apo LBD (Fig. 3B). The protection from HDX observed in helix 12 in particular appears to correlate with the formation of hydrogen bond between the ligand and a residue in helix 12 (e.g., PPAR_γ Tyr473). HDX on other receptor LBDs has shown a similar stabilization upon ligand binding, although the specific regions can differ, including data on CAR/RXRα (Wright et al., 2011), ERs (Dai et al., 2008, 2009), GR (Frego and Davidson, 2006), PPARγ (Hamuro et al., 2006; Bruning et al., 2007; Choi et al., 2010, 2011b; Hughes et al., 2012; Malapaka et al., 2012), PPARγ/RXRα (Chalmers et al., 2006; Chandra et al., 2008), OR (Solt et al., 2011; Kumar et al., 2012), RXRα (Yan et al., 2004, 2006, 2007), and VDR/RXRα (Zhang et al., 2010, 2011; Chalmers et al., 2011).

Fluorescence anisotropy has also been used to study the effect of ligand binding on the dynamics of helix 12. This technique involves labeling the LBD with a fluorophore at site-specific locations, allowing for the measurement of the anisotropy of the fluorophore. The anisotropy signal is inversely proportional to mobility (dynamics). Thus, this technique is useful to determine how ligand binding affects the dynamics of different sites on the LBD. When a fluorophore was coupled to the C-terminus of helix 12 on the PPAR_γ LBD, the PPAR_γ full agonist rosiglitazone caused a dose-dependent increase in helix 12 anisotropy (Kallenberger et al., 2003). Due to the inverse relationship between anisotropy and mobility, this revealed that rosiglitazone reduces the motion of helix 12 relative to apo LBD. Time-resolved fluorescence anisotropy decay measurements also revealed that rosiglitazone stabilizes helix 12 on a fast motion time scale, revealing that ligand binding resulted in reduced helix 12 mobility. In addition, a significant increase in intermediate motion was observed upon binding rosiglitazone, a motion that approximates the overall motion of the LBD. This suggests that in the apo LBD, helix 12 possesses independent motion compared with the core of the LBD. Rosiglitazone binding immobilizes helix 12, tethering helix 12 to the core of the LBD via the hydrogen bond between the ligand and Tyr473, and thus helix 12 tumbles with core LBD.

In summary, data from these studies are consistent with structural interpretations suggesting that the apo nuclear receptor LBD is a dynamic, molten globule-like domain (Nagy and Schwabe, 2004). In the absence of ligand, there is native-like helical secondary structure but the LBD possesses a dynamic ligand-binding pocket, which perturbs a conformational disorder to nearby functional surfaces, such as the PPAR_γ AF-2, and negatively affects coactivator protein interaction. In the case of PPAR_γ, binding of an agonist ligand stabilizes the molten globule-like nature of the ligand-binding pocket and also stabilizes the AF-2 surface to adopt a conformation favoring coactivator protein interaction. To some degree, the dynamic molten globule-like nature of apo LBD is a characteristic of an intrinsically disordered protein domain; however, in this case, it is one that possesses the ability to be fully stabilized by ligands—or, as described below, partially stabilized resulting in graded receptor activity.

**Dynamic Features of Graded Receptor Agonism**

Although the helix 12 structure-function model derived from nuclear receptor LBD crystal structures details the relationship between the two major and opposing functional states, in which the active state is associated with transcriptional agonism and the inactive state is associated with antagonism, it does not explain very well several features of nuclear receptor function. This includes graded transactivation (also called intermediate or partial agonism) as well as non-classic helix 12-independent activation; selective nuclear receptor modulators fall within this class of ligands. On the basis of ligand-receptor cocrystal structures, several mechanisms have been suggested for graded/partial agonists.

This includes an intermediate/quasi-antagonist conformation in which coregulator interaction can switch helix 12 between different helix 12 positions, either by inducing a conformation that is like the agonist conformation but different enough that it does not induce a robust coregulator interaction, therefore providing a reduced transactivation response (Piko et al., 1999, 2000b), or by the ligand binding in different binding modes, perhaps allowing the receptor to sample active and inactive conformations (Bruning et al., 2010; Hughes et al., 2012). However, partial agonist bound nuclear receptor LBDs have in general been difficult to crystallize (Bruning et al., 2010), and therefore the low sampling of partial agonist bound LBD crystal structures has limited these interpretations. Furthermore, these structural models describing possible mechanisms for providing a partial agonist response were primarily derived from ER crystallography studies and may not universally apply to other nuclear receptors. As discussed previously for PPAR_γ, nearly all crystal structures of its LBD—whether in the apo form or bound to any ligand of graded agonist activity—show the same three-dimensional fold with helix 12 in the “active” conformation (Fig. 3A) (Nolte et al., 1998; Uppenberg et al., 1998; Bruning et al., 2007). This includes a study on an intact nuclear receptor complex, where full-length PPAR_γ/RXRα cocrystallized with a PPAR_γ full agonist, partial agonist, or antagonist, all show helix 12 in the active conformation (Chandra et al., 2008).

Protein NMR studies were among the first to provide a glimpse into the mechanism of partial agonism. The PPAR_γ partial agonist nTZDp, a potent EC_{50} = 57 nM yet weak partial agonist (25% efficacy versus rosiglitazone), only partially stabilized the PPAR_γ LBD, affording a partial increase in NMR resonances when compared with NMR data for apo PPAR_γ LBD (Berger et al., 2003). Subsequent HDX mass spectrometry studies revealed additional insight for
a variety of PPARγ ligands with graded response profiles (Hamuro et al., 2006; Bruning et al., 2007). Unlike full agonists, PPARγ partial agonists do not stabilize helix 12 and differentially stabilize other portions of receptor ligand-binding pocket, including the β-sheet surface and helix 3 (Fig. 3B). Protein NMR studies have also revealed that stabilization of the receptor from intermediate conformational exchange is correlated with the graded response of ligand (Fig. 3B) (Hughes et al., 2012). Full PPARγ agonists provide robust stabilization compared with apo LBD, and weak partial PPARγ agonists provide less stabilization. Interestingly, the PPARγ NMR studies also revealed that PPARγ ligands can bind to the LBD in more than one conformation, suggesting added complexity to the structure-function relationship of PPARγ ligands.

When the PPARγ NMR and HDX studies are considered with cocrystal structures of the PPARγ LBD complexed to these ligands, a fuller picture of the mechanism of action is realized for ligands with graded response profiles. Although the crystal structures for the PPARγ LBD complexed to MRL20 and MRL24 show helix 12 in “active” conformation, only MRL20 is observed to make a hydrogen bond with Tyr-473. This explains the protection from HDX on helix 12 observed for MRL20, as well as the slight increase in stabilization resulting in the appearance of data in the protein NMR experiments for residues in helix 12. On the other hand, MRL24 does hydrogen bond to Tyr-473, does not afford much protection from HDX in helix 12, and does not stabilize the receptor as much as MRL20, resulting in no assigned NMR resonances for residues in helix 12. When discussing the use of HDX to study ligands with graded response profiles, it is noteworthy that the gradations in HDX observed for these compounds are not a reflection of ligand affinity. This is most apparent when comparing rosiglitazone, a PPARγ full agonist, which has a weaker binding affinity, on the order of one tenth compared with partial agonists such as rosiglitazone and MRL20, ER ligands do not stabilize helix 12 through hydrogen bond formation with a residue on helix 12 but rather through interactions with other residues lining the ligand-binding pocket, including His-524 on helix 11. Furthermore, apo ER LBD has been difficult to crystalize and was only possible by making mutations that introduce a hydrogen bond observed in the agonist- and antagonist-bound helix 12 conformations to stabilize the receptor conformation (Nettles et al., 2008). This is in contrast to studies on PPARγ. Crystal structures of the PPARγ-LBD in the apo form or when bound to agonist all show helix 12 in the active conformation with helix 12.

These techniques that report on the conformational dynamics of nuclear receptor LBDs have provided unique insight into the mechanism of action of nuclear receptor ligands, in particular those with graded function. The protein disorder imparted, or rather not stabilized, by graded or partial agonists may allow the receptor to search for different binding partners. This is supported by the studies on PPARγ showing that, unlike full agonists that facilitate binding of coactivators and disfavor binding of corepressors, partial agonists allow coactivator binding while retaining the ability to interact with corepressors (Lee et al., 2002; Leesnitzer et al., 2002; Motani et al., 2009).

Conformational Dynamics as a Guide for Nuclear Receptor Drug Discovery

Several recent studies support the incorporation of dynamic information to help explain the mechanism of action of subclasses of ligands, including the use of dynamic information during structure-activity relationship (SAR) analysis in the drug discovery pipeline. HDX analyses for a variety of SERMs revealed dynamic profiles that correlated with the pharmacological profiles of the ligands with respect to ERα activity (Dai et al., 2008). Similar to the analysis of graded PPARγ agonists (Bruning et al., 2007), SERMs displaying various graded response profiles also display gradations in the degree of protection from HDX in several structural regions. Interestingly, clustering analysis of the ligand-induced HDX profiles allowed for the prediction of the tissue specificity of the ER compounds, suggesting this relatively high-throughput HDX structural assay, compared with a low-throughput method such as crystallography, may be useful to generate new SERMs with specific tissue specificity and thus reduce side effects. This study was extended to compare HDX profiles between the two ER subtypes, ERα and ERβ (Dai et al., 2009). These receptors bind many of the same ligands, but have different tissue expression profiles, different affinities for the same ligand, and they are thought to oppositely regulate one another (i.e., ERα is thought to oppose the functions of ERβ and vice versa) (Matthews and Gustafsson, 2003; Zhao et al., 2008). Interestingly, the HDX studies revealed that ERβ undergoes different structural changes when compared with ERα for the same ligand, giving support to the notion that the same ligand can affect the function of ERα and ERβ differently. What was further interesting in both of these studies is that the dynamics of helix 12 in the ER LBD is not altered when compared with apo receptor upon binding agonist or antagonist ligands.

Crystal structures of ER LBDs show a clear repositioning of helix 12 when bound to agonists or antagonists. However, unlike PPARγ agonists such as rosiglitazone and MRL20, ER ligands do not stabilize helix 12 through hydrogen bond formation with a residue on helix 12 but rather through interactions with other residues lining the ligand-binding pocket, including Ser-273. This explains the protection from HDX on helix 12 observed for MRL20, as well as the slight increase in stabilization resulting in the appearance of data in the protein NMR experiments for residues in helix 12. When discussing the use of HDX to study ligands with graded response profiles, it is noteworthy that the gradations in HDX observed for these compounds are not a reflection of ligand affinity. This is most apparent when comparing rosiglitazone, a PPARγ full agonist, which has a weaker binding affinity, on the order of one tenth compared with partial agonists such as rosiglitazone and MRL20, ER ligands do not stabilize helix 12 through hydrogen bond formation with a residue on helix 12 but rather through interactions with other residues lining the ligand-binding pocket, including His-524 on helix 11. Furthermore, apo ER LBD has been difficult to crystalize and was only possible by making mutations that introduce a hydrogen bond observed in the agonist- and antagonist-bound helix 12 conformations to stabilize the receptor conformation (Nettles et al., 2008). This is in contrast to studies on PPARγ. Crystal structures of the PPARγ-LBD in the apo form or when bound to agonist all show helix 12 in the active conformation with helix 12.

These studies suggest that although ER ligands may not directly stabilize helix 12 through hydrogen bond formation with a residue on helix 12, they may stabilize helix 12 through other interactions within the ligand-binding pocket and in concert with coregulator proteins that bind to the AF-2 surface, which includes helix 12. Related to ER, a similar and larger profiling study on 87 VDR modulators has revealed statistically significant trends in HDX profiles that correlate with the selective activation properties of the ligands (Chalmers et al., 2011) (P. Griffin, personal communication).

HDX studies have also been used as a guide for SAR analysis in PPARγ drug discovery. It was recently revealed that the antidiabetic effect of synthetic PPARγ ligands involves a novel mechanism: stabilization of dynamics around LBD residue Ser273, which inhibits Ser273 phosphorylation by the protein kinase Cdk5 (Choi et al., 2010). Notably, blocking of Ser273 phosphorylation by PPARγ ligands was
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found to be independent of the transcriptional activation properties of the ligand afforded by helix 12 stabilization. In this study, two synthetic PPARγ compounds were studied: the full agonist, rosiglitazone, and the partial agonist, MRL24. Although both ligands displayed a similar level in their ability to block Ser273 phosphorylation, the lower transcriptional activity profile of MRL24 correlated with reduced helix 12 stabilization as well as a better gene expression profile when compared with rosiglitazone. Microarray analysis revealed an overlapping set of genes affected by rosiglitazone and MRL24, which included genes dysregulated in type 2 diabetes and obesity, including adiponectin and adipin. However, the expression of a large set of genes corresponding to the classic adipogenic genes was increased by rosiglitazone but not by MRL24. These principles provided a pathway whereby classic transcriptional activation could be dissociated from antidiabetic efficacy on the molecular level via HDX profiles. In a subsequent study, HDX was among several SAR assays used in the design of a proof-of-concept PPARγ nonagonist ligand, SR1664 (Choi et al., 2011a). SR1664 binds directly to the PPARγ LBD and, like MRL24 but unlike rosiglitazone, SR1664 does not stabilize helix 12. Although SR1664 is devoid of transcriptional activation, it displays potent antidiabetic activity with a reduced side effect profile compared with rosiglitazone in terms of reduced fluid retention, weight gain, and interference with bone formation.

Summary

The studies discussed above reveal the powerful synergy in combining different approaches focused on structure and conformational dynamics to provide a fuller understanding of the mechanism of action of nuclear receptor ligands. Although X-ray crystallography studies have played an important role in understanding nuclear receptor structure-function relationships, and will continue to do so in the future, they do not readily report on solution-state conformational dynamics. This is quite important, because most biomacromolecules are dynamic and the inherent dynamics of the protein play an important role in function. As can be appreciated from the studies reviewed here, modulation of conformational dynamics represents a potentially exploitable feature for drug discovery. Thus, techniques to study biomacromolecular structure and dynamics are critical in understanding the molecular basis of biologic function and mechanism of action of ligands.

Authorship Contributions

Wrote or contributed to the writing of the manuscript: Kojetin, Burris.

References


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