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Elusive Conformational Dynamics of PPARγ Inactivation Tied Down by Chemical Cross-Linking

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In this issue of *Structure*, Zheng et al. (2018) have described the dynamics of PPARγ in complex with a non-agonist by exploring its solution-phase conformational landscape through chemical cross-linking in combination with a multitude of different treatment conditions, including their new synthetic anti-diabetic non-agonist, revealing the physical mechanism of PPARγ inactivation.

Peroxisome proliferator-activated receptor gamma (PPARγ) plays a central role in activating hundreds of genes to drive glucose metabolism and adipogenesis and has been one of the prime pharmacological targets in the treatment of type 2 diabetes. Ligands that bind PPARγ have provided a therapeutic option for individuals whose type 2 diabetes symptoms cannot be managed by lifestyle modifications alone. In the late 1990s, a class of PPARγ agonists known as thiazolidinediones (TZDs) was identified that was successful in reducing insulin resistance in diabetic patients. Unfortunately, many TZDs cause a variety of terrible side effects (Bermúdez et al., 2010) including edema, weight gain, and loss of bone density that have led to a decline in their usage. Consequently, there exists a strong drive to understand why TZDs lead to these side effects, and how can their therapeutic benefits be realized separate from these effects. This drive led to the discovery that the side effects of TZDs seem to be dependent upon a high rate of transactivation of PPARγ (Larsen et al., 2008) and downstream metabolic effects caused by increased transactivation. The second vital step in setting the stage for improved ligand design was the discovery that insulin sensitization caused by PPARγ ligands is correlated with blocking phosphorylation of S273 (Choi et al., 2010), rather than any increase in transactivation of PPARγ. These two discoveries taken together suggested that the essential features of a therapeutic PPARγ ligand should include potent inhibition of pS273 with minimal transactivation of PPARγ.

Developing a new ligand of multiple specific functions is no trivial task, but Griffin's lab produced one of the first potent antidiabetics of desired specification, SR1664 (Choi et al., 2011). It was a clear case of a non-agonist capable of inhibiting pS273 and showed promise in mouse studies as a novel therapeutic. Despite these characteristic improvements, SR1664 suffered from poor pharmacokinetics, necessitating further improvements in ligand design to improve its efficacy. Large-scale structure-activity relation studies were carried out (Asteian et al., 2015) to optimize SR1664 to produce a more efficacious ligand, which eventually gave rise to SR11023, which displays similar activity in cells as SR1664 with improved pharmacokinetics. In this issue of *Structure*, Zheng and co-workers present not only an optimized antidiabetic drug but answer many questions about the structural dynamics of PPARγ binding of both agonist and non-agonist ligands (Zheng et al., 2018). In turn, these structural studies inform how to better inhibit transactivation of PPARγ while maintaining antidiabetic effects, which will provide guidance in future ligand design studies.

Studying the structural dynamics of a protein upon ligand binding is a difficult task. Co-crystallization of a protein and ligand has historically been the method of choice for studying protein-ligand interactions. However, crystallography is less amenable to highly flexible, dynamic regions of proteins that might be relevant to protein function. This was the problem that faced Zheng and co-workers, as it was known that the C-terminal H12 helix in PPARγ is important for modulating its gene regulatory functions (Heldring et al., 2007), but the H12 helix is not resolved in the presence of repressive ligands. To determine what characteristics optimized non-agonists should possess, it was necessary to determine where H12 shifted when PPARγ was in a repressed state. Instead of relying on traditional crystallographic methods, Griffin and colleagues combined a number of orthogonal solution phase probes in which PPARγ is treated with a TZD agonist versus their new non-agonist, SR11023 (Zheng et al., 2018).

Initial application of HDX and NMR studies revealed a striking difference between agonist and non-agonist binding: H12 is stabilized by agonist treatment and highly mobile in the presence of non-agonists. Interestingly, both agonists and non-agonists bind strongly to H3, protecting it from solvent exchange, but agonists additionally block H12 (Zheng et al., 2018). While these techniques demonstrate that a difference exists in the portions of the protein affected by different classes of ligands, they do not provide much insight into the nature of this difference. Griffin and co-workers (Zheng et al., 2018), through an extensive combinatory array of ligands, co-activators, and co-repressors combined with quantitative chemical cross-linking, illuminate the nature of this difference and pin down the dynamics of H12.

Chemical cross-linking provides a low-resolution method to identify if two residues are close enough in space to become bridged by a cross-linker, and these distance constraints have been used for decades as a way to study

protein conformations (Cohen and Sternberg, 1980). Chemical cross-linking is particularly useful when studying highly dynamic portions of proteins, as it can measure the relative abundance of different conformers of the protein, making it ideal for studying the dynamic ligand binding domain of PPARγ (Johnson et al., 2000). In this study, the cross-linker BS3 provides the authors a way to determine if two residues are within approximately 24 Å of each other. While this resolution is a far cry from crystallographic precision, when examining the highly dynamic H12, it proves sufficient for localizing it. To probe the location of H12, they monitor the relative abundance of cross-links produced between H12, H11, and near H3, the central helix proximal to the ligand binding pocket. Upon treatment with an agonist, the interaction between H12 and H11 is significantly increased, indicating that a greater fraction of the PPARγ population exists in a conformation with H12 near H11. In the same treatment condition, cross-links between H12 and every lysine on H3 are greatly reduced compared to the apo-protein, providing further evidence that agonists lock H12 against H11, away from H3 (Figure 1). These results are unsurprising, as they point to a conformation identical for known co-crystal structures of PPARγ in complex with an agonist, but they demonstrate that low resolution structural data can be used to localize H12.

Where their cross-linking data shine are in determining what happens to H12 interactions upon non-agonist binding (Zheng et al., 2018). The addition of a non-agonist significantly reduces the intensity of the cross-link between H12 and the C-terminal portion of H3 compared to the apo protein, but other monitored cross-links show comparatively small changes. These shifts in cross-link intensity suggest that non-agonist binding displaces H12, but do not make it clear where it localizes in the non-agonist bound state. Significant shifts in abundance at multiple cross-link sites come only when treating with both a non-agonist and a co-repressor, namely NCOR13 or SMRT2. The combination of a non-agonist and a co-repressor causes a significant increase in the relative intensity of an interaction between H12 and a site near the H2-H3 loop region. This site is approximately 35 Å away in agonist bound crystal structures, indicating that H12 must have undergone a significant localization change to bring those residues into close enough proximity to be cross-linked (Figure 1). The authors surmise from the synergistic effect of these two ligands that non-agonists displace H12, which opens the C-terminal portion of H3 for binding to a co-repressor. This switch-like mechanism described by Zheng and coworkers has now also been visualized with higher-resolution methods (Frkic et al., 2018), corroborating their cross-linking experiments. Ultimately this is the physical cause of the low rate of transactivation observed from SR11023: non-agonists improve access to PPARγ for co-repressor binding, which reduces activation of downstream targets.

The authors (Zheng et al., 2018) work to elucidate the dynamics of H12 and its effect on the transactivation of PPARγ, providing significant impact, both in terms of the technical value in demonstrating a method to quantify protein dynamics across a diverse set of conditions as well as the clinical value in discovering how these dynamics can inform the design of future antidiabetic therapeutics.

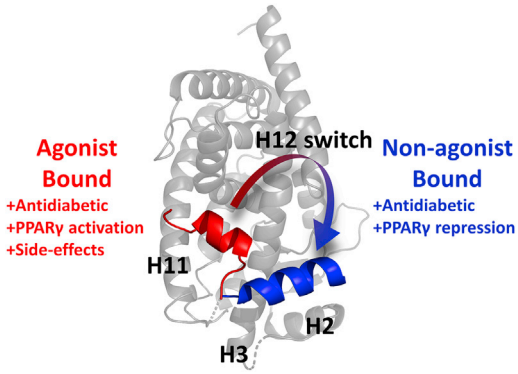

Figure 1. The Molecular Switch Showing the Change in Localization of H12 in the Agonist and Non-agonist Bound Forms of PPARγ

In the agonist bound conformation (red; PDB: 1PRG), H12 is located near H11, which leads to activation of PPARγ, causing the side effects commonly associated with TZDs. Upon non-agonist binding, H12 moves toward the H2-H3 loop region (blue; PDB: 6C5T), which facilitates the binding of co-repressors and inactivation of PPARγ.

The use of diverse, orthogonal techniques reinforces the idea that the solution phase structure and dynamics of proteins are critical to their function and need to be considered. Cross-linking experiments demonstrate that in contrast to the singular conformation observed in co-complexes of PPARγ, it exists across a plethora of ligand-dependent conformations that affect its ability to recruit co-activators or co-repressors. Although in this study Zheng and co-workers present a new non-agonist SR11023 that shows promise as a potent antidiabetic, their analysis of PPARγ dynamics is no less of a contribution of clinical significance. Thanks to their work, we now understand the functional importance of the physical interaction between non-agonists and H12, and future SAR studies for improved ligands will benefit from the knowledge of how non-agonists enact their function.

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Unique Characteristics of the Parasite Polyamine Pathway

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A critical function of spermidine is in the formation of hypusine, an essential post-translational modification of eukaryotic initiation factor eIF5A. In this issue of *Structure*, Afandor et al. (2018) determine the crystal structure of trypanosomal deoxyhypusine synthase, which shows that gene duplication and subsequent mutations provide significant differences from the mammalian equivalent exploitable for drug design.

Polyamines and Growth and Drug Design

Polyamines are small basic molecules that bind to many cellular components including nucleic acids, membranes, and proteins. By virtue of these interactions, polyamines have a wide variety of cellular functions. The two polyamines present in mammals are spermidine and spermine. Spermidine is well established to be essential for the viability of eukaryotes. Inactivation of any one of the genes needed for its synthesis prevents development past the early embryonic stages. It has a unique feature, described below, its action as a precursor of hypusine. Spermine cannot take part in this reaction but performs many similar functions to spermidine with different potency. It is essential for normal growth and development in mammals. The phenotypes of *Gyro* mice and human patients with the X-linked genetic condition Snyder-Robinson syndrome, both lacking normal spermine synthase activity, show clearly that the correct spermine:spermidine ratio is critical (Pegg and Michael, 2010).

It is now 40 years since the discovery that polyamines are needed for mammalian cell growth. Reports that their synthesis and content were enhanced in proliferative diseases led to the search for inhibitors of polyamine synthesis, which might prove to be useful drugs (Pegg and McCann, 1982). Although there are potent inactivators of every step in the polyamine biosynthesis pathway, by far the most widely studied of these inhibitors is DFMO (Eflornithine), a mechanism-based inactivator of ornithine decarboxylase (ODC) first described in 1978. Although at that time no structural information was available on the enzymes involved in polyamine metabolism, DFMO (and many other inhibitors) were conceived and synthesized by scientists in the Merrell Dow Research Institute directed by Al Sjoerdsma to provide potent inactivators based on known enzyme mechanisms. Studies with cultured tumor cells showed that DFMO was profoundly antiproliferative, but initial clinical trials in malignant diseases were not promising. More recently, some success has been achieved in clinical trials for some cancers such as malignant gliomas and neuroblastoma and for chemoprevention in patients at high risk for gastrointestinal cancer (Casero et al., 2018).

Antiparasitic Effects

Despite the lack of success of DFMO for treating malignancies, studies showed that it was effective against African sleeping sickness caused by *T. b. gambiense*. It is an important part of the current treatments, particularly when combined with nifurtimox that generates reactive oxygen species (Kennedy, 2013). This finding has led to increased interest in targeting the polyamine pathway for treatment of other forms of trypanosomiasis and other parasitic diseases including malaria, leshmaniasis, and Chagas disease. However, the studies have not yet advanced to major clinical trials. Several reasons for this include the opportunity to take up endogenous polyamines that are available to parasites that have intracellular locations, although it is likely that a major reason is the lack of highly species-specific compounds.