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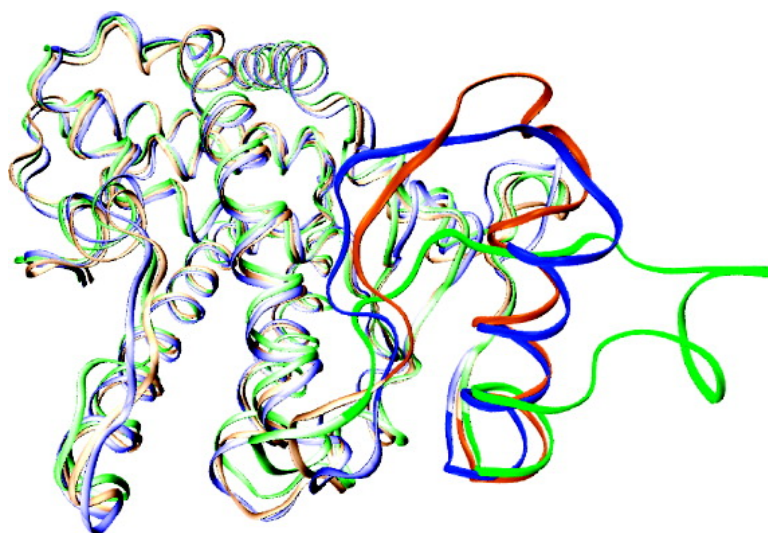
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Protein Polarization Is Critical to Stabilizing AF-2 and Helix-2′ Domains in Ligand Binding to PPAR-γ

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Abstract: The peroxisome proliferator-activated receptor (PPAR-γ) is a ligand-dependent transcription factor that is important in adipocyte differentiation and glucose homeostasis. This paper presents a detailed dynamics study of PPAR-γ and its binding to the agonist rosiglitazone using both polarized and unpolarized force fields. The numerical result revealed the critical role of protein polarization in stabilizing the activation function-2 (AF-2) in ligand binding to PPAR-γ and a helix structure (helix-2′). Specifically when nonpolarized force field is used, a critical H-bond in PPAR-γ binding is broken, which caused AF-2 to adopt random structures. In addition, helix-2′ is partially denatured during the MD simulation, due to the breaking of a backbone hydrogen bond. In contrast, when polarized force field is employed in MD simulation, the PPAR-γ ligand binding structure is stabilized and the local structure of helix-2′ remains folded, both being in excellent agreement with experimental observations. The current result demonstrates the importance of electronic polarization of protein in stabilizing hydrogen bonding, which is critical to preserving the native structure of local helices and protein−ligand binding in PPAR-γ.

The peroxisome proliferator-activated receptors (PPARs) belong to the family of nuclear receptors[1] that require the ligand-binding recruitment of coactivator proteins to stimulate gene transcription.[2−7] PPARs govern numerous biological processes, including energy metabolism, cell proliferation, and inflammation.[8] Since nuclear hormone receptors govern numerous important biological processes and their activity can be influenced by small molecules, they are considered as prime targets for drug development. There are three known human PPAR subtypes, α, γ, and δ.[9,10] Because PPAR-γ is important in adipocyte differentiation and glucose homeostasis, it has drawn much attention from medical researcher in recent years as a primary target to develop drug treatment for type 2 diabetes.[11,12]

The ligand-dependent transcription requires a highly conserved motif, termed activating function-2 (AF-2), located at the far C terminus of the LBD (ligand binding domain), which depends on interactions with coactivators. Without binding to an agonist, the C-terminal helix H-12 (in AF-2) adopts some random structures. From NMR and fluorescence anisotropy techniques,[13,14] it was established that stabilization of H-12 by agonist plays an important role in recruiting coactivators. It is noted that the most important interaction between H-12 and agonist is a hydrogen bond. Figure 1 shows the relative positions of PPAR-γ, agonist coactivator, and the helix-2′.

The agonist rosiglitazone,[15] designed to mimic PPAR-γ's native ligand, has a hydrophilic head and a long hydrophobic tail. The hydrophilic head, forms three hydrogen bond with PPAR-γ, which makes dominant contributions to the stabilization of PPAR-γ/agonist complex. In addition, the agonist's headgroup forms one hydrogen bond with Tyr473 residue, which lies in the important AF-2 domain (Figure 2). This hydrogen-bonding interaction was recognized as playing the most important role in biological function of the agonist. Without the agonist, AF-2 domain adopts some random structures and prevents recruitment of coactivators due to unfavorable entropic effect. This additional hydrogen bonding stabilizes AF-2 and

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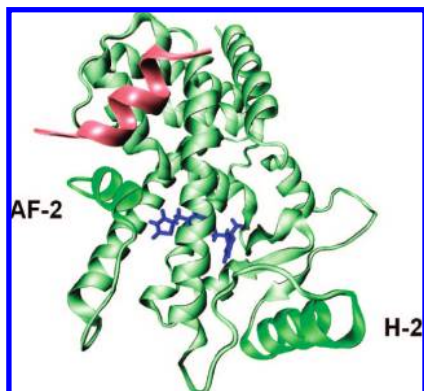
Figure 1. Structure of PPAR-γ/agonist/coactivator (PPAR-LBD, green; agonist, blue; coactivator, pink; AF-2 and H-2′ domains are highlighted).
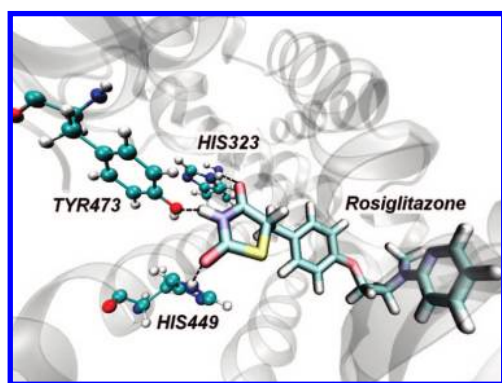
Figure 2. Interaction network between rosiglitazone and PPAR-γ LBD.

makes it possible to recruit coactivators. This argument was validated by NMR experiments, fluorescence anisotropy techniques, and H-D exchange experiments.[13,16]

Methods

In this study, the crystal structure of PPAR-γ/agonist/coactivator determined by Nolte et al.[17] was used as the starting structure. Protein was solvated in an octahedron-like box and the system is neutralized by adding counterions. After heating and equilibration, another 20 ns simulation was done at 300 K (NPT). The simulation was done by employing two force fields: Amber99SB[18] and PPC (polarized protein-specific charges).[19] In PPC, the corresponding AMBER force field parameters[18] are retained, except for the atomic charges that are replaced by PPC. For the ligand rosiglitazone, Amber GAFF parameters were used.[20]

Since the procedure to compute PPC for a given protein structure is already reported elsewhere,[19] we give only a brief review of the method here. The basic procedures in fitting atomic charges of protein in our approach can be described as follows: First, gas-phase quantum chemistry calculation of protein is performed with the MFCC approach to obtain initial electron density of the protein through fragment calculation for the given structure as described

earlier.[21−23] The calculated electron density is used to fit atomic charges using the RESP procedure. The charge fitting philosophy used here was the same as that used in the Amber force field and this guarantees that PPC charge is consistent with other parameters of the Amber force field. Solution of the PB equation is then carried out to obtain reaction field, from which discrete surface charges on the cavity surface are generated. The quantum chemistry calculation of protein fragment is performed again but now with the protein embedded in an external electrostatic potential produced by induced solvent surface charges and other fragments of the protein.[19,23] The newly calculated protein atomic charges are used again to calculate new solvent-induced charges, and such processes are repeated until convergence is reached. Typically, about five such iterations are sufficient to reach desired convergence.

A comment on the possible dependence of the PPC on the specific quantum chemistry method used to compute ESP is in order here. Since DFT is expected to do a decent job of providing the electrostatic interaction of molecular systems, it should be reasonably accurate to be used in charge fitting. Higher level correlation methods certainly provide more accurate electronic energy, but the improvement in electrostatic potential over DFT may be limited. Of course, more investigation is needed to fully address this point in future studies.

Results and Discussion

Stability of AF-2 in Ligand Binding. Originally, we tried to investigate entropic changes accompanied with the agonist binding using the standard AMBER force field. However, unexpected results happened and the most important hydrogen bond between the ligand's headgroup and Tyr473 breaks during the simulation. We therefore applied a recently developed approach (MFCC-PB)[19] to calculate PPC for the protein system. The PPC is derived from efficient quantum mechanical calculation of protein in solution using a fragment-based approach.[21,23] Specifically, the PPC are atomic charges that represent the polarized electronic state of the protein at a given (or native) structure. Different from amino acid−based AMBER charges, the PPC are protein-specific and need to be calculated for a given protein structure, usually the native structure.[19] Because PPC explicitly includes electronic polarization of the protein at its native structure, it should give more accurate representation of the electrostatic interaction near the native structure, as was shown recently for the accurate prediction of the pK_a shift of Asp26 in thioredoxin.[19] We thus can compare MD results resulting from the application of both AMBER and PPC potentials for the current system. Except for atomic charges of the protein, all other force field parameters are exactly the same in both AMBER and PPC potentials. Thus, any difference in dynamical properties must result from an electrostatic effect.

Figure 3 plots the time evolution of the hydrogen bond between rosiglitazone (agonist) and Tyr473 during MD simulation in both force fields. As seen clearly in the figure, this hydrogen bond breaks after about 8 ns simulation under the Amber force field. On the other hand, the same hydrogen bond is well preserved under PPC, as shown in Figure 3 by the red curves. This result clearly demonstrates that polarization helps stabilize hydrogen bonding and therefore plays important roles in protein−ligand interaction, especially for interactions in which electrostatic interactions are strongly weighted. Previous work

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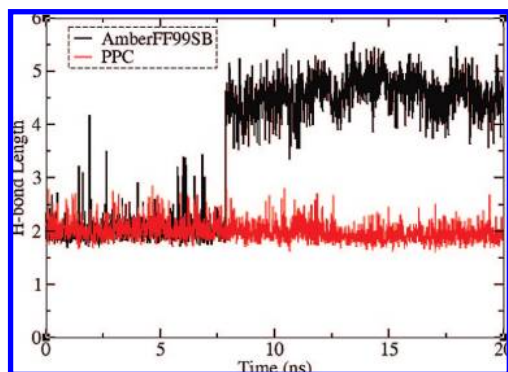(19) Ji, C. G.; Mei, Y.; Zhang, J. Z. H. *Biophys. J.* **2008**, *95*, 1080.
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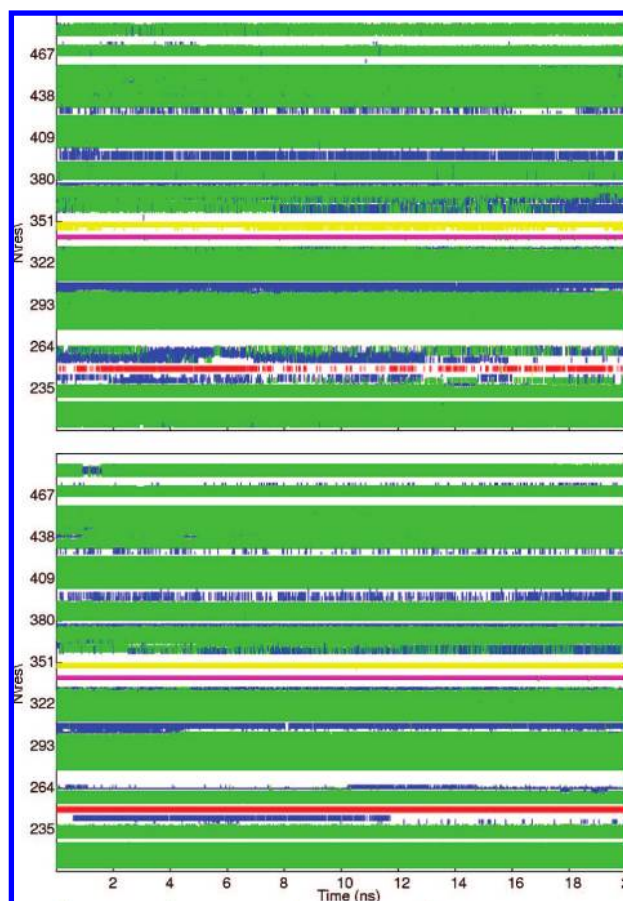
Figure 3. Evolution of hydrogen bond length between rosiglitazone and Tyr473.

of Fresiner and co-workers[24] showed that inclusion of polarization in ligand improved protein−ligand binding structure significantly.

Stability of Helix-2′. To further understand the influence of force field parameters on protein structure during MD simulation, the evolution of the secondary structure of PPAR-γ was computed by DSSP analysis.[25] Figure 4 shows that some disordered structures appeared under the AMBER potential, especially for residues between 251 and 266 (upper figure). This segment of the protein corresponds to a helix structure (helix-2′). In contrast, the DSSP under PPC potential shows ordered structures, including the helix-2′ segment as shown in Figure 4 (lower figure). More detailed analysis for this phenomenon is given below.

The partially denatured structure from AMBER MD in Figure 4 was plotted against the native structure (the starting structure) in Figure 5. We can see that helix-2′ is denatured under the Amber potential (Figure 5.). This part of the protein corresponds to the disordered sequences reflected in DSSP plots in Figure 4. It will be shown later that the breaking of backbone H-bonds in the helix structure under AMBER potential is responsible for this artificial denaturing of the helix. In contract, the helix is preserved under PPC potential in which the protein polarization effect is embedded in the atomic charges.

It is well-known that a protein's secondary structure is mainly determined by noncovalent bonding interactions between residues. The magnitude of these interactions is relatively small compared to chemical bond interactions. A protein's stable three-dimensional structure is a result of a detailed balance of all these weak interactions, with hydrogen-bonding often being the dominant interactions, especially for secondary structures. Perturbation of a small part of these interactions may introduce large conformational change on a protein's structure. Such an idea has been widely recognized for years in the mutation study of proteins. Thus, a high-quality force field for MD simulation is required due to the high sensitivity of a protein's structure to inter- and intraprotein interactions. An inaccurate force field may drive the system away from its native state. The final structure during MD simulation would rely heavily on newly established equilibrium between intraprotein interaction and other interactions determined by the force field employed. Both the breaking of the important hydrogen bond between rosiglitazone and PPAR-γ and denaturing of helix-2′ under the

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Figure 4. Secondary structure evolution during MD simulation. The upper figure corresponds to the AMBER force field and the lower figure represents the result from PPC (parallel sheet, red; mixed P/A sheet, yellow; double antiparalel sheet, purple; 3-helix, blue; 4-helix, green).
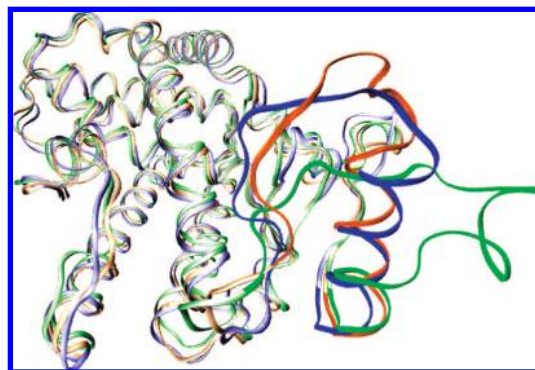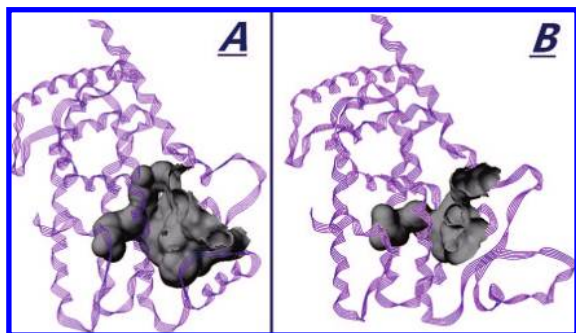

Figure 5. Superposition of the native structure (blue) with the final structure under, respectively, the Amber potential (green) and PPC potential (orange) from MD simulation. The partly denatured structure is seen under the AMBER potential (green).

AMBER potential are a result of rebalancing intraprotein and protein−ligand interactions. Such an equilibrium structure is a pseudonative structure specific to the force field employed.

Important pockets for binding or enzyme reaction in proteins were formed by steady building blocks (such as helix or sheets). The above-mentioned helix-2′ is part of the agonist-binding pocket. Local hydrophobic collapse occurred and is accompanied by denaturing part of the secondary structure under the AMBER potential. This local hydrophobic collapse would block the important binding pocket. For example, Figure 6 shows that

Figure 6. Volume of the binding pocket: (A) native structure and (B) partially denatured structure under the Amber potential.
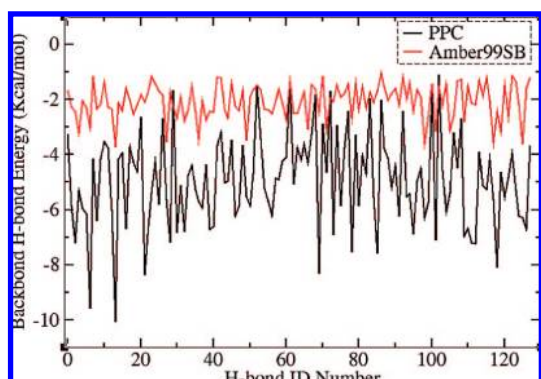
Figure 7. Strength of the backbone hydrogen-bonding energy under AMBER and PPC potentials.

Figure 8. Time evolution of the H-bond interaction energy between Lys261 and Met257 under AMBER and PPC potentials, respectively.

Figure 9. Breaking of the backbone hydrogen bond between Lys261 and Met257 in helix-2′: (A) native structure and (B) partially denatured structure of helix-2′ from MD simulation with the AMBER force field in which the backbone H-bond between Lys261 and Met257 is broken. Instead, the side chain of Lys261 forms a hydrogen bond with the CO group of Met257. (C) The NH group of Lys261 forms a backbone hydrogen bond with Gly258.

the size of the ligand binding pocket, which is reduced significantly due to the unexpected hydrophobic collapse in the AMBER potential.
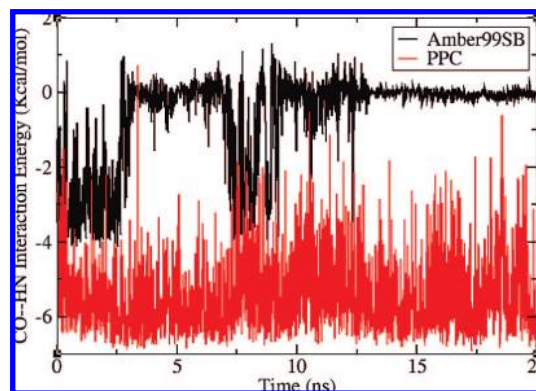
Effect of Polarization on Hydrogen-Bonding Strength. To give a more quantitative measure in difference between two sets of charges, we calculated the H-bond energy of the backbone hydrogen bonds (the CO—HN pairs) at the native structure. Due to the lack of polarization effect, the hydrogen-bonding energy in AMBER is smaller than that given by PPC (Figure 7). The average H-bond strength is 2.1 kcal/mol under AMBER vs 4.9 kcal/mol under PPC. This result is also in good agreement with a previous DFT study on β-sheets.[26] The relatively weak H-bond under Amber99SB may not be able to stabilize some naturally occurring secondary structures during MD simulations.

To further investigate this issue and to understand the physical origin for the denaturing of helix-2′ under AMBER, we plotted the energy change of the backbone H-bond between Lys261 and Met257 in helix-2′ (Figure 8). This H-bond breaks during simulation and these two residues finally formed hydrogen bonds with water molecules under the AMBER potential. In contrast, the same H-bond is well-preserved under the PPC potential, as shown in Figure 8. The formation of the helix is recognized as an enthalpy-driven process. Whether the peptide folds to a helix or adopts a coil state is mostly determined by the free energy change during the hydrogen-bond exchange process:[27]
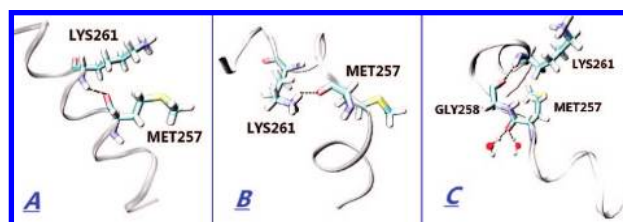
$$\Delta H(\text{helix} \rightarrow \text{coil}) = \Delta H(\text{hb}_H) + \Delta H(\text{hb}_{w-w}) - \Delta H(\text{hb}_{c-w})$$

(1)

Here hb_H stands for the hydrogen bond in the helix, hb_{w-w} for hydrogen bonds between water molecules, and hb_{c-w} for hydrogen bond between coiled peptide and water molecules. The nonpolarized force field may underestimate $\Delta H(\text{hb}_H)$ and drive the native helix to a coil structure.

Backbone Hydrogen Bonding in Helix-2′. Detailed interaction networks around Lys261 and Met257 are pictured in Figure 9. In the native structure, the NH group of Lys261 forms a hydrogen bond with the backbone CO group of Met257, as shown in Figure 9A. After long simulation under the AMBER potential, this hydrogen bond breaks and we picked two representative coil structures to show in Figure 9. Figure 9B shows that instead of the original backbone hydrogen bond, the side chain of Lys261 forms a hydrogen bond with Met257. In Figure 9C, the NH group of Lys261 forms a backbone hydrogen bond with another amino acid Gly258 and the CO group of Met257 is liganded by two water molecules.

Some force fields try to balance electrostatic interactions with water models by using larger partial atomic charges than would be found in a gas-phase residue. Amber99SB adopts charges fitted from an HF/6-31G* gas phase calculation that systematically overestimates the polarity of residues.[28] Amber03 fits charges from condensed phase calculation with dielectric constant $\varepsilon = 4$.[29] This may reflect the average protein's environment. However, it could not reflect overpolarization of surface residues by water and underpolarization of inner

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residues. PPC solves the balance problem by combining quantum chemistry calculation of the entire protein with the continuum solvation model. High-resolution XRD experiments by Lario et al.[30] showed that amino acids have different polarity under different environments. The inhomogeneous character of protein implied that the polarization effect of each amino acid cannot be described correctly by some empirical mean field protocol. For example, amino acids on the surface may be more polarized than the one in the internal residues, due to the strong polarity of water. And due to a cooperative effect, those residues involved in helix formation are more polarized than those in loops.

Conclusion

An old question one usually asks in routine MD simulation is, when will the system reach equilibrium? There is no definite answer here, for that is dependent on the particular force field used and also the specific system being studied. An inaccurate force field could drive the whole system to its "pseudonative state". If the force field specific pseudonative state is near its real native state, then one is lucky, because it would not cost too much simulation time before equilibrium. However, if the force field specific pseudonative state is far away from its real native state, one could be trapped into thinking about how long the MD should be run before equilibrium is reached. In this case, the rmsd often rises slowly and one may never reach the correct equilibrium or be trapped in some pseudonative states.

It is important to point out that PPC is created to represent accurate electrostatic interaction by explicitly including the electronic polarization effect of a protein at a given structure (usually the native structure). Thus, its accuracy is limited to studying properties of protein near the native structure. It is expected that the larger the deviation of the protein conformation from the reference structure used to determine PPC, the less accurate the PPC will be. An extreme example is the difference between AMBER and PPC. The standard AMBER charges represent fully extended structure(s) well, while PPC correctly represents the native structure (or reference structure chosen).

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