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Flexible ligand recognition of peroxisome proliferator-activated receptor-γ (PPARγ)

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ABSTRACT

The peroxisome proliferator-activated receptor-γ (PPARγ) is a direct pharmacological target for drugs that enhance insulin sensitivity and are used clinically for the treatment of type II diabetes. Because the specificity of ligand recognition is lower for PPARγ than for other nuclear receptors, PPARγ can bind a larger variety of ligand types. In order to elucidate why the ligand recognition of PPARγ is so flexible, we performed correlated fragment molecular orbital calculations for complexes of PPARγ and each of two distinctive ligands, rosiglitazone and farglitazar. We found quite different patterns of ligand binding for these two ligands. The ligand-binding system of rosiglitazone, a drug in common clinical use, is based mainly on local electrostatic interactions around the thiazolidine ring, whereas both electrostatic interactions and van der Waals dispersion interactions with hydrophobic residues are required for the binding of farglitazar to PPARγ. We suggest that the development of novel ligands will require adequately hydrophobic pharmacophores.

In recent years, the use of in silico drug design has rapidly increased the demand for accurate and reliable molecular orbital (MO) calculations of large biomolecules. Recently, we predicted theoretical binding affinities of ligands to a nuclear receptor (NR).[1] Using coupled experimental alanine scanning mutational analysis with the ab initio fragment molecular orbital (FMO) calculations for the vitamin D receptor (VDR),[2–5] we determined that electrostatic interactions are the major determinant of ligand binding activity and ligand recognition specificity, whereas van der Waals interactions are important for protein folding and, in turn, for cofactor binding.[6] All electron calculations based on the FMO method[7–10] enable us to correctly evaluate not only the electrostatic interactions but also van der Waals dispersion interactions between a ligand (or substrate) and hydrophobic residues of its receptor (or enzyme), making the FMO method an efficient tool for in silico drug design.[11–13]

Peroxisome proliferator-activated receptor-γ (PPARγ) is a member of the NR superfamily that functions as a ligand-dependent transcription factor.[14,15] PPARγ mediates several bioactivities (adipocyte differentiation, lipid and glucose homeostasis, and accumulation of lipids[16,17]) and is a target protein for the treatment of type II diabetes.[18,19] The ligand-binding pocket (LBP) of PPARγ has a larger hydrophobic pocket than that of other NRs.[20–22] Therefore, the specificity of ligand recognition for PPARγ is lower than that for other NRs, and PPARγ can therefore bind a larger variety of ligand types. The thiazolidinediones (TZDs), such as rosiglitazone, pioglitazone, and troglitazone are agonists for PPARγ and have been used clinically as insulin sensitizers.[20,23] Farglitazar is a tyrosine-based PPARγ agonist. It was developed as a non-thiazolidine derivative, and its affinity is 2–3 orders of magnitude stronger than that of previous agonists.[21] Yamamoto and Yamada, two authors of the current Letter, have developed novel agonists for PPARγ[24,25] and determined the structures of PPARγ bound to oxidized fatty acids.[26] In this study, we investigated the origin of the flexible ligand recognition of PPARγ by applying the FMO method at the correlated Møller–Plesset second-order perturbation (MP2) level to complexes between PPARγ and each of two distinctive ligands, rosiglitazone and farglitazar.

In the FMO calculations, PPARγ was divided into one-residue fragments, with cutoff points at the Cα of each residue, and each ligand, rosiglitazone and farglitazar, was treated as a single fragment. All FMO calculations were performed on a cluster computer system using the ABINIT-MP program (available from http://www.ciss.iis.u-tokyo.ac.jp/fsis/en/theme/molecula/index.html).

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The three-dimensional data for the human PPARγ-ligand-binding domain (LBD)/rosiglitazone and PPARγ-LBD/farglitazar complexes (Fig. 1) were retrieved from the Protein Data Bank (PDB; code numbers 2prg[20] and 1fm9,[21] respectively). The structural defects of the PDB data were amended using the molecular graphics software InsightII (Molecular Simulations Inc., San Diego, CA, USA) and refined by the CHARMM force field incorporated in the package. The positions of the hydrogen atoms forming the hydrogen bonds between PPARγ and the ligand in the LBP were optimized at the traditional HF/6-31G** level for partial model systems with the GAUSSIAN 03 program package.[27]

We carried out ab initio FMO calculations at the correlated MP2/6-31G* level for the PPARγ/rosiglitazone complex. The MP2 method can be used to correctly evaluate both electrostatic and van der Waals dispersion interactions between the ligands and residues in the receptors,[6] and it determines these interaction energies separately. We also analyzed the energies of the interaction between each residue and rosiglitazone by using an interfragment interaction energy (IFIE) analysis[6] based on the FMO calculation. The FMO-IFIE analysis can be used to evaluate the energy of interactions with the ligand and assign the functions of key residues in PPARγ. FMO-IFIE analysis is, then, a powerful tool for analysis of pharmacophores of target receptors and their ligands. Each IFIE result can be separated into electrostatic and van der Waals energies.

Figure 2a shows the energies of interaction between rosiglitazone and all residues located within 4 Å of it. Six hydrophilic residues (Gln286, Ser289, His323, Tyr327, His449, and Tyr473) were within 3 Å of rosiglitazone and had large interaction energies with it. These six residues are located around the thiazolidine ring of rosiglitazone (Fig. 1c). Even though the distances between the residues and the thiazolidine ring were similar, the interaction energies of each residue with rosiglitazone were quite different. This calculated result gives us vital information about the function of

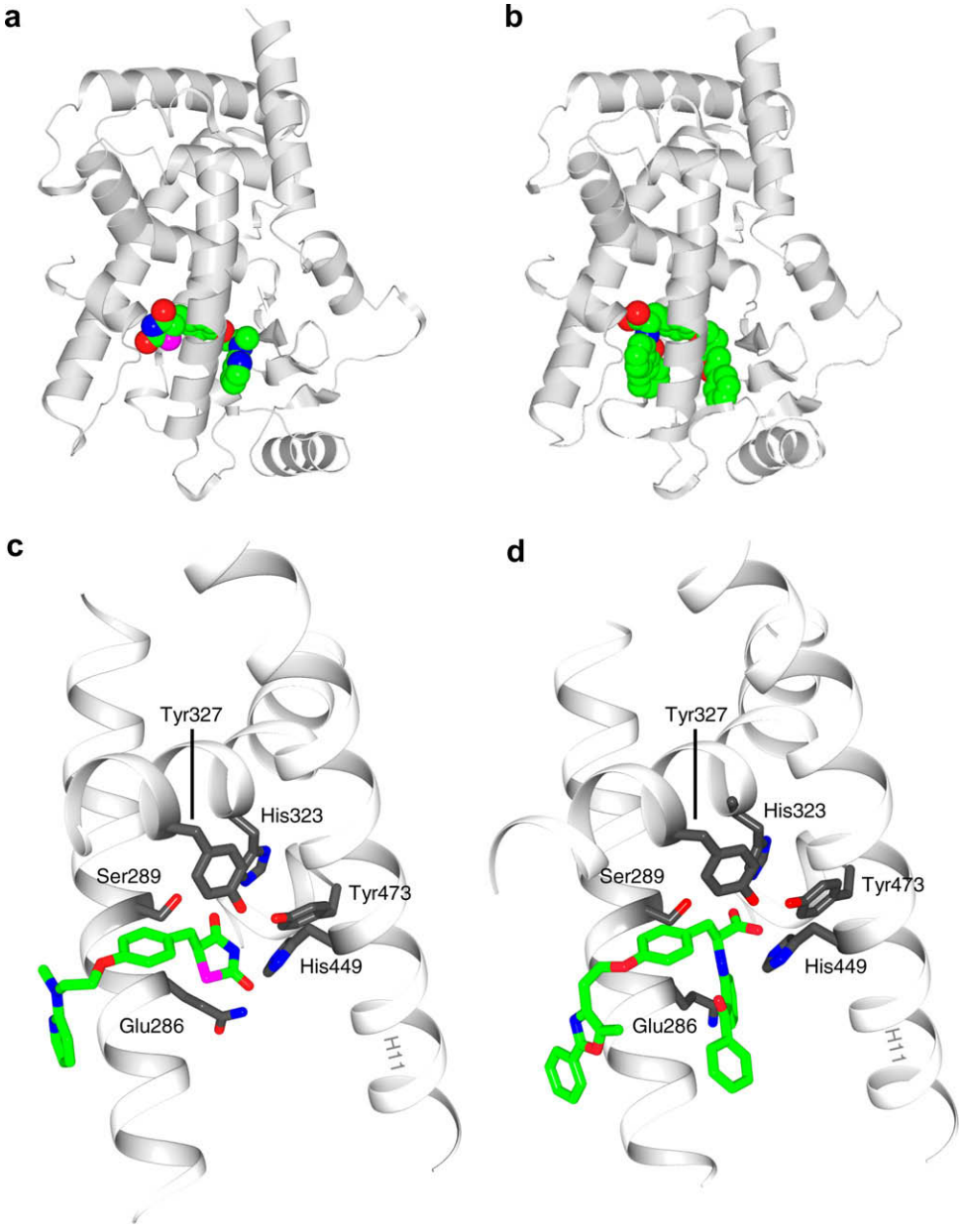

Figure 1. (a and b) Three-dimensional structures of PPARγ/ligand complexes. The ligands are shown with the CPK model (carbon, green; oxygen, red; nitrogen, blue; sulfur, pink). (a) rosiglitazone, (b) farglitazar. (c and d) Close-up view around the LBP of PPARγ. The ligands are shown with the stick model (carbon, green; oxygen, red; nitrogen, blue; sulfur, pink). Selected hydrophilic residues are indicated. (c) rosiglitazone, (d) farglitazar.

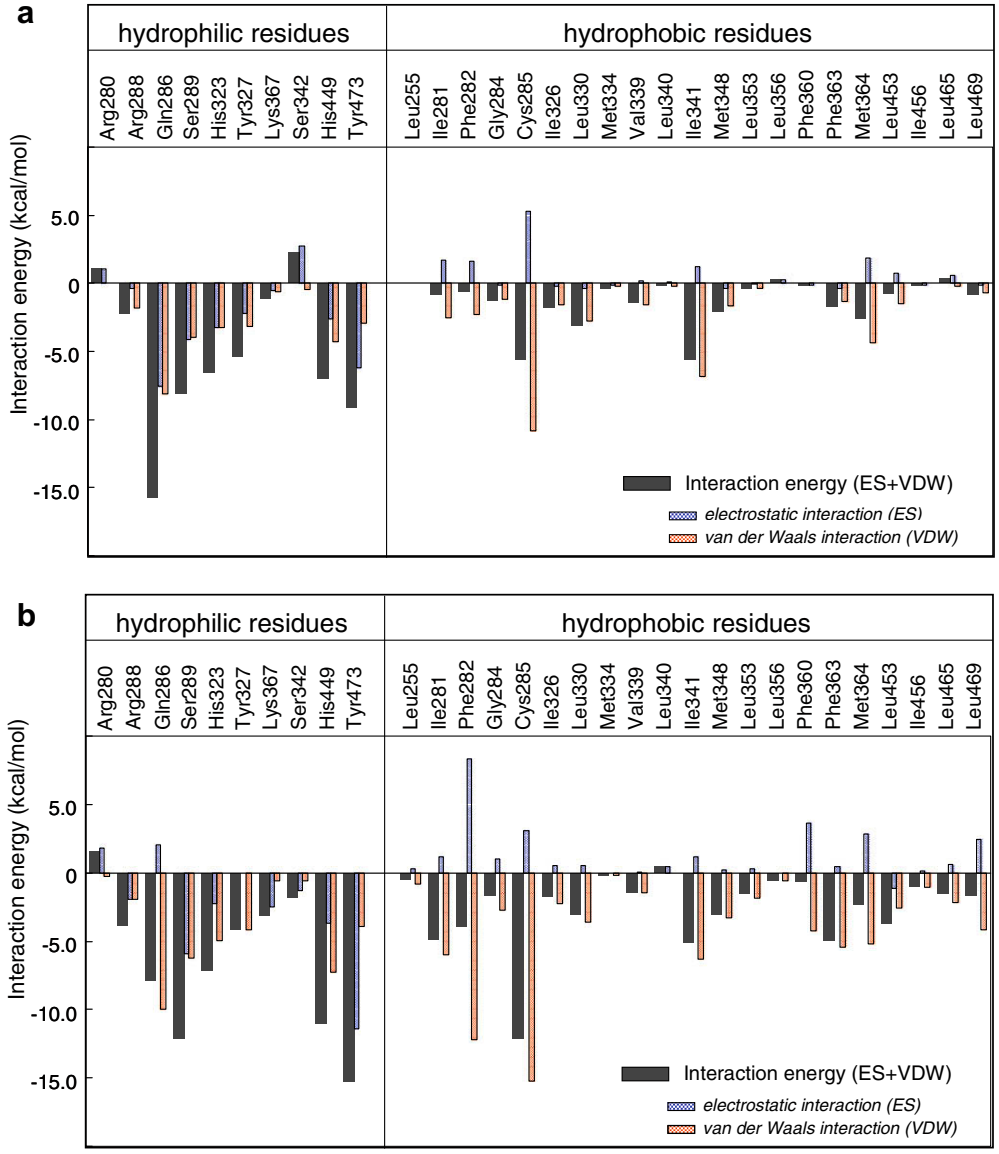

Figure 2. Energies of interaction between ligands and residues of PPARγ. The interaction energies (kcal/mol) calculated by the FMO-IFIE analysis at the MP2/6-31G* levels are shown by the dark gray bars. The electrostatic (blue bars) and van der Waals (red bars) interaction energies calculated with FMO-IFIE analysis are shown separately. (**a**) rosiglitazone, (**b**) farglitazar.

the residues. Glu286 had the most stable energy of the interaction with rosiglitazone. We found that the van der Waals interaction energies between the six hydrogen-bonding residues and rosiglitazone were similar, and that the substantial differences between the overall interaction energies were due to electrostatic interactions. These findings indicate that local electrostatic interactions around the thiazolidine ring are dominant in the ligand binding of rosiglitazone to PPARγ.

We also carried out FMO calculations at the MP2/6-31G* level for the PPARγ/farglitazar complex and analyzed the energies of the interaction between each residue and farglitazar by using an FMO-IFIE analysis. Figure 2b shows the energies of interaction between farglitazar and all residues located within 4 Å of it. Five hydrophilic residues (Ser289, His323, Lys367, His449, and Tyr473) were within 3 Å of farglitazar (Fig. 1d) and had large interaction energies with farglitazar (Fig. 2b); however, the interaction energy between Gln286 and farglitazar was only −7.94 kcal/mol, half of that between Gln286 and rosiglitazone. Similarly, the energy of interaction with Tyr327 decreased to −4.16 kcal/mol from the −5.43 kcal/mol of rosiglitazone. Thus, no hydrogen bonds are created between farglitazar and these residues, and the stabilization energy calculated from the electrostatic interactions was markedly lower than that for rosiglitazone. In contrast, the interaction with His449 increased to −11.0 kcal/mol for farglitazar from −6.96 kcal/mol for rosiglitazone, and the interaction with Tyr473 increased to −15.3 kcal/mol for farglitazar from −9.13 kcal/mol for rosiglitazone. This analysis suggests that the unstable electrostatic interactions of many residues could become stable through van der Waals interactions. Although each interaction between a hydrophobic residue and the ligand is small, the sum of the interactions is large enough to confer stability. Because farglitazar is bigger than rosiglitazone, it occupies a larger space in the LBP of PPARγ and can substantially interact with many more hydrophilic and hydrophobic residues in the LBP than rosiglitazone. These results clearly show that both electrostatic interactions and van der Waals interactions are required for the binding of farglitazar to PPARγ. This property also results in higher activity for farglitazar than for rosiglitazone, because farglitazar binds tightly to PPARγ through both electrostatic and van der Waals interactions.

Table 1
Theoretical binding score and experimental transcription

Ligand	Binding score[a]	Transactivation[b] PPARγ pEC_{50}
Rosiglitazone	−70.0	7.05 ± 0.19
Farglitazar	−107.4	9.47 ± 0.44
Ratio[c]	1.53	1.34

[a] Theoretical binding score at the FMO-MP2/6-31G* level (kcal/mol).
[b] pEC50, −log of the concentration of a test compound required to induce 50% of the maximum alkaline phosphatase activity ± standard error (Ref. 18).
[c] Ratio of farglitazar to rosiglitazone.

We estimated the theoretical binding score (BS) between PPARγ and the ligands from the following equation:

$$BS = E_{\text{complex}} - (E_{\text{receptor}} + E_{\text{ligand}}), \quad (1)$$

where E_{complex}, E_{receptor}, and E_{ligand} are the energy of the complex, receptor, and ligand, respectively.

The BS values for the two ligands were calculated to be −70.0 and −107.4 kcal/mol for rosiglitazone and farglitazar, respectively (Table 1). The BS value is closely related to the genetic transcription activity initiated by the ligand–receptor binding. Our theoretical ratio of binding affinities for farglitazar/rosiglitazone (1.53) is in good agreement with a recent experimental ratio (1.34) obtained for the corresponding effective concentration, pEC_{50} (Table 1).[18]

PPARγ has a larger LBP than other NRs, enabling it to flexibly recognize various types of ligands through different binding systems. We used theoretical calculations to analyze the nature of the flexible ligand recognition of PPARγ. Our FMO calculations clearly indicate that the binding systems for two typical ligand–PPARγ complexes have different dominant factors. The ligand-binding system of the drug most frequently used for diabetes treatment, rosiglitazone, is based mainly on electrostatic interactions around the thiazolidine ring. In the case of farglitazar, the molecular size is bigger, so that farglitazar can substantially interact with many more hydrophilic and hydrophobic residues in the LBP than rosiglitazone. Therefore, in addition to electrostatic interactions, van der Waals interactions between farglitazar and the hydrophobic residues of PPARγ are also important for its stable binding. We conclude that the large LBP of PPARγ permits different binding systems for typical ligands and suggest that the development of novel ligands of sufficient binding affinity will require adequately hydrophobic pharmacophores.

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