Specific interactions between vitamin D receptor and ligand depending on its chirality: \textit{ab initio} fragment molecular orbital calculations

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Abstract

The chirality of a compound affects its biochemical and pharmaceutical properties. It was found that the binding affinity between vitamin D receptor (VDR) and its ligand depends significantly on the chirality of the ligand. To elucidate the reason for this dependence, we here investigated the specific interactions between VDR and two types of ligands with different chirality, using \textit{ab initio} fragment molecular orbital (FMO) calculations. The FMO results reveal that the part of ligand with different chirality interacts strongly with the imidazole ring of histidine side-chain in VDR, and that the binding affinity between VDR and the ligands depends on the protonation state of the histidine. This finding indicates the possibility that ligands with different chirality can assign the protonation state of VDR histidine residues existing near the ligand.

Key Words: Chirality, Specific interactions, Molecular simulation, Fragment molecular orbital, Vitamin D receptor, Binding affinity

Area of Interest: \textit{In silico} drug discovery
1. Introduction

Many biologically active molecules are chiral, including naturally occurring amino acids and sugars. In biological systems such as proteins and DNAs, most of these component compounds are of the same chirality. In contrast, synthesized compounds can have different chirality, and their biochemical and pharmaceutical properties depend on their chirality. For example, the binding affinity between vitamin D receptor (VDR) and its ligand was found to depend significantly on the chirality of the ligand [1].

VDR has been recognized to exist in almost all cell tissues in vivo, and the relationship between various pathological conditions and genetic mutations was analyzed [2]. It was also found that VDR is expressed in many cancer cells, and that some vitamin D$_3$ (VD3) derivatives can inhibit the proliferation of these cancer cells [3]. Therefore, numerous VD3 derivatives have been synthesized as candidate agents for the treatment of cancer, and their beneficial effects have been investigated in clinical medicine [4].

It is indispensable to determine the structures of VDR complexed with VD3 derivatives for elucidating the physiological effect of VDR and proposing novel agents as a potent ligand to VDR. In the X-ray structure analysis [5], the structures of human VDR complexed with VD3 derivatives were determined. The results clarified that the VD3 derivatives form hydrogen bonds to the Arg274 residue of VDR and have a high binding affinity for VDR. It was also elucidated that the difference in chirality of VD3 derivative affects significantly on its binding affinity with VDR. However, the reason why the difference in chirality of the VD3 derivatives affects their binding affinity with VDR was not explained.

In the previous ab initio molecular simulations [6], we explained the reason for the significant difference in biological effect on the VDR activity between the two VD3 derivatives, whose structures are almost the same as each other but for the CH position at the terminal tetrazole ring. In the present study, we employed two types of VD3 derivatives, which have different chirality at each other at the carbon atom of the terminal furan ring as shown in Figure 1.

![Figure 1](image_url)

**Figure 1.** Chemical structures of ligands employed in the present study

The structure of ligands 1 and 2 are obtained from their complexes with VDR registered in PDB. PDB ID is 3CS4 (ligand 1) and 3CS6 (ligand 2), respectively.
2. Details of molecular simulations

To elucidate the dependence of chirality of VD3 derivative on its specific interactions with VDR, the structures of VDR+VD3 complexes were optimized in water by the classical molecular mechanics (MM) method, and the binding properties between VDR and the VD3 derivatives were investigated by \textit{ab initio} fragment molecular orbital (FMO) calculations \cite{7} at an electronic level. This method has been widely used for investigating the interactions between biomolecules such as protein, DNA and ligand at an electronic level. Recently, in order to realize the \textit{in silico} drug design based on FMO calculations, FMO Drug Design consortium \cite{8} was established, and a database on the specific interactions between proteins related with the onset of diseases and the ligands was created. Based on this database, it might be possible to classify the ligands into some categories depending on their interactions and propose novel agents having high-affinity for binding to the protein related to the target disease. In the present study, we investigated the specific interactions between VDR and the ligands shown in Figure 1 by taking the following steps.

2.1 Structure optimization and charge distribution of two VD3 derivatives

The chemical structures of two VD3 derivatives employed in the present study are shown in Figure 1. It is noted that only the chirality at the carbon atom of the terminal furan ring is different in the two structures, although their binding affinity to VDR are significantly different to each other \cite{5}. Their initial structures were cut out from the PDB structures (PDB ID: 3CS4 for ligand 1, 3CS6 for ligand 2). These structures were optimized by the B3LYP/6-31G(d,p) method of \textit{ab initio} MO program package Gaussian09 (G09) \cite{9}. In addition, the charge distributions of the VD3 derivatives were evaluated by the RESP (Restrained Electrostatic Potential) analysis of G09, using the HF/6-31G(d) method, because the same method for making charge parameters of ligands in AMBER is employed. These charges were employed for constructing the force fields \cite{10} of these derivatives in the MM method.

2.2 Construction of initial structure of VDR

As for the initial structure of VDR, we here employed the PDB structures \cite{11} of the complexes of human VDR (hVDR) with each of the VD3 derivatives. The PDB IDs are 3CS4 for ligand 1 and 3CS6 for ligand 2. These PDB structures have no information on the structure of the residues 163-215. Since these residues build a long loop domain of hVDR, this domain is so flexible that the structure cannot be determined by the X-ray analysis. Therefore, Rochel \textit{et al.} \cite{12} engineered a ligand-binding domain (LBD) of hVDR without its flexible loop composed with the residues 165-215, which exist far away from the amino acids involved in the binding of the ligand or the interaction with corepressors or coactivators. The dissociation constants of the wild-type and the engineered LBDs were observed to find that the deletion of the loop domain has no major effect on the ligand binding. Accordingly, we here ignored the loop domain and connected the 164 and the 216 residues by a peptide bond. The sterical hindrance of artificial peptide bond caused the disorder with Gly163 and Gly164 residues existing near the bond. As a result, the information on these residues is missing in the PDB file. In addition, to determine the protonation states of His residues included in VDR, we analyzed the pKa values around each His residue and assigned the His protonation state based on the pKa values obtained by PROPKA 3.0 \cite{13}\cite{14}.

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2.3 Structure optimization of VDR+VD3-derivative complexes

To obtain stable structures of the VDR+VD3-derivative complexes, we added hydrogen atoms to the PDB structures, by use of the tleap command of the MM and molecular dynamics (MD) simulation program AMBER12 [15]. Only the positions of the hydrogen atoms were optimized by the MM method, and subsequently, the structure around the complemented residues was optimized, considering the crystal water molecules included in the PDB structure. In the MM optimizations, the AMBER99SB-ILDN force field [16], the TIP3P model [17] and the general AMBER force field (GAFF) [18] were used for VDR, water molecules and VD3 derivatives, respectively. The criterion for the convergence of structure optimization was set as 0.0001 kcal/mol/Å.

2.4 Analysis of specific interactions between VDR and VD3-derivatives by FMO calculations

Finally, we investigated the electronic properties of the VDR+VD3-derivative complexes in explicit waters, using the ab initio FMO method [7]. The MP2 [19][20]/6-31G(d) method of the FMO calculation program ABINIT-MP Ver6.0 [21] was used. Each amino acid residue of VDR, VD3-derivative and each water molecule was assigned as a fragment, since this fragmentation makes it possible to analyze the interactions between each VDR residue and the VD3-derivative. In addition, to highlight the important VDR residues for the ligand binding, we investigated the inter fragment interaction energies (IFIEs) [22] obtained by the FMO calculations, and IFIEs were divided into electrostatic (ES), exchange repulsion (EX), charge transfer (CT+mix) and dispersion interaction (DI) components by use of the Pair Interaction Energy Decomposition Analysis (PIEDA) [23]. PIEDA calculation was done by using MIZUHO/BioStation software package [24]. Furthermore, to investigate the binding affinity between VDR and VD3 derivatives, the binding energy (BE) between VDR and the derivative was estimated from the total energies (TEs) using the following equation.

\[ \text{BE} = \text{TE}(\text{VDR+VD3+water}) - \text{TE}(\text{VDR+water}) - \text{TE}(\text{VD3+water}) + \text{TE}(\text{water}). \]

3. Results and discussion

3.1 Determination of His protonation by FMO calculations

VDR contains nine His residues. To determine their protonation state, pKa value around each His residue was evaluated. His residues with a pKa value of larger than 6 are assigned to Hip+ protonation, while the other His residues are assigned to Hid or Hie protonation. It is not easy to determine which protonation Hid or Hie is more preferable. In the present study, two kinds of VDR structures are considered; one has Hip+ and Hid protonations and the other has Hip+ and Hie protonations. They are defined as VDR(Hid) and VDR(Hie), respectively.

Based on total energies evaluated by ab initio FMO calculations, more preferable protonation of His residues in VDR was determined. The total energies for the VDR+ligand complexes are listed in Table 1, indicating that VDR(Hie)+ligand is more stable than VDR(Hid)+ligand for the both ligands.

Moreover, to estimate the binding affinity between VDR and the ligands, the BE between VDR and ligand was calculated by FMO method. In Table 2, the calculated BEs are compared with the binding affinity obtained by the experiment [5]. The BE between VDR(Hie) and ligand 1 is 2.5 kcal/mol larger than that between VDR(Hie) and ligand 2. This result is qualitatively consistent with the trend of the binding affinity. On the other hand, the BE between of VDR(Hid) and ligand 1
is 3.8 kcal/mol smaller than that between VDR(Hid) and ligand 2, being inconsistent with the results of the experiment. Therefore, it can be concluded from Tables 1 and 2 that the VDR(Hie) protonation is more preferable and that the BEs between VDR(Hie) and the two ligands shown in Figure 1 are comparable to the experimental results [5].

**Table 1.** Total energies (Hartree) for VDR+ligand complexes depending on His protonation

<table>
<thead>
<tr>
<th>Complex</th>
<th>Total energy (Hartree)</th>
<th>Complex</th>
<th>Total energy (Hartree)</th>
</tr>
</thead>
<tbody>
<tr>
<td>VDR(Hie)+ligand 1</td>
<td>−116213.8714</td>
<td>VDR(Hie)+ligand 2</td>
<td>−118805.5264</td>
</tr>
<tr>
<td>VDR(Hid)+ligand 1</td>
<td>−116213.8435</td>
<td>VDR(Hid)+ligand 2</td>
<td>−118805.4867</td>
</tr>
<tr>
<td>Difference (kcal/mol)</td>
<td>−17.5</td>
<td>Difference (kcal/mol)</td>
<td>−24.9</td>
</tr>
</tbody>
</table>

**Table 2.** Binding energies (BE, kcal/mol) and between VDR and ligand, and the binding affinity between VDR and ligand obtained by the previous experiment [5]. BEs were evaluated for two types of VDRs, which have Hie or Hid protonation state of histidine residues.

<table>
<thead>
<tr>
<th>Complex</th>
<th>Binding affinity</th>
<th>BE</th>
<th>Complex</th>
<th>Binding affinity</th>
<th>BE</th>
</tr>
</thead>
<tbody>
<tr>
<td>VDR(Hie)+ligand 1</td>
<td>2.0</td>
<td>−118.7</td>
<td>VDR(Hid)+ligand 1</td>
<td>2.0</td>
<td>−115.2</td>
</tr>
<tr>
<td>VDR(Hie)+ligand 2</td>
<td>1.0</td>
<td>−116.2</td>
<td>VDR(Hid)+ligand 2</td>
<td>1.0</td>
<td>−119.0</td>
</tr>
<tr>
<td>Difference</td>
<td>–</td>
<td>−2.5</td>
<td>Difference</td>
<td>–</td>
<td>3.8</td>
</tr>
</tbody>
</table>

3.2 Specific interactions between VDR(Hie) and ligand

In order to elucidate the reason for the change in BEs between VDR(Hie) and ligands, we investigated the IFIEs between each amino acid residue of VDR(Hie) and the ligands. In Figures 2(a) and 2(b), the VDR(Hie) residues, which have attractive IFIE larger than −10 kcal/mol, are indicated by the red lines. The both ligands interact most strongly with Arg274 of VDR(Hie). In addition, the ligands interact with the amino acids Thr143, Ser278, and Trp286. This result is comparable to that for the VDR+1α,25(OH)2D3 complex calculated by the previous FMO calculations (MP2/6-31G**) [25]. To make clear the difference in interactions with VDR(Hie) for the ligands, we analyzed the difference in IFIEs between the ligands 1 and 2. As shown in Figure 2(c), the difference in IFIEs is very small, indicating the similar interaction with VDR(Hie) for both the ligands. It is also revealed from Figure 2(c) that the IFIEs between the ligand and Hie305 or Hie397 of VDR(Hie) are slightly different for the ligands 1 and 2. Therefore, Hie305 and Hie397 are expected to be main factor for detecting the difference in chirality of the two ligands.
We moreover investigated the origin of these interactions by use of PIEDA [23] for the IFIEs obtained by the FMO calculations. Table 3 lists the values of the components divided into ES, EX, CT+mix and DI terms. Table 3(a) indicates that the ES terms for Hie305 and Hie397 are significantly different for the two ligands, and that this ES is a main cause for the difference in IFIE.

To clarify the difference in interactions between VDR residues (Hie305 and Hie397) and ligands 1 and 2, we analyzed their interacting structures. As shown in Figure 3, in the both complexes, the oxygen atom of the hydroxyl group of the ligand forms two hydrogen bonds with the hydrogen atoms of the imidazole rings of Hie305 and Hie397. Since Hie has a hydrogen atom at the ε position of the ring, this hydrogen atom can form a hydrogen bond with the OH group of the ligands, as shown in Figure 3. The distances of the hydrogen bonds for ligand 1 are shorter than those for ligand 2, resulting in the larger BE between VDR(Hie) and ligand 1 compared with that for ligand 2, as listed in Table 2.
Table 3. IFIEs (kcal/mol) between ligand and VDR residues (His305 and His397)

IFIEs are divided into four components by PIEDA [23] analysis; electrostatic (ES), exchange repulsion (EX), charge transfer (CT+mix), and dispersion (DI) terms. The results evaluated for two types of VDRs are compared; (a) VDR with Hie protonation and (b) VDR with Hid protonation of histidine residues.

(a) VDR(Hie)

<table>
<thead>
<tr>
<th>Complex</th>
<th>Amino acid</th>
<th>IFIE</th>
<th>ES</th>
<th>EX</th>
<th>CT+mix</th>
<th>DI</th>
</tr>
</thead>
<tbody>
<tr>
<td>VDR(Hie)+ligand 1</td>
<td>Hie305</td>
<td>−9.5</td>
<td>−14.4</td>
<td>16.9</td>
<td>−4.6</td>
<td>−7.3</td>
</tr>
<tr>
<td>VDR(Hie)+ligand 2</td>
<td>Hie397</td>
<td>−7.3</td>
<td>−9.8</td>
<td>13.8</td>
<td>−3.8</td>
<td>−7.5</td>
</tr>
</tbody>
</table>

(b) VDR(Hid)

<table>
<thead>
<tr>
<th>Complex</th>
<th>Amino acid</th>
<th>IFIE</th>
<th>ES</th>
<th>EX</th>
<th>CT+mix</th>
<th>DI</th>
</tr>
</thead>
<tbody>
<tr>
<td>VDR(Hid)+ligand 1</td>
<td>Hid305</td>
<td>−1.5</td>
<td>−0.2</td>
<td>7.1</td>
<td>−2.0</td>
<td>−6.5</td>
</tr>
<tr>
<td>VDR(Hid)+ligand 2</td>
<td>Hid397</td>
<td>−16.2</td>
<td>−18.4</td>
<td>14.1</td>
<td>−4.1</td>
<td>−7.8</td>
</tr>
</tbody>
</table>

Figure 3 also elucidates the possibility that the change in the protonation state of Hie305 and Hie397 residues can cause a significant change in the interactions between these residues and ligand. If Hie is changed to Hid, the hydrogen atoms contributing to the hydrogen bonds are shifted to the δ position of the imidazole ring. As a consequence, it is expected that the hydrogen bonds shown in Figure 3 disappear, resulting in a significant change in interactions between these residues and the ligands.
Figure 3. Interacting structures between VDR(Hie) residues (Hie305 and Hie397) and ligand
Red dashed lines indicate hydrogen-bonding interactions.

3.3 Specific interactions between VDR(Hid) and ligand

In order to elucidate the influence of the His protonation on the interact between VDR and the
ligands, we conducted the same analysis for the VDR(Hid)-ligand complexes, in which His residues have Hip⁺ or Hid protonation. As show in Figures 4(a) and 4(b), the IFIEs between ligand and each amino acid residue of VDR(Hid) are similar to those for VDR(Hie)+ligand shown in Figure 2. The both ligands interact most strongly with Arg274 and have strong attractive interactions with the Thr143, Arg274, Ser278, and Trp286 residues. In addition, ligand 1 strongly interacts with Hid397, while the ligand 2 strongly interacts with Hid305. To clarify the difference in interactions between VDR(Hid) and the ligands, we analyzed the difference in IFIEs. As shown in Figure 4(c), the interactions between ligand and Hid305, Hid397 are significantly different for the ligands 1 and 2. The difference is larger than 12.6 kcal/mol and more significantly than that for the VDR(Hie)+ligand complexes shown in Figure 2(c). It is therefore elucidated that the change of the protonation states for His305 and His397 in VDR causes a remarkable change in the IFIEs between these His residues and the ligands 1 and 2.
Figure 4. IFIEs between each amino acid residue of VDR(Hid) and ligand; (a) ligand 1, (b) ligand 2, and (c) difference in IFIEs for ligands 1 and 2

To elucidate the reason IFIEs between these His residues and ligand are significantly changed by the change of their protonation states, we analyzed the interacting structures between Hid305 and Hid397 and ligand. As shown in Figure 5, since the imidazole rings of Hid305 and Hid397 has no hydrogen atom at the ε position of the ring, the hydrogen bonds between the oxygen atom of the hydroxyl group of the ligands and the hydrogen atom of Hie305 and Hie397 shown in Figure 3 are not formed in the VDR(Hid)+ligand complexes. In contrast, the hydrogen atom of the hydroxyl group of the ligand forms a hydrogen bond with the nitrogen atom of the imidazole ring of Hid305 or Hid397, as shown in Figure 5. Ligand 1 also interacts weekly with the nitrogen atom of Hid305, while three hydrogen atoms of ligand 2 have significant attractive interactions with the nitrogen atom of the imidazole ring of Hid305. These differences in interactions cause the difference in IFIE (15 kcal/mol) between Hid305 and the ligands shown in Figure 4(c). On the other hand, the imidazole ring of Hid397 interacts more strongly with the hydrogen atom of ligand 1 than that of ligand 2, resulting in a larger IFIE between Hid397 and ligand 1. It is therefore elucidated from Figures 3 and 5 that the specific interactions between VDR(Hie) and ligands 1 and 2 are similar to
each other, while those between VDR(Hid) and the ligands are remarkably different to each other. This result indicates the possibility that the protonation states of His305 and His397 can be determined by the docking of the ligands 1 and 2 having different chirality to VDR.

![Figure 5. Interacting structures between VDR(Hid) residues (Hid305 and Hid397) and ligand. Red, blue and green dashed lines indicate hydrogen-bonding, electrostatic interaction and distance, respectively.]

### 4. Conclusions

In the present \textit{ab initio} FMO study, we investigated the specific interactions between VDR and two types of ligands, whose structures are the same but the chirality at the carbon atom of the furan ring is different. To reveal the effect of the His protonation states of VDR on the interactions, we considered two types of VDR structures. VDR(Hie) and VDR(Hid) have Hie and Hid protonations, respectively. The FMO results for these VDR+ligand complexes elucidate the following points.

1. Both ligands 1 and 2 interact most strongly to Arg274 of VDR.
2. VDR(Hie)+ligand is more stable than VDR(Hid)+ligand for the both ligands, and BEs between VDR(Hie) and the ligands are consistent with the trend of binding affinity between VDR and the ligands obtained by the experiment [5].
3. In the VDR(Hie)+ligand complexes, the ligands 1 and 2 have almost the same binding mode with VDR(Hie).
4. By changing the protonation states of His residues of VDR to Hid, the interactions between the ligand and Hid305 and Hid397 are remarkably changed.
5. The protonation states of histidine residues in VDR can be assigned by interacting the ligands 1 and 2 to VDR.
Acknowledgments

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References


