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Ligand-dependent conformation change reflects steric structure and interactions of a vitamin D receptor/ligand complex: A fragment molecular orbital study☆

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ABSTRACT

We used an *in silico* computational method to theoretically analyze important residue-ligand interactions as well as ligand conformation changes in the vitamin D receptor (VDR). The ligand used for analysis was 1α,25-dihydroxy-19-nor-vitamin D_3 [1α,25-19-nor-$(OH)_2D_3$] [1,2], whose crystal structure has not been solved. To estimate amino acid residue-ligand interactions with chemical accuracy, we adopted the fragment molecular orbital (FMO) method [3,4], which is based on the nonempirical total electronic quantum calculation. The docking of the ligand to the VDR was controlled by hydrophilic and hydrophobic interactions between amino acid residues and the ligand in the ligand binding pocket (LBP) [5–8]. These molecular interactions changed when the conformation of the ligand in the VDR was changed [5,9,10]. This conformation change is important to consider in computational, *in silico,* approaches for analyzing the mechanism of ligand-docking to the VDR.

The position of the 1α,25-19-nor-$(OH)_2D_3$ ligand in the VDR-LBP was related to the hydrophobic interaction that occurred between the Ile271 residue of the VDR-LBP and the ligand. The interaction between Ile271 and 1α,25-19-nor-$(OH)_2D_3$ was repulsive, whereas, that between Ile271 and the natural ligand, 1α,25-$(OH)_2D_3$, is stable. The orientation change in the isopropyl group of Ile271 affected the residue's interaction with 1α,25-19-nor-$(OH)_2D_3$. We also found that conformation changes in the A-ring affected electrostatic (hydrophilic) interactions between the VDR and the ligand.

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1. Introduction

Vitamin D analogues have been widely used for the treatment of osteoporosis [15]. These analogues also have been prepared for use in treatment of some cancers and immune disease [11–15]. However, the clinical use of these analogues is limited, because their therapeutic doses cause serious side-effects [15]. Among these analogues, 1α,25-19-nor-$(OH)_2D_3$, which lacks an exomethylene group at C19, is highly stable and easily synthesized, and binds to the vitamin D receptor (VDR) with a lower affinity than that of the natural ligand, 1α,25-$(OH)_2D_3$ [1]. In the ease of 1α,25-19-nor-$(OH)_2D_3$, the calcemic effects are less intense than those observed in the natural ligand, whereas the cell differentiation activity is similar between the natural ligand and the analogue [1]. Therefore, 1α,25-19-nor-$(OH)_2D_3$ is expected to be a candidate for the treatment of the above-mentioned diseases [15]. However, the origin of the unique biological activity of 1α,25-19-nor-$(OH)_2D_3$ is unknown. We believe that a computational, *in silico,* approach is useful to reveal the origin of the molecular mechanisms of the activity of 1α,25-19-nor-$(OH)_2D_3$.

The fragment molecular orbital (FMO) method, which is based on the nonempirical total electronic quantum calculation, has been successfully applied to various protein-ligand interaction analyses and has been demonstrated to estimate amino acid residues-ligand interactions with sufficient chemical accuracy [16–20]. We have already used the FMO method to various biological functions in the ligand-binding pocket (LBP) of the VDR, as well as to identify key residues responsible for these functions [18,19].

To analyze the mechanism of ligand-docking to the VDR, it is necessary to determine the effect of the conformation change of the ligand and of the receptor by means of such a computational, *in silico,* approach. In the previous FMO studies [16–20], however, these effects were neglected. In particular, weak inter-

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actions between hydrophobic amino acid residues in the LBP and the ligand strongly depend on the conformation change of both the ligand and the receptor. In this study, we used a correlated FMO method to theoretically analyze the important interactions between these residues and 1α,25-19-nor-$(OH)_2D_3$, accounting for the conformation change of both the ligand and the receptor.

2. Methods

2.1. Construction of the VDR/1α,25-19-nor-$(OH)_2D_3$ complex

The X-ray structure of the VDR/1α,25-19-nor-$(OH)_2D_3$ complex has not been reported, so we constructed a VDR/1α,25-19-nor-$(OH)_2D_3$ complex from the experimental structure of the VDR with the natural ligand (PDB ID:1db1) [5]. The exomethylene group at C19 was removed, and two hydrogen atoms were added to C10 by means of the ViewPro 4.2 software package [21]. For the isolated ligand system, we used the Gaussian03 program [22] to partially optimize the A-ring at the Hartree–Fock (HF)/6-31G** level. The position of the hydrogen atoms of 1α-OH and 3β-OH on the ligand, of OH on Tyr143, Ser237, and Ser278, and of NH_2 on Arg274, were refined by partial optimization at the same level for the small model system, which consisted of the A-ring of the ligand, Tye143, Ser237, Arg274, Ser275, Asn276, Ser278. These amino acid residues are located nearby the A-ring. The modified ligand was superimposed on the position of the natural ligand in the VDR.

2.2. Interaction analysis based on the fragment molecular orbital calculation

We performed the FMO calculations at the standard HF and correlated Møller–Presset second-order (MP2) levels with the 6-31G basis set for the complex. In the FMO calculations, each amino acid residue in the VDR was treated as a single fragment. The ligand was also treated as a single fragment. The interfragment interaction energies (IFIE) based on the FMO method were used to estimate the residue-ligand interactions. All FMO calculations were done with the ABINIT-MP package [23].

3. Results and discussion

We performed correlated FMO-MP2 calculations at the 6-31G level to estimate the hydrophobic interaction energies between Leu233, Ile271, Ser275, Trp286, and the ligand. The results are shown in Fig. 1. The conjugated triene connecting the A- and C-rings is effectively contacted with these four residues by means of hydrophobic interactions [5]. Black bars in Fig. 1 show the hydrophobic interaction energies between the residues and 1α,25-$(OH)_2D_3$ at the geometry of our previous study [19]. Gray bars show the hydrophobic interaction energies between the same residues and 1α,25-19-nor-$(OH)_2D_3$ without the conformation change of the receptor, *that is*, the orientations of all residues in the LBP were fixed to the 1α,25-$(OH)_2D_3$ case. In this conformation, only the interaction between Ile271 and 1α,25-19-nor-$(OH)_2D_3$ was substantially different from the interaction observed between the same residue and 1α,25-$(OH)_2D_3$: a repulsive interaction was observed for the 1α,25-19-nor-$(OH)_2D_3$ case. Choi and Yamamoto et al. pointed out that Ile271 is essential for transcriptional activity [24], and the conformation change of Ile271 must be included in the interaction analysis. We estimated the interaction energies between Ile271 and 1α,25-19-nor-$(OH)_2D_3$ with internal rotation of the isopropyl group of Ile271, for which the C–C–C–C torsion angle was changed from 60.0° to 80.0°. The hydrophobic interaction energy was most stable at 67.0°. The white bars of Fig. 1 show the interaction energies between Ile271 and 1α,25-19-nor-$(OH)_2D_3$ at the most stable geometry obtained in this study (Fig. 2). Ile271 had a significantly attractive interaction with 1α,25-19-nor-$(OH)_2D_3$ at the most stable conformation, but the interaction energies between the ligand and Leu233, Ser275, and Trp286 were not almost changed (Fig. 1, gray and white bars). Note that the ligand conformation change that occurred upon docking to the VDR caused the difference in observed interactions between amino acid residues and the ligand. The ligand-binding energy was estimated by conventional supermolecular calculation same as the recent studies [16,17,20]. The binding energies of the VDR/1α,25-19-nor-$(OH)_2D_3$ with and without modification of the orientation of Ile271 calculated at the MP2/6-31G level were −107.2 and −109.1 kcal/mol, respectively. The energy difference −1.9 kcal/mol was well related to the change of the interaction between Ile271 and the ligand (Fig. 1, the gray and white bars).

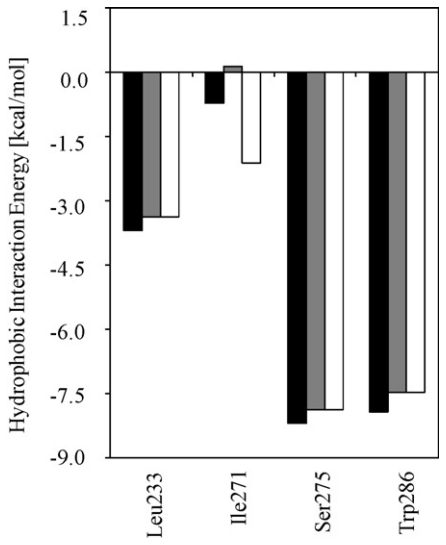

Fig. 1. Hydrophobic interaction energies between the residues and 1α,25-$(OH)_2D_3$ (black) or 1α,25-19-nor-$(OH)_2D_3$ (gray and white). Leu233, Ile271, Ser275, and Trp286 are located around the seco-B-ring within 4.0 Å of the ligand.

The positions of hydrogen atoms within the residues, Tyr143, Ser237, Arg274, and Ser278, and the A-ring are shown in Fig. 3. We performed standard FMO-HF calculations at the 6-31G level

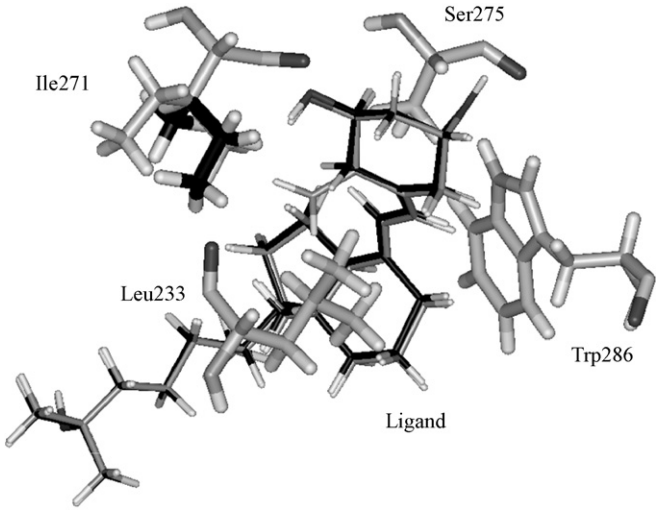

Fig. 2. The most stable geometry of the Ile271 and 1α,25-19-nor-$(OH)_2D_3$ (black) was superimposed on the geometry of the Leu233, Ile271, Ser275, Trp286 and 1α,25-$(OH)_2D_3$ (gray).

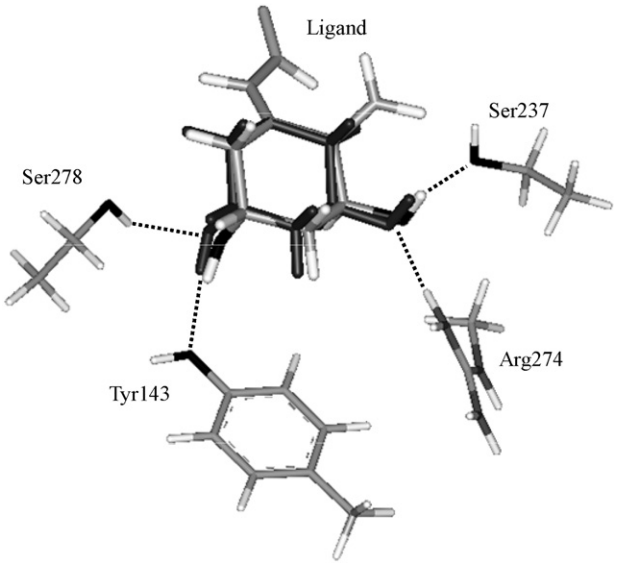

Fig. 3. Positions of hydrogen atoms within the residues and the ligand. The conformation of 1α,25-19-nor-$(OH)_2D_3$ (black) is superimposed on that of 1α,25-$(OH)_2D_3$ (gray).

to estimate the electrostatic interaction energies between these residues and the ligand. The results are shown in Fig. 4. Black bars in this figure show the electrostatic (i.e., hydrophilic) interaction energies between the residues and 1α,25-$(OH)_2D_3$ at the geometry of our previous study [19], whereas the gray bars show the electrostatic interaction energies between the same residues and 1α,25-19-nor-$(OH)_2D_3$. These interactions changed from attractive to repulsive only for Ser278, due to the conformation change of the ligand that occurred upon changing from 1α,25-19-nor-$(OH)_2D_3$ to 1α,25-19-nor-$(OH)_2D_3$.

Considering the induced-fit effect, *that is*, the conformation changes of not only the ligand but also the receptor, we used the FMO method to theoretically analyze important interactions between the VDR and 1α,25-19-nor-$(OH)_2D_3$. The position change of the ligand in the VDR-LBP was strongly related to the hydrophobic interaction between Ile271 and the ligand. The natural ligand

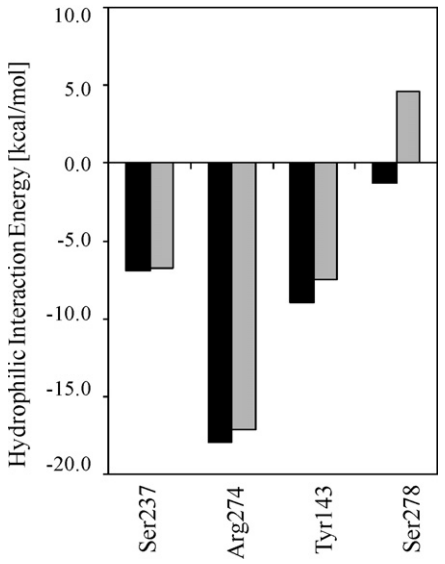

Fig. 4. Hydrophilic (electrostatic) interaction energies between the residues and 1α,25-$(OH)_2D_3$ (black) or 1α,25-19-nor-$(OH)_2D_3$ (gray). Ser237, Arg274, Tyr143, and Ser278 are located around the A-ring within 3.0 Å of the ligand.

1α,25-$(OH)_2D_3$ interacted stably with Ile271, whereas 1α,25-19-nor-$(OH)_2D_3$ had repulsive interaction with the same residue. The orientation change of the isopropyl group of Ile271 was relevant to the residue's hydrophobic interaction with 1α,25-19-nor-$(OH)_2D_3$. Note that the ligand conformation change that occurred upon docking to the VDR LBP caused the interaction between Ile271 and the ligand to change from repulsive to attractive. We also found that the modified-A-ring affected the electrostatic interactions between Ser278 and the ligand. 1α,25-19-nor-$(OH)_2D_3$ with a modified-A-ring was closer to Ser278. In the modified-A-ring, the electrostatic interaction between Ser278 and the ligand was repulsive.

Ligand-docking to the VDR is the initial stage of multiple hormone functions. The conformation of the ligand-dependent transcriptional activation domain (AF-2) changes after the ligand docks to the VDR [5,25]. We are currently analyzing the conformation of AF-2 in a rat VDR/ligand complex [6,8].

References

[1] K.L. Perlman, R.R. Sicinski, H.K. Schnoes, H.F. DeLuca, 1α,25-dyhidroxy-19-nor-vitamin D_3, a novel vitamin D-related compound with potential therapeutic activity, Tetrahedron Lett. 31 (13) (1990) 1823–1824.
[2] K.L. Perlman, R.E. Swenson, H.E. Paaren, H.K. Schoes, H.F. DeLuca, Novel synthesis of 19-nor-vitamin D compounds, Tetrahedron Lett. 32 (52) (1991) 7663–7666.
[3] K. Kitaura, et al., Pair interaction molecular orbital method: an approximate computational method for molecular interactions, Chem. Phys. Lett. 312 (1996) 319–324.
[4] K. Kitaura, et al., Fragment molecular orbital method: an approximate computational method for large molecule, Chem. Phys. Lett. 313 (1999) 701–706.
[5] N. Rochel, J.W. Wurtz, A. Mitschler, B. Klaholz, D. Moras, The crystal structure of the nuclear receptor for vitamin D bound to its natural ligand, Mol. Cell. 5 (2000) 173–179.
[6] J.L. Vanhooke, M.M. Benning, C.B. Bauer, J.W. Pike, H.F. DeLuca, Molucular structure of the rat vitamin D receptor ligand binding domain complexed with 2-carbon-substitutd vitamin D_3 hormone analogues and a LXXLL-containing coactivator peptide, Biochemistry 43 (2004) 4101–4110.
[7] G. Tocchini-Valentini, N. Rochel, J.M. Wurtz, D. Moras, Crystal structures of the vitamin D nuclear receptor liganded with the vitamin D side chain analogues calcipotriol and seocalcitol, receptor agonists of clinical importance. Insights into a structural basis for the switching of calcipotriol to a receptor antagonist by further side chain modification, J. Med. Chem. 47 (8) (2004) 1956–1961.
[8] H.F. DeLuca, T. Ikura, N. Ito, et al., 2-substituted-16-ene-22-this-1,25-dihydroxy- 26,27-dimethyl-19-norvitamin D_3 analogues: synthesis, biological evaluation, and crystal structure, Bioorg. Med. Chem 16 (2008) 6949–6964.
[9] S. Nayeri, C. Carlberg, Functional conformation of the nuclear 1α,25-dyhidroxy vitamin D_3 receptor, Biochem. J. 327 (1997) 561–568.
[10] L.L. Issa, G.M. Leong, R.L. Shutherland, J.A. Eisman, et al., Vitamin D analogue-specific recruitment of vitamin D receptor coactivaors, J. Bone. Miner. Res. 17 (5) (2002) 879–890.
[11] H. Abe, C. Miyaura, H. Sakagami, M. Takeda, K. Konnno, T. Tamazaki, S. Yoshiki, T. Suda, Differntiation of mouse myeloid leukemia cells induced by 1α,25-dihydroxyvitamin D_3, Proc. Natl. Acad. Sci. U.S.A. 78 (8) (1981) 4990–4994.
[12] V.K. Ostrem, Y. Tanaka, J. Prahl, H.F. DeLuca, N. Ikekawa, 24-and 26-homo-1,25-dihydroxyvitamin D_3: Preferential activity in inducing differentiation of human leukemia cells HL-60 in vitro, Proc. Natl. Acad. Sci. U.S.A. 84 (1987) 2610–5614.
[13] R. Bouillon, W.H. Okamura, A.W. Norman, et al., Structure-function relationships in the vitamin D endocrine system, Endocr. Rev. 16 (2) (1995) 200–257.
[14] H.F. DeLuca, C. Zierold, et al., Mechanisms and functions of vitamin D, Nutr. Rev. (56) (1998) 54–75.
[15] D. Feldman, F.H. Glorieux, J.W. Pike (Eds.), Vitamin D, Academic Press, New York, 1997, pp. 1213–1225.
[16] K. Fukuzawa, K. Kitaura, M. Uebayasi, K. Nakata, T. Kaminuma, T. Nakano, Ab initio quantum mechanical study of the binding energies of human estrogen receptor with its ligands: an application of fragment molecular orbital method, J. Comp. Chem. 26 (1) (2005) 1–10.
[17] K. Fukuzawa, Y. Mochizuki, S. Tanaka, K. Kitaura, T. Nakano, Molecular interactions between estrogen receptor and its ligand studied by the ab initio fragment molecular orbital method, J. Phys. Chem. B 110 (32) (2006) 16102–16110;
K. Fukuzawa, Y. Mochizuki, S. Tanaka, K. Kitaura, T. Nakano, Molecular interactions between estrogen receptor and its ligand studied by the ab Initio fragment molecular orbital method, J. Phys. Chem. B 110 (47) (2006) 24276.
[18] K. Yamamoto, S. Yamada, et al., Vitamin D receptor: ligand recognition and allosteric network, J. Med. Chem. 49 (2006) 1313–1324.
[19] K. Yamagishi, H. Tokiwa, et al., Functions of key residues in the ligand-binding pocket of vitamin D receptor: fragment molecular orbital-interfragment interaction energy analysis, Chem. Phys. Lett. 420 (2006) 465–468.

[20] T. Harada, K. Yamagishi, T. Nakano, K. Kitaura, H. Tokiwa, Ab initio fragment molecular orbital study of ligand binding to human progesterone receptor ligand-binding domain, Naunyn-Schmiedeberg's Arch. Pharmacol. 377 (2008) 607–615.
[21] ViewerPro 4.2, Accelrys Inc., San Diego, CA, 2001.
[22] M.J. Frisch et al., Gaussian 03 Program, Gaussian, Inc., Wallingford, CT, 2004.
[23] ABINIT-MP package is available from: http://www.ciss.iis.u-tokyo.ac.jp/rss21/result/download/index.php.
[24] M. Choi, K. Yamamoto, et al., Interaction between vitamin D receptor and vitamin D ligands: two-dimentional alanine scanning mutational analysis, Chem. Biol. 10 (2003) 261–270.
[25] S. Nayeri, J.P. Kahlen, C. Carlberg, et al., The high affinity ligand binding conformation of the nuclear 1,25-dihydroxyvitamin D3 receptor is functionally linked to the transcription domain2 (AF-2), Nucleic Acids Res. 24 (22) (1996) 4513–4518.