Molecular Dynamics Simulation Studies on the Modulation of Vitamin D Receptor Activity by Agonists and Antagonists

Mikael Peräkylä

Department of Chemistry, University of Kuopio, P.O. BOX 1627, FIN-70211, Kuopio, Finland
Mikael.Perakyla@uku.fi

Abstract. MD simulations of vitamin D receptor (VDR) complexed with ligands having structurally and functionally varying properties have been carried out to investigate atomic level mechanisms responsible for the ligands' functional behavior. It was shown that the degree of structural order in the carboxy-terminal α-helix inversely correlated with the strength of the antagonistic activity of the ligand and that a two-side chain analog of vitamin D functions as a potent agonist to the VDR despite its significantly increased volume. Simulations showed that the second side chain can choose between two binding positions. Binding of novel nonsteroidal VDR agonists was also investigated. Simulation results were combined with extensive experimental data. This work nicely demonstrates that MD simulations are capable of revealing the subtle differences there exists between VDR activating and deactivating ligands, and how theoretical and experimental work may be fruitfully combined to study complex receptor regulation.

Introduction

The nuclear receptor (NR) for 1α,25-dihydroxyvitamin D_3 ($1\alpha,25(OH)_2D_3$), the vitamin D receptor (VDR), binds its ligand with high affinity (K_d is 1 nM or lower). The ligand binding domain (LBD) of VDR is formed of 12 α-helices and its overall architecture is similar for all NRs. A crucial step in the regulation of the biological activity of VDR is the stabilization of the agonistic conformation of the LBD via repositioning of the most carboxy-terminal α-helix (helix 12, Fig. 1.). This conformational change is initiated by agonist binding. Natural and synthetic molecules that selectively activate or inhibit VDR or other NRs are of considerable biological significance and may have important clinical applications.

The complexes formed between a number structurally and functionally different ligands (Fig. 2) and the LBD of VDR were studied using MD simulations. Special emphasis was placed on finding connections between the structural changes induced by ligand binding and ligand's functional properties (agonism/antagonism). Simulation results were used to suggest specific mutagenesis experiments and to provide structural data that could be used to rationalize experimental observations.

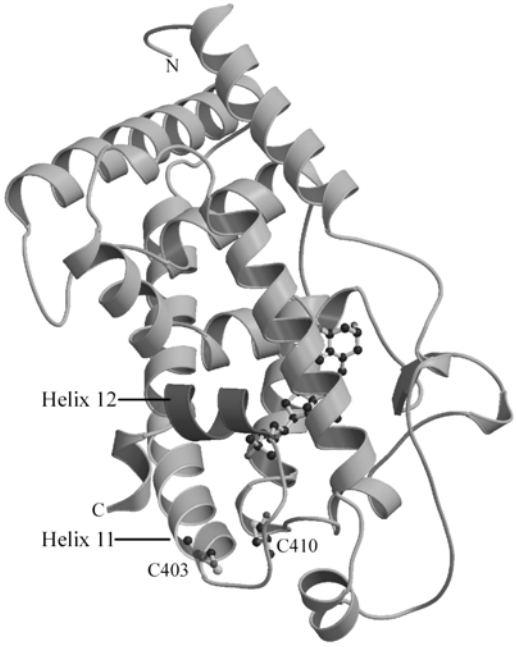

Fig. 1. The structure of the VDR-LBD-1α,25(OH)$_2$D$_3$ complex.

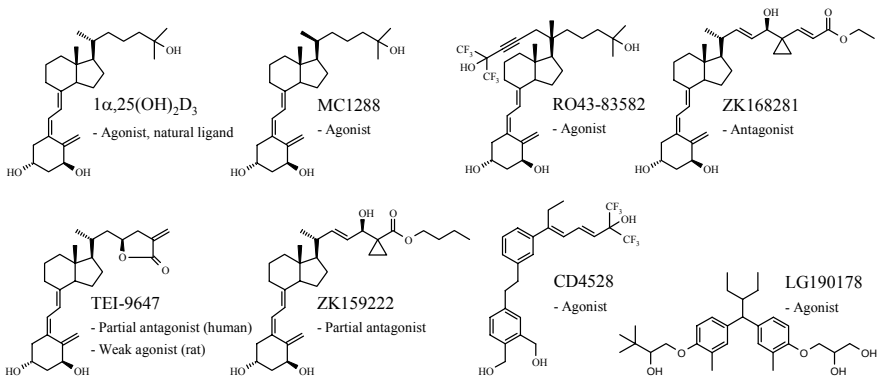

Fig. 2. Structures of agonistic and antagonistic VDR ligands. RO43-83582 is a double side chain agonist, TEI-9647 a partial antagonist for human, but a weak agonist for rodent VDR, and CD4528 and LG190178 are potent nonsteroidal VDR agonists.

Computational Details

The initial coordinates of VDR were obtained from the X-ray crystal structure of the VDR-LBD-1α,25$(OH)_2D_3$ complex (Brookhaven Protein Data Bank code 1DB1).[1] Coactivator peptide KNHPMLMNLLKDN was added to the simulation system and placed on the surface of the VDR-LBD on the basis of (rat) VDR-LBD-1α,25$(OH)_2D_3$ complex x-ray structure (1RK3).[2] Ligands were placed to the ligand-binding site using the VDR-LBD-1α,25$(OH)_2D_3$ crystal structure as a model. The two-side chain analog RO43-83582 was docked to the ligand-binding pocket using the locally enhanced sampling (LES) method with five copies of ligand side chains. The structures obtained from the LES simulations were studied further with long MD simulations.

For the molecular dynamics simulations VDR complexes were solvated by TIP3P water molecules in a periodic box of ~61×69×86 Å. Crystallographic water molecules were included in the simulation systems. In the production simulations of 2-10 ns the electrostatics were treated using the particle-mesh Ewald method. A timestep of 1.5 fs was used and bonds involving hydrogen atoms were constrained to their equilibrium lengths using the SHAKE algorithm. The simulations were done using the AMBER 7.0/8.0 simulation package and the parm99 parameter set of AMBER. The parameters of the ligands were generated with the Antechamber suite of AMBER in conjunction with the general amber force field. The atomic point charges of the ligands were calculated with the two-stage RESP fit at the HF/6-31G* level using ligand geometries optimized with the semi-empirical PM3 method using the Gaussian03 program.

Results and Discussion

The carboxy-terminal α-helix, helix 12, of VDR contains a critical ligand-modulated interface for the interaction with coactivator proteins. MD simulation were done for the natural VDR agonist 1α,25$(OH)_2D_3$, a partial antagonist ZK159222 and a complete antagonist ZK168281. It was observed that, as expected, helix 12 stayed in the agonistic conformation during the VDR-1α,25$(OH)_2D_3$ simulation. An x-ray structure of this complex has been determined.[1] MD simulations could explain the different action of the two antagonists by demonstrating a more drastic displacement of helix 12 through ZK168281 than through ZK159222.[3]

RO43-83582 is an exceptional vitamin D_3 analog with two side chains that, despite about 25 % increased volume, binds to the VDR and can function as a potent agonist. Twelve different LBD-RO43-83582 conformations were simulated by rotating the side chains in steps of 20°. This conformation analysis resulted in two possible positions of the second side chain of RO43-83582. The first side chain keeps the same position than the single side chain of 1α,25$(OH)_2D_3$. This structural prediction was challenged by mutating the residues closest to the binding positions of the extra side chains into bulky phenylalanines. It could be demonstrated that filling both binding sites of the extra side chain with one bulky phenylalanine is more severe than placing two phenylalanines together into one or the other binding site. In addition, mutations

were found to disturb the action of RO43-83582 significantly more than that of $1\alpha,25(OH)_2D_3$. Thus, it was demonstrated that the second side chain can choose between two binding positions within the LBP of the VDR.[4-6] There is a preliminary information on the zebrafish VDR-LBD - two side chain analog x-ray structure, in which the second side chain occupies one of the predicted binding pockets.[7]

The 26,23-lactone derivative of $1\alpha,25(OH)_2D_3$, TEI-9647, is a partial antagonist of the human VDR, but in rat cells it behaves as a weak agonist. This action could be mimicked in human cells by the double mutagenesis (Cys403Ser and Cys410Asn) of human VDR. MD simulations showed that TEI-9647 decreases the stability of helix 12 of human VDR. In contrast, Asn410 of the rat VDR stabilizes, via backbone contacts, the critical interactions between helices 11 and 12.[8]

MD simulations have also been used to understand how four selected nonsteroidal VDR agonists bind to the LBD of the VDR. It was demonstrated that the nonsteroidal ligands take a shape within the LBP that is very similar to that of the natural ligand and that each of the three hydroxyl groups of the ligands formed hydogen bonds with the residues of the LBP. Simulated structures showed that the more exactly the nonsteroidal ligands place their hydroxyl groups, the more potent VDR agonists they seem to be. This observation was confirmed by mutagenesis experiments.[9]

References

1. Rochel, N., Wurtz, J.M., Mitschler, A., Klaholz, B. and Moras, D. Mol. Cell, 2000, 5, 173-179.
2. Vanhooke, J.L., Benning, M.M., Bauer, C.B., Pike, J.W. and DeLuca, H.F. Biochemsitry, 2004, 43, 4101-4110.
3. Väisänen, S., Peräkylä, M., Kärkkäinen, J.I., Steinmeyer, A. and Carlberg, C. J. Mol. Biol., 2002, 315, 229-238.
4. Väisänen, S., Peräkylä, M., Kärkkäinen, J.I., Uskokovic, M.R. and Carlberg, C. Mol. Pharmacol., 2003, 63, 1230-1237.
5. Gonzalez, M.M., Samenfeld, P., Peräkylä, M. and Carlberg, C. Mol. Endocrinol., 2003, 17, 2028-2038.
6. Molnár, F., Peräkylä, M. and Carlberg, C. J. Biol. Chem., 2006, in press.
7. Ciesielski, F., Rochel, N., Mitschler, A., Kouzmenko, A. and Moras, D. J. Steroid Biochem. Mol. Biol., 2004, 89-90, 2004.
8. Peräkylä, M., Molnar, F. and Carlberg, C. Chem. Biol., 2004, 11, 1147-1156.
9. Peräkylä, M., Malinen, M., Herzig, K.H. and Carlberg, C. Mol. Endocrinol., 2005, 19, 2060-2073.