Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Steroid Biochemistry & Molecular Biology 89–90 (2004) 107–110
The Journal of Steroid Biochemistry & Molecular Biology
www.elsevier.com/locate/jsbmb

Model of three-dimensional structure of VDR bound with Vitamin D_3 analogs substituted at carbon-2☆

Wanda Sicinska [a], Piotr Rotkiewicz [b], Hector F. DeLuca [a,*]

[a] *Department of Biochemistry, University of Wisconsin-Madison, 433 Babcock Drive, Madison, WI 53706, USA*
[b] *Department of Chemistry, University of Warsaw, Pasteura 1, 02-093 Warsaw, Poland*

Abstract

All Vitamin D analogs possessing the A ring modified at C-2 and showing calcemic activities nest themselves in the VDR binding pocket, oriented towards Tyr 143. Such topology resembles the position of the Vitamin D hormone in hVDRmt [Proc. Natl. Acad. Sci. U.S.A. 98 (2001) 5491]. Conversely, inactive 2β-methyl-19-nor-analogs anchor the receptor cavity in a distinguishably different manner, namely by their side chain. Moreover, these inactive vitamins have a different conformation around C(6)–C(7) bond. Topology of modeled complexes suggests that a Vitamin D analog will be biologically active if its intercyclic 5,7-diene moiety assumes parallel position to tryptophan aromatic rings; such orientation allows for creating π–π interactions. The broad comparison of calcemic activities of the analogs, and their interactions with VDR, revealed that specific hydrophobic contacts are involved in bone calcium mobilization (BCM). These contacts occur between 21-methyl group and a few amino acids (V296, L305 and L309), conserved in the nuclear receptor superfamily. In the inactive 2β-methyl-19-nor analogs such contacts do not exist. We speculate that two hydrophobic receptor patches, being in close contact with ligand methyl groups, might influence interaction with co-modulators involved in calcium homeostasis.

Keywords: Vitamin D receptor; Ligand binding domain of rat VDR; Docking of Vitamin D analogs

1. Introduction

At present, only complexes of the hVDRmt with Vitamin D analogs possessing an unmodified A ring have been crystallized [1]. It was found that irrespectively of the configuration at C-20 or other side chain modifications, ligands anchored in the binding pocket in a similar fashion to that of 1α,25-$(OH)_2D_3$. In this work, intensive docking experiments were performed to establish how the full-length ligand binding domain of rat VDR accommodates analogs substituted at C-2 (showing highly differentiated calcemic activities) [2].

2. Material and methods

Docking simulations were performed by FlexiDock software from TRIPOS [3], using procedures described earlier [4]. Internal rotations around single bonds (except those forming the ring system) of the ligands were allowed in the simulations of the six theoretical Vitamin D conformers (two 6-s-*trans* and four 6-s-*cis*). For each vitamin (compounds **3** –**9**, Table 1), the docking procedures were repeated several times, for various arbitrarily chosen initial positions (and conformations) of the ligand in a vicinity of the binding pocket.

3. Results and discussion

Our docking experiments revealed that most Vitamin D analogs occupy VDR binding pocket in 6-s-*trans* conformation of diene system and equatorial orientation of 1α–OH group; the only exemption are both 2β-methyl-19-norvitamins (compound **7** and **8**). The analog **7** with natural configuration at C-20 anchors the receptor pocket as s-*trans* rotamer with axial 1α-OH substituent, while its 20*S*-counterpart is situated in binding pocket having 6-s-*cis* conformation of diene moiety, *M* (−) chirality and equatorial orientation of 1α-OH group. It appears that parallel position of Trp (Figs. 1 and 2) relatively to an intercyclic 5,7-diene moiety (found also in the hormone) is a prerequisite for biological activity. Such orientation

☆ Presented at the 12th Workshop on Vitamin D (Maastricht, The Netherlands, 6–10 July 2003).

* Corresponding author. Tel.: +1-608-262-1620; fax: +1-608-262-7122.
E-mail address: deluca@biochem.wisc.edu (H.F. DeLuca).

0960-0760/$ – see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.jsbmb.2004.03.102

Table 1
Specific hydrophobic contacts (in Å) between vitamin 21-CH_3 group and conserved VDR amino acids

Compound	V296	L305	L309
1α,25-$(OH)_2D_3$ (**1**)[a]	3.3	2.7	3.3
20-Epi-1α,25-$(OH)_2D_3$ (**2**)[a]	4.1	2.4	3.1
2-Methylene-19-nor-1α,25-$(OH)_2D_3$ (**3**)	–	1.7	1.5
2-Methylene-19-nor-(20*S*)-1α,25-$(OH)_2D_3$ (**4**)	2.8	3.1	2.0
2α-Methyl-19-nor-1α,25-$(OH)_2D_3$ (**5**)	2.1	3.1	3.5
2α-methyl-19-nor-(20*S*)-1α,25-$(OH)_2D_3$ (**6**)	2.1	3.6	2.2
2β-Methyl-19-nor-1α,25-$(OH)_2D_3$ (**7**)	–	–	–
2β-Methyl-(20*S*)-19-nor-1α,25-$(OH)_2D_3$ (**8**)	–	–	–
2β-(3-Hydroxypropoxy)-1α,25-$(OH)_2D_3$ (**9**)	1.8	2.4	1.7

[a] Literature data taken from ref. [1].

was found in all complexes listed in Table 2. Within this group, only complexes with the distances between aromatic and diene planes shorter than 5 Å (allowing for π–π interactions) were active. Moreover, our docking experiments showed that all ligands revealing calcemic action anchored the rVDR as s-*trans* rotamers directed towards Tyr 143 (Fig. 1). Although this orientation resembles the position of the hormone in hVDRmt, specific contacts differ slightly from those found in complexes with parental ligand. For example, none of 19-nor analogs creates strong hydrogen bonds with Ser 233 (Table 2). The lack of this hydrophilic interaction was detected in mutational experiments with (20-*S*)-1α,25-$(OH)_2D_3$ as a

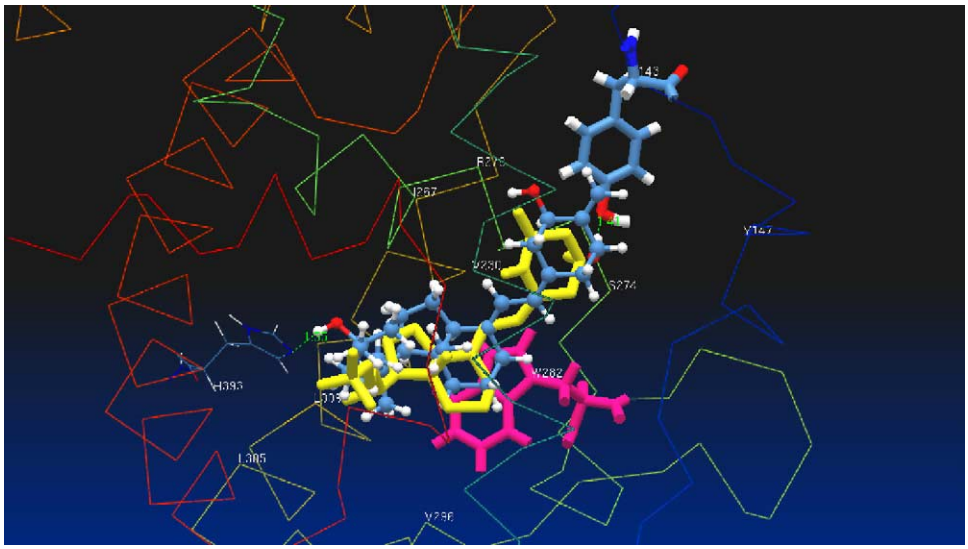

Fig. 1. Superimposition of 2MD (ball and stick presentation) and 1α,25-$(OH)_2D_3$ (yellow) in the VDR cavity. They are indicated by two amino acids (Y143 and H393) forming the shortest hydrogen bonds (1.41 and 1.55 Å) with 3-OH and 25-OH, respectively. Trp 282 (pink) is parallelly oriented in respect to 5,7-diene moiety.

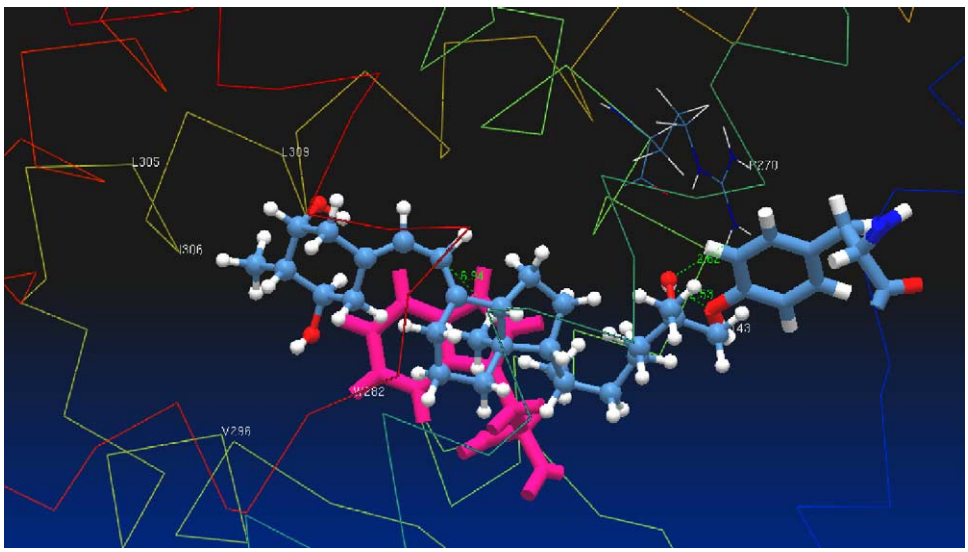

Fig. 2. The inactive analog 2β-methyl-(20*S*)-19-nor-1α,25-$(OH)_2D_3$ (**8**) anchors in the receptor cavity in distinguishably different manner than the hormone. In this complex the side chain of (**8**) (not the A ring) is oriented toward Y143. The intercyclic 5,7-diene fragment possesses s-*cis* conformation and 1-OH group is equatorially oriented. In this model, hydrogen bonds were found only to one of the three hydroxyls: 25-OH group creates HB with Y143 (2.53 Å) and R270 (2.62 Å).

Table 2
Specific contacts (<3.5 Å) between Vitamin D analogs and rVDR (118–423)[a]

(1)[b]	**Y143** L223 L226 L229 V230 **S233** I267 M268 **R270 S274** W282 (4.3 Å) V296 A299 **H301** L305 L309 **H393** L400 L410 V414 F418
(2)[b]	**Y143** L223 L226 L229 V230 **S233** I264 I267 M268 **R270 S274** W282 (3.8 Å) **H301** L305 L309 **H393** L400 V414 F418
(3)	**Y143 Y147** L226 V230 I264 I267 M268 **R270 S274** W282 (4.5 Å) **H301** L305 L309 **H393**
(4)	**Y143 Y147** V230 I267 **R270 S274** W282 (4.7 Å) V296 L305 L309 **H393**
(5)	**Y143** L223 L226 A227 L229 V230 **R270 S274** W282 (4.8 Å) V296 A299 **H301** L305 I306 L309 L400 L410 V414
(6)	**Y143 Y147** L226 V230 I267 **R270 S274** W282 (4.6 Å) V296 A299 **H301** L309
(7)	I264 **R270** W282 (7.1 Å) **H301** L305
(8)	**Y143 R270** W282 (6.9 Å) V296 L305 I306 L309
(9)	**Y143** L226 V230 **S233** I264 R270 **S274** W282 (5.1 Å) V296 L305 I306 L309 **H393**

[a] The amino acids creating hydrogen bonds with vitamins are marked in bold. The parallel orientation of tryptophan ring and vitamin diene system was found in all listed complexes; the distances between aromatic and diene planes are marked in parentheses.

[b] The contact sites between hVDR and hormone or its 20-epi analog were found after adding protons to published X-ray structures [1]. The amino acids being in (3.5 Å) contacts with ligands were renumbered according the rVDR sequence.

ligand [5]. There is a general tendency for 2-alkylidene vitamins, or compounds substituted with aromatic rings, to create contact sites by π–π interactions. Such interactions, previously observed in 2-ethylidene vitamins [6], were found in 2-methylene-19-nor-(20*S*)-1α,25-$(OH)_2D_3$ (2MD) and in 19-nor analog with 2α-(benzyloxy) substituent [7]. According to our models, the π–π interactions are likely to occur between a C-2 methylene group and Y143 (compound **4)** and between a phenyl ring of C-2 substituent (in 2α-(benzyloxy)-19-nor-1α,25-$(OH)_2D_3$) and of the aromatic rings of F150 (2.73 Å distance) and Y232 (3.32 Å distance). Worth noting is that 2β-methyl 19-nor-analogs, that are inactive in calcium assays (both ICA and BCM), anchor the receptor differently. They enter the VDR binding cavity by a side chain and have different conformation around C(6)–C(7) bond (Fig. 2). Interestingly, such topology is not common for all 2β-substituted vitamins. In our preliminary docking experiment, the 2β-(3-hydroxypropoxy)-1α,25-$(OH)_2D_3$ analog, that is more effective in treatment of osteoporosis than calcitriol [8], occupies VDR cavity in the hormone fashion. Comparison of specific ligand/VDR interactions and calcemic activity of the analogs sheds light on the question of which contact sites are involved in bone calcium mobilization (BCM). We found that short hydrophobic contacts between Vitamin D methyl group at C-20 and conserved amino acids: V 296, L 305, L309 occur in complexes of compounds possessing elevated BCM activity in comparison with 1α,25-$(OH)_2D_3$. This conclusion is in agreement with observed reduced calcium mobilizing activity of the hormone analog with deleted 21-methyl group [9] or substituted at C-17 with a small alkyl substituent [10]. Detailed analysis of VDR binding sites creating contacts with 18- and 21-methyl groups revealed the existence of two hydrophobic patches (on the receptor surface) that are responsible for specific methyl-methyl interactions (Fig. 3) The patches are situated on the VDR surface very close to each other, and they consist of four (L226, L229, V230, V296) and five (I264, I267, M268, L305, L309) amino acids. Some amino acids creating this hydrophobic surface are far apart in the sequence and belong to different helices (3, 5 and 7). As demonstrated elsewhere [11], a tight contact between helices 3 and 5 induced by the ligand is necessary to keep the nuclear receptors in transcriptionally active conformations. It is also accepted that co-modulators interact with VDR and RXR through highly hydrophobic peptide motifs (LXXLL and LIM) [12]. Therefore, we believe that specific hydrophobic interactions between the VDR and methyl groups of Vitamin D analogs might modulate interaction with co-activators or co-repressors involved in calcium homeostasis.

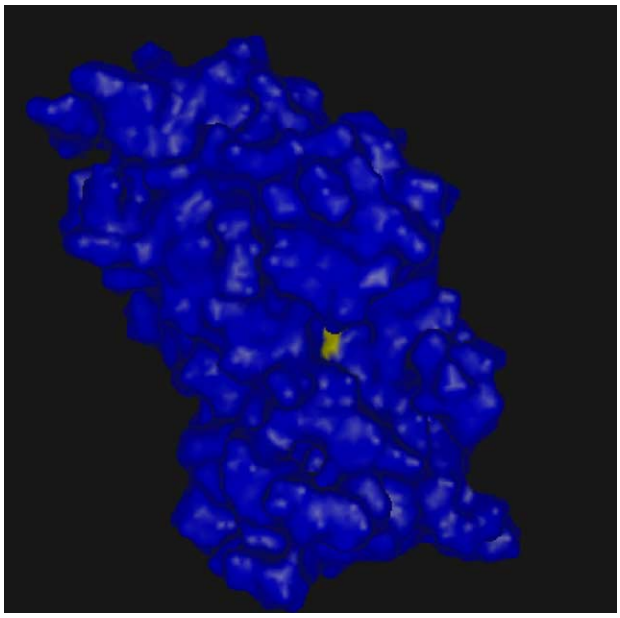

Fig. 3. The amino acids being in specific hydrophobic contacts with 18- and 21-methyl groups create on the VDR surface two hydrophobic patches (marked in yellow) situated very closely to each other.

4. Conclusion

Docking of Vitamin D analogs, possessing varying calcemic activities, into rVDR revealed that inactive vitamins anchor in the receptor in a distinguishably different manner than the hormone. Analysis of specific binding sites allowed us to find the amino acids responsible for specific interactions with the ligand's 18- and 21-methyl groups. On the VDR surface, these residues (L226, L229, V230, V296, and I264, I267, M268, L305, L309) create two hydrophobic patches (situated very closely to each other) that may interact with co-modulators involved in calcium homeostasis.

Acknowledgements

W.S. thanks Dr. Andrzej Kolinski for many helpful discussions. Coordinates of the molecular models discussed in this work can be found on page: http//biocomp.chem.uw.edu.pl.

References

[1] G.D. Tocchini-Valentini, N. Rochel, J.M. Wurtz, A. Mitschler, D. Moras, Crystal structures of the Vitamin D receptor complexed to superagonist 20-epi ligands, Proc. Natl. Acad. Sci. U.S.A. 98 (2001) 5491–5496.

[2] R.R. Sicinski, J.M. Prahl, C.N. Smith, H.F. DeLuca, New 1α,25-dihydroxy-19-norVitamin D_3 compounds of high biological activity: synthesis and biological evaluation of 2-hydroxymethyl, 2-methyl, and 2-methylene analogues, J. Med. Chem. 41 (1998) 4662–4674.

[3] SYBYL Modeling Program, 6.7 edition, Tripos Inc., St. Louis, MO.

[4] P. Rotkiewicz, W. Sicinska, A. Kolinski, H.F. DeLuca, Model of three-dimensional structure of Vitamin D receptor and its binding mechanism with 1α,25-dihydroxyvitamin D_3, Proteins 44 (2001) 188–199.

[5] P.H. Maenpaa, S. Vaisanen, T. Jaaskelainen, Vitamin D_3 analogs (MC 1288, KH 1060, EB 1089, CS 1558, and CB 1093): studies on their mechanism of action, Steroids 66 (2001) 223–225.

[6] R.R. Sicinski, P. Rotkiewicz, A. Kolinski, W. Sicinska, J.M. Prahl, C.M. Smith, H.F. DeLuca, 2-Ethyl and 2-ethylidene analogues of 1α,25-dihydroxy-19-norvitamin D_3: synthesis, conformational analysis, biological activities, and docking to the modeled rVDR ligand binding domain, J. Med. Chem. 45 (2002) 3366–3380.

[7] W. Sicinska, H.F. DeLuca, unpublished results.

[8] T. Okano, N. Tsugawa, S. Masuda, A. Takeuchi, T. Kobayashi, Y. Takita, Y. Nishii, Regulatory activities of 2β-(3-hydroxypropoxy)-1α,25-dihydroxy-vitamin D_3. A novel synthetic vitamin D_3 derivative, on calcium metabolism, Biochem. Biophys. Res. Commun. 163 (1989) 1444–1449.

[9] H.F. DeLuca, J. Wicha, 21-nor-vitamin D compounds, US Patent 5,847,173 (1998).

[10] H.F. DeLuca, R.R. Sicinski, S. Gowlugari, L.A. Plum, M. Clagett-Dame, 1α-hydroxy-2-methylene-19-nor-homopreganacalciferol and its uses, US Patent 6,440,953 (2002).

[11] S. Yamada, M. Shimizu, K. Yamamoto, Structure-function relationships of Vitamin D including ligand recognition by the Vitamin D receptor, Med. Res. Rev. 23 (2003) 89–115.

[12] P. Pathrose, O. Barmina, Ch-Y. Chang, D.P. McDonnell, N.K. Shevde, W. Pike, Inhibition of 1,25-dihydroxyvitamin D_3-dependent transcription by synthetic LXXLL peptide antagonists that target the activation domains of the Vitamin D and retinoid X receptors, J. Bone Miner. Res. 17 (2002) 2196–2205.