

Model of three-dimensional structure of VDR bound with Vitamin D₃ analogs substituted at carbon-2[☆]

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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 2 β -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 intericyclic 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 2 β -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.

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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 1 α ,25-(OH)₂D₃. 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 1 α -OH group; the only exemption are both 2 β -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 1 α -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 1 α -OH group. It appears that parallel position of Trp (Figs. 1 and 2) relatively to an intericyclic 5,7-diene moiety (found also in the hormone) is a prerequisite for biological activity. Such orientation

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Table 1
Specific hydrophobic contacts (in Å) between vitamin 21-CH₃ group and conserved VDR amino acids

Compound	V296	L305	L309
1 α ,25-(OH) ₂ D ₃ (1) ^a	3.3	2.7	3.3
20-Epi-1 α ,25-(OH) ₂ D ₃ (2) ^a	4.1	2.4	3.1
2-Methylene-19-nor-1 α ,25-(OH) ₂ D ₃ (3)	–	1.7	1.5
2-Methylene-19-nor-(20 <i>S</i>)-1 α ,25-(OH) ₂ D ₃ (4)	2.8	3.1	2.0
2 α -Methyl-19-nor-1 α ,25-(OH) ₂ D ₃ (5)	2.1	3.1	3.5
2 α -methyl-19-nor-(20 <i>S</i>)-1 α ,25-(OH) ₂ D ₃ (6)	2.1	3.6	2.2
2 β -Methyl-19-nor-1 α ,25-(OH) ₂ D ₃ (7)	–	–	–
2 β -Methyl-(20 <i>S</i>)-19-nor-1 α ,25-(OH) ₂ D ₃ (8)	–	–	–
2 β -(3-Hydroxypropoxy)-1 α ,25-(OH) ₂ D 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*)-1 α ,25-(OH)₂D₃ as a

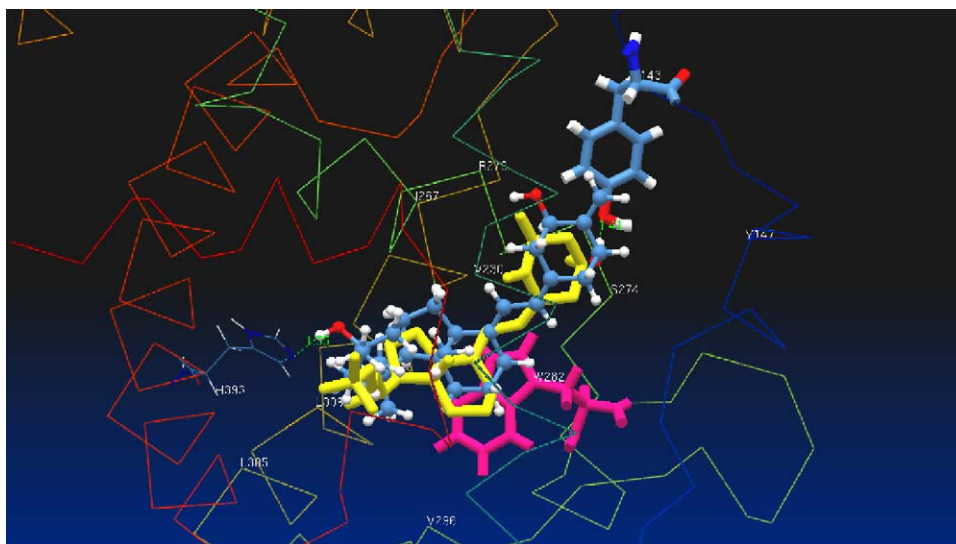


Fig. 1. Superimposition of 2MD (ball and stick presentation) and 1 α ,25-(OH)₂D₃ (yellow) in the VDR cavity. They are indicated by two amino acids (Y143 and H393) forming the shortest hydrogen bonds (1.41 and 1.55 Å) with 3-OH and 25-OH, respectively. Trp 282 (pink) is parallelly oriented in respect to 5,7-diene moiety.

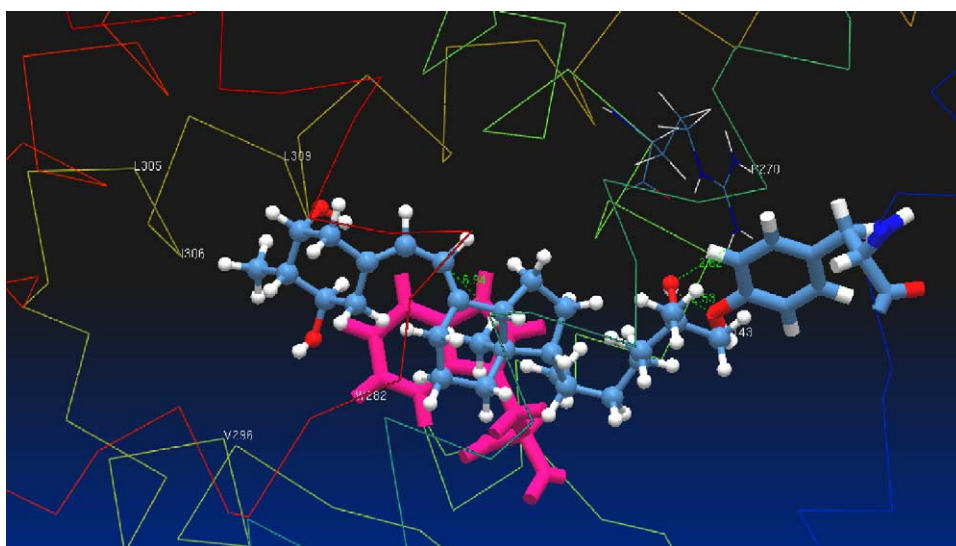


Fig. 2. The inactive analog 2 β -methyl-(20*S*)-19-nor-1 α ,25-(OH)₂D₃ (**8**) anchors in the receptor cavity in distinguishably different manner than the hormone. In this complex the side chain of (**8**) (not the A ring) is oriented toward Y143. The intericyclic 5,7-diene fragment possesses *s-cis* 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 to 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-(20S)-1 α ,25-(OH)₂D₃ (2MD) and in 19-nor analog with 2 α -(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 2 α -(benzyloxy)-19-nor-1 α ,25-(OH)₂D₃) and of the aromatic rings of F150 (2.73 Å distance) and Y232 (3.32 Å distance). Worth noting is that 2 β -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 2 β -substituted vitamins. In our preliminary docking experiment, the 2 β -(3-hydroxypropoxy)-1 α ,25-(OH)₂D₃ 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 1 α ,25-(OH)₂D₃. 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.

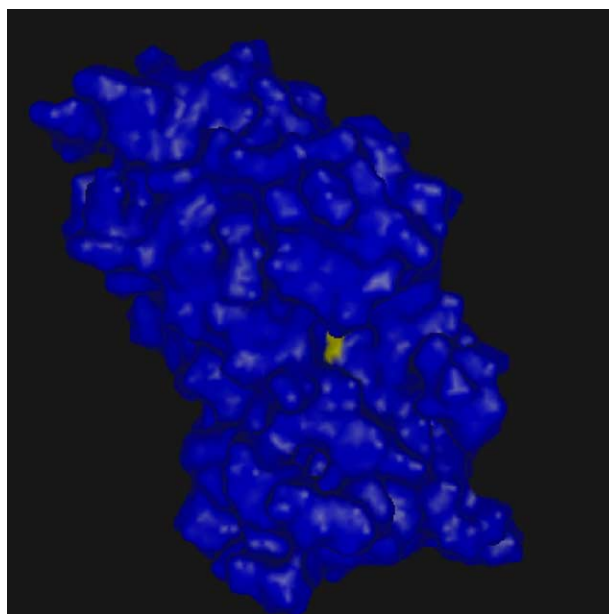


Fig. 3. The amino acids being in specific hydrophobic contacts with 18- and 21-methyl groups create on the VDR surface two hydrophobic patches (marked in yellow) situated very closely to each other.

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.

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