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Model of Three-Dimensional Structure of Vitamin D Receptor and Its Binding Mechanism With 1α,25-Dihydroxyvitamin D_3

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ABSTRACT **Comparative modeling of the vitamin D receptor three-dimensional structure and computational docking of 1α,25-dihydroxyvitamin D_3 into the putative binding pocket of the two deletion mutant receptors: (207–423) and (120–422, Δ [164–207]) are reported and evaluated in the context of extensive mutagenic analysis and crystal structure of holo hVDR deletion protein published recently. The obtained molecular model agrees well with the experimentally determined structure. Six different conformers of 1α,25-dihydroxyvitamin D_3 were used to study flexible docking to the receptor. On the basis of values of conformational energy of various complexes and their consistency with functional activity, it appears that 1α,25-dihydroxyvitamin D_3 binds the receptor in its 6-s-*trans* form. The two lowest energy complexes obtained from docking the hormone into the deletion protein (207–423) differ in conformation of ring A and orientation of the ligand molecule in the VDR pocket. 1α,25-Dihydroxyvitamin D_3 possessing the A-ring conformation with axially oriented 1α-hydroxy group binds receptor with its 25-hydroxy substituent oriented toward the center of the receptor cavity, whereas ligand possessing equatorial conformation of 1α-hydroxy enters the pocket with A ring directed inward. The latter conformation and orientation of the ligand is consistent with the crystal structure of hVDR deletion mutant (118–425, Δ [165–215]). The lattice model of rVDR (120–422, Δ [164–207]) shows excellent agreement with the crystal structure of the hVDR mutant. The complex obtained from docking the hormone into the receptor has lower energy than complexes for which homology modeling was used. Thus, a simple model of vitamin D receptor with the first two helices deleted can be potentially useful for designing a general structure of ligand, whereas the advanced lattice model is suitable for examining binding sites in the pocket. Proteins 2001;44:188–199.** © 2001 Wiley-Liss, Inc.

Key words: vitamin D receptor; steroid hormone receptor; docking of 1α,25-dihydroxyvitamin D; nuclear receptor; 6-s-*trans*-1α,25-dihydroxyvitamin D_3

INTRODUCTION

The vitamin D receptor (VDR) belongs to the nuclear receptor (NR) superfamily including receptors for the steroid, retinoid, and thyroid hormones.[1,2] Although DNA binding domains (DBD) of these receptors[1,2] are generally highly conserved (sequence identity > 40%), ligand binding domains (LBD) exhibit limited similarity.[3] Crystal structures of liganded TR,[4] RAR,[5] and unliganded RXR[6] receptors show well-conserved architecture (11 or 12 antiparallel helices composed in three layers) of the LBD. The recently published crystal structure of deletion mutant of complexed hVDR (118–425, Δ [165–215]) revealed its greatest similarity to RAR and conservancy of sandwiched structure of helices packed in three layers.[7] Most hydrogen bonds formed between three hydroxy groups and six amino acids are supported with mutation experiments. It was found that 1α-OH group contacts Ser 237 and Arg 274, 3β-OH group contacts Ser 278 and Tyr 143, whereas 25-OH is hydrogen bonded to His 397 and His 305. Only the mutant S278A[8] does not show significant reduction of binding and transcriptional activity. It is worth mentioning that contacts between the receptor and ligand include amino acids that span from helix 3 onward (Tyr 143 (H2) being the only exception).

The amino acid sequences in human and rat receptors display considerable similarity; the DBD is identical between the two species and the LBD is 93% conserved. The rat receptor is four amino acids shorter than the human receptor, the difference being in the region between DBD and LBD.[9] The LBD is the most diverse domain in the NR superfamily; in the VDR, it is responsible for binding. It also upregulates gene transcription and heterodimeriza-

Abbreviations: VDR, vitamin D receptor; hVDR, human vitamin D receptor; rVDR, rat vitamin D receptor; NR, nuclear receptor superfamily; LBD, ligand binding domain; DBD, DNA binding domain; ER, estrogen receptor; PR, progesterone receptor; RAR, all-*trans*- and 9-*cis*-retinoic acid receptor; PPAR, peroxisome proliferator activated receptor; SICHO, SIde CHain Only high coordination lattice molecular modeling tool.

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tion with the RXR.[10] Based on the complementary experiments, mutations (see ref. in Table III), resistance of mutants to digestion,[11] affinity labeling,[12–14] and comparison of binding capabilities of 1α,25-$(OH)_2D_3$ with its analogs,[10,15,16] a hormone pocket has been located between helices H-3, H-5, H-11, and H-12. This prediction was confirmed by the crystal structure of liganded hVDR mutant.

Three models of the ligand pocket, created by manual docking of the hormone into the VDR, have been reported recently.[8,17,18] Wurtz et al.[17] modeled a complex with the hormone being in 6-s-*trans* conformation and entering the pocket with its side chain oriented inside, whereas Norman et al.[18] proposed a 6-s-*cis* (steroidal) form for the docked ligand with the A ring directed toward the interior. In the Yamamoto model,[8] 1α,25-$(OH)_2D_3$ is docked in the 6-s *trans* conformation and the A-ring is oriented toward the cavity. All three amino acids forming hydrogen bonds with 25-OH (His 397) and 1-OH (Arg 274 and Ser 237) were predicted correctly, but conformation of the A ring and, consequently, orientation of 1α-OH, was not. In this model, the 1α-OH group adopted axial instead of equatorial orientation.

To gain a better insight into the binding mechanism of the ligand to the receptor, the most accurate structure of the VDR is needed. Fortunately, high-resolution structures of five highly homologous proteins are already known. All of them have been used in the present work as a multiple template for comparative modeling of the VDR structure. Because the calculations reported in this article were accomplished before the crystal structure of hVDR construct was published, this template is missing in our model. However, this fact should not affect our result significantly, because the template proteins are highly homologous with hVDR. Knowing the putative structure of the VDR receptor as well as the experimental structures of receptor-ligand complexes of five homologous proteins, we were able to predict with relatively high fidelity the set of residues that constitute the binding pocket of the VDR. This was achieved by a conservative analysis of the multiple sequence alignment of the five template proteins and the VDR sequence. With the calculated VDR structure, docking of six distinct conformers of 1α, 25-$(OH)_2D_3$ was studied and evaluated.

MATERIALS AND METHODS

Part of the docking simulations were performed by using the computing facilities of Interdisciplinary Center for Mathematical and Computational Modeling of the University of Warsaw (ICM) and the Donald Danforth Plant Science Center in St. Louis.

Two models of rVDR that differ in length were calculated in this work. To compute highly homologous parts of the receptor (207– 423) MODELLER 4 was used, whereas building of the elongated protein (120–422, Δ [164–207]) required involvement of lattice modeling.

The computational part of this work consists of several steps. First, and crucial for the entire procedure, is the identification of the template for the comparative modeling and generating of appropriate multiple sequence alignment. Five highly homologous proteins of known structure were identified. These were subsequently used in VDR model building by using an automated molecular modeling procedure, MODELLER 4. The resulting molecular model was refined by using molecular dynamics and the SYBYL force field. Finally, six forms of 1α,25-$(OH)_2D_3$ were inserted into the VDR binding pocket by means of a flexible docking procedure. For each form of the ligand, the docking procedures were performed several times, starting from various arbitrary chosen initial positions (and conformations) of the ligand in a vicinity of the binding pocket.

Below the protocols are described in detail.

Multiple Sequence Alignment

The sequence of VDR was aligned with all sequences of NCBI nonredundant (nr) sequence database. By using the PSI-BLAST procedure,[19] the sequence database was searched until convergence of multiple sequence alignment was achieved. It required three iterations. The use of the whole sequence database allowed a statistical sequence profile to be created. Therefore, the sensitivity of the search and quality of alignments could be improved. Ten sequences of high similarity and known crystallographic structure were found. Five of these proteins covered almost the entire LBD of the VDR. These were included in our set of structural templates. For these five proteins, the sequence to structure alignments were produced by using the BLAST procedure. The alignments covering residues 207–422 of the VDR are shown in Figure 1. Table I shows PDB codes of the proteins used for homology modeling.

Modeling of the VDR Three-Dimensional Structure

The alignment of the VDR sequence to the sequences of the five nuclear receptors of known crystallographic structures was used as input for MODELLER 4, an automatic comparative modeling program designed by Sali and Blundell.[20] Based on the sequence alignments, MODELLER extracts a large number of spatial restraints from the template structures and builds molecular model of the query protein. To ensure reasonable packing of the side chains, the obtained model was subsequently subjected to a long molecular dynamics minimization procedure ("Geometry Optimization" module of the Tripos force field). For this purpose, we used SYBYL force field by Tripos.[21] During the model optimization simulations the main-chain coordinates changed very little, by tenths of an Angstrom. Table II contains the results of the best pairwise structural superposition of the five template proteins and the obtained model of VDR ligand-binding domain. The deviation of the model structure from the templates is on the same level as the deviation between various template structures. This suggests that the obtained molecular model is of relatively high accuracy, probably close to the accuracy of crystallographic structures.

Docking Procedure

Docking simulations were performed by FlexiDock software from TRIPOS,[22] which uses generic algorithm for the search of conformational space of the ligand in respect to

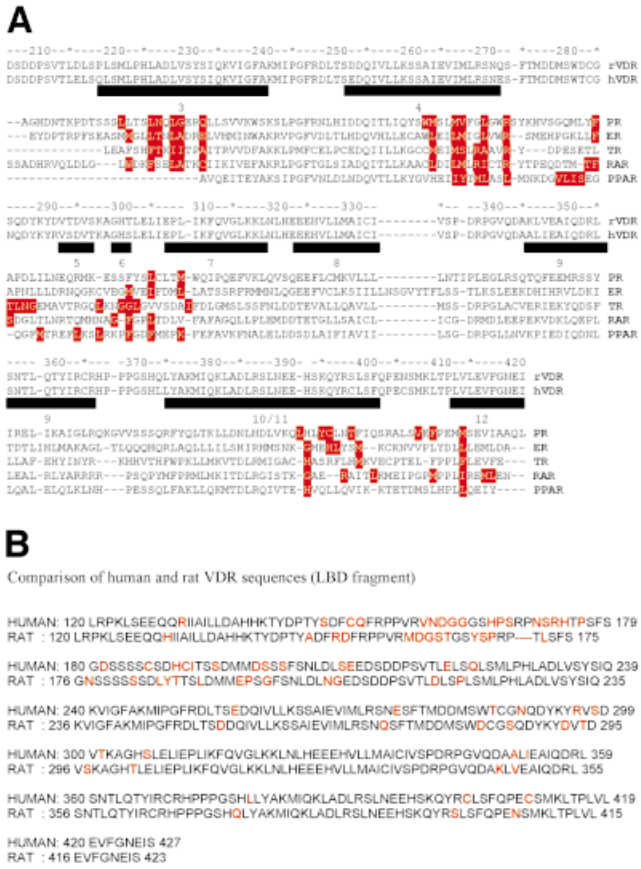

Fig. 1. **A:** Alignments of homologous proteins to the VDR sequence generated by PSI-BLAST. The black bars represent helices numbered in accordance with the notation used for RAR. Residues that participate in ligand binding are shown in red. **B:** Alignments of highly homologous human and rat VDR sequence generated by PSI-BLAST.

given structure of the receptor's binding site. We considered the six conformers (two 6-s-*trans* and four 6-s-*cis* forms) of the ligand (A–F), shown in Figure 2. Internal rotations around the single bonds of the ligand were allowed during the simulations. FlexiDock requires an approximate starting position of the ligand to be provided. Several simulations, of 20,000 steps each, were performed with various initial positions of the ligand in respect to the binding pocket of the receptor. All simulations for particular forms of the ligand converged to approximately the same structure within the binding site. For final consideration, the structures of the lowest conformational energy of the ligand-receptor complex were selected. A similar protocol was used to calculate conformation of the ligand complexed with the extended receptor (120–422, Δ [164–207]).

Building the Extended Model

As mentioned above, the elongated protein model cannot be built by means of conventional comparative modeling algorithms nor is it practical to assembly such large protein fragments by means of standard molecular dynamics simulations. Therefore, we used the recently developed lattice model for protein folding and distant homology modeling developed by Kolinski et al.[23–25] This algorithm enables simulations of the entire folding process of small proteins, large-scale relaxation of poor-quality models originated from sparse alignments (or threading alignments), and "docking" of relatively large protein fragments to partially determined protein structures. The last is exactly the case of the present application. Starting from an arbitrary expanded conformation, the missing part (residues 120–164) of the model structure (the elongated protein model, residues 120–422, Δ [164–207]) was assembled on the scaffold of the model built by a more standard approach, that is, MODELLER 4 for the fragment consisting of residues 207–423. As a result of several independent lattice simulations, the N-terminal helix always assembled at the same position in respect to the "smaller" model. The inherent accuracy of the SICHO (SIde CHain Only lattice protein model)[24] model for such models is between 2 and 4 Å, depending on the size of modeled fragment. Subsequent (off-latice) refinement by MODELLER improves local details and usually decreases the error by about 0.5 Å of RMSD from the "true" structure.

RESULTS AND DISCUSSION

Single-Point Mutations in the VDR

Inspection of Table III reveals several single point mutations which considerably decreases (10 times or more) the ability of the VDR to bind vitamin D hormone: Ser 233, Ile 244, Arg 270, Trp 282, Cys 284, His 393, Val 414 and Phe 418. Particularly interesting are the amino acids in contact with the ligand molecule. We considered that contacts occur when distances shorter than 4 Å were found between any atom of the ligand and receptor. The fragments that are in contact can interact directly by creation of hydrogen bonds, or indirectly, through hydrophobic or sandwich-type interactions. Analysis of the binding pocket geometry suggests that some mutations (His 225 and Asp 228), involving amino acids not in contact with the VDR, can nonetheless decrease binding ability indirectly by changing geometry of the active site. Although several residues of the last helix (H-12) do not interact directly with the ligand, this helix is crucial for closing the ligand cavity. Therefore, the mutations in the C-terminus part of the chain (Val 414, Leu 413, Glu 416, and Phe 418) also affect binding. Analysis of the crystal structure of the hVDR also supports conclusions drawn from modeling and mutagenesis studies. The position of helix 12 in the crystal complex is stabilized by several hydrophobic contacts involving Thr 415, Leu 417, Val 418, Leu 419, Val 421, Phe 422, and residues of H3 (Asp 232, Val 234, Ser 235, Ile 238, Gln 239), H5 (Ala 267 and Ile 268), and H11 (His 397 and Tyr 401). Because two amino acids (Val 418 and Phe 422) contact (Van der Waals interaction) the methyl group of ligand, helix 12 is found to be crucial for hormone binding.

Ligand-Binding Domain of the VDR

LBD of the rVDR spans between amino acids 116 and 423. This fragment binds the hormone, $1\alpha,25\text{-}(OH)_2D_3$, with high affinity (0.1–0.3 nM), similar to that of the full

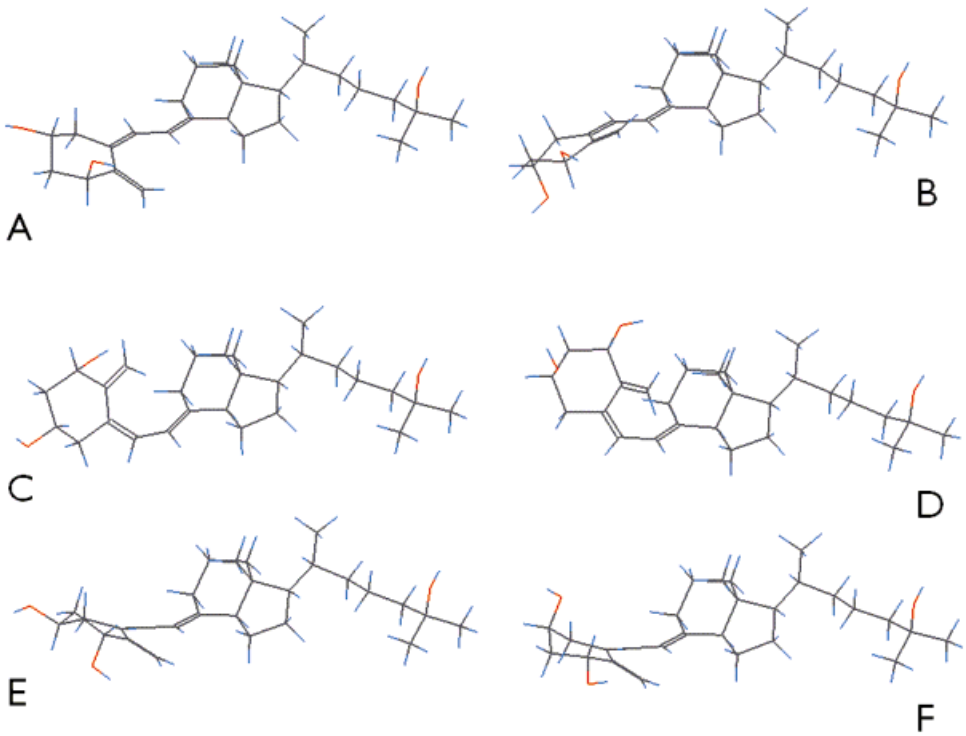

Fig. 2. Six conformers (**A–F**) of 1α,25-$(OH)_2D_3$ docked into the VDR-LBD. Structures A and B represent hormone in its 6-s-*trans* conformation and the remaining C–F in the 6-s-*cis* conformation. Orientation of the 1α-hydroxy group is axial in the forms A, C, and E, whereas equatorial in the other conformers. 6-s-*Cis*-conformers C and D have left-handed (*M*) chirality of the triene, whereas E and F are characterized by right-handed (*P*) chirality of the double bond system.

TABLE I. PDB Codes of the Proteins Used in Homology Modeling, PSI-BLAST Scores, and Sequence Identity to the Aligned Part of the VDR Sequence

PDB code	VDR seq. identity (%)	PSI-BLAST score	Name of protein
1a28	17	150	Human progesterone receptor ligand binding domain
1bsx	26	212	Human thyroid hormone receptor beta ligand binding domain
1ere	21	196	Human estrogen receptor ligand binding domain
2lbd	28	235	Human retinoic acid receptor ligand binding domain
4prg	31	190	Human peroxisome proliferator activated receptor gamma ligand binding domain

TABLE II. RMS Deviations (in Å) Between Alpha Carbon Atoms of the Best Structural Superimpositions of the Template Proteins and the Constructed Model of VDR

Protein	1a28	1bsx	2lbd	4prg	1ere	VDR MODEL
PROG 1a28	0.00					
THYR 1bsx	1.77	0.00				
RAR 2lbd	1.65	1.63	0.00			
PERO 4prg	1.96	1.90	1.38	0.00		
ERGO 1ere	1.33	1.61	1.61	2.00	0.00	
VDR MODEL	1.60	1.00	0.62	1.00	1.51	0.00

length protein.[26] It has been established that the carboxy terminus plays a crucial role in ligand binding. Consequently, removal of 20 amino acids from the COOH terminal of the hVDR resulted in a 10-fold decrease of binding affinity for the ligand.[27] The product of rVDR-LBD degradation (expressed as an amino-terminal His-tagged protein), truncated by about 20 amino acids, exhibited binding affinity reduced by a factor of 10 compared with that of the full-length receptor (unpublished results). Both models of rVDR calculated in this article encompass all

TABLE III. Experimental Mutations in Vitamin D Receptor LBD

hVDR Mutant*	Hormone binding	Heterodimer with RXR	Transactivat.†	Ref.
S**225**A	WT			(53)
S**225**A *(S221)*		WT	◆	(54)
H**229**A	◆◆◆			(53)
H**229**A *(H225)*	◆◆	◆	◆◆◆	(54)
D**232**A	◆◆◆			(53)
D**232**A (D228)	◆◆	◆◆◆	◆◆◆	(54)
V**234**A *(V230)*	◆			(53)
S**235**A	◆			(53)
S**235**A		◆	◆	(54)
S**235**R *(S231)*	◆			(55)
Y**236**A	◆			(53)
Y**236**A *(Y232)*	WT	◆	◆◆◆	(54)
S**237**A	◆◆◆			(53)
S237A *(S233)*	◆◆		◆◆	(18)
K**240**A	◆			(53)
K**240**A *(K236)*		◆	◆	(54)
I**242**A	WT			(53)
I**242**R *(I238)*	WT			(55)
F**244**G *(F240)*	WT		◆◆	(56)
K**246**A	◆			(53)
K**246**E	WT			(55)
K**246**G *(K242)*	WT	WT	◆◆◆	(56)
K**246**R *(K242)*			◆◆	(56)
I**248**S *(I244)*	◆◆◆◆	◆◆◆◆		(57)
D**253**R *(D249)*	WT			(55)
L**254**G *(L250)*	WT		◆◆	(56)
D**258**A		◆◆	◆	(57)
Q**259**G *(Q255)*	WT	◆	◆◆	(56)
I**260**R *(I256)*	WT			(55)
L**262**G *(L258)*	WT		◆◆◆	(56)
L**263**R[#] *(L259)*				(55)
K**264**A *(K260)*	WT	WT	◆◆	(34)
R**274**L¶* *(R270)*	◆◆◆◆		◆◆◆	(58)
S**275**A	◆			(53)
S275A *(S271)*	◆		◆	(18)
S278A *(S274)*[&]	◆		WT	(18)
M**284**A	◆			(11)
M**284**S *(M280)*	◆			(11)
W**286**F	◆◆◆◆			(11)
W**286**S	◆◆◆◆			(11)
W**282**F[‡] *(W28)2*	◆◆◆			(38)
C**288**G	◆◆◆	WT	◆◆◆	(59)
C288A *(C284)*	◆◆		◆◆	(18)
H**305**Q[&] *(H301)*	◆◆	WT	◆◆	(60)
I**314**S[&] (I310)	WT		WT	(61)
L**325**R *(L321)*	WT	WT	WT	(62)
L**332**R *(L328)*	WT	◆◆	◆◆◆	(62)
C**337**G *(C333)*	◆◆	WT	◆	(59)
C**369**G *(C365)*	◆	WT	WT	(59)
K**382**E *(K378)*	WT	◆◆	◆◆◆	(62)
K**382**Q *(K378)*	WT	WT	◆	(62)
M**383**G *(M379)*	WT	◆◆	◆◆◆	(62)
Q**385**K *(Q381)*	WT	◆◆	◆◆	(62)
K**386**I *(K382)*	WT	WT	◆◆	(62)
K**386**Q *(K382)*	◆	◆◆	◆	(33)
A**388**T *(A384)*	WT	WT	WT	(62)
L**390**A *(L386)*	WT	WT	WT	(62)
L**390**G *(L386)*	WT	◆◆	◆◆	(62)
R**391**C[&] *(R387)*	◆	◆◆	◆	(61)
R**391**Q *(R387)*	WT	◆◆	◆◆	(33)
H397A *(H393)*	◆◆◆		◆◆◆	(18)
L**417**A	WT	WT	◆◆◆	(30)
L**417**R	WT			(55)
L**417**S *(L413)*	◆	WT	◆◆◆	(33)
V**418**A *(V414)*	◆◆◆◆			(10)

TABLE III. Continued

hVDR Mutant*	Hormone binding	Heterodimer with RXR	Transactivat.†	Ref.
L**419**S			◆◆	(32)
L**419**R *(L415)*	WT			(55)
E**420**A	◆		◆◆	(31)
E**420**A			◆◆	(32)
E**420**A	WT	WT	◆◆◆	(30)
E**420**K *(E416)*	WT			(55)
E**420**Q	◆	◆	◆◆◆	(33)
E**420**Q *(E416)*	WT	WT	◆◆	(34)
F**422**A *(F418)*	◆◆◆◆			(10)
E**425**Q	WT		WT	(31)
E**425**Q *(E421)*	WT	◆	◆	(33)

*The mutations are listed in sequential order. The natural mutants are marked by (&). Relative activities of mutants are compared to wild type (WT) as follows: WT-mutant acts as wild-type receptor; symbols ◆, ◆◆, ◆◆◆, ◆◆◆◆ denote that adequate action is decreased 1.2–3 times, 3–10 times, 10–100 times, and > 100 times, respectively.
†Transactivation or transcriptional activity.
‡The mutation W282F was performed in rat VDR.
#The mutation L263R increases binding affinity by factor 1.4.

amino acids that are important for hormone binding (Table III and crystal structure of hVDR construct). The comparison of the rVDR sequence to the sequences of five nuclear receptors, RAR, TR, ER, PR, and PPAR, revealed its highest similarity to the RAR. Unlike the other members of the family, RAR exists as a monomer in both solution and in crystal form.[5] It is worth noting that native gels performed for the rVDR-LBD solutions (concentrated up to 1 mg/mL) in different buffers (20 mM Tris-HCl, pH 7.5; 50 mM NaH_2PO_4, pH 8.0) remains as a monomer (unpublished results). These results are in agreement with the data published recently for hVDR.[28,29]

All-*trans*-retinoic acid enters the binding pocket of the RAR with the aliphatic side chain. Electrostatic interactions between E414, E417, and K264 are responsible for repositioning, upon ligand binding, of the last helix sealing the cavity.[5] Glutamic acid in helix 12 (at position 414 in retinoic acid receptor) is highly conserved in the NR superfamily[30] and seems to be responsible for transcriptional events regulated by all these receptors. Mutations of hVDR in position E420, which corresponds to E414 in RAR, were performed by several laboratories[30,31–34] and confirmed the importance of a carboxy group in this particular position. The "mouse trap" mechanism, proposed for closing the RAR cavity,[5] seems to be universal in the nuclear receptor superfamily and was found in hVDR mutant.[7] The polar interactions of helix 12 involve the salt bridge Lys 264-Glu 420 and a hydrogen bond between Ser 235 and Thr 415.[7] The structures of two lowest energy complexes, generated by homology modeling in this work, are shown in Figure 3(A) and (B). The architecture of helices, which are packed in three antiparallel layers, is very similar to crystal structures of RAR,[5] RXR,[6] TR,[4] PR,[35] ER,[36] and PPAR.[37] Our modeled receptor (207–423) is very closely related to the crystal structure of complexed hVDR [Fig. 4(A)]. The proteins superimpose with an RMSD of 2.4 Å over 205 residues (218–423); exclusion of the loop with residues Ser 281–Tyr 291 decreases RMSD to 1.8 Å approaching the resolution value of crystal structure of hVDR complex.[7] In the modeled receptor helix 12,* which closes the cavity on hormone binding, contacts helices 3 (V230) and 4 (K260) in the same places as in the RAR. The proximity (4.79 Å) of a carboxyl group from E416 and an amino side-chain group from K260 allows for strong electrostatic interactions, which can be responsible for repositioning of H-12. Relevance of this position on transcription was confirmed by mutations of Glu416 to Ala or Gln (Table III). The second contact between H-12 and the protein has a different hydrophobic nature. A distance of 5.01 Å between Cγ (V414) and Cα (V230) allows for such interactions. A single-point mutation V414A[11] probably destroys the hydrophobic interaction, causes repositioning of H-12, and indirectly influences the binding by altering the architecture of active site. It is known that transcription is a $1\alpha,25\text{-}(OH)_2D_3$-mediated process and the RXR is a required participant in all known transcriptions activated by the VDR.[38] The established fact (see Table III) that all mutations that impair heterodimerization decrease transcription capability, as well, strongly supports this thesis. There are also some mutations that have no apparent effect on ligand binding and/or heterodimerization with RXR, but they abolish transcription. Our receptor model suggests that mutations, E416A, E416Q, L413S, and K260A, impair interactions between H-12 and H-3/H-4, leading to repositioning of the last helix—the lid of the hormone pocket. This implies that this particular position of H-12, that is, closing of the cavity, is responsible for creation of transactivation surfaces. Some indirect evidence suggests that the ligand, on complexing, changes the architecture of VDR. First of all, the protein involved in the complex is more stable than the unliganded receptor.[39,40] Complexing also changes the VDR digestion pattern.[41]

Even though the simplified model of rVDR, built by MODELLER 4, shows high consistency with established biological function of receptor (Table III), it could not be

*The last helix is numbered 12 in accordance with the notation used for the NR superfamily.

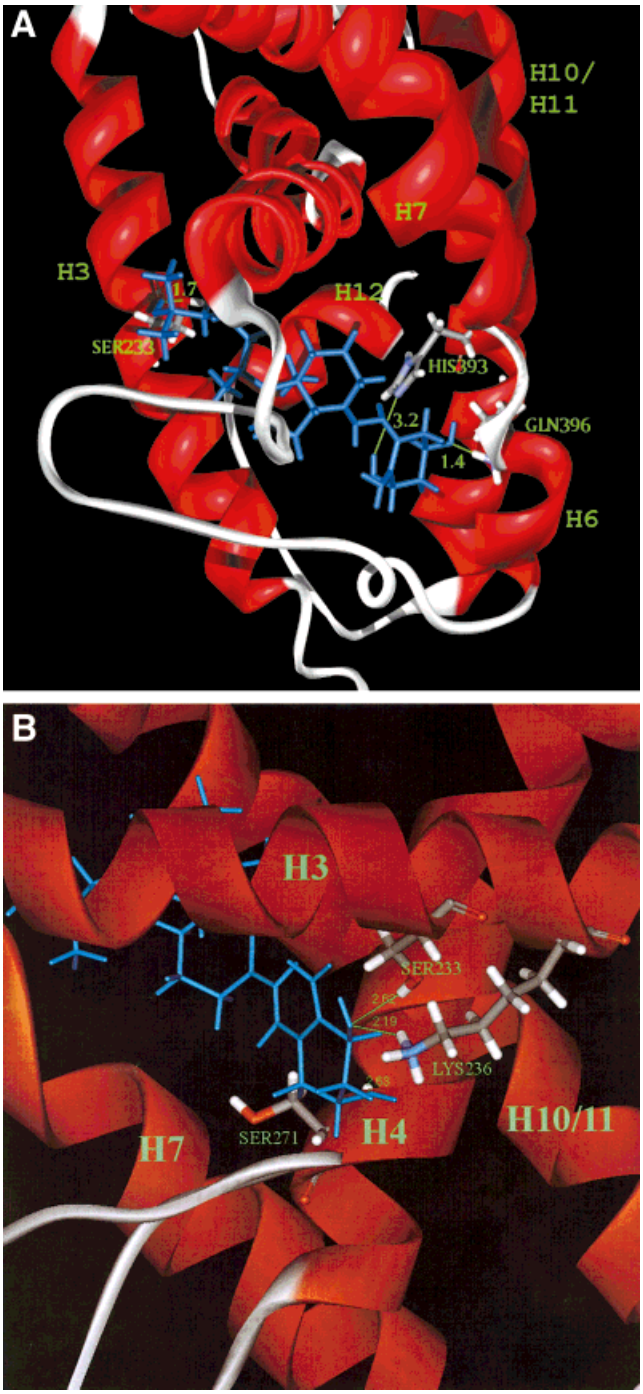

Fig. 3. **A:** View of the three-dimensional structure of ligand binding cavity for 1α,25-$(OH)_2D_3$ docked in A form. The hormone is colored in blue. They are indicated three amino acids (His 393, Qln 396, and Ser 233) forming the shortest hydrogen bonds (3.2 Å, 1.4 Å and 1.7 Å) with 1-OH, 3-OH, and 25-OH, respectively. **B:** View of the three-dimensional structure of ligand binding cavity for 1α,25-$(OH)_2D_3$ docked in B form. The hormone is colored in blue. They are indicated three amino acids (Lys 236, Ser 233, and Ser 271) forming hydrogen bonds (2.19 Å, 2.62 Å, and 2.63 Å) with 1-OH and 3-OH, respectively.

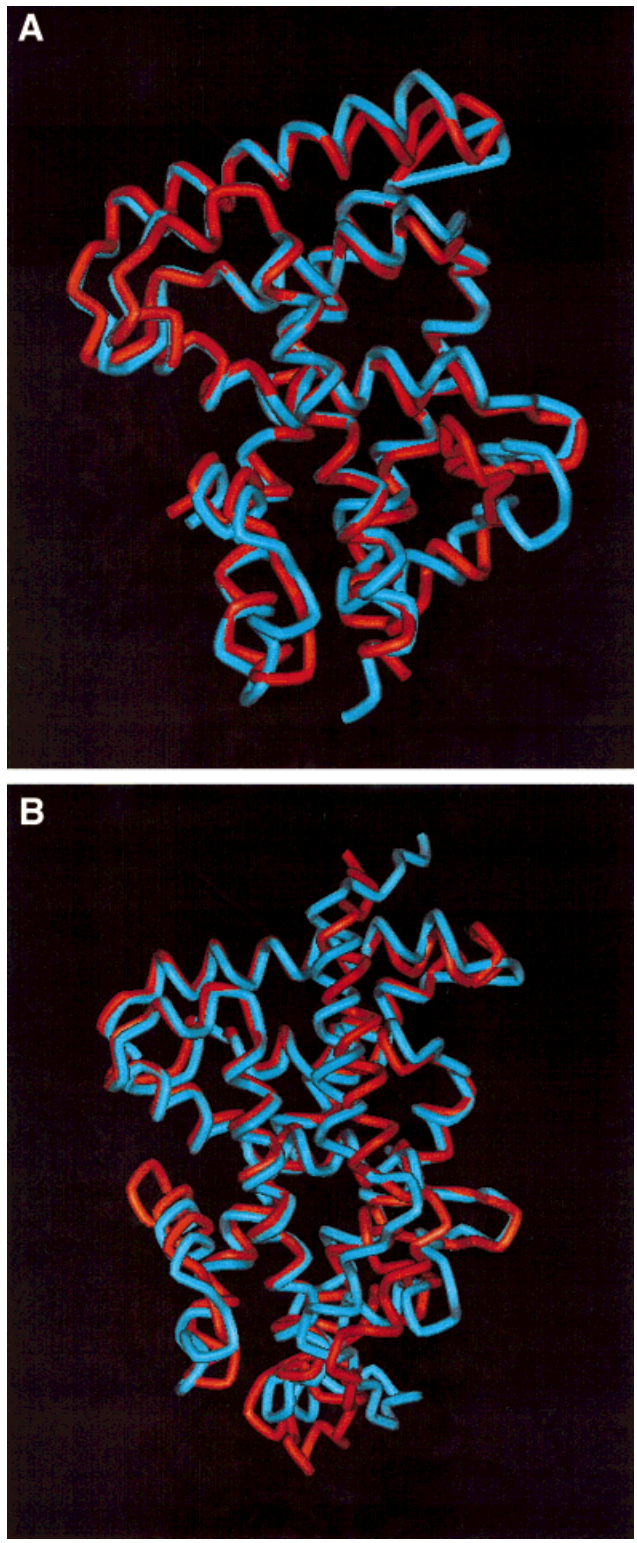

Fig. 4. **A:** Superimposition of rat VDR-LBD model (red, residues 207–423) and human VDR X-ray structure (blue) of construct (118–425, Δ [164–207]). **B:** Superimposition of rat VDR-LBD model (red, 120–422, Δ [164–207]) and human VDR X-ray structure (blue) of construct (118–425, Δ [164–207]).

used to examine detailed contacts existing in complex on docking hormone into shortened LBD (207–423). The recently published crystal structure of hVDR deletion

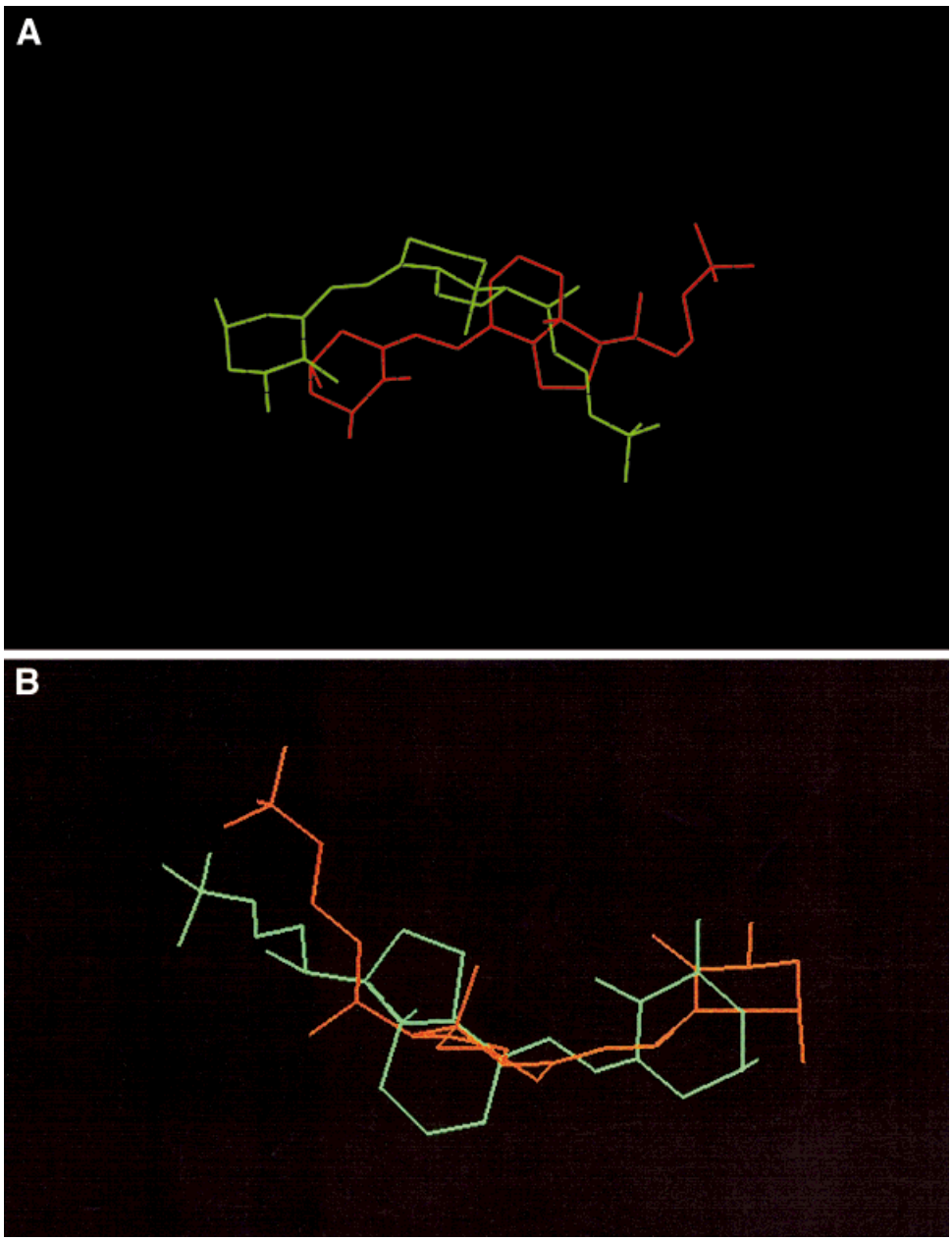

Fig. 5. **A:** Comparison of positions of ligand inside ligand-binding cavities of rat VDR-LBD model (red, residues 207–423) and human VDR construct (118–425, Δ [164–207]; in green X-ray structure). **B:** Comparison of positions of ligand inside ligand-binding cavities of rat VDR-LBD model (red, residues 120–422, Δ [164–207]) and human VDR construct (118–425, Δ [164–207]; in green X-ray structure).

mutant (118–425, Δ [165–215])[7] revealed that Tyr 143 (positioned in helices omitted in homology model) creates hydrogen bond with hormone hydroxyl group situated at C–3.

To model a fragment of rVDR (120–164), we have used a side chain only lattice protein folding method according to the procedure described in Materials and Methods. The resulting model can be easily compared with the X-ray structure of hVDR. The modeling program (SICHO) used the secondary structure predictions by PHD method.[42]

Subsequently, the lattice model was refined (MODELLER) and compared with the experimental structure of the modeled ligand binding domain. Figure 4(B) shows optimal superimposition of corresponding residues of rat and human receptors mentioned above. The DRMS deviation between alpha carbons is 3.4 Å. This finding proves that combining classical comparative modeling and lattice modeling opens the possibility of a model with reasonable accuracy even in the case when a part of the target protein is homologous to known protein structure(s).

Ligand of the VDR

It is well known that 1α,25-$(OH)_2D_3$ is a molecule of high conformational flexibility.[43] Inversion of the A ring and rotation around the C(6)-C(7) bond produces six basic conformers [Fig. 2(A)–(F)]. Rotation around the five side-chain bonds increases the number of possible conformers. Vitamin D hormone in the lowest energy form (global minimum), calculated by Geometry Optimizer from SYBYL,[22] has its side chain oriented in the "northeastern" direction.[18,44] It is commonly accepted that 1α,25-$(OH)_2D_3$ exists as a mixture of two rapidly equilibrating A-ring chair conformers abbreviated as α and β forms, in which 1α-OH occupies axial or equatorial orientation, respectively.[45–47] We performed docking procedures for all six vitamin D conformations (A–F). The lowest energy complexes were obtained for the ligand docked in forms A and B, possessing nearly planar diene C(5)═C(6)—(C(7)═C(8) moiety in the s-*trans* conformation. The ligand in form A enters the pocket with 1α-OH group axially oriented and side chain directed toward the cavity interior. The ligand in form B enters the cavity with 1α-OH group equatorially oriented and ring A faces toward the pocket interior. On the basis of mutagenesis analysis (Table III), it is not possible to rule out either of these two forms. Differences in energy are too small to be conclusive. It is worth noting that there is close similarity between the modeled complex possessing ligand in form B and the crystal structure of holo hVDR construct. Figure 5(A) shows superimposed structures of ligands from overlaid complexes mentioned above. It is apparent that general orientation of ligands is similar, and the conformation of ring A with equatorial 1α-OH substituent is identical. The hydroxyl at position 1, which is more important for hormone binding than 3β-hydroxy group, is hydrogen bonded to Ser 233 in the modeled complex. Such contact was also found in the crystal structure of the human VDR mutant. It is worth mentioning that all complexes having the hormone docked in the s-*cis* conformation of C(5)═C(6)—(C(7)═C(8) moiety are characterized by much higher energy and could be ruled out on the basis of energetic criteria. Thus, it is possible that the modeled structure of rVDR (207–423), with its first two helices removed, can be useful for prediction of a general shape of analogs (6-s-*cis* or 6-s-*trans* forms) docked into the VDR.

Superimposed structures of ligand from the crystal complex (118–425, Δ [165–215]) and that obtained from calculations (120–422, Δ [164–207]) are shown in Figure 5(B). For the elongated complex (calculated by SICHO), orientation of the ligand in the cavity (A ring facing toward the interior) as well as conformation of its ring A (with equatorial orientation of 1-OH group) are very similar to the hormone structure found in the crystal mutant and in the complex calculated by simple homology modeling (MODELLER). Attention is drawn to the fact that of the six active sites existing in crystal form, four of them were found in the elongated holo rVDR. Inspection of Table IV reveals that each of three hormone hydroxyl groups forms at least one hydrogen bond with amino acids, creating contacts in crystals: 1-OH contacts R270, 3-OH contacts S274 and Y143, and 25-OH contacts H393. Tryptophan, known as residuum crucial for VDR activity, is located almost parallel with the diene moiety of the ligand molecule. Such orientation of tryptophan closely resembles its situation in crystals. High similarity found between contacts in crystals and modeled complexes shows that lattice model, used for folding the part of VDR characterized by poor homology, creates a structure consistent with the real one.

There is no simple relationship between hormone-binding capability and its chemical structure.[10,15] The intriguing possibility that some analogs can be complexed in the 6-s-*cis* (steroid) form should also be taken into consideration. Thus, for example, it can be hypothesized that 20-oxopregnacalciferol, synthesized in our laboratory,[48] binds the progesterone receptor in 6-s-*cis* conformation. Inspection of Table IV reveals that three of the four steroid conformers of 1α,25-$(OH)_2D_3$, when docked into the VDR, do not form hydrogen bonds with 25-OH substituent. It was shown in our laboratory that hormone analogs with considerably shortened hydrocarbon side chains (*iso*-Pr or *iso*-Bu groups at 17β-position) bind to the full-length VDR with rather high affinity, only 4 and 11 times, respectively, lower than that of 1α,25-$(OH)_2D_3$.[49] This striking result can be explained by assuming that these analogs enter the hormone pocket in one of their 6-s-*cis* conformations.

Ligand-Binding Pocket of the VDR

The rVDR ligand binding pocket is bordered by helices 3, 5, 10/11, and the loop encompassing residues Pro 404–Pro 412. The calculated pocket volume (610 $Å^3$) is 1.7 times greater than that of the ligand (360 $Å^3$). Helix 12 seals the cavity, interacting with residues from helices 3 and 4. The rVDR pocket is lined mostly by 31 hydrophobic amino acids that surround hydrophobic parts of the vitamin D molecule in close proximity to its C-11, C-18, C-21, and C-22.[15,50]

Significant effort was made in our laboratory to elucidate a role of tryptophan[†] in the binding of 1α,25-$(OH)_2D_3$. It was established that mutation of Trp 282 to Phe greatly reduced (up to 100 times) ligand-binding capabilities of the rVDR.[38] Recently presented mutagenesis data,[12] describing single-point mutations of Trp 286 (hVDR) to Phe and Ser, are in agreement with our results. Furthermore, NMR data from our laboratory indicate that a proton resonance at 11.7 ppm undergoes a shift to 12.2 ppm on hormone binding to LBD.[38] A possible source of this resonance could be amino moiety from tryptophan or histidine rings. The 90% tryptophan fluorescence quenching on ligand binding further confirms that Trp belongs to active site.[26] The lack of binding activity of 6,7-diaza-19-norvitamin D compounds, synthesized in our laboratory, seems to indicate parallel orientation of the tryptophan residue to C(5)═C(6)—(C(7)═C(8) moiety. These diaza analogs[51] showed binding affinity to porcine intestinal nuclear receptor reduced by several orders of magnitude, compared with

[†]In the RAR, tryptophan was also found in close proximity to the β-ionone ring of retinoic acid, when ligand was bound to the receptor.[5]

TABLE IV. Ligand Positions Important for Docking to the VDR (207–423)[a]

Position	A (*trans*-ax)	B (*trans*-eq)	B[b] (*trans*-eq)	E (*P*) (*cis*-ax)	C (*M*) (*cis*-ax)	F (*P*) (*cis*-eq)	D (*M*) (*cis*-eq)
1-OH	3.27 Å H393 (−N═,SC)	2.19 Å K236 (NH,SC) 2.62 Å S233 (OH,SC)	3.36 Å R270 (N-ϵ,SC)	2.61 Å G285 (NH,bb)	2.67 Å S233 (OH,SC)	2.58 Å S286 (NH,bb)	2.16 Å C284 (NH,bb)
3-OH	1.42 Å Q396 (CONH2,SC) 3.54 Å H393 (C═O,bb) 3.28 Å S286 (OH,SC)	2.63 Å S271 (NH,bb)	2.60 Å S274 (OH,SC) 4.03 Å Y143 (C═O,bb)	2.47 Å S274 (NH,bb) 2.52 Å C284 (C═O,bb)	1.63 Å S286 (NH,bb) 2.66 Å S231 (OH,SC)		2.01 Å S233 (OH,SC)
25-OH	1.71 Å S233 (OH,SC) 2.10 Å K236 (NH,SC) 2.19 Å K236 (NH,SC) 2.66 Å S233 (OH,SC)		2.62 Å H393 (N1,SC)		2.40 Å L305 (C═O,bb)		
W-282	5.87 Å (NH,SC)-C8	8.78 Å (NH,SC)-C26	9.45 Å (C5,SC)-C7	8.41 Å (NH,SC)-C15	5.71 Å (NH,SC)-C16	6.01 Å (NH,SC)-C16	5.82 Å (NH,SC)-25-OH
C-11	3.34 Å M268 4.87 Å I267 2.99 Å L309 5.07 Å M268 Å 5.87 Å L389 Å	3.76 Å L226		2.65 Å I267	5.65 Å I267	2.60 Å I267 5.01 Å S271	2.71 Å L226 2.72 Å V230 3.69 Å
C-18	2.91 Å L226 3.76 Å I267 5.95 Å M268 Å 4.27 Å V230	2.81 Å L226	5.22 Å I267	2.62 Å I267 4.14 I264 4.29 Å V230 5.48 Å M268	2.69 Å I267 5.88 Å I264	5.28 Å M268 5.38 Å L226	
C-22	3.04 Å I267 5.21 Å L229	4.05 Å H393 4.19 Å L223	5.33 Å M268	2.28 Å F418	3.21 Å M268 5.35 Å I264	4.90 Å M268	5.10 Å L223
C-21	3.01 Å L229 4.24 Å L226 4.36 Å V230	3.70 Å F418 3.84 Å V230	6.10 Å L226	3.45 Å V230 4.13 Å V414 4.68 Å I264	3.93 Å I264	4.05 Å M268	
A/SC	SC	A	A	A	A	A	A
Energy (kcal/mol)	−50	30	−200	177	147	369	141

[a]In the table the nearest distance between a respective position of the ligand and the indicated amino acid is given. In the abbreviations of the ligand conformation (in parentheses) *cis* and *trans* denote conformation around C(6)-C(7), ax and eq describe orientation of the 1α-hydroxyl. *P* and *M* denote respectively, right- and left-handed chirality of the vitamin D triene system in the 6-s-*cis* conformation. Side-chain and backbone positions are marked by SC and bb, respectively. A/SC denotes that ligand enters the pocket having A ring or side chain positioned toward the cavity.

[b]Complex with the hormone docked into calculated deletion construct of rVDR 120-422, Δ[164-207].

1α,25-$(OH)_2D_3$. It is known from microwave data[52] that C═N—N═C moiety of aliphatic diaza compounds can considerably depart from planarity, the corresponding dihedral angle being larger than 60°. It is therefore possible, that 6,7-diaza-19-norvitamin D analogs have somewhat twisted diene fragment, which changes the architecture of their active sites and destroys binding capability. The unique role of single tryptophan residue was confirmed by analysis of the crystal structure of hVDR construct. In the human vitamin D deletion mutant tryptophan indole rings are situated almost parallel to C(5)═C(6)—C(7)═C(8) diene and distances between the corresponding carbons from these two moieties cluster around 4.9 Å, allowing for sandwich-type interactions.

CONCLUSIONS

Ligand-docking experiments were performed several times for each of the ligand forms presented in Figure 2. The results of particular simulations are reproducible; qualitatively, the same structure of the receptor-ligand complex was obtained for a given form of the ligand. Various conformers of the ligand bind in different ways, but the energy differences are too small to be conclusive without comparison with experimental data.

On the basis of energetic criteria and consistency with experimental data, we conclude that 1α,25-$(OH)_2D_3$ is complexed by the VDR in its 6-s-*trans* form with 1α-OH oriented equatorially. Two models of rVDR, simplified (207–423,) and elongated (120–422, Δ [164–207]), show high agreement with crystal structure of hVDR construct (118–425, Δ [165–215]), but only the latter calculated by SICHO retrieves the most active sites on docking hormone. Therefore, we can expect that lattice modeling of the other poorly homologous part of rVDR (164–207) could be a precise method for building the full-length receptor (116–423) and creating a complex with the hormone. It should be noted that in the complexed full-length human receptor, mutation S278A[8] does not confirm involvement of Ser 278 in hydrogen bonding with the ligand hydroxyl groups. Therefore, it shall be taken into consideration that the real orientation of 1α,25-$(OH)_2D_3$ in the VDR might be slightly different from that found in crystals of hVDR deletion mutant (118–425, Δ [165–215]). Until the architecture of the receptor is known from NMR spectra performed in solution, modeling of the VDR structure and docking experiments, similar to those described in this work, can be potentially useful as the tools for rational design of new vitamin D analogs.

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