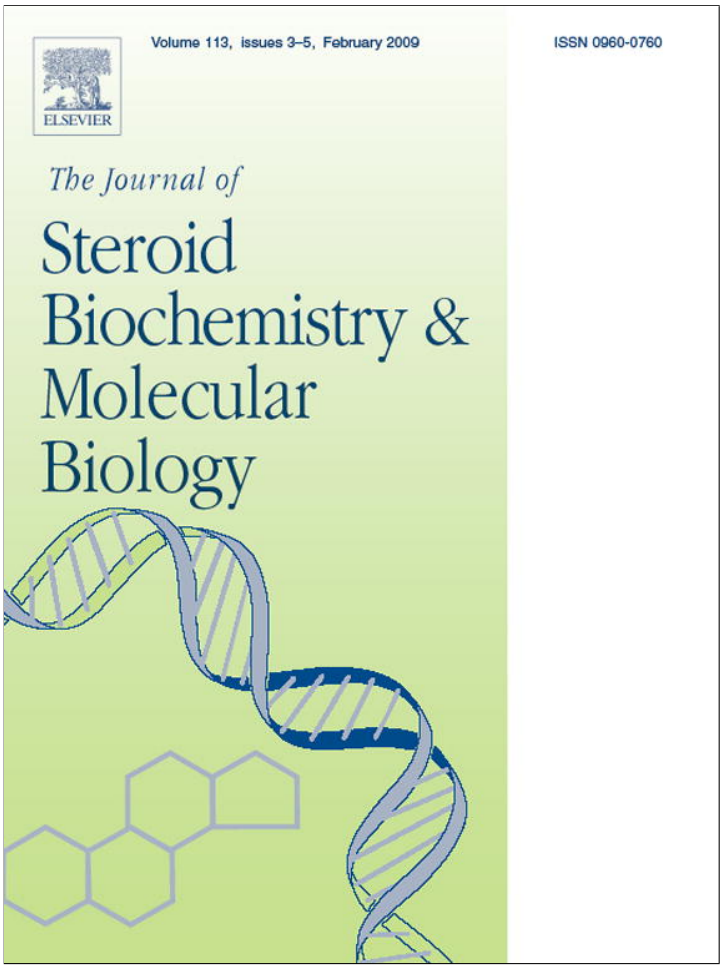

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Structural changes of vitamin D receptor induced by 20-*epi*-1α,25-$(OH)_2D_3$: An insight from a computational analysis

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ABSTRACT

We employ a new computational tool CCOMP for the comparison of side chain (SC) conformations between crystal structures of homologous protein complexes. The program is applied to the vitamin D receptor (VDR) liganded with 1α,25-$(OH)_2D_3$ (in 1DB1) or its 20-*epi* (in 1IE9) analog with an inverted C-20 configuration. This modification yields no detectable changes in the backbone configuration or ligand topology in the receptor binding cavity, yet it dramatically increases transcription, differentiation and antiproliferation activity of the VDR. We applied very stringent criteria during the comparison process. To eliminate errors arising from the different packing of investigated crystals and the thermal flexibility of atoms, we studied complexes belonging to the same space group, having a low *R* value (0.2) and a *B*-factor below 40 for compared residues. We find that 20-*epi*-1α,25-$(OH)_2D_3$ changes side chain conformation of amino acids residing far away from direct ligand–VDR contacts. We further verify that a number of the reoriented residues were identified in mutational experiments as important for interaction with SRC-1, GRIP, TAFs co-activators and VDR-RXR heterodimerization. Thus, CCOMP analysis of protein complexes may be used for identifying amino acids that could serve as targets for genetic engineering, such as mutagenesis.

1. Introduction

The vitamin D receptor is a ligand-activated transcription factor belonging to the nuclear receptor (NR) superfamily [1]. During the last eight years several complexes of the human, rat and zebrafish holoVDR have been successfully crystallized in the absence and presence of peptides which mimic the sequence of SRC-1 and DRIP co-activators [2–5]. Superimposition of these liganded VDR complexes demonstrated that conformations of receptor backbones and the topology of ligands in the VDR binding cavity do not vary among crystals, even when the vitamin D compounds differ drastically in their biological potency [6].

Modifications of the VDR natural ligand, 1α,25-$(OH)_2D_3$ ($1,25D_3$), led to the development of drugs with selective therapeutic activity [7]. So far the most important modification of $1,25D_3$ consists of a configuration change at carbon 20 from *R* to *S*. Analogs with such side chain structure (called 20-*epi*-) reveal much higher activity than $1,25D_3$ in transcription, antiproliferation, differentiation and bone calcium mobilization (Table 1S), [8–10]. Interestingly, the synthetic vitamin D analog with two identical side chains anchored at carbon 20 (KH, parental Gemini) exhibits biological activities (Table 1S) resembling $1,25D_3$ not its 20-epimer [11]. X-ray studies revealed that backbones of zVDR in complexes with Gemini (2HCD.pdb) and $1,25D_3$ (2HC4.pdb) have nearly identical overall structure with the root mean square deviation (RMSD) of 0.37 Å. The binding of the additional 20-*epi*-vitamin chain is made by the rotation of zL337 (hL309) side chain that opens a hydrophobic channel and increases the LBP (ligand binding pocket) by 25%. Superimposition of the natural and Gemini ligands shows that the "normal branch" of the KH side chain (equivalent to the side chain in $1,25D_3$) and A, seco-B and C/D rings perfectly overlap except a small (0.6 Å) shift of the C/D moiety [4]. The fact that 20-*epi*-1α,25-$(OH)_2D_3$, 1α,25-$(OH)_2D_3$ and Gemini with the "normal" branch of its side chain occupy the same part of VDR binding cavity, yet these compounds differ drastically in their biological activities, points on necessity of looking for differences between protein complexes on the level of atomic not residue length scale. Small alteration in VDR structure, like rotation of amino acid's side chains in response to the ligands might result in a conformation that preferentially accomodates co-activators responsible for selected biological potency [12].

The sequence of events leading to transcription is a complex, multistep process and is far from being understood. Ligand binding is the first step of molecular action that switches the VDR to an active state in which the receptor is able to attract RXR and co-modulators [1]. It is difficult to identify subtle changes in the

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Table 1
PDB codes of the crystal human VDR complexes.

PDB code	Resolution[a] in Å	Ligand	Reference	Construct	Space group	Missing residues[b]
1DB1	1.80 (0.191)	1,25-D_3 (1)	[2]	h118–425, Δ[165–215]	$P2_12_12_1$	(118–119, 375–377, 424–425)
1IE9	1.40 (0.214)	MC1288 (2)	[3]	h118–427, Δ[165–215]	$P2_12_12_1$	(424–427)

[a] *R* values are in parenthesis. *B*-factors for most VDR amino acids are below 40.
[b] Missing residues are present in the construct but not detected.

receptor's structure which result from ligand or co-factors binding. Recently we described a preliminary version 3.30 of the Complex COMParison (CCOMP) program [12], which aided the comparison process by calculating values of the local RMSD between α-carbon coordinates of amino acids in two protein complexes.

The purpose of this work is to identify subtle changes in the side chains of VDR residues caused by the presence of 20-*epi* compound. We utilize an expanded version of the CCOMP program [version 3.70, http://www.icho.edu.pl/ENG/Achievements/Achievements.htm] which distinguishes between center-of-mass deviation C-RMSD and distance D-RMSD per residuum as opposed to the single local L-RMSD measure reported by the previous version. Comparisons of binary VDR complexes are the focus of this work.

2. Computational methods

2.1. Computational approaches

Complex COMParison version 3.70 is a software tool designed to speed up the cumbersome process of comparing molecular complexes. The program performs several consecutive steps: aligning protein sequences, superimposing structures of the aligned proteins, transforming ligands according to the protein superposition, calculating differences in orientation and conformation between individual amino acids and between the ligand molecules. While all these steps can be done with the help of an interactive molecular visualization program, such as Biodesigner [13], CCOMP automates the process and finally lists amino acids with considerably reoriented side chains, for whose $\Delta\chi_{SC} \geq 10^\circ$. Additionally, the program can resolve data errors commonly encountered in Protein Data Bank (PDB) files, such as missing atoms or duplicated residues.

The preliminary version 3.30 of CCOMP program [12,14] http://www.pirx.com/ccomp calculated just coordinate root mean square deviation for all atoms of the individual amino acids. The amino acids with their RMSD exceeding the average value by 30%, or more, were subjected to further analysis. Interestingly, a careful comparison of these residues revealed that the average RMSD actually measures in fact two different effects: shifting of the side chain and change of the side chain internal conformation. In this work we introduce a new, more versatile version of the CCOMP program, allowing for effective separation of the above mentioned effects [http://www.icho.edu.pl/ENG/Achievements/Achievements.htm].

The 3.70 version of CCOMP calculates several difference measures: local all-atom RMSD (L-RMSD), amino acid center-of-mass deviation (C-RMSD) and distance RMSD (D-RMSD) per residue, for whole amino acids and for side chains only. The L-RMSD reflects both spatial differences and conformational changes found in the compared complexes. The C-RMSD averages the atomic coordinates of particular amino acids, and therefore reflects mostly the spatial (translational) differences. On the other hand, the D-RMSD ignores translations and rotations and reflects only intra-residue conformational changes. To assess statistical significance of the difference measures, distributions of the RMSD values are first calculated and subsequently normalized according to their standard deviations. The normalized values (*z*-scores) are considered significant if they exceed a certain threshold. The default *z*-score (z-score $= (x - x_{av})/\text{S.D.}$) threshold in CCOMP is equal to 1.0. As shown in Figure 1S (example of CCOMP version 3.70 output) only amino acids with at least one *z*-score over the threshold are reported by the program. VDR complexes analyzed in this work are listed in Table 1. Figure 2S depicts equivalent amino acid positions in aligned (Clustal W) [15] sequences of human, rat and zebrafish VDR. Figure 3S shows aligned sequences of rat VDR, mouse RXRα, human RARγ and human PPARγ. Yet another amino acid comparison measure implemented in CCOMP is a difference between values of side chain χ angles. These differences can reflect more subtle changes in side chain conformations than the D-RMSD values themselves. By default, all amino acids with χ angle differences exceeding 10° are listed by CCOMP. Taking into account the quality (*R* and *B* values) of the studied complexes, only differences exceeding 40° are considered significant and discussed in this paper.

2.2. Validation of crystal complexes and setting cut off value for χ angle in CCOMP

Crystal packing forces can stabilize a protein structure that is characteristic only for the solid state. Usually it is not the case for rigid part of proteins, easy detectable in X-ray and NMR experiments. Nuclear receptor family, to which VDR belongs, shows well-conserved architecture of LBD (ligand binding domain). Eleven or twelve helices [6] are arranged in three layered sandwich structure. Liganding makes the receptor's structure more rigid by repositioning the last helix 12 in a "mouse trap mechanism". The biggest conformation differences are observed between NR members in the location and length of flexible loops or "unbound" helix 12, sometimes undetectable even in NMR spectra [16]. Vitamin D receptor is very mobile in comparison with other nuclear receptors. The flexible long loop (72–81 amino acids between helices 1 and 3) hampers crystallization of the full-length LBD-VDR. For this reason all reported VDR X-ray structures are obtained for liganded VDR deletion mutants (holoVDRΔ). Recently published data showed that crucial VDR amino acid Trp 286, which appears just once in the VDR sequence and occupies the center of the ligand binding pocket, conserves its orientation with respect to the vitamin double bond C(7)═C(8) in solid state [2], vacuum [17] and in buffered aqueous solutions [18], being a *milieu* closest to physiological conditions. Very often results derived from analysis of crystal structures become also confirmed by *in vivo* studies. Genetic engineering offers many methods of verifying proteins activity predicted from crystal analysis. There is no question that crystalographic model**s** are invaluable in understanding structure-function relationship of protein multicomplexes. Sometimes receptor–modulator interactions are critically dependent on just a few amino acids, located at the binding interface.

The first challenge for protein complex comparisons arises in choosing the procedure for crystal structure validation. Crystal structures entered in databases meet criteria set by the scientific community; they have resolution below 3 Å and *R*-factor of about 0.2. However, one should keep in mind that in general proteins are fairly flexible. Even in crystals detected with good resolution and showing *R*-factors in the 0.15–0.25 range, the structure of flexible molecule parts has low reliability. The *B*-factor, also known as the "temperature factor," reflects the mobility of flexible substructures.

Table 2
VDR residues significantly changing their side chain orientation in 20-*epi* analog of 1α,25 $(OH)_2D_3$.

Compared complexes[a]														
1DB1-1IE9														
$\Delta\chi_{1(2/3)} > 40^\circ$	E128	(H139)	H140	K141	R158	S222	(I238)	Q259	I260	K264	P312	(M334)	E353	(I355)
$\Delta\chi$ value	176	174	178	116	50	−109	119	142	−104	103	81	85	−42	105
Helix		H1				H3n	H3		H4		H7	H8		H9
SASA[b] (%)														
In 1DB1	17	1	52	65	35	62	0	17	53	32	36	0	52	3
In 1IE9	19	3	51	63	29	69	0	20	58	32	20	2	51	7
Important residues[c] for:														
CoA				+			+	+	+	+		+		
Transcription				−			+	+		+				
Dimerization with RXR													+	
Other					+									

[a] All residues are numbered according to the hVDR sequence. Amino acids creating direct contacts with ligands are not considered in this table. Crystals of 1DB1 (*R* value 0.19) and 1IE9 (*R* value 0.21) belong to the same space group $P2_12_12_1$. For all investigated amino acids *B*-factor is below 40.

[b] SASA denotes normalized solvent accessible surface area. Buried residues with SASA value below 10% are in parenthesis.

[c] Mutational experiments are available for eight of the fourteen residues found by CCOMP. Amino acid sensitive/not sensitive to the listed biological events are marked by **+**/−, respectively; blank space denotes that experiments are unknown. Literature data cited here are taken from references [4,5,22–26]. R158 reveals different digestion pattern (c_{LPD}) in 1,25D_3 and 20-*epi*-1,25D_3 VDR complexes [25].

High *B* values (exceeding 60) imply high uncertainty in a specific part of the model. Side chains of amino acids are the most flexible units in protein structures. For the creation of rotamer libraries only amino acids with *B*-factors below 40 are considered [19]. It is commonly accepted that the position of a residuum is well ascertained if the *B*-factor of each atom of the studied protein fragment is below 40 [20]. VDR amino acids compared in this paper fulfill these rather stringent criteria. The fact that 1α,25-$(OH)_2D_3$-VDR and 20-*epi*-1α,25-$(OH)_2D_3$-VDR complexes crystallize in the same space group (Table 1) eliminates errors arising from different crystal order [21]. Table 2 [4,5,22–26] shows amino acids with markedly reoriented side chains in the presence of 1α,25-$(OH)_2D_3$ modified at C-20.

It is accepted [27] that a *B*-factor of 20 corresponds to an error range of 0.5 Å in superimposed structures. *B*-factors of residue atoms exceeding 60 imply that side chain orientation is unknown. Taking these facts into account we set a cut off value of 40 for SC orientation comparisons. Unfortunately, ternary VDR-SRC-1 complexes 2HC4 and 2HCD have *B*-factor values exceeding 60 for almost all their atoms, and therefore could not be reliably analyzed.

2.3. SASA (solvent accessible surface area)

It is conceivable that rearrangement of some side chains upon co-factor binding prepares protein complexes for subsequent biological events leading to transcription of genes regulated by this particular receptor. Therefore, knowledge whether reoriented amino acids are exposed to a solvent and thus capable of attracting co-modulators is of significant interest. In this work, values of SASA were calculated using the DSSP program [28] and then normalized by the average amino acid surface. The average surface value per amino acid is defined as the surface of the central residue in reference tripeptides [29]. Table 2 contains normalized SASA values for hVDRmt residues differently oriented in 1DB1 and 1IE9 crystals.

2.4. Docking of SRC-1 motif to VDR deletion mutants

To provide theoretical proof that CCOMP indeed identifies surface amino acids interacting with co-modulators, peptide-protein docking was performed. SRC-1 co-activator (R686, H687, K688, I689, L690, H691, R692, L693, L694, Q695) taken from crystal structure of 1α,25-$(OH)_2D_3$-zVDRΔmt-SRC-1part (2HC4.pdb) was flexibly docked to rigid VDR, extracted from three complexes: 1α,25-$(OH)_2D_3$-zVDRΔmt-SRC-1part (2HC4.pdb), 1α,25-$(OH)_2D_3$-hVDRΔmt (1DB1.pdb), 20-*epi*-1α,25-$(OH)_2D_3$-hVDRΔmt (1IE9.pdb). Docking simulations were performed by FlexiDock software (package of Sybyl7.1) from Tripos [30]. FlexiDock requires an approximate starting position of the ligand to be provided. For each binary complex, simulations (of 100,000 steps each) were repeated several times, for various arbitrarily chosen initial positions of the peptide (ligand) in the vicinity of the receptor binding pocket (hVDR: I242, K246, R252, L263, K264, E420). Internal rotations around 28 single bonds of SRC-1 side chains were allowed in the simulations of human/zebrafish VDR-SRC-1 complexes. Two hydrogen bonding sites (hK246-L693, hE420-L690 or zK274-L693, zE446-L690) were always marked in Setup FlexiDock dialog box. The lowest energy complexes were selected for final consideration.

3. Results and discussion

The vitamin D receptor acts as a ligand-dependent transcription factor. As far as biological activity is concerned, 20-*epi* is the most important modification till now. It is well documented that 20-*epi* compounds have significantly increased transcription, cell differentiating and antiproliferative potency in comparison with 1,25D_3 (Table 1S). While this elevated activity can be linked to higher affinity for the VDR, more efficient uptake into target cells, different metabolism and catabolism than that of 1α,25-$(OH)_2D_3$-VDR, these factors alone cannot explain the increase in biological potency of 20-*epi* compounds [31]. Further studies [9] revealed that ligand-dependent recruitment of RXR or GRIP1 and DRIP205 co-activators to VDR elevate the ability of the receptor to activate transcription of genes regulated by MC1288. According to Norman et al. [31], stimulation of transcription by 20-*epi* D vitamins lies in ligand–VDR interactions generating unique conformational changes of the receptor that compel VDR interaction with other transcription factors like RXR or co-activators.

Thus, to quantify changes in the VDR structure caused by the presence of analogs with inverted configuration at C-20, we chose two VDR complexes for comparison, namely those accommodating 1,25D_3 (1DB1) and MC1288 (1IE9) as ligands (Fig. 1). Analysis of contacts in these two crystalline structures shows that vitamins interact with the same amino acids (Y143, Y147, F150, L227, L230, A231, L233, V234, S237, I268, I271, M272, R274, S275, S278, W286, C288, Y295, V300, A303, H305, L309, L313, H397, Y401, L404, L414, V418 and F422). Surprisingly, these residues have very similar con-

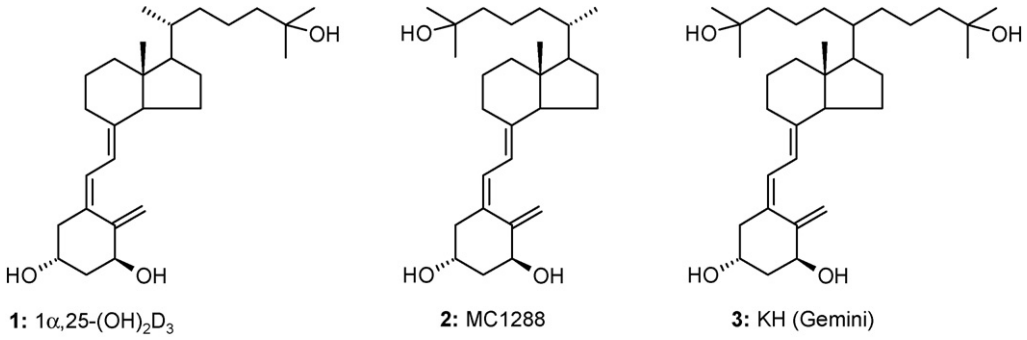

Fig. 1. . Chemical structure of (1) 1α,25-$(OH)_2D_3$ (1,25D_3), (2) MC1288 (20-*epi*-1α,25-$(OH)_2D_3$) and (3) KH (21-(3OH-3-methyl-butyl)1α,25-$(OH)_2D_3$).

formation of their side chains in both crystals ($\Delta\chi$ clusters around 10°). Most of these residues have *B*-factor values below 30. This indicates that direct ligand–amino acid contacts cannot be responsible for the augmented transcription of 1IE9 (Fig. 2).

Comparison of 1DB1 and 1IE9 structures by CCOMP (Table 2) revealed that 14 amino acids, residing mainly in helices H1 and H4, have their side chains considerably reoriented ($\Delta\chi \geq 100°$). None of them belong to helix 12, known as a main transcriptional platform. Most of these 14 residues are hydrophilic, so they can play an important role in molecular recognition. Four years ago Camacho and co-workers [32] identified anchor/latches amino acids for 39 protein–protein complexes. Mainly these residues appeared to be easily accessible by a solvent. The authors also studied the structure of the interface and found it was not flat, but rather included residue side chains deeply protruding into well-defined cavities on the counterpart protein.

Reorientation of residues that are polar/charged and exposed to a solvent can have significant biochemical consequences in creating a receptor–modulator platform. This prompted us to consider VDR amino acids with reoriented side chains as potential interface units. In Table 2 residues, whose affinity to co-activators was verified by mutational experiments, are marked by +. For others reoriented VDR residues interactions between mutated receptor and co-activators were not investigated. The prevalence of +'s among the identified by CCOMP residues raises confidence in the program's usefulness.

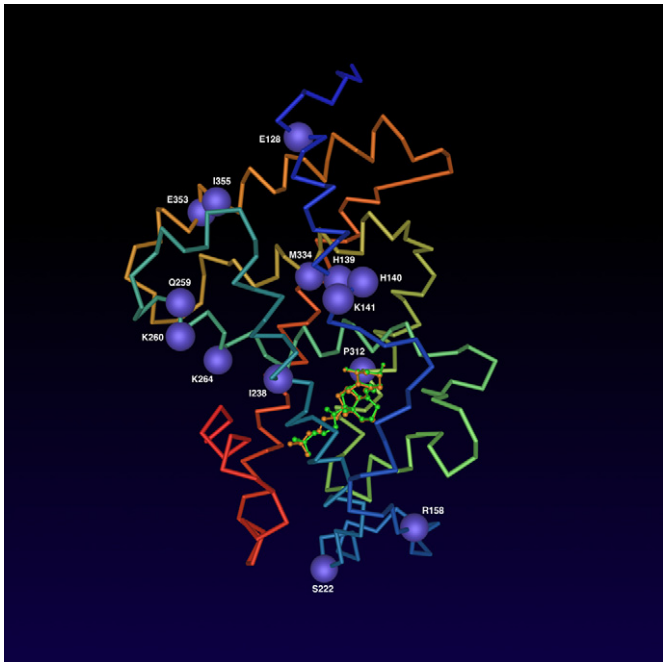

Fig. 2. . Comparison of three-dimensional structures of binary VDR complexes, liganded with 1,25D_3 (1DB1) and its 20-*epi*-analog MC1288 (1IE9). Fourteen amino acids (E128, H139, H140, K141, R158, S222, I238, Q259, I260, K264, P312, M334, E353, I355) differing significantly theirs side chains orientation are labeled.

Four (H139, I238, M334, I355) of the fourteen amino acids reoriented in 1DB1/1IE9 have normalized SASA values below 7% (Table 2). Therefore, they are effectively buried and cannot directly interact with co-factors involved in the events following VDR liganding. The remaining ten amino acids are accessible by a solvent; for seven (H140, K141, R158, S222, I260, K264, E353) of them normalized values of SASA exceed 30% (Table 2). These constitute the most likely candidates for co-factor recognition. Mutational experiment data are available for eight out of the ten reoriented and exposed to solvent amino acids. Six of the residues (K141, I238, Q259, I260, K264, M334) identified by CCOMP can interact with co-activators like SRC-1 [22,23], GRIP [22] or TAF [22,24]. Two amino acids, namely I260 and K264, bind SRC-1 in the ternary complex 2HC4 (1,25D_3-zVDR-SRC-1) [4] whereas K264 creates also specific contact with DRIP co-activator (1,25D_3-rVDR-DRIP) [5]. Only one residue H141 from eight mutated did not influence transcription [26]. Given that every amino acid identified by CCOMP for which mutational data are available, proves involvement in the biological processes leading to transcription, we believe that remaining two residues might also be important and constitute good targets for mutagenesis.

It is commonly accepted that co-modulators bound with the VDR significantly influence the selective activity of vitamin D analogs. It is reasonable to postulate that reoriented residues can augment protein-co-activator interactions, and in that way increase transactivation potency of the receptor. It is well documented that mutations revealing reduced binding to the above mentioned co-modulators hamper transactivation [22,33–35]. For example, a triple mutation in hVDR (K141L-T142W-Y143A) which diminishes the receptor interaction with DRIP (regulatory panel for RNA polymerase II) [36] results in the development of HVDRR (human VDR resistance ricket) without alopecia [26]. It is also known that MC1288 interacts with DRIP hundred times more efficiently than 1,25D_3 and shows 500 times higher potency than the natural hormone in cell antiproliferation [8–10].

In order to provide evidence other than mutations that CCOMP has indeed identified surface residues whose change in conformation lead to a higher stability of the vitamin D receptor liganded by 20-*epi*-1α,25-$(OH)_2D_3$ in comparison to the receptor bound to 1α,25-$(OH)_2D_3$, we performed peptide-protein docking experiments. The fragment of SRC-1 co-activator (H687, K688, I689, L690, H691, R692, L693, L694) containing LxxLL motif was flexibly docked to the receptor surface under conditions described in Section 2.4. Analysis of 2HC4 crystals revealed that six of zVDR residues create eight strong (below 3.5 Å) specific contacts (hydrogen bonding or hydrophobic interactions between CH_3

groups) with five SRC-1 amino acids: zI270-L693, zI270-L694, zK274-L693, zR280-L694, zL291-L694, zK292-H687, zE446-I689 and zE446-L690. The fact that in the simulated SRC-1-zVDR$_{2HC4}$ complex six specific contacts were reproduced with accuracy 0.02–0.45 Å and two were reproduced with accuracy about 1 Å, verify the correctness of chosen docking conditions. In the computed SRC-1-hVDR$_{1DB1}$ complex only three (hI242-L693, hI242-L694, hK246-L693) of eight specific interactions were reproduced (accuracy 0.6–0.9 Å). In the case of simulated SRC-1-hVDR$_{1IE9}$ complex six of eight specific CoA-receptor interactions were retrieved; four (hI242-L693, hK246-L693, hL263-L694 and hK264-H687) with 0.1–0.6 Å accuracy and two (hI242-L694, hE420-L690) with significantly lower accuracy: 1.4 Å and 1.8 Å, respectively. The fact that energy of SRC-1-hVDR$_{1IE9}$ complex (-343 kcal/mol) is lower than energy of SRC-1-hVDR$_{1DB1}$ complex (−337 kcal/mol) also indicates that receptor reoriented by 20-*epi*-1α,25-$(OH)_2D_3$ has higher affinity to SRC-1 than VDR liganded by parental vitamin D.

Among the most important VDR's amino acids is hK264 (zK292). In NR family this conserved lysine interacts with co-activators like GRIP [37], DRIP [5] and β-catenin [38]. In the vitamin D receptor hK264 creates salt bridge with hE420, joining helices H4 and H12 [2]. This residue is exposed to solvent (32% of normalized SASA) and possesses different side chain conformation in 1DB1 and 1IE9 complexes (Table 2) but similar in 1IE9 and 2HC4 crystals. The maximum reorientations of K_{SC} ($\Delta\chi$ value) in compared 1DB1/2HC4, 1IE9/2HC4, 1DB1/1IE9 pairs are equal to 112°, −21° and 103°, respectively. It indicates that 20-*epi*-vitamin reorients lysine side chain like SRC-1 co-activator upon binding to 1,25D_3-zVDR complex. Thus, K264$_{1IE9}$ should interact with SRC-1 with less energetic cost than K264$_{1DB1}$. Taking into account that hK264 is highly involved in transcription process and SRC-1 in up-regulation of osteocalcin (OC) gene [39] at least two potent 20-*epi*-1α,25-$(OH)_2D_3$ functions (transcription and bone calcium mobilization) could be better understood.

Summarizing, the influence of 20-*epi*-1α,25-$(OH)_2D_3$ on the vitamin D receptor appears to lie in changes to side chain conformations of amino acids which are important for biological activities. The novel tool proposed in this paper facilitates the comparison of SC conformations of homologous proteins. We believe that the type of analysis conducted in this work can also be used for identifying a set of viable targets for alanine scanning mutational experiments or other genetic engineering methods.

4. Conclusions

In the process of computer-aided drug design it is usually assumed that a ligand or co-factor does not change the overall structure of the host molecule upon binding. Sometimes it is an acceptable approximation, although in general the receptors' structure could be significantly modified upon the binding of a ligand or other molecules. Detailed comparison of protein complexes is a cumbersome process, but it can be very informative. We show that 20-*epi* modification in 1α,25$(OH)_2D_3$ does not influence the conformation of residues creating direct contacts with ligands, but change side chain orientation of more distant amino acids. These reoriented residues are known to interact with co-activators or influence VDR-RXR heterodimerization. It is likely that 20-*epi* analog MC1288 augments (through these repositioned units) VDR interactions with co-factors and in this way increase transcription of genes controlled by the receptor. To automate the process of identifying these reoriented residues we created a versatile program for analysis of protein complexes. CCOMP version 3.70 calculates several measures of structural differences: local all-atom RMSD (L-RMSD), amino acid center-of-mass deviation (C-RMSD) and distance RMSD (D-RMSD) per residue, for whole amino acids and for side chains only. Since D-RMSD ignores translations and rotations, it reflects only internal conformational changes. To facilitate the process of identifying reoriented side chains, CCOMP lists residues for which values of $\Delta\chi_{SC}$ angle exceeds 10°.

The robust features of CCOMP could be used for comparison of any liganded/unliganded protein complexes. We hope that CCOMP can be useful as a tool for comprehensive analysis of biologically active complexes and in elucidating the nature of NR responses to natural ligands and their analogs.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jsbmb.2009.01.007.

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