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Computational analysis of the active sites in binary and ternary complexes of the vitamin D receptor

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Abstract

We have developed a program CCOMP that compares overlapping fragments of two protein complexes and identifies differently oriented amino acids. CCOMP initially performs a sequence alignment of the analyzed receptors, then superimposes the corresponding aligned residues, and finally calculates the root mean square deviation (RMSD) of individual atoms, every amino acid and the entire complex. Thus, amino acids important for functional differences between both complexes can be detected. Application of CCOMP to 1α,25-$(OH)_2D_3$-hVDR (1DB1) [Proc. Natl. Acad. Sci. U.S.A. 98 (2001) 5491] and 1α,25-$(OH)_2D_3$-rVDR-peptide (1RK3) [Biochemistry 43 (2004) 4101] complexes revealed that the peptide (KNHPMLMNLLKDN) mimicking a co-activator sequence significantly changes the side chain conformation of 35 amino acids. Four of these residues (K242, I256, K260, E416) actually contact the peptide, but all of them are essential for biological activity. Only two (L309 and L400) of the 35 differently oriented amino acids contact the ligand. Interestingly, when the peptide is present (1RK3) leucine 400 shifts closer (0.7 Å) to the vitamin D 26-methyl group. Applying the CCOMP and DSSP programs to binary and ternary VDR complexes also resulted in establishing that seven amino acids (I238, S252, I256, L413, L415, E416, V417) exhibit significant differences in solvent accessibility and are capable of interacting with co-activators.

Keywords: Vitamin D receptor; Interactions with co-modulators; Changes of residue orientations upon ligand and peptide binding

1. Introduction

During the last 6 years several complexes of the human and rat vitamin D receptor have been successfully crystallized in the absence (hVDR) and presence (rVDR) of a peptide (KNHPMLMNLLKDN), which mimics the co-activator sequence (Table 1) [1–3]. Superimposition of different ligand–VDR complexes shows that conformations of the receptor and the peptide backbones do not vary among complexes, even when the ligands differ drastically in their biological potency [4,5]. These results suggest that co-activators might influence the conformation of the receptor's side chains, thus modifying active sites responsible for biological activity. To verify this hypothesis we have developed a program that identifies amino acids, which change orientation upon ligand or co-modulator binding.

2. Computational details

The CCOMP program originates from the Biodesigner software package [6]. In the first step CCOMP performs global sequence alignment of the receptor sequences using a standard dynamic programming algorithm. The alignment step employs BLOSUM62 mutation matrix and −11, −1 penalties for gap opening and extending, respectively. Subsequently, the corresponding alpha-carbon coordinates of the aligned receptors are subjected to structural superposition. Next, the ligand molecules are transformed according to the computed superposition. Finally, root mean standard deviations (RMSD) between coordinates of all atoms of both complexes are calculated and reported. The deviations can be calculated independently for each residuum. Amino acids that change orientation have values of the local RMSD significantly higher than the value of the all-atom RMSD (Tables 2 and 3). Visualization of residues with changed side chain conformation was performed in Sybyl [7]. To

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Table 1
PDB codes of the VDR complexes compared by the CCOMP program[a]

	PDB code					
	1DB1	1TXI	1RK3	1RKH	1RJK	1RKG
Resolution (Å)	1.80	1.90	2.20	2.28	1.99	1.90
Ligand	1,25-D_3	TX522	1,25-D_3	2AM20R	2MD	2MbisP

[a] Crystallographic data are taken from Refs. [1–3]. The co-activator peptide (KNHPMLMNLLKDN) is present in 1RK3, 1RKH, 1RJK and 1RKG crystals.

Table 2
Changes of amino acid orientations[a] upon ligand or peptide[b] binding computed by CCOMP program

	Compared complexes					
	1DB1-1TXI	1DB1-1RK3	1RK3-1RKH	1RK3-1RJK	1RK3-1RKG	1RJK-1RKG
Number of compared residues	246	216	249	249	249	249
All-atom RMSD (in Å)	0.320	1.013	0.779	0.699	0.828	0.830
Number of differently oriented residues (in %)	12 (4.9)	35 (16.2)	12 (4.8)	13 (5.2)	15 (6.0)	19 (7.6)

[a] Amino acids are assumed to be differently oriented if they have values of the local RMSD significantly higher than the value of the all-atom RMSD.
[b] The peptide mimicking a co-activator has the following sequence: KNHPMLMNLLKDN.

establish solvent accessible surface of amino acids (Table 3) we employed the DSSP software package [8].

3. Results and discussion

To estimate the influence of a co-activator on the activity of the vitamin D receptor, we applied the CCOMP procedure to analyze binary and ternary VDR complexes (Table 1 and Fig. 1) holding as a ligand the parent vitamin 1α,25-$(OH)_2D_3$ and its analogs TX522, 2MD, 2MbisP, 2AM20R. We started our studies from comparing the three-dimensional structure of 1α,25-$(OH)_2D_3$–VDR complexes in the presence (1RK3) and absence (1DB1) of a peptide (KNHPMLMNLLKDN) mimicking the co-activator sequence. It was established by using the Biodesigner program [6] that the peptide contacts 13 amino acids on the VDR surface; none of which are situated near the vitamin D hormone. However, a comparison performed by the CCOMP program revealed that 35 amino acids (ca.16% of all residues in the receptor) changed the

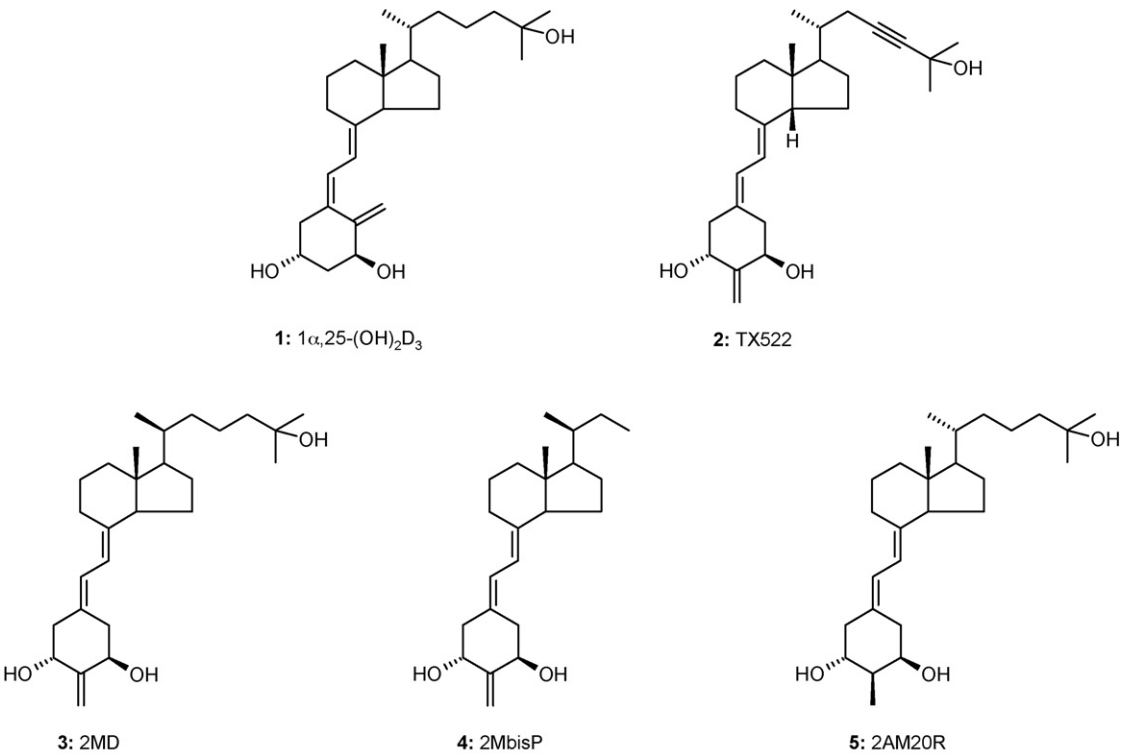

Fig. 1. Chemical structure of (1) 1α,25-$(OH)_2D_3$, (2) 19-nor-23-yne-1α,25-$(OH)_2D_3$ (TX522), (3) (20*S*)-2-methylene-19-nor-1α,25-$(OH)_2D_3$ (2MD), (4) (20*S*)-2-methylene-19-nor-1α-(OH)-bishomopregnacalciferol (2MbisP) and (5) 2α-methyl-19nor-1α,25-$(OH)_2D_3$ (2AM20R).

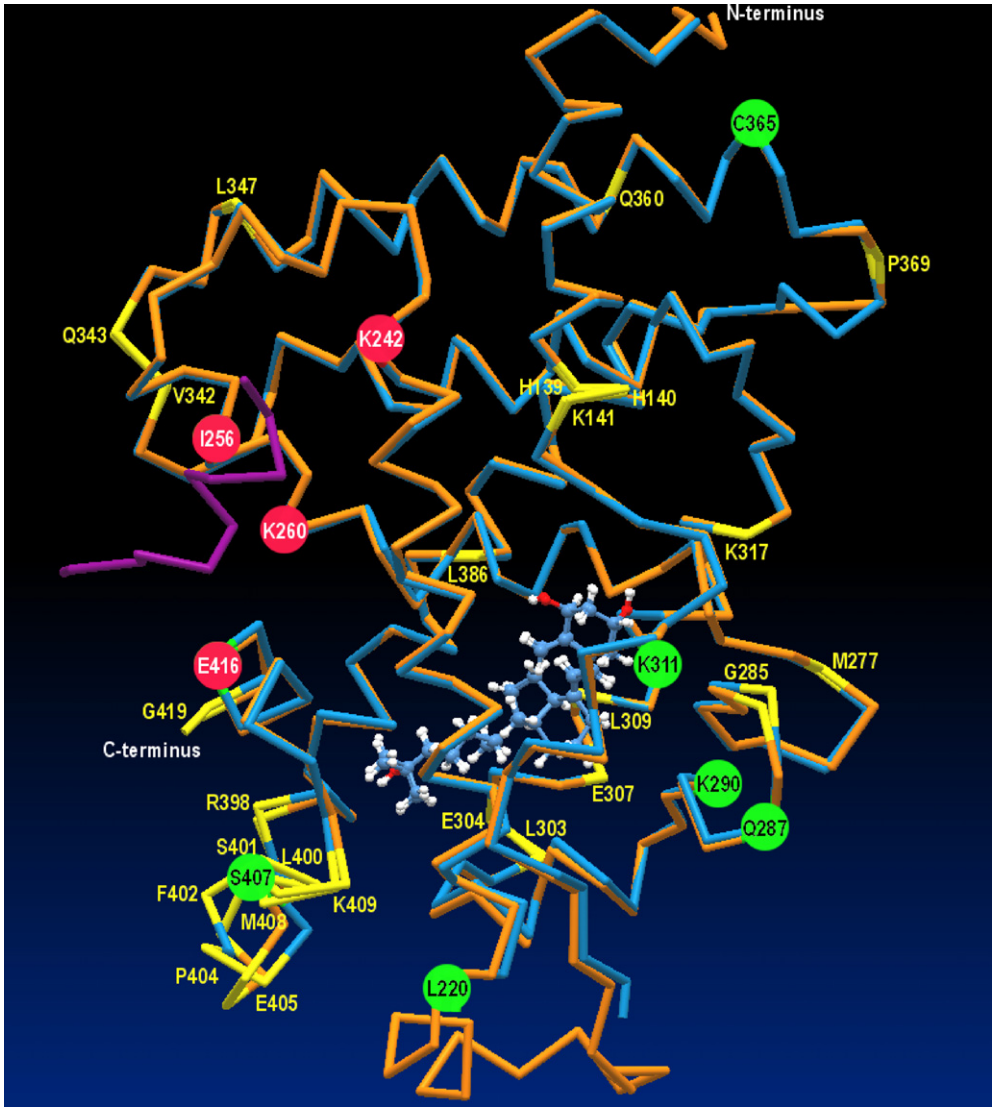

Fig. 2. Superimposition of two VDR complexes: hormone-hVDRmt (1DB1 in orange) and hormone-rVDRmt-peptide mimicking co-activator (1RK3 in light blue) reveals that 35 amino acids (marked as circle or ordinary labeled) have different orientation: H139, H140, K141, L220, K242, I256, K260, M277, G285, Q287, K290, L303, E304, E307, L309, K311, K317, V342, Q343, L347, Q360, C365, P369, L386, R398, L400, S401, F402, P404, E405, S407, M408, K409, E416, G419. Four of these 35 amino acids (red circle) being contacted by the peptide (K242, I256, K260, E416) are essential for biological action. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of the article.)

orientation of their side chains under the influence of the peptide (Table 2 and Fig. 2). Only four of these residues (K242, I256, K260, E416) actually contact the peptide, but all of them are essential for biological activity [9]. They create a salt bridge, "charge clamp" or are indispensable for interactions with co-activators. Further analysis of 1RK3 and 1DB1 complexes revealed that only two (L309 and L400) of the discussed amino acids contact the hormone. Interestingly, leucine 400 in the 1RK3 complex shifts closer (0.7 Å) to the vitamin D 26-methyl group. Drawing on studies of the nuclear receptor family it could be expected that 15 amino acids: Y143, I234, I238, K242, S252, Q255, I256, L259, K260, L413, V414, L415, E416, V417 and F418 interact with co-activators (Fig. 3). Seven of above residues (I238, S252, I256, L413, L415, E416, V417) exhibit significant differences in solvent accessibility when the co-activator peptide is absent (1DB1). Calculations (DSSP program) of solvent accessibility of the VDR complexes revealed that 10 receptor residues (L220, I256, Q287, K290, K311, C365, S401, S407, E416, G419) significantly change both their side chain orientation and solvent accessibility (Table 3). Two of them contact the co-activator peptide (I256 and E416) and two occupy the protein active site (Q287, C365). Using the DeepView program [10] we found that lysine 290 constitutes the receptor's KYD motif. This sequence is characteristic for SMRT co-repressor structure [11]. Inspection of 1DB1 and 1RK3 complexes strongly suggests that the CoA peptide attached to the vitamin D receptor changes the conformation and solvent accessibility of amino acids of great biological importance. Further comparison of VDR complexes (1DB1-1TXI,

Table 3
VDR residues significantly changing their side chain orientations and their solvent accessibility (in Å^2) in the presence of the peptide mimicking a co-activator

Amino acid[a]	Local RMSD (Å) for compared residues[b]	Solvent VDR accessibility of residues[c]	
		in 1DB1	in 1RK3
L220	1.97	3	45
I256	1.93	93	7
Q287	1.41	115	167
K290	1.73	117	33
K311	2.19	162	127
C365	1.75	31	56
S401	1.86	60	5
S407	2.73	59	16
E416	1.30	90	25
G419	2.80	84	20

[a] Amino acids are renumbered according to the rat VDR sequence.
[b] Positions of residues in crystals of 1DB1 and 1RK3 were compared by the CCOMP program; the all-atom RMSD is 1.013 Å.
[c] Solvent accessibility (in Å^2) calculated by the DSSP program; the total accessible surface for VDR from the complexes 1DB1 and 1RK3 is equal to 12,319 Å^2 and 12,148 Å^2, respectively.

1RK3-1RKH), having as ligands vitamin D analogs exhibiting similar biological activity, showed that only 12 residues have altered side chain orientation. In contrast, superimposition of two ternary VDR–peptide complexes, possessing ligands with dramatically different calcemic [4,5] functions (2MD and 2MbisP in 1RJK and 1RKG crystals, respectively), revealed 19 amino acids with changed side chain orientations (Table 2). It should be mentioned that the 2MD ligand contacts 29 receptor residues while 2MbisP contacts only 22. Lack of contacts between the latter compound and amino acids: L223, A227, Y397, L400, L410, V414 and F418 could be responsible for calcemic inactivity of this vitamin. It is worth noting that asparagine 420 from helix 12 (which closes the binding cavity) bridges two residua: K260 and E416 only in the complex with the inactive ligand (1RKG). This results in the disturbance of the main electrostatic contact stabilizing the VDR structure. There is no doubt that interactions between the vitamin D compound and the protein residues modulate biological activity of the liganded vitamin D receptor.

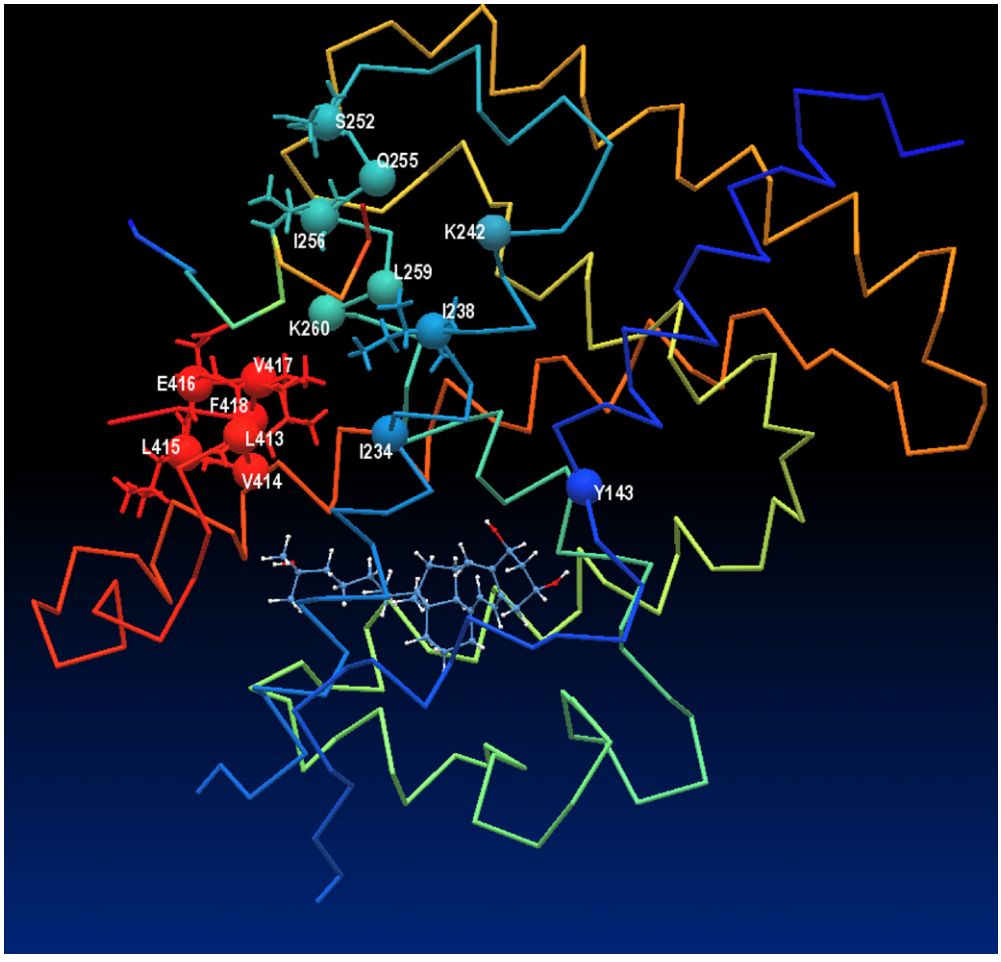

Fig. 3. View of the three-dimensional structure of 1RK3 complex. The 15 amino acids (Y143, I234, I238, K242, S252, Q255, I256, L259, K260, L413, V414, L415, E416, V417, F418) known as interacting in NR family with co-activators are labeled. Seven (I238, S252, I256, L413, L415, E416, V417) of above 15 amino acids exhibit significant differences in solvent accessibility when peptide mimicking co-activator is removed from 1RK3. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of the article.)

4. Conclusion

Developed by us the CCOMP program is useful for identifying amino acids, which are differently oriented in protein multicomplexes containing various ligands. Applying the CCOMP analysis to vitamin D receptor allowed us to establish the changes in the architecture of the VDR active sites, emerging upon binding of co-activator and/or different vitamin D analogs.

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