# SHORT COMMUNICATION

# NMR Assignments of Tryptophan Residue in Apo and Holo LBD-rVDR

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***ABSTRACT*** **Binding sites in the full-length, ligand-binding domain of rat vitamin D receptor (LBD-rVDR) for an active hormone derived from vitamin D (1α,25-dihydroxyvitamin $D_3$) and three of its C-2 substituted analogs were compared by nuclear magnetic resonance (NMR) spectroscopy. Specific residue labeled with [UL]-$^{15}N_2$ Trp allowed assignment of the side-chain $H^{\epsilon 1}$ and $N^{\epsilon 1}$ resonances of the single tryptophan residue at position 282 in LBD-rVDR. Comparison of $^1H[^{15}N]$ Heteronuclear Single Quantum Correlation (HSQC) spectra of apo and holo LBD-rVDR revealed that the position of the Trp282 $H^{\epsilon 1}$ and $N^{\epsilon 1}$ signals are sensitive to the presence of the ligand in the receptor cavity. Binding of the ligands to LBD-rVDR results in a shift of both Trp $H^{\epsilon 1}$ and $N^{\epsilon 1}$ resonances to lower frequencies. The results indicate that the interaction between the ligands and Trp282 is not responsible for differences in calcemic activity observed in vitamin D analogs. Proteins 2005;61:461–467.**


**Key words: steroid hormone receptors; nuclear receptors; protein–ligand interactions; transcription factors; orphan receptors**

## INTRODUCTION

Calcitriol and its analogs constitute a versatile system, responsible for calcium and phosphorous homeostasis, regulation of apoptosis and immune system, as well as cell proliferation and differentiation.[1–4] Such diverse biological activity of vitamin D receptor (VDR) ligands is mediated through their ability to change the receptor structure[5–8] and, as a result, its interactions with comodulators.[9] Over a thousand analogs of the vitamin D hormone have been synthesized to date, and their therapeutic value has been evaluated.[1,3] Most modifications have been made to the side chain of 1α,25-dihydroxyvitamin $D_3$ (1α,25-$(OH)_2D_3$), some resulting in drastic changes in activity.[10] It is well known that epimerization at position 20 (20-epi) elevates the transcriptional and cell differentiation potency of analogs,[3] while truncation of the side chain to small alkyl substituents decreases or even completely erases calcemic activity (unpublished data).[11] A-ring modifications that markedly and selectively change the biological potency of vitamin D analogs involve substitution at the C-2 position and removal of the exomethylene group at C-10.[12-13] The introduction of a 2-methylene group into 19-nor-1α,25-$(OH)_2D_3$, combined with epimerization at C-20, preferentially elevates bone calcium mobilization (BCM) activity of the resulting analog, named 2MD.[8,14] It has been recently established that the presence of a 2α-(hydroxypropyl) substituent in calcitriol increases bone calcium mobilization activity 500 times,[15] while the introduction of a 2α-(hydroxymethyl) group in the 19-nor-(20*S*) analog impairs both calcemic functions.[12] Although it is evident that the C-2 position plays a key role in vitamin D analogs, further studies are required to explain why modifications at this particular position have such a significant effect on biological activity.

It was anticipated that knowledge of the three-dimensional crystal structure of VDR/vitamin D complexes would provide indispensable information for our understanding of the structure–function relationship (SFR) of the ligands. Recently, several complexes of human and rat VDR mutants with vitamin D analogs, possessing various side-chain and A-ring modifications, have been successfully crystallized.[16–18] Unexpectedly, it was determined that both the overall structure of VDR and the position of amino-acid residues lining the binding pocket are identical in all analyzed complexes. Even though vitamin D compounds are flexible molecules,[19] all ligands bind to the receptor as s-*trans* rotamers in conformations characteristic for 1α,25-$(OH)_2D_3$ in its crystalline state.[20] The first apparent difference between the crystallized complexes appears in the localization of their flexible side chains. In order to accommodate the receptor pocket, the side chains of analogs with unmodified A-rings change their conformation in a fashion that allows for the preservation of all

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anchoring hydrogen bonds and similar positioning of the A, B-seco, C, and D rings. By contrast, when the superpotent 2MD analog (with an A-ring modification at C-2) occupies the VDR cavity, its C and D rings are notably tilted in comparison to the natural ligand.[18] The fact that in the crystal holo-VDR maintains the same conformation in complexes with analogs that differ markedly in their biological activities potentially indicates that crystal packing forces stabilize a structure that is not characteristic for the active receptor *in vivo*. Further evidence supporting the above hypothesis comes from limited protease digestion studies[21] and two-dimensional alanine-scanning mutational analysis.[7]

Although crystal phase analysis has failed to uncover the SFR of vitamin D analogs, it is possible that disparities between the binding sites of holo-VDR will be observable in aqueous solution, a milieu closer to physiological conditions. In this work, we use nuclear magnetic resonance (NMR) spectroscopy to examine the binding site of vitamin D analogs with respect to the single tryptophan residue of full-length rat VDR.

A natural mutation of tryptophan to arginine leads to severe hereditary vitamin D-resistant rickets (HVDRR).[22] With the tryptophan residue having such important biological functions, we might expect an involvement in ligand binding. Trp282 lines the binding pocket of VDR.[23-24] Studies of VDR mutations[5,7,25–27] have corroborated the importance of Trp282 for anchoring the ligand in the binding pocket and for transactivation of 1α,25-$(OH)_2D_3$ target genes. Most relevant from the perspective of this study, in the crystal structures,[16–18] the indole group of tryptophan is always stacked with the vitamin D C(6)-C(7)═C(8) moiety. A question arises whether this regularity also occurs in aqueous solution. Results obtained from molecular modeling suggest that it might not be so. It was observed in the docking of the C-2-modified vitamins to full-length LBD-rVDR that contact sites between Trp and ligands of varied calcemic potency were not conserved.[28] Although the modeled binding of analogs resembled that of the natural ligand with the aromatic rings of tryptophan oriented parallel to the intercyclic 5,7-diene system of the ligands, the tryptophan residue was shifted along the seco-B ring.

In the work described here, we employed the $^{15}$N-NMR signals from the indole moiety of VDR Trp282 to study the binding of various analogs of 1α,25-$(OH)_2D_3$ to VDR. With these results at hand, we will be able to address the question of whether this unique amino acid conserves its orientation with respect to the anchored ligands under physiological conditions.

## MATERIALS AND METHODS

### Protein Production

*Escherichia coli* BL21(DE3)/NK7420 cells auxotrophic for *L*-tryptophan (gift from Dr. Brian Fox, University of Wisconsin-Madison) were prepared according to the described procedure.[29] For expression of the VDR, the plasmid p29lbdwt (derivative of pET-29b, Novagen, Madison, WI, USA), conferring kanamycin resistance to the cells and coded for the production of wild-type LBD-rVDR (116–423) with a C-terminal hexahistidine tag, was introduced into competent cells using heat shock procedure (42°C for 45 s). The obtained transfected *E. coli* cells were then grown at 37°C in 5 L of M9 medium (pH 7.0) supplemented with [UL]-$^{15}N_2$ Trp and unlabeled L-amino acid mixture, trace metal solution, $Mg^{+2}$, $Ca^{+2}$, $Fe^{+2}$ ions, 0.4% glucose, vitamin mix:thiamine, nicotinic acid and biotin, nucleic acids mix:guanosine, thymine, uracil, cytosine and 50 mg/L kanamycin. When the optical density of 600 nm reached 0.8 AU, the bacterial culture was cooled to 22°C, and LBD-rVDR expression was induced with 0.4 m*M* isopropylthio-β-galactoside (IPTG). After 6 h, cells were harvested by centrifugation and frozen in liquid nitrogen. The typical yield of bacteria obtained from a 5 L culture was 55 g. For selective incorporation of $^{15}N_2$-labeled tryptophan, M9 minimal medium containing a mixture of unlabeled L-amino acids and 50 mg/L of isotopically enriched Trp was prepared according to the procedure described by Cheng.[30] To check the expression system, an aliquot of cells was lysed with lysozyme (in 50 m*M* Tris buffer, pH 8.0, containing 25% sucrose (w/v), 2 m*M* EDTA, 5 m*M* $NaN_3$) and then treated with DNAse; soluble LBD-rVDR appeared as a major band on a sodium-dodecylsulfate-polyacrylamide gel in SDS-PAGE electrophoresis of cell lysate. In large-scale experiments, following sonication in 50 m*M* Tris buffer, pH 8.0, containing 10 m*M* imidazole, 0.5*M* NaCl, 0.02% $NaN_3$, 10 m*M* 2-mercaptoethanol (MCE), 1 m*M* phenylmethylsulfonyl fluoride (PMSF), with traces of pepstatin A, the expressed protein was purified on metal affinity superflow resin with nitriolotriacetic acid (Ni-NTA) and cation exchange (Sepharose) columns as described by Vanhooke.[18] The average yield of the protein (purity above 95% according to SDS-PAGE chromatography) was 50 mg from 5 L of bacterial culture. The activity of Trp ($^{15}N_2$)-labeled LBD-rVDR was verified by comparison of 1α,25-$(OH)_2D_3$ affinity to the expressed protein and to the porcine intestinal VDR.[31]

### NMR Samples of LBD-rVDR

The solution conditions used in the NMR experiments of apo- and holo-LBD-rVDR were 35 m*M* phosphate buffer (pH 7.5, containing 100 m*M* NaCl, 1 m*M* MCE, 0.015% $NaN_3$) supplemented with β-octyl glucoside (20 m*M*) and ammonium sulfate (130 m*M* for apo- and 320 m*M* for holo-LBD-rVDR samples). Electrophoresis on native gels showed that LBD-rVDR exists mainly in the monomeric state under such conditions. Continuous electrophoresis on nondenaturating polyacrylamide gels (native gels) was performed according to the published procedure.[32] Typically, a 15-mA current was applied to a polyacrylamide gel (layer thickness 0.75 mm, ammonium persulfate solution [APS]:Temed = 10). For the holo-LBD-rVDR samples, vitamin D hormone 1α,25-$(OH)_2D_3$ and its three 19-nor analogs were used as the ligands. Two analogs (compounds 2 and 4 in Fig. 1) were synthesized according to the published procedures,[12] while 1α,25-$(OH)_2D_3$ and (20*S*)-2-methylene-19-nor-1α-(OH)-bishomopregnacalciferol were purchased from Tetrionics (Madison, WI). To prepare the

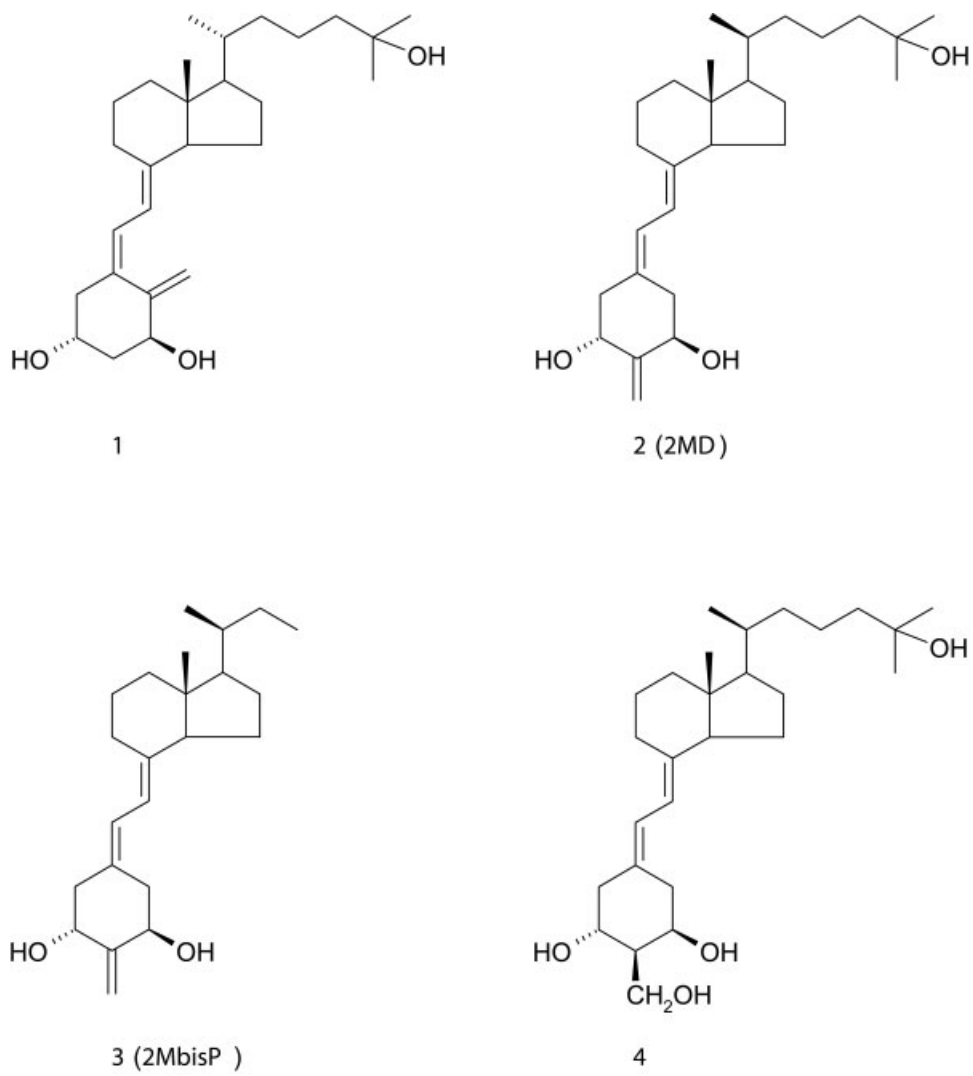


Fig. 1. Chemical structure of (**1**) 1α,25-dihydroxyvitamin $D_3$ and its 20-epi analogues: (**2**) (20*S*)-2-methylene-19-nor-1α,25-$(OH)_2D_3$, (**3**) (20*S*)-2-methylene-19-nor-1α-(OH)-bishomopregnacalciferol, and (**4**) (20*S*)-2α-(hydroxymethyl)-19-nor-1α,25-$(OH)_2D_3$.

LBD-rVDR/ligand complexes, a solution of the LBD-rVDR in phosphate buffer was slowly added to a solution of the analog in 2 μL of ethanol (1.3 molar ratio of vitamin to LBD-rVDR). The mixture was then incubated for 2 h at 0°C. LBD-rVDR complexes with peptide KNHPMLMN-LLKDN were prepared according to the procedure published by Vanhooke.[18]

### NMR Experiments

Samples used for NMR spectroscopy were dissolved in phosphate buffer and concentrated to 0.15 m*M*. Ten percent $^2H_2O$ was used as the lock. $^1H\{^{15}N\}$ Heteronuclear Single Quantum Correlation (HSQC)[33] spectra of the [UL]-$^{15}N_2$ Trp VDR were recorded on a Varian Inova 800 MHz and a Bruker Biospin 600 MHz spectrometer. Both spectrometers were equipped with 5 mm triple-resonance cryogenic probes. The temperature for all experiments was 10°C. The chemical shift reference for $^1H$ was internal sodium-3-(trimethylsilyl)-1-propane sulfonate (DSS). The $^{15}N$ shifts were determined indirectly from the absolute frequency of DSS[34] (Table I).

Knowledge of the chemical shifts of the tryptophan indole moiety allowed us to reassign the downfield NH signal, observed previously in a $^1H$-$^{15}N$ correlation spectrum of the free VDR and its complex with 1α,25-$(OH)_2D_3$. The proton signal at 11.77 ppm, found in apo-LBD-rVDR,[26] probably arises from a histidine residue. This signal is shifted to 12.26 ppm upon hormone binding to the receptor, and the observed deshielding is characteristic of the histidine $NH_{sc}$ group's involvement in hydrogen bonding.

## RESULTS AND DISCUSSION

LBD-rVDR (37.4 kDa) is rather large for the use of NMR techniques.[35] The limiting factors for structural studies of molecules with sizes above 25 kDa are sensitivity, overlap,

**TABLE I. Chemical Shifts of Tryptophan Indole Moiety (in ppm)[a] in Apo- and Holo-LBD-rVDR**

| Complex | $^1H^{\epsilon 1}$ | $^{15}N^{\epsilon 1}$ |
|---|---|---|
| apo LBD-rVDR[b] | 10.11 | 129.5 |
| apo LBD-rVDR[c] | 10.09 | 129.8 |
| apo LBD-rVDR/peptide[b,d] | 10.11 | 129.8 |
| 1α,25-$(OH)_2D_3$/LBD-rVDR | 8.91 | 125.6 |
| 1α,25-$(OH)_2D_3$/LBD-rVDR/peptide[c,d] | 9.09 | 125.9 |
| 2MD/LBD-rVDR | 9.00 | 125.9 |
| 2MbisP/LBD-rVDR | 9.17 | 125.8 |
| 2α-(hydroxymethyl)-19-nor-(20S)-1α,25-$(OH)_2D_3$/LBD-rVDR | 9.17 | 125.6 |

[a]Spectra were recorded at +10°C with DSS [sodium 3-(trimethylsilyl)-1-propane sulfonate (15 μ*M*)] as the internal proton chemical shift standard. $^{15}N$ chemical shifts were indirectly referenced to liquid $NH_3$ according to ref.[34] The line broadening of proton and nitrogen signals was about 0.1 and 0.6 ppm respectively. Concentrations of all protein samples were 0.15 m***M;*** the phosphate buffer we used is described in detail in the experimental section.
[b]Concentration of $(NH_4)_2SO_4$ amounted 130 m***M.***
[c]Concentration of $(NH_4)_2SO_4$ amounted 320 m***M.***
[d]The peptide KNHPMLMNLLKDN (FW 1567), used as a cofactor in crystallization of rVDRmt [116–423, Δ (165–211)], was added (in 1.5 molar excess) to LBD-rVDR.

and line widths. To reduce these problems, we selectively labeled LBD-rVDR with [UL]-$^{15}N_2$ Trp.[36] LBD-rVDR has a single tryptophan residue at position 282, and the chemical shifts of the $H^{\epsilon 1}$ and $N^{\epsilon 1}$ are usually shifted away from the signals arising from the backbone amide nitrogens (Fig. 2).

The main contributions to line broadening come from rapid transverse spin relaxation and intermolecular exchange between LBD-rVDR multimeric forms. In order to alleviate this problem, it was essential to find a solvent in which the VDR exists predominantly as a monomer. After testing several candidate solvents, we found that phosphate buffer is most suitable for this purpose. We further determined that when the $NaH_2PO_4$ buffer is supplemented with sodium chloride, β-octyl glucoside (BOG) and ammonium sulfate, holo-LBD-rVDR exists exclusively in the monomeric state, while the monomer/multimer equilibrium for the ligand-free receptor is shifted largely to the monomeric (80%) form.

The NMR literature shows that double carbon–carbon bonds, due to the magnetic-susceptibility anisotropy of their π system, cause significant shielding of the nuclei lying above and below the C═C plane.[37] In the crystal structure of VDR,[16–18] Trp282 is oriented parallel to the intercyclic 5,7-diene of the vitamin D moiety; the chemical shifts of Trp $H^{\epsilon 1}$ and $N^{\epsilon 1}$ nuclei should be sensitive to the detailed position of the ligand with respect to Trp282. This, combined with the fact that tryptophan appears in the VDR sequence only once, motivated us to choose this amino acid as a convenient probe for evaluating binding sites in holo-LBD-rVDR. The natural vitamin D hormone 1α,25-$(OH)_2D_3$ (Fig.1, structure 1) and its three analogs (Fig. 1, structures 2–4) show marked difference in bone calcium mobilization and intestinal calcium transport (Table II). With large differences in activities, it was proposed that these ligands may show differences in binding geometry and were thus chosen as the ligands in this study. Specific labeling of the VDR with tryptophan ($^{15}N_2$) greatly simplified its two-dimensional NMR spectra; only the proton–nitrogen correlation arising from the indole moiety was resolved in the $^1H$-$^{15}N$ correlation experiment. This allowed for unequivocal assignment of $H^{\epsilon 1}$ and $N^{\epsilon 1}$ chemical shifts in the tryptophan indole fragment (Table I, Fig. 2) of LBD-rVDR. The expected signal from the backbone amide group of Trp282 was not resolved from the background signals that arise from the natural abundance $^{15}N$ signals of the backbone amides in LBD-rVDR. To retain a monomeric state, different concentrations of ammonium sulfate are required for apo- and holo-LBD-rVDR. Salt concentration influences electrostatic and hydrophobic interactions as well as the solubility of organic compounds and their hydrogen-bonding capability.[38-39] To exclude the possibility that different salt concentrations might influence the chemical shifts of the Trp282 of LBD-rVDR, $^1H\{^{15}N\}$ HSQC spectra of the ligand-free receptor were collected at both concentrations of ammonium sulfate. The chemical shifts of Trp $H^{\epsilon 1}$ and Trp $N^{\epsilon 1}$ in ligand-free VDR were identical in 130 m*M* and 320 m*M* ammonium sulfate.

The peptide KNHPMLMNLLKDN was used as a cofactor in the crystallization of LBD-rVDR [116–423, Δ (165–211)].[18] The crystallographic structure shows that the synthetic peptide binds to VDR too far from the ligand-binding pocket to influence the Trp–vitamin D binding region. According to our expectations, the NMR spectrum of the complex of the peptide with LBD-rVDR shows no changes in the chemical shift of the Trp282 resonances. Analysis of the data in Table I clearly reveals that the chemical shifts of the tryptophan indole signals are sensitive to the presence of the ligand in the binding pocket. The binding of the vitamin D analogs to the receptor causes shielding of both $H^{\epsilon 1}$ and $N^{\epsilon 1}$ nuclei. This shift apparently arises from the shielding effect of the 5,7-diene system of vitamin D on the nuclei in the aromatic ring of tryptophan.[37] When the NH moiety in the tryptophan side chain forms hydrogen bonds to solvent, the nitrogen nucleus of indole ring is significantly deshielded (up to 14 ppm).[40-41] The evidence presented here suggests that the single tryptophan molecule in VDR is the contact site for binding to vitamin D analogs.

In the crystal structure, the intercyclic 5,7-diene of the vitamin D analogs is located in the same position with respect to the tryptophan indole moiety. Nitrogen shielding is influenced by a number of factors, including solvent effects and interactions with proximal amino acids. These effects can produce secondary $^{15}N$ chemical shifts of up to 20 ppm.[42] Any subtle differences in the position of the ligand with respect to the indole moiety would be expected to be observable by NMR spectroscopy. However, the experimental data show that the chemical shifts for the $H^{\epsilon 1}$ and $N^{\epsilon 1}$ nuclei are virtually identical in all the vitamin D/LBD-rVDR complexes (9.0 ± 0.2 ppm for $H^{\epsilon 1}$ and 125.7 ± 0.2 ppm for $N^{\epsilon 1}$). The sensitivity of the $^{15}N$ chemical shift to its environment and the constancy of the shifts in all of the complexes studied here suggest that the

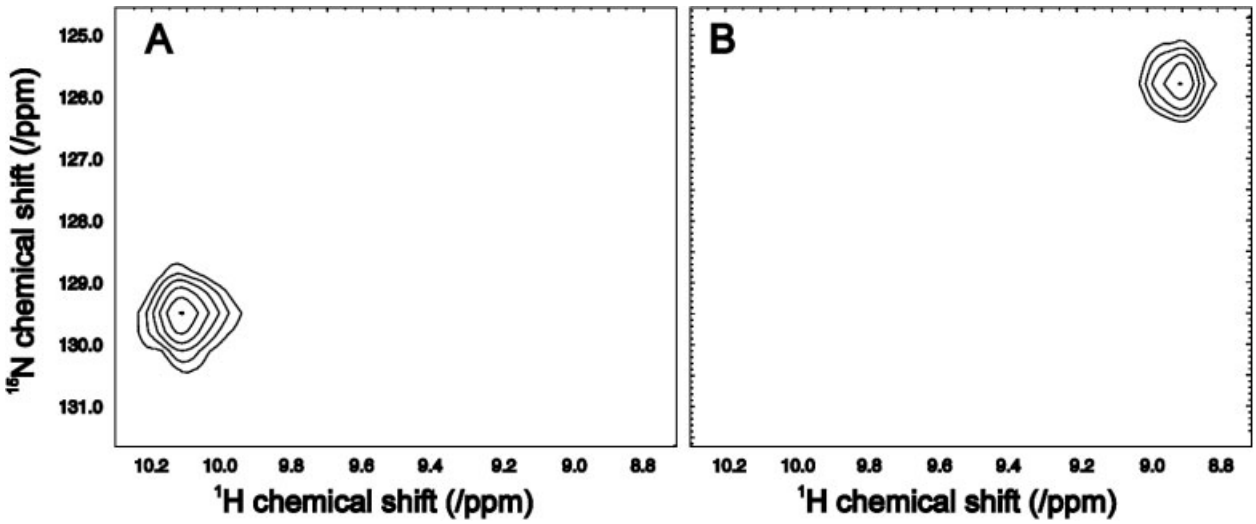


Fig. 2. 800 MHz $^{1}H\{^{15}N\}$HSQC NMR spectra of (**A**) apo-LBD-rVDR and (**B**) LBD-rVDR complexed with 1α,25-$(OH)_2D_3$.

**TABLE II. Literature Data[a] for Biological Activities of Analogs**

| Compound (number) | VDR binding ratio[b] | Amount (pmol) | Ca transport S/M (ICA) | Serum Ca (BCM) |
|---|---|---|---|---|
| None (control) | | 0 | 5.5 ± 0.2 | 5.1 ± 0.2 |
| 1α,25-$(OH)_2$ $D_3$ (Fig. 1, compound 1) | 1.0 | 260 | 6.2 ± 0.4 | 7.2 ± 0.5 |
| 2MD (Fig. 1, compound 2) | 1.3 | 260 | 4.6 ± 0.7 | 14.4 ± 0.6 |
| None (control) | | 0 | - | 9.3 ± 0.4 |
| 1α,25-$(OH)_2$ $D_3$ (Fig. 1, compound 1)[c] | 1.0 | 0.2 | - | 10.6 ± 0.1 |
| 2MbisP (Fig. 1, compound 3) | 8.6 | 70 | - | 9.5 ± 0.1 |
| None (control) | | 0 | 4.0 ± 0.3 | 3.8 ± 0.1 |
| 1α,25-$(OH)_2$ $D_3$ (Fig. 1, compound 1) | 1.0 | 260 | 6.6 ± 0.5 | 5.2 ± 0.1 |
| Compound (Fig. 1, compound 4) | 1.3 | 260 | 5.8 ± 0.4 | 3.9 ± 0.1 |

[a]Literature data are taken from ref. 12 (Fig. 1, compounds 2 and 4) and from unpublished results (Fig. 1, compound 3).
[b]Competitive binding of 1α,25-$(OH)_2$ $D_3$ (Fig. 1, compound 1) and the vitamin D analogs to the porcine intestinal VDR. Binding ratio is the ratio of the analog average $ED_{50}$ to the $ED_{50}$ for 1α,25-$(OH)_2$ $D_3$. The $ED_{50}$ values are derived from dose- response curves and represent the analog concentration required for 50% displacement of the radiolabeled 1α,25-$(OH)_2$ $D_3$ from the receptor protein.
[c]Dose level in μg/kg

interaction of all complexes between the vitamin D analogs and LBD-rVDR are identical within the resolution of the NMR experiment.

Data obtained from X-ray crystallography, NMR spectroscopy, and mutational studies suggest that the binding site on the receptor for the vitamin D analogs is preserved. Therefore, the interactions between the vitamin D 5,7-diene fragment and the tryptophan aromatic ring cannot account for differences in calcemic activity of vitamin D analogs. It is commonly accepted that the structures of ternary complexes of VDR, retinoid X receptor and vitamin D response element (VDR-RXR-VDRE) vary with different vitamin D agonists and antagonists, which then influences the ability of VDR to stabilize the active receptor conformation and association with comodulators.[5-6] In the nuclear receptor (NR) family, helix H12 and the hydrophobic clefts from helices H11[43] and H10[44] were identified as interacting with coactivators.[45] It is interesting that coactivators contact nuclear receptors through helical hydrophobic motifs containing LXXLL,[45] FFFKLI,[43] and LIM[46] signatures. This piece of evidence strongly suggests the involvement of hydrophobic interactions in the VDR functions. During our recent studies on docking of vitamin D analogs with differentiated calcemic activities into LBD-rVDR, we found two hydrophobic patches situated on the surface that are responsible for specific interactions with the 18- and 21-methyl groups of the ligand.[28] Thus, it is possible that specific hydrophobic interactions between the VDR and the methyl groups of vitamin D analogs may play a role in calcemic activities of the vitamin D hormone. The biological importance of the vitamin 18-methyl group/VDR contacts was recently suggested by Mourino.[47] He showed that the introduction of different substituents at C-12, which is close to the 18-$CH_3$ group, dramatically changes the affinity of such hormone analogs for the VDR. However, deletion of the 18-$CH_3$ did not eliminate but did reduce calcemic activity of 1,25-$(OH)_2D_3$.[48] Hydrophobic contacts of the side-chain terminal methyl groups (at C-26,27) also seem to play an important role because additional carbons at these positions increase biological activity.[49]

These data and the results of our NMR studies suggest that contacts between VDR and the central 5,7-diene part (seco-B ring) of vitamin D ligands are not responsible for calcium homeostasis. It appears that contacts of LBD-rVDR with ring A of vitamin D and its hydrophobic methyl groups may be responsible for the calcemic functions of vitamin D analogs.

## CONCLUSIONS

NMR studies of large molecules are compromised by low sensitivity and line broadening. By establishing buffer conditions under which the VDR exists in monomeric form, we were able to successfully collect spectra of LBD-rVDR both in its free form and in complexes with vitamin D analogs. Specific labeling of the VDR with [UL] $^{15}N_2$ Trp enabled us to verify contact sites between analogs of the vitamin D hormone and the $^{15}$N-enriched Trp282 residue in solution. The chemical shifts of Trp $H^{\epsilon 1}$ and Trp $N^{\epsilon 1}$ were found to be sensitive to the binding of ligand molecules in the LBD-rVDR binding cavity. The observed shielding of the indole resonances upon complexation with ligand suggests that the intercyclic 5,7- vitamin D diene moiety of the ligand binds close to Trp282. Even though the analyzed ligands differ markedly in their biological potency, Trp $H^{\epsilon 1}$ and Trp $N^{\epsilon 1}$ chemical shifts in the complexes are virtually identical. This suggests that ligand–tryptophan contacts are not responsible for variations in the calcemic activities of vitamin D analogs. It appears that the indole ring of the tryptophan residue acts as a common binding site for the intercyclic 5,7 diene moiety of the vitamin analogs in the LBD-rVDR binding pocket for the ligands.

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