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ON THE SIDE CHAIN CONFORMATION OF 1α,25-DIHYDROXYVITAMIN D_3 RESPONSIBLE FOR BINDING TO THE RECEPTOR

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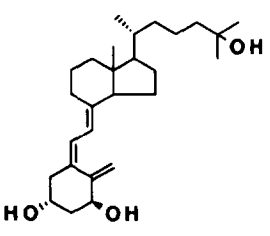
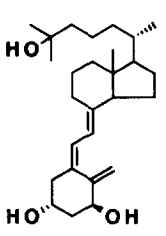
Abstract: Side chain mobility of $1,25(OH)_2D_3$ (**1**) and its 20-epimer (**2**) was analyzed and shown in a three-dimensional dot map that indicates the two distinct spatial regions accessible by the terminal 25-hydroxyl group for each vitamin. The biological activities of two analogs (**3** and **4**) of **1**, which possess restricted side chain flexibility, were tested and the results suggest the spatial region of **1** responsible for binding to VDR and DBP.

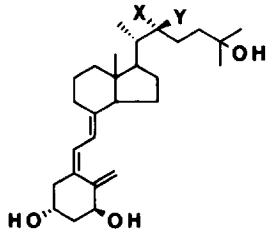
1α,25-Dihydroxyvitamin D_3 [$1,25(OH)_2D_3$,**1**] is the hormonal form of vitamin D_3 that regulates calcium metabolism,[1] cell differentiation,[2] and immune system.[3] It is believed that most of the vitamin D actions are expressed via genomic mechanism through the formation of the complex between vitamin D and its nuclear receptor (VDR).[4] The gene of VDR has been cloned and the amino acid sequence has been determined.[5] Vitamin D transporting protein in serum (vitamin D binding protein, DBP) is also shown to play an important role for vitamin D to express its activities in vivo.[6] By extensive structure-function studies on analogs of $1,25(OH)_2D_3$ (**1**), it is now clear that discrimination of the various actions of vitamin D is possible.[7] However, the mechanism by which the discrimination occurs is not clear.[7] Many of the analogs that have differential activity, are modified on the side chain. For example, 22-oxa-1α,25-dihydroxyvitamin D_3 (OCT)[7a] and Δ^{22}-26,27-cyclo-1α,24*S*-dihydroxyvitamin D_3 (MC903)[7b] have a selective activity in inducing cell differentiation, and 20-epi-analogs of $1,25(OH)_2D_3$ (**1**), such as 20-epi-1α,25-dihydroxyvitamin D_3 (**2**) and 20-epi-22-oxa-1α,25-dihydroxy-24,26,27-trihomovitamin D_3 (KH1060), have a remarkably strong activity in inducing cell differentiation and regulating immune response.[7c] The 20-epi-compounds are especially interesting series of analogs because they bind to VDR with approximately the same affinity as **1** in spite of the unnatural stereochemistry at C(20). These results indicate that the side chain plays an important role in discriminating various vitamin D actions. As a part of continuing studies on the conformation and function of $1,25(OH)_2D_3$ (**1**),[8] which has much flexibility in the side chain, the seco-B ring, and the A-ring part, we conducted conformational analysis of the side chain, and designed and synthesized analogs suitable for the study. We here disclose the evidence to indicate which side chain conformation of $1,25(OH)_2D_3$ (**1**) is important to bind to VDR and DBP and to express differentiating activity in HL-60 cells.



1



2



3: X=Me,Y=H
4: X=H, Y=Me

Conformational Analysis

We analyzed the side chain mobility of $1,25(OH)_2D_3$ (**1**) and its 20-epimer (**2**) by conformational systematic search. Initial structure of $1,25(OH)_2D_3$ (**1**) was constructed based on x-ray crystal structure of 25-hydroxyvitamin D_3 [$25(OH)D_3$]:[9] the 1α-hydrogen of $25(OH)D_3$ was replaced with a hydroxyl group and the resulting structure was optimized by molecular mechanics using the conjugate gradient method on Consistent Valence force field (Discover)[10] and then on Tripos force field (MAXMIN2 routine in SYBYL).[11] The initial structure of 20-epi compound (**2**) was constructed by modifying **1** with molecular modeling system (Insight II)[10] and subsequent energy minimization to a global minimum on the force field described above. Conformational analysis was performed by the systematic search using a search routine of SYBYL.[11] Each of the 17,20-, 20,22-, 22,23-, 23,24- and 24,25-bonds in the initial structures of **1** and **2** was rotated 360° with 30° step to yield a total of $(360/30)^5$=248832 conformers for each compound. From these, infeasible conformers with intramolecular van der Waals repulsion were eliminated by applying van der Waals bump coefficient of 0.95. The most stable structures of the two vitamins (**1**, black and **2**, green) and these possible side chain conformers are shown in Figure 1 as a stereoview. In the Figure the most stable conformers are shown in the wire and are superimposed at the skeletal part. The side chain conformers generated as above are shown with dots at the position of their 25-oxygen. Thus the dot map shows not only the spatial regions to which the 25-hydroxyl group of each vitamin can reach but also the position of the VDR cavity where the side chain fits in. The hydroxyl groups of $1,25(OH)_2D_3$ (**1**) play an important role in binding to VDR since an absence of either 1α-, or 25-hydroxyl group reduces the VDR binding activity several hundred times. Okamura's group has reported a dot map for models of **1** and its analogs to study the structure-activity relationships.[12]

Inspecting the dot map with the aid of a stereoscope we notice that both $1,25(OH)_2D_3$ (**1**) and 20-epi-$1,25(OH)_2D_3$ (**2**) have two major densely populated regions. We defined these spatial regions as **A** and **G** for **1** and **EA** and **EG** for **2**. The terms **A** and **G** refer to anti and gauche, and **E** to epi. Thus **A** and **G** are the regions where the side chain of $1,25(OH)_2D_3$ (**1**) occupies when its dihedral angle at C(17,20,22,23) is anti and gauche(+), respectively, while **EA** and **EG** are those regions for 20-epi-$1,25(OH)_2D_3$ (**2**) when the same dihedral angle is anti and gauche(-), respectively. It should be noted that the regions occupied by the side chains of $1,25(OH)_2D_3$ (**1**) and its 20-epimer (**2**) do not overlap with each other: **A** and **G** regions do not overlap with **EA** and **EG** regions. These results point out that if the skeletal part of the vitamins (**1** and **2**) occupies the same portion of VDR, the side chain of each vitamin D must occupy different portion of the VDR.

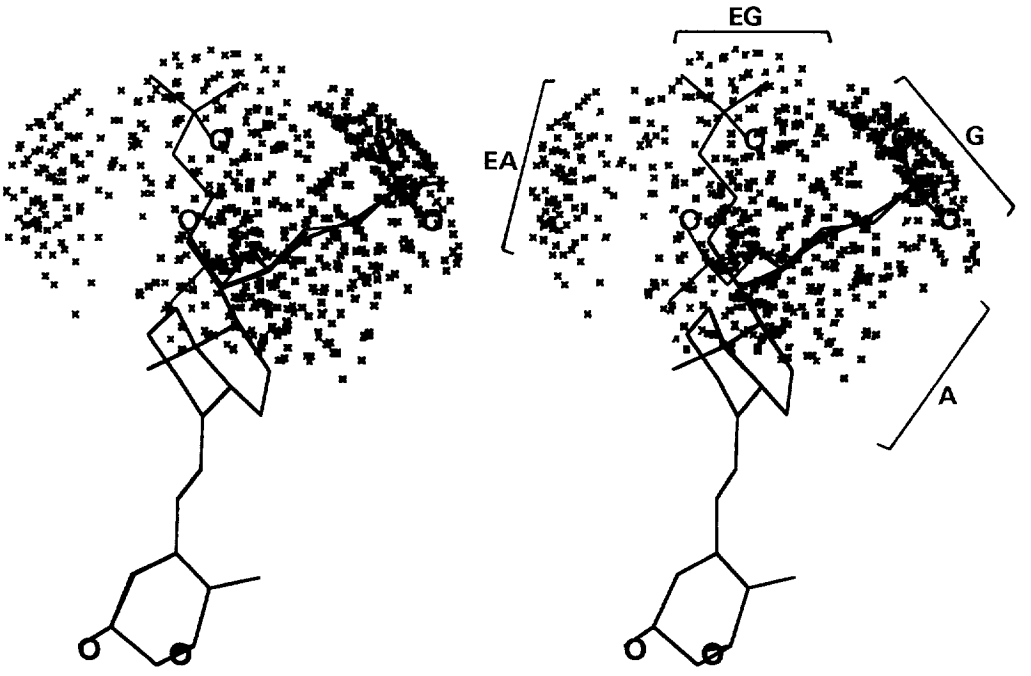


Figure 1. Stereoview of the dot map for $1,25(OH)_2D_3$ (**1**) and its analogs (**2-4**): black, $1,25(OH)_2D_3$ (**1**); green, 20-epi-$1,25(OH)_2D_3$ (**2**); red, (22*R*)-22-methyl-$1,25(OH)_2D_3$ (**3**); blue, (22*S*)-22-methyl-$1,25(OH)_2D_3$ (**4**). Wire represents the most stable conformation of each vitamin D. Dots are the regions to which the 25-oxygen of vitamin D can reach. **A** and **G** are the two distinct spatial regions occupied by the 25-hydroxyl group of **1**, and **EA** and **EG** are those regions occupied by that of **2**.

Design and Synthesis of Analogs with Restricted Side Chain Flexibility

The flexibility of the side chain can be restricted by introducing a methyl group at the 22-position. The 22-position is the key position to determine the side chain conformation since it is adjacent to the chiral center at C(20).[13] Modifications of this position have been known to cause considerable effect in the biological activity of vitamin D as the above mentioned examples show. The construction of the initial structures, the most stable conformers, of (22*R*)-22-methyl-1,25(OH)$_2$D$_3$ (**3**) and (22*S*)-22-methyl-1,25(OH)$_2$D$_3$ (**4**) and their conformational analysis were done by the similar procedure described above. When a methyl group is introduced to the *pro-R*-position, the side chain is confined to occupy only **G** region (**3**, red in Figure 1), whereas when it is introduced to the *pro-S*-position, the side chain is restricted in **A** region (**4**, blue in Figure 1). Methyl substituent is expected to cause minimum effect on the size, hydrophilicity, and hydrophobicity of the molecule when compared to the parent molecule (**1**). Thus if only one of the two 22-methylated analogs binds to VDR, the space where the active isomer occupies would be the right space where the side chain of 1,25(OH)$_2$D$_3$ (**1**) occupies when it binds to VDR. Accordingly these two 22-methyl compounds are appropriate analogs to study the side chain conformation contributing in the formation of VDR-vitamin D complex and the expression of activities. We successfully synthesized the two 22-methylated vitamin D (**3** and **4**) in a stereoselective manner as reported.[8a,14]

Biological Activity

Biological activities of (22*R*)-22-methyl-1,25(OH)$_2$D$_3$ (**3**) and (22*S*)-22-methyl-1,25(OH)$_2$D$_3$ (**4**) were evaluated in compared with 1,25(OH)$_2$D$_3$ (**1**). Binding affinity for VDR was examined by competitive binding assay using the porcine intestinal VDR and the bovine thymus VDR.[15] The two 22-methylated analogs (**3** and **4**) showed distinct activities. In the porcine VDR assay, 22*R*-epimer (**3**) was 1/60 as potent as **1**, whereas 22*S*-isomer (**4**) was 1/3 as active as **1** (Figure 2). The results with thymus VDR were similar: the activities of **3** and **4** compared to **1** were 1/50 and 1/3, respectively (Figure 3). Thus it is clear that only 22*S*-epimer (**4**) mimics the action of **1**. The results suggest that the **A** region of **1** is responsible for binding to VDR and the C(17,20,22,23) anti conformation contributes to the formation of VDR-vitamin D complex.

Activity in differentiating HL-60 cells was examined by nitro blue tetrazolium (NBT) reduction.[16] As shown in Figure 4, 22*S*-isomer (**4**) showed approximately the same potency as the native hormone (**1**), whereas that of 22*R*-isomer (**3**) was 1/80 as potent as **1**. Thus only 22*S*-methyl isomer (**4**) imitated the in vitro differentiating action of **1**. The data are consistent with the affinities of **3** and **4** for VDR.

The same trend was observed in the abilities of the two 22-methyl analogs (**3** and **4**) in binding to DBP. The binding activity was determined by competitive displacement of [^{3}H]-25(OH)D$_3$ for DBP in vitamin D-deficient rat serum[15b] and the results are shown in Figure 5. The activities of **3** and **4** compared to **1** were 1/220 and 2/3, respectively. The results suggest that the **A** region of **1** is also responsible for binding to DBP.

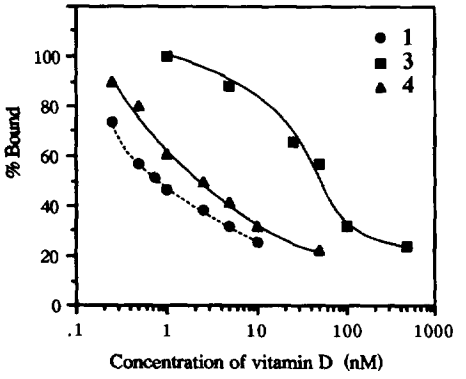


Figure 2. Binding affinity of 22-methyl analogs for specific $1,25(OH)_2D_3$ receptors in porcine intestine.

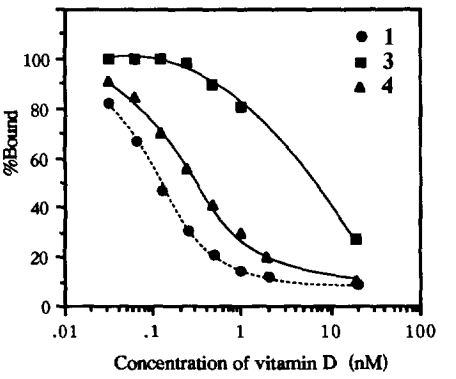


Figure 3. Binding affinity of 22-methyl analogs for specific $1,25(OH)_2D_3$ receptors in bovine thymus.

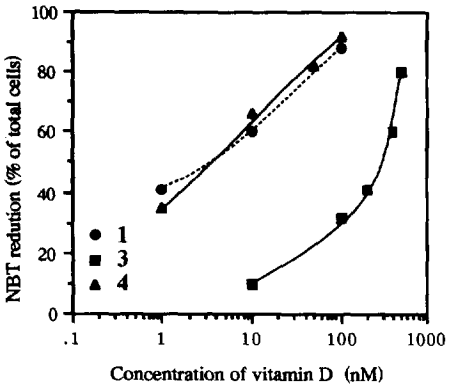


Figure 4. Activity of 22-methyl analogs in inducing NBT reducing activity in HL-60 cells.

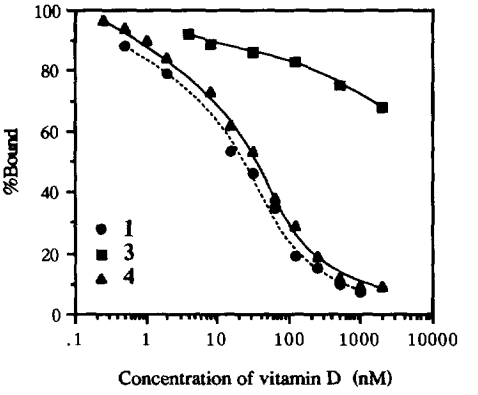


Figure 5. Binding affinity of 22-methyl analogs for rat serum DBP.

In conclusion, we presented a line of evidence to indicate that the side chain of $1,25(OH)_2D_3$ (**1**) is oriented in the A-region when it binds to either VDR or DBP. The synthesis and biological testing of the corresponding 22-methylated analogs of 20-epi-$1,25(OH)_2D_3$ (**2**) would provide further evidence to answer why 20-epi-$1,25(OH)_2D_3$ (**2**) can strongly bind to VDR, which of EG and EA regions is responsible for VDR binding, and how the remarkable differential action results.

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