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DNA Bending Is Induced by Binding of Vitamin D Receptor-Retinoid X Receptor Heterodimers to Vitamin D Response Elements

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Abstract The ability of vitamin D receptor-retinoid X receptor (VDR-RXR) heterodimers to induce a DNA bend upon binding to various vitamin D response elements (VDRE) has been investigated by circular permutation and phasing analysis. Recombinant rat VDR expressed in the baculovirus system and purified recombinant human RXRβ have been used. The VDREs were from 1,25-dihydroxyvitamin D_3 (1,25-$[OH]_2D_3$) enhanced genes (rat osteocalcin, rOC; mouse osteopontin, mOP, and rat 1,25-dihydroxyvitamin D_3-24-hydroxylase, r24-OHase), and a 1,25-$(OH)_2D_3$ repressed gene (human parathyroid hormone, hPTH). As shown by circular permutation analysis, VDR-RXR induced a distortion in DNA fragments containing various VDREs. Calculated distortion angles were similar in magnitude (57°, 56°, 61°, and 59°, respectively for rOC, mOP, r24-Ohase, and hPTH). The distortions took place with or without a 1,25-$(OH)_2D_3$ ligand. The centers of the apparent bend were found in the vicinity of the midpoint of all VDREs, except for rOC VDRE which was found 4 bp upstream. Phasing analysis was performed with DNA fragments containing mOP VDRE and revealed that VDR-RXR heterodimers induced a directed bend of 26°, not influenced by the presence of hormone. In this study we report that similar to other members of the steroid and thyroid nuclear receptor superfamily, VDR-RXR heterodimers induce DNA bending. J. Cell. Biochem. 74:220–228, 1999.

Key words: vitamin D receptor; DNA bending; VDR-RXR heterodimers; vitamin D response elements

The role of 1,25-dihydroxyvitamin D_3 (1,25-$[OH]_2D_3$) in calcium homeostasis is mediated by the vitamin D receptor (VDR). This receptor belongs to the steroid and thyroid hormone receptor (SHR) superfamily of ligand inducible transcription factors [Evans, 1988]. Members of this family have in common structural and functional domains. The DNA binding domain, highly conserved among members of the superfamily, is known to interact specifically with *cis*-acting sequences called hormone response elements (HRE), present in target genes. The VDR requires retinoid X receptors (RXR) as a partner to bind with high affinity to its response element [Kliewer et al., 1992; Munder et al., 1995; Sone et al., 1991; Yu et al., 1991]. Vitamin D response element (VDRE) sequences consist of two direct repeats spaced by three nonspecified nucleotides (DR-3) [Umesono et al., 1991]. VDREs have been located in 1,25-$(OH)_2D_3$ regulated genes such as mouse osteopontin [Noda et al., 1990], human [Ozono et al., 1990], and rat osteocalcin [Demay et al., 1990], rat [Zierold et al., 1995], human 25-hydroxyvitamin D_3 24-hydroxylase [Chen and DeLuca, 1995], rat calbindin D-9k [Darwish and DeLuca, 1992], and mouse calbindin D-28k [Gill and Christakos, 1993]. Recent work [Darwish and DeLuca, 1996] indicates that the negatively regulated human PTH gene contains also a DR-3 type VDRE and not a single half site [Demay et al., 1992]. The mechanism whereby nuclear receptor-DNA interaction modulates transcription is not entirely understood. It likely involves protein-protein interactions with the

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transcription initiation complex directly or indirectly mediated by transcription-intermediary factors [Beato and Sanchez-Pacheco, 1996; Tsai and O'Malley, 1994]. Contacts between proteins bound to separated sites on DNA require distortion of the DNA helix. When proteins are bound to sites in close proximity (several hundred bp) looping of the straight DNA helix is not energetically favorable [Horowitz and Wang, 1984]. Therefore, DNA bending has been proposed as a mechanism to facilitate the process of assembling the transcription complex. An increasing number of prokaryotic and eukaryotic transcription factors have been shown to induce a DNA bend by binding to their recognition sites [van der Vliet and Verrijzer, 1993].

The most widely used methods to detect protein-induced DNA bending rely on the anomalous electrophoretic mobility of bent DNA fragments. According to gel electrophoresis convention, the electrophoretic mobility of a DNA fragment is dependent on its end to end distance [Lumpkin et al., 1985]. Therefore, a DNA fragment with a bend at its center will migrate slower than a DNA fragment with a bend at its end [Wu and Crothers, 1984]. Among members of the steroid and thyroid hormone receptor superfamily, estrogen receptor (ER) [Nardulli and Shapiro, 1992; Nardulli et al., 1995; Sabbah et al., 1992], progesterone receptors (PR) [Petz et al., 1997; Prendergast et al., 1996], glucocorticoid receptor DNA-binding domain [Petz et al., 1997], thyroid hormone receptor (TR) [King et al., 1993; Shulemovich et al., 1995], and various RXR heterodimers [Lu et al., 1993; Shulemovich et al., 1995] have been found to bend DNA. We have investigated DNA conformational alterations induced by VDR-RXR binding to different VDREs, in absence or presence of 1,25-$(OH)_2D_3$, using circular permutation and phasing analysis.

MATERIALS AND METHODS

Plasmid Construction and Probe Preparation

The circularly permuted probes were constructed by inserting synthetic oligonucleotides containing the rat osteocalcin rOC-VDRE [Demay et al., 1990], the mouse osteopontin mOP-VDRE [Noda et al., 1990], the distal rat 25-hydroxyvitamin D_3 24-hydroxylase r24-OHase VDRE [Zierold et al., 1995], or the human PTH-VDRE [Darwish and DeLuca, 1996] between XbaI-SalI restrictions sites in plasmid pBend2 [Kim et al., 1989]. Phasing plasmids mOP-22, mOP-24, mOP-26, mOP-29, and mOP-31 were constructed by inserting the following oligonucleotides between the same sites in the pBend2 vector:

CTAGAGGTTCACGAGGTTCAGGCA$_5$CGGGCA$_5$CGGGCA$_5$CGGTCGAG;
CTAGAGGTTCACGAGGTTCAGTCGCA$_5$CGGGCA$_5$CGGGCA$_5$CGGTCGAG;
CTAGAGGTTCACGAGGTTCAGTCACGCA$_5$CGGGCA$_5$CGGGCA$_5$CTCGAG;
CTAGAGGTTCACGAGGTTCAGTCGACACGCA$_5$CGGGCA$_5$CGGGCA$_5$CTCGAG;
and
CTAGAGGTTCACGAGGTTCAGTCGACTGACGCA$_5$CGGGCA$_5$CGGGCA$_5$CTCGAG,

respectively. All DNA constructs were analyzed by sequencing.

For preparation of DNA probes for circular permutation analysis, the tandem duplication, located between EcoRI and HindIII, was amplified by PCR using primers complementary to flanking sequences. The PCR amplification product was purified by isolation from a polyacrylamide gel and digested with the appropriate restriction endonuclease (Fig. 1). Each probe had the same size of 136 bp (except 147 bp for hPTH VDRE set). DNA probes for phasing analysis were prepared by digesting each plasmid with EcoRI and HindIII, yielding a set of DNA fragments of 292 to 300 bp. The DNA probes were labeled either by polynucleotide kinase with [γ-^{32}P]ATP for blunt-end fragments or by Klenow fragment with [α-^{32}P]dCTP or [α-^{32}P]dGTP for cohesive-end fragments. Further gel purification was performed to isolate each set of labeled DNA fragments for circular permutation and phasing analyses.

Preparation of Receptor Protein

Recombinant rat VDR was expressed as full-length protein in a baculovirus system as previously described [Ross et al., 1991]. Purified recombinant human RXRβ was purchased from Affinity BioReagents (Neshanic Station, NJ).

Electrophoretic Mobility Shift Assay (EMSA)

For binding reactions 500 fmol VDR from crude cell extracts and 220 fmol RXRβ were incubated 1 h on ice in 12 µl of 10 mM Tris HCl pH = 7.5, 2.7 mM dithiothreitol (DTT), 150 mM KCl (80 mM KCl for hPTH VDRE probes), 8% glycerol, 1 µg poly dI-dC, and 1 µl ethanol vehicle or 1 µl ethanol containing 1,25-(OH)2D3 (Tetrionics, Inc., Madison, WI) to give a final concentration of 10^{-6} M. Another incubation of 15 min with the set of ^{32}P-labeled probes was performed at room temperature. The reaction mixtures were loaded in a 5% non-denaturing

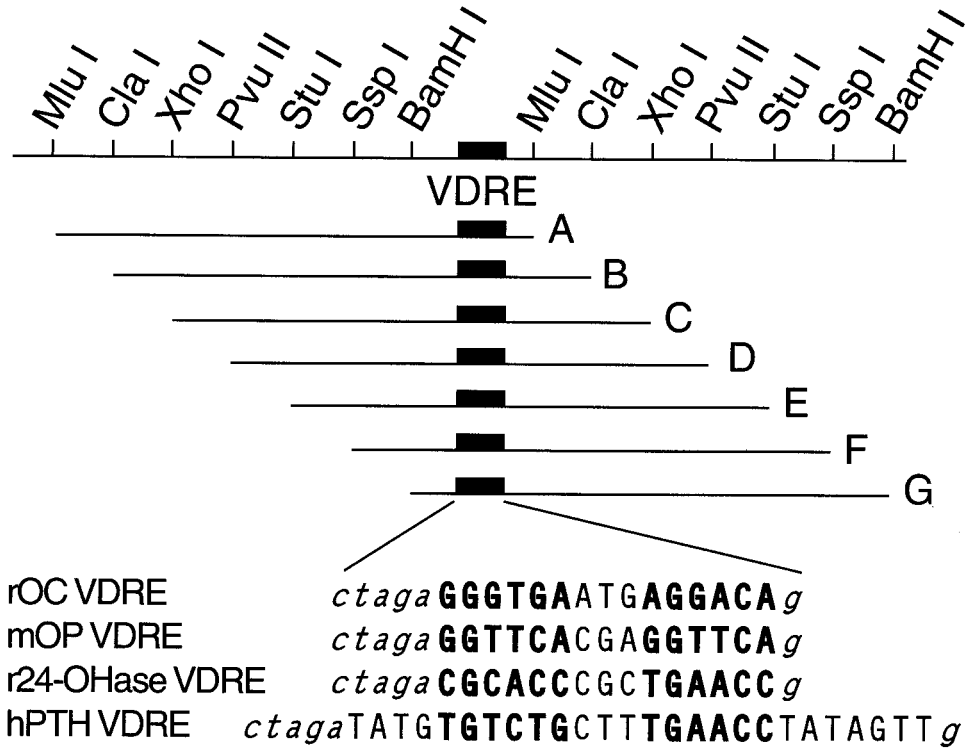

Fig. 1. Schematic representation of circularly permuted probes. Solid boxes represent the location of the VDRE. The two half sites of the VDRE are indicated in bold. Probes were generated by serial restriction digests of the pBend2 polylinker resulting in variable VDRE position relative to the ends of the DNA. All DNA fragments were of identical size: 136 bp for rOC, mOP, and r24-OHase VDRE probes, and 147 bp for hPTH VDRE probes.

polyacrylamide gel, submerged at 4°C in 1X TBE (90 mM Tris borate, 2 mM EDTA), prerun 1 h at 100 V. Migration was performed at constant voltage (160 V) during 7 h. Gels were dried and scanned in a PhosphoImager (Molecular Dynamics, Sunnyvale, CA) and then submitted to autoradiography.

Calculation of DNA Parameters

The distance of migration of the complexes and the free probes were measured either manually or by automatic band recognition of PhosphorImager data or Densitometer data (Molecular Dynamics, Sunnyvale, CA).

For circular permutation assays, the complex mobilities were corrected for slight variation in probe mobilities and normalized to the complex with the fastest mobility. Relative mobilities were plotted as a function of the distance from the center of the VDRE to the ends of the probe. The best fit of the relative mobilities to a cosine function (termed circular permutation function) was determined through SigmaPlot® software (Jandel Scientific). The DNA distortion angle (α_D) was calculated from the amplitude (ACP) of the circular permutation function using the following formula ACP = 1-cos(k$\alpha_{D/}$2) [Kerppola and Curran, 1991]. ACP reflects the differences between the probes with the lowest mobility and theoretically the highest mobility, k is a coefficient reflecting factors that influence the relative mobilities (temperature, gel composition, and field strength). The coefficient k was calculated under our electrophoresis conditions using a set of standards containing two to nine phased A-T tracts (plasmid pJT170-n; n = 2–9) generously provided by J. Thompson and A. Landy. Phased A-T tracts bend DNA toward the minor groove and each A-T tract bends DNA by approximately 18° [Thompson and Landy, 1988]. The relative mobilities (μM/μE) of the A-T tract DNA under our electrophoretic conditions were plotted versus the predicted bending angle. μM is the relative mobility of the standard with the A-T tract positioned in the middle and μE the relative mobility when positioned at the end of the DNA fragment. From the relationship μM/μE = coskα/2 [Kerppola and Curran, 1991] we found a k value of 1.02.

For phasing analysis, the complex mobilities were corrected for variations in probe mobili-

ties and then normalized to the average mobility of all complexes and plotted as a function of the length of the spacer between the center of the mOP-VDRE and the center of the intrinsic DNA bend. The best fit of the relative mobilities to a cosine function was determined. The protein-induced bend angle (α_B) was calculated from the amplitude of the phasing function (A_{PH}) using the empirical equation introduced by Kerppola and Curran [1991] $\tan(k\alpha_B/2) = A_{PH}/\tan(k\alpha_C/2)$, where α_C is the intrinsic DNA bend angle. In these experiments, the reference angle was 54°. To determine the orientation of the DNA bend, the helix periodicity was assumed to be 10.5 bp per turn. The lowest complex mobility will have a helix periodicity that cooperates with the intrinsic bend, and the fastest mobility a helix periodicity that counteracts the intrinsic bend. Since the A-T tract bends the DNA toward the minor groove this allows the determination of the orientation of the protein-induced bend.

RESULTS

VDR/RXR Heterodimers Induce a Significant Distortion When They Bind to VDRE

The circular permutation analysis was used first to detect protein-induced DNA bending. This assay is based on the idea that a DNA fragment with a bend at its center will migrate slower than a DNA fragment with a bend at its end [Wu and Crothers, 1984]. Electrophoretic mobility shift assay (EMSA) was performed using different sets of probes of identical lengths, containing VDREs at various positions within the ends (Fig 1). The VDREs analyzed were from 1,25-$(OH)_2D_3$ stimulated genes (rat osteocalcin, rOC; mouse osteopontin, mOP; rat 25-hydroxyvitamin D_3 24-hydroxylase, r24-OHase) and a 1,25-$(OH)_2D_3$ repressed gene (hPTH). Baculovirus expressed rat VDR and recombinant human RXRβ were used in the binding reaction, in the absence or presence of 1,25-$(OH)_2D_3$. Binding reactions were performed at 150 mM KCl, a salt concentration that presumably mimics physiologic nuclear levels [Paine et al., 1981]. At this salt concentration, the hormone stabilizes the complex formation [Kimmel-Jehan et al., 1997; and data not shown]. However, for hPTH VDRE we used 80 mM KCl because no complexes were formed at 150 mM KCl in absence of ligand [Kimmel-Jehan et al., 1997; and data not shown]. Since in circular permutation assay, the mobility anomalies seen may not result entirely from a directed bend in DNA, but also to increased flexibility or from other perturbations in the DNA helix, the angle measured will be referred as distortion angle (α_D). Representative experiments are shown in Figure 2. In all cases, VDR-RXRβ heterodimer complexes bound to probes on which the VDRE is located closer to the center of the DNA fragment (probe D) migrated more slowly than when the VDRE is located closer to the ends (probe A and G; Fig. 2). The relative mobilities were plotted against the distance between the center of the VDRE and the ends of the probe. DNA distortion angles were calculated from the amplitude of the best-fit cosine function for each set of data and reported in Table I. The magnitude of the DNA distortion angle was similar for all VDREs, in the range of 56 to 61°. There was no significant change in magnitude in the presence of 1,25-$(OH)_2D_3$. The center of distortion (C_D) was determined from the amplitude (A_{CP}) of the circular permutation function, as described in Materials and Methods, and indicates the position in the sequence which when localized at the end of the fragment would theoretically give maximum mobility. For mOP, r24-Ohase, and hPTH VDREs the center of distortion mapped in the vicinity of the midpoint between the two half sites. In the case of the rOC VDRE this center was positioned 4 bp 5′ from the center of the VDRE (Table I). However the location of the centers of distortion is not defined at the precise base because of the limit in resolution of the assay.

VDR-RXR Binding of mOP VDRE Induces A Directed Bend

Phasing analysis was used to determine what proportion of the DNA distortion introduced by VDR-RXRβ was due to a directed bend in the DNA. This assay is based on the fact that two directed bends located on a single DNA molecule create an overall bend which is determined by the size, direction, and helical phasing of the two component bends [Zinkel and Crothers, 1987]. A set of probes was constructed by placing a standard DNA bend (A-T tract) of known magnitude and orientation at different distances from the mOP-VDRE. The distance between the centers of the mOP-VDRE and the intrinsic bend was varied over one turn of DNA helix (10.5 bp), from 22 to 31 bp. Binding reactions were performed as above, using 150 mM

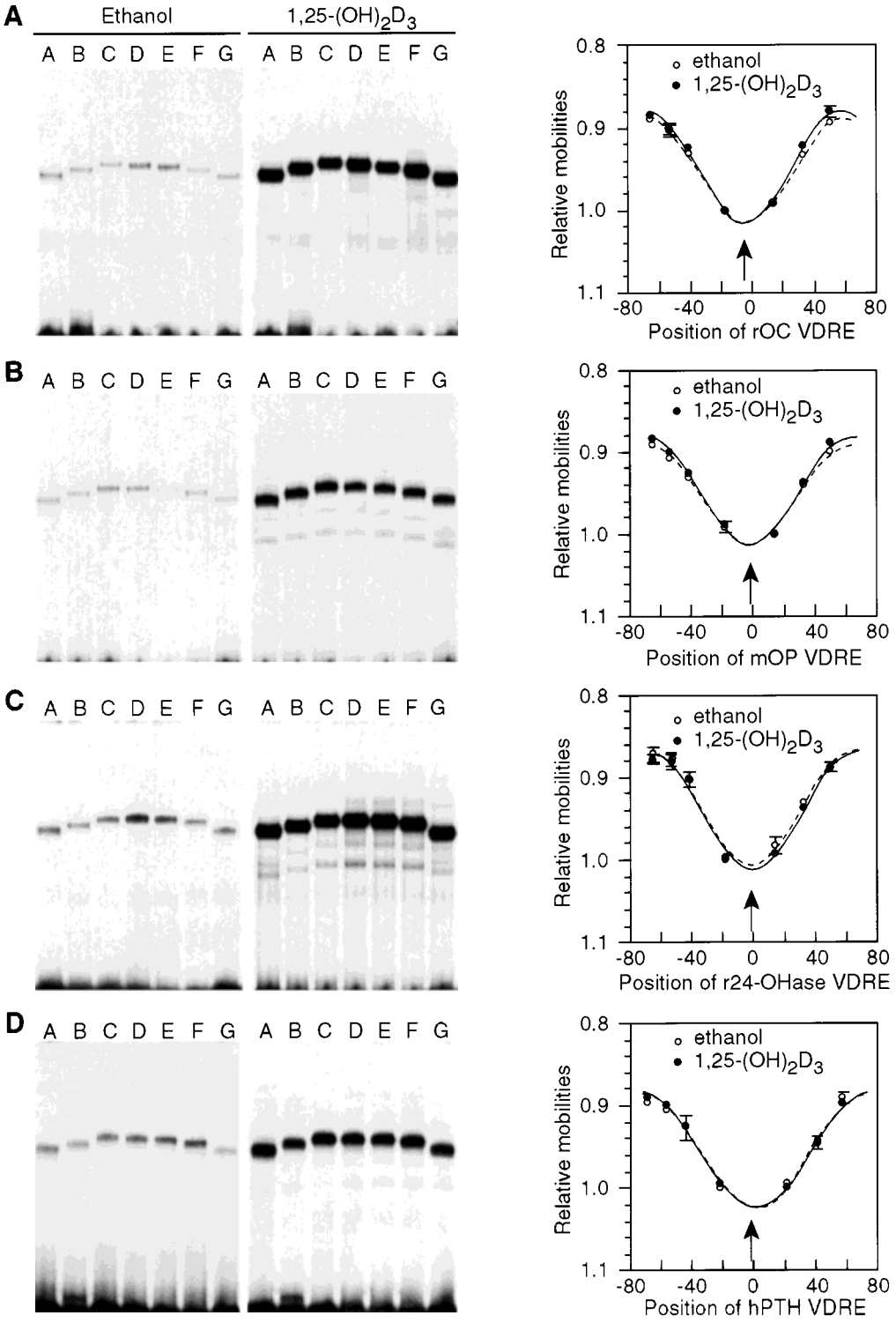

Fig. 2. Circular permutation analysis demonstrates that VDR-RXRβ heterodimers mediate distortion of target DNA. Left: Band mobility shift analysis of VDR-RXRβ heterodimers bound to circularly permuted probes, in which the distance between the center of **(A)** rOC VDRE, **(B)** mOP VDRE, **(C)** r24-OHase VDRE, or **(D)** hPTH VDRE and the ends of the DNA was varied. Binding reactions were performed with baculovirus expressed rat VDR and purified recombinant human RXRβ, in absence or presence of 10^{-6} M 1,25-$(OH)_2D_3$. Labeled probes added are shown above the lanes. Right: Relative mobilities of VDR-RXRβ complexes, calculated as indicated in Materials and Methods, are plotted against the position of the center of the VDRE relative to the ends of the probe. Each point is an average value of three independent experiments, and the SDs are shown as vertical bars. The points are connected by the calculated best fit of a cosine function to each set of data. Arrows indicate the maximum relative mobility of the best fit and represent the positions of the center of distortion.

TABLE I. DNA Distortion and Bending Mediated by VDR-RXRβ Heterodimers to Different VDREs

	Circular permutation analysis			Phasing analysis	
	Circular permutation amplitude[a] (A_{CP})	Distortion angle[b] (α_D)	Center of distortion[c] (C_D)	Phasing amplitude[a] (A_{PH})	Directed bend angle[b] (α_B)
rOC VDRE					
−D	0.13 ± 0.01	57° ± 1	−4 ± 1	NT	NT
+D	0.14 ± 0.01	59° ± 1	−5 ± 1	NT	NT
mOP VDRE					
−D	0.12 ± 0.01	56° ± 1	−2 ± 1	0.24 ± 0.04	26° ± 3
+D	0.13 ± 0.01	58° ± 1	−2 ± 1	0.23 ± 0.04	24° ± 4
r24-OHase VDRE					
−D	0.14 ± 0.01	61° ± 3	−1 ± 2	NT	NT
+D	0.14 ± 0.01	61° ± 3	−1 ± 1	NT	NT
hPTH VDRE					
−D	0.14 ± 0.01	59° ± 2	−1 ± 1	NT	NT
+D	0.14 ± 0.01	60° ± 1	0 ± 1	NT	NT

[a]The amplitudes of circular permutation (A_{CP}) and phasing (A_{PH}) functions were determined as described in Materials and Methods.

[b]The distortion angles (α_D) and bend angles (α_B) were calculated from the amplitude of the best-fit cosine function for each set of data.

[c]The centers of distortion (C_D) were determined from the circular permutation functions. The sequence is numbered from the midpoint of the two half sites of the VDRE, which is designated as 0. The values represent the averages and SDs derived from three (circular permutation) or six (phasing) independent experiments. NT, not tested.

KCl, in presence or absence of 1,25-$(OH)_2D_3$. VDR-RXRβ heterodimers were bound to the phasing probes and subjected to polyacrylamide gel electrophoresis. As shown in Figure 3, mobilities of VDR-RXRβ complexes varied depending on the spacing between the mOP-VDRE and the intrinsic DNA bend. This confirms that VDR-RXRβheterodimers induce a directed bend. The relative mobilities were plotted against the spacer length. The directed bend angles (α_B) determined from the amplitude of the best-fit cosine function are 26° ± 3 in absence and 24° ± 4 in presence of 1,25-$(OH)_2D_3$ (Table I). The absolute orientation of DNA bending was determined from the phasing between the intrinsic bend and the protein-induced bend. The five A-T tracts used as an intrinsic bend have been reported to bend the DNA toward the minor groove at the center of the third A-T tract [Salvo and Grindley, 1987; Zinkel and Crothers, 1987]. When the two bends cooperate, the end-to-end distance of the fragment will be smaller and will have the lowest mobility. This is observed when the spacer length between the center of VDRE and the center of the A-T tract is 28 bases (Fig. 3). We have found equivalent results using bacterially expressed rat RXRα in the phasing analysis with mOP-VDRE probes (data not shown).

DISCUSSION

We have shown by circular permutation analysis that the binding of VDR-RXRβ heterodimer complexes to different VDREs induces a distortion in DNA structure. The values of the distortion angles were similar for VDREs from 1,25-$(OH)_2D_3$-enhanced genes (57°, 56°, and 61°, respectively for rOC, mOP, and r24-OHase VDREs) and from a 1,25-$(OH)_2D_3$-repressed gene (59° for hPTH VDRE). The hPTH VDRE has recently been identified as a classical DR-3 [Darwish and DeLuca, 1996]. The center of distortion was positioned within 1 base pair from the midpoint of the two hPTH VDRE half sites. For all VDREs, the magnitude of the DNA distortion was not significantly influenced by the presence of 1,25-$(OH)_2D_3$. Phasing analysis using mOP VDRE containing probes demonstrated that VDR-RXRβ heterodimers induce a directed DNA bend which is not influenced in magnitude or orientation by 1,25-$(OH)_2D_3$. The degree of the distortion angle (56°) determined by circular permutation assay was larger than the directed bend angle (26°) determined by phasing analysis. Similar quantitative discrepancies have been reported for several other sequence-specific transcription factors including members of the SHR super-

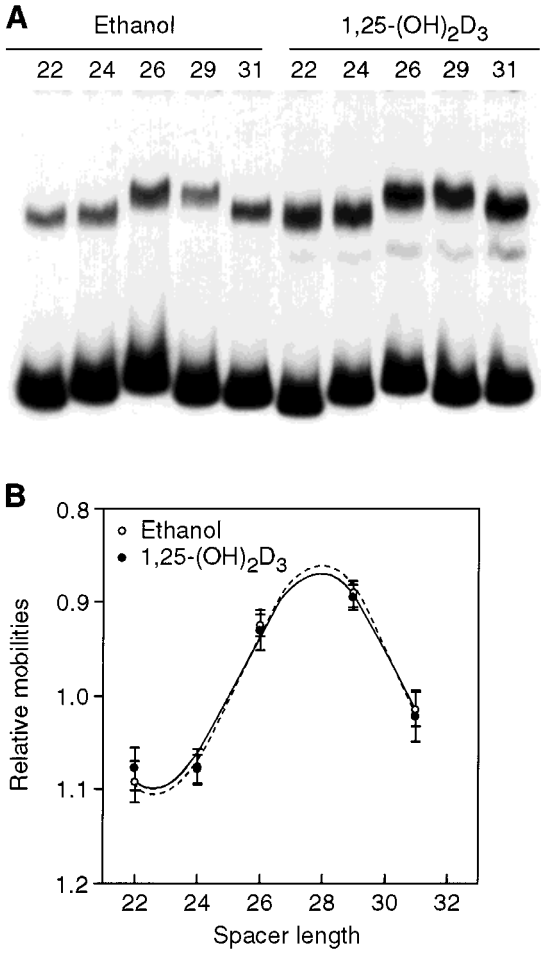

Fig. 3. VDR-RXRβ heterodimers induce a directed bend as detected by phasing analysis. **A:** Electrophoretic mobility shift analysis of VDR-RXRβ heterodimers bound to phasing analysis probes, in which spacing between the centers of mOP VDRE and the intrinsic DNA bend (A-T tract) is varied through a helical turn (10.5 bp). Binding reactions were performed at 150 mM KCl, in absence or presence of 10^{-6} M 1,25-$(OH)_2D_3$, and the probes shown above the lanes were added. The probe names indicate the spacer length. **B:** Relative mobilities of complexes are plotted as a function of the spacer length. Results are the average of six independent experiments, and the SDs are shown as vertical bars. Lines represent the best calculated fit of a cosine function.

family [Kerppola and Curran, 1991, 1993; Lu et al., 1993; Prendergast et al., 1996; Shulemovich et al., 1995]. The reason is that circular permutation analysis is sensitive to conditions other than DNA bend, such as increased DNA flexibility [Kerppola and Curran, 1991]. Therefore in this assay the apparent bend angle is overestimated.

Retinoid X receptors (RXR) have been shown to heterodimerize with retinoid acid receptor (RAR), TR, and VDR and to mediate a variety of biological functions upon binding to their cognate HRE [Kliewer et al., 1992; Yu et al., 1991]. DNA bending induced by RXR-containing complexes has been previously reported. Similar to our results, the distortion angles induced by various RXRα-RAR heterodimers were found in the range of 57°to 64° as determined by circular permutation assay, and the DNA bend angles, calculated from phasing analysis, in the range of 22° to 31° [Lu et al., 1993]. However for TRα1-RXRs heterodimers the magnitudes of the DNA bend angles were smaller (11°–13°) indicating that only a small part of the distortion of the DNA upon binding to DR-4 thyroid hormone response element (TRE) was due to DNA bending [Lu et al., 1993; Shulemovich et al., 1995]. DNA distortions caused by RXR-RARs and RXR-TR complexes were not significantly influenced by the presence of specific ligands 9-*cis* retinoic acid and T_3 [Lu et al., 1993].

The orientation of the DNA bend is usually determined relative to that of the intrinsic bend and depends upon an accurate estimation of the bend center. The center of the bend induced by VDR-RXRβ heterodimers was estimated by circular permutation assay at the position −2 bp relative to the center of the mOP VDRE, designated as 0. The resolution of this assay does not allow a determination at the precise base pair because it is sensitive to factors other than DNA bending. In the phasing analysis, the spacer length was determined from the midpoint of the two VDRE half sites to the center of the intrinsic bend and therefore may not be accurate. Unfortunately, the distance of 28 bp, at which the two bends have been found to cooperate, cannot be related to a multiple of helical turn (10.5 bp) or half turn. Thus, the orientation of the DNA bend induced by VDR-RXRβ heterodimers cannot be determined relative to the major or minor groove from our data. It is of interest that members of the SHR family reported so far to induce DNA bending mediate a bend toward the major groove [Lu et al., 1993; Nardulli and Shapiro, 1992; Prendergast et al., 1996]. However opposite directions of the bend induced by TRα1-RXRs heterodimers upon binding to DR-4 TRE have been reported in two different studies [Lu et al., 1993; Shulemovich et al., 1995].

It has been difficult to design experiments that test the physiologic role of DNA bending in transcriptional function. Either deleting or mu-

tating VDR responsive elements eliminates function as has already been clearly demonstrated [Demay et al., 1990; Noda et al., 1990; Ozono et al., 1990]. These changes also eliminate heterodimer binding to DNA, thus eliminating any study of DNA bending by these techniques.

Although conformational changes in the receptor induced by ligand binding are important for transcription activation [Allan et al., 1992], in almost all studies reported to date, including our own, no changes in the magnitude or orientation in DNA bending induced by SHR by the presence of hormone have been found [King et al., 1993; Lu et al., 1993; Nardulli and Shapiro, 1992; Prendergast et al., 1996]. One report indicated a smaller DNA bend induced by the wild-type ER in the presence of estradiol [Lazennec et al., 1997]. The physiological relevance of the bending in the regulation of transcription is still unknown. Direct binding with transcription factor IIB, a component of the general transcription machinery, has been reported for the VDR and other SHR [Blanco et al., 1995; Ing et al., 1992; MacDonald et al., 1995]. DNA bending may assist stabilization of components of the preinitiation complex. Other transcription intermediary factors have been reported to interact with both SHR and general transcription factors [reviewed in Beato and Sanchez-Pacheco, 1996]. Whether coactivators enhance DNA bending is not known. Coactivators such as steroid receptor coactivator 1 (SRC-1A) and CREB (cAMP regulatory element-binding protein)-binding protein (CBP) have been shown to enhance SHR transactivation and to possess intrinsic histone acetyltransferase (HAT) activities [Jenster et al., 1997]. In the cell nucleus, the DNA is organized in chromatin. The remodeling of the chromatin is also important in the regulation of transcription. The exact role, if any, played by induced DNA bending in the regulation of transcription, therefore, is still to be learned.

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