Proc. Natl. Acad. Sci. USA
Vol. 90, pp. 10648–10652, November 1993
Biochemistry

Strand-invasion of duplex DNA by peptide nucleic acid oligomers

(D-loop/transcription/DNA-bending)

NANCY J. PEFFER, JEFFERY C. HANVEY, JOHN E. BISI, STEPHEN A. THOMSON, C. FRED HASSMAN, STEWART A. NOBLE, AND LEE E. BABISS*

Departments of Cell Biology and Medicinal Chemistry, Glaxo, Inc., Research Institute, Research Triangle Park, NC 27709

Communicated by Harold S. Ginsberg, August 2, 1993

ABSTRACT **Polyamide oligomers, termed peptide nucleic acids (PNAs), bind with high affinity to both DNA and RNA and offer both antisense and antigene approaches for regulating gene expression. When a PNA binds to a complementary sequence in a double-stranded DNA, one strand of the duplex is displaced, and a stable D-loop is formed. Unlike oligodeoxynucleotides for which binding polarity is determined by the deoxyribose sugar, the unrestrained polyamide backbone of the PNA could permit binding to a DNA target in an orientation-independent manner. We now provide evidence that PNAs can, in fact, bind to their complementary sequence in DNA independent of the DNA-strand polarity—that is, a PNA binds to DNA in both "parallel" and "antiparallel" fashion. With a mixed-sequence 15-mer PNA, kinetic studies of PNA·DNA interactions revealed that D-loop formation was rapid and the complex was stable for several hours. However, when measured either by gel-mobility-shift analysis or RNA polymerase II-elongation termination, D-loop formation was salt dependent, but PNA-strand dissociation was not salt dependent. We observed that D-loop-containing DNA fragments had anomalous gel mobilities that varied as a function of the position of the D-loop relative to the DNA termini. On the basis of permutation analysis, the decreased mobility of the PNA·DNA complex was attributed to a bend in the DNA at or near the D-loop.**

The inhibition of gene expression mediated by oligodeoxynucleotides is a powerful research tool that has been exploited to study the function of specific gene products in complex biological pathways (for reviews, see refs. 1 and 2). Although technically challenging, inhibiting gene expression at the level of transcriptional initiation and/or RNA polymerase elongation (antigene approaches) may represent the most fundamental means of ablating gene function. Two mechanisms by which gene-specific inhibition of transcription could be achieved *in vivo* are oligomer binding in the major groove of duplex DNA to form a triple helix (3–5) or by strand-invasion of duplex DNA, resulting in the formation of a stable D-loop [using peptide nucleic acids (PNAs) (6–10)]. In the case of triplex formation, recognition is mediated by Hoogsteen base pairing (11). The nonphysiologic conditions necessary to promote third-strand binding of an oligomer to a duplex target has largely precluded the use of this approach for *in vivo* studies. However, the requirement for low pH or specific ionic conditions to stabilize cytosine protonation (12, 13) has recently been obviated by pH-independent cytosine surrogates (14, 15).

PNAs consist of achiral monomer subunits derived from N-ethylaminoglycine bearing appendent nucleoside heterocyclic bases, which are coupled by standard methods of amide bond formation. We and others (6, 9, 10) have shown that thymidine-rich PNAs recognize complimentary sequences in duplex DNA by strand-invasion, which results in local strand displacement (formation of a D-loop). Because nucleases and chemical probes are unsuitable for measuring the extent of strand-invasion, we developed two quantitative assays for PNA binding. (*i*) We measured the inhibition of restriction enzyme cleavage (*Hin*dIII) at a site overlapping the PNA target sequence (9). Using this approach we demonstrated a PNA dose-dependent inhibition of *Hin*dIII cleavage at the PNA target sequence. (*ii*) We measured the efficiency and stability of PNA strand-invasion by studying PNA-dependent inhibition of RNA polymerase II (pol II) transcription elongation *in vitro* (9). Binding of PNAs to the transcribed strand of a guanine-free transcription cassette completely inhibited transcription elongation, precisely at the position of the D-loop. Unlike the transient nature of pol II-elongation inhibition seen by oligodeoxynucleotide triplex formation (14), the inhibition we observed persisted for several hours.

In this paper we have used inhibition of transcription elongation and a recently described quantitative mobility-shift assay (based on observations that DNA·PNA complexes have anomalous gel-migration properties) to address several properties of PNA strand-invasion of duplex DNA. Because PNAs do not have the conformational and configuration restraints about a glycosidic bond, their binding to a target sequence has been postulated to occur in an orientation-independent manner (8). Using the transcription assay, we now show that a PNA can strand-invade duplex DNA and bind to its complementary sequence aligned in either an $NH_2 \rightarrow 5'$ or $NH_2 \rightarrow 3'$ orientation.

We have exploited the mobility-shift assay to determine how the position of the D-loop relative to the DNA termini affects the extent of the band-shift. We find that the altered mobility is more pronounced when the D-loop is localized symmetrically between the DNA termini, and the extent of band shift decreases as the D-loop approaches a DNA terminus. These results strongly suggest that the D-loop is inducing a bend or kink in the DNA helix, which may provide an additional means of inhibiting cellular gene transcription.

MATERIALS AND METHODS

Plasmids. Plasmid constructs used in the transcription assay contained the target sequence for PNA binding downstream of a guanine-lacking cassette sequence in the plasmid pMLP (16). Transcription was driven by the adenovirus major late promoter, and all PNA-binding targets were cloned into the transcribed strand of the guanine-lacking cassette. The plasmid pMLP-SV40 (where SV40 represents simian virus 40) contained a 20-bp SV40 large tumor (T) antigen sequence (5′-GAAGAAAAAATGAAGAAAAT) cloned into pMLP (9), and plasmids pN5 and pN3 contained the sequences 5′-$(AG)_5A_5$ and 5′-$A_5(GA)_5$, respectively, cloned

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: PNA, peptide nucleic acid; SV40, simian virus 40; T, large tumor; pol II, RNA polymerase II.

*To whom reprint requests should be addressed.

into the same site of pMLP as the SV40 sequence. All of these plasmid constructs contained an additional 55 bp of guanine-less cassette sequence downstream of the target sequence. The plasmid pCXAB was prepared by cloning a 127-bp sequence containing sites for *Cla* I, *Xho* I, Asp-718, and *Bss*HII into the *Sst* I site of pBSA10, a derivative of pBluescript II KS+ containing an A_{10} site (9).

***In Vitro* Transcription.** Unless otherwise indicated, strand-invasion was done for 1 hr at 37°C in 1 mM Tris·HCl, pH 8.0/0.1 mM EDTA using 0.2 μg (0.09 pmol) of plasmid DNA and 8 pmol of PNA. Transcription was initiated by the addition of transcription buffer, NTPs (with [^{32}P]UTP) and HeLa cell nuclear extract (Promega) as the source of transcription factors (16). The radiolabeled RNA transcripts were resolved on a 6% denaturing polyacrylamide gel. Sequence-specific transcription termination was determined by quantitating the amount of radioactivity in the full-length and truncated fragments with a Molecular Dynamics PhosphorImager model 400E.

Gel-Retardation Assay. The ability of a PNA·DNA D-loop to decrease gel migration was assessed by PAGE of restriction fragments of pCXAB. To measure how salt affects the efficiency of PNA strand-invasion, PNA T_{10}-Lys (2 μM) was incubated with 0.4 μg (13 nM) of pCXAB in 1 mM Tris·HCl, pH 8.0/0.1 mM EDTA plus various concentrations of NaCl for 1 hr at room temperature. After the addition of NaCl to a final concentration of 100 mM and React 1 buffer (BRL) to one times standard concentration, the complex was digested with *Pvu* II and *Bss*HII for 1 hr at 37°C. The products were analyzed on a 6% polyacrylamide gel. Quantitation was done by using photographic negatives of the ethidium bromide-stained gel on a Molecular Dynamics computing densitometer model 300B.

The ability of the D-loop to bend the DNA helix was assessed by using restriction fragments of pCXAB in which the PNA-binding site was positioned at variable lengths from the end of the fragment. PNA T_{10}-Lys was incubated with pCXAB as above. After the addition of React 2 buffer (BRL) to standard concentration (1×), or buffer B (Boehringer Mannheim) to standard concentration (1×) for the Asp-718 reaction, the complex was digested with *Cla* I, *Xho* I, Asp-718, or *Bss*HII for 1 hr at 37°C. The products were analyzed by a 6% polyacrylamide gel, and the migration distance was determined after scanning the photographic negatives of the ethidium bromide-stained gels as above.

RESULTS

Detection of PNA·DNA Interactions. PNA oligomers were synthesized containing a 10-mer homothymidine (T_{10}-Lys) or two 15-mer sequences; $(TC)_5T_5$-Lys and $CTTCAT_6CTTC$-Lys, all with a lysine group at the carboxyl terminus (Fig. 1*A*). The interactions of these PNAs with plasmids containing complementary target sequences were assayed by the termination of pol II-directed transcription (9) and by a band-shift assay. It has been reported (9, 10) that a PNA·DNA complex has an anomalous gel migration. Both assays allow us to quantitate the extent of D-loop formation as a consequence of PNA strand-invasion.

PNA Strand-Invasion Is Orientation Independent. PNA molecules could theoretically invade a strand of duplex DNA and form a D-loop in either of two orientations, with the NH_2 terminus of the PNA molecule aligned to either the 5′ or 3′ position of DNA (Fig. 1*A*). To test the binding orientation, an asymmetric PNA, $(TC)_5T_5$-Lys, was prepared, and its complementary sequence was cloned in both orientations into the transcribed strand of a guanine-free transcription cassette of plasmid pMLP. The plasmid pN5 contains the sequence $5'(AG)_5A_5$, where the PNA would bind in the $NH_2 \rightarrow 5'$ orientation; whereas plasmid pN3 contains the sequence

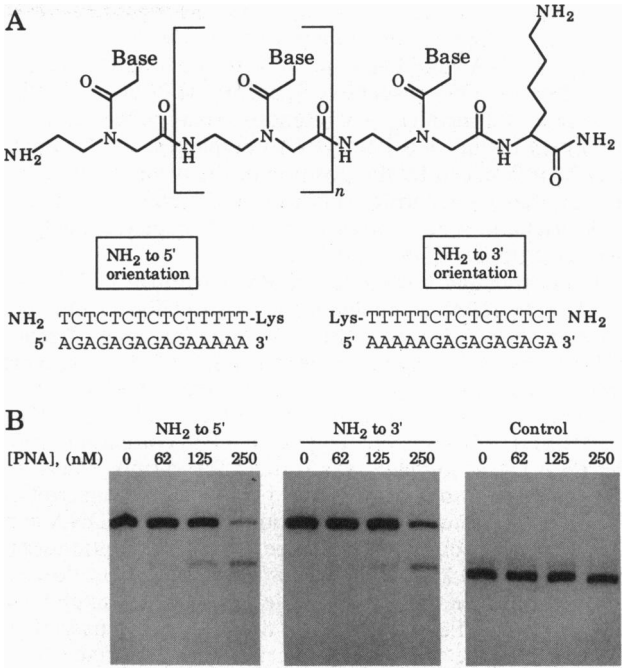


FIG. 1. PNAs bind to complementary DNA sequences independent of DNA strand polarity. (*A*) General structure of a PNA backbone with a carboxyl-terminal lysine residue and the potential interactions of the same PNA, $(TC)_5T_5$-Lys (top strand) with either of two DNA sequences (bottom strand). These interactions are termed $NH_2 \rightarrow 5'$ and $NH_2 \rightarrow 3'$. (*B*) Transcription inhibition is independent of binding orientation. The potential targets for PNA binding in *A* were cloned into transcription vector pMLP. Transcription from both plasmids (pN5 and pN3) is specifically inhibited by PNA NH_2-$(TC)_5T_5$-Lys in a dose-dependent manner (*Middle* and *Left* lanes). Control reactions with pMLP are not inhibited (*Right* lanes). The full-length transcripts for pN5 and pN3 are longer than for pMLP (448 compared to 383 bases). Specific termination of transcription at the PNA-binding site of pN5 and pN3 would give a transcript of 388 bases, essentially the same size as that from pMLP.

$5'\text{-}A_5(GA)_5$, in which the binding would be $NH_2 \rightarrow 3'$. If one binding orientation were preferred, then four mismatched base pairs (two T·G and two A·C pairs) would be generated in the incorrect orientation and likely preclude complex formation (7, 8).

After incubation of various concentrations of $(TC)_5T_5$-Lys with plasmids pN5 and pN3 in a low-salt buffer, transcription reactions were done by using a HeLa cell nuclear extract as the source of the transcription factors. Full-length transcription products from pN5 and pN3 would be 448 bases, whereas that from the control plasmid pMLP, without the insert would produce a transcript of 383 nt. If transcription terminated specifically at the PNA-binding site, the transcripts would be 388 nt long.

Nonspecific inhibition of transcription, indicated by a decrease of all transcript bands, was observed at concentrations of $(TC)_5T_5$-Lys at or above 0.5 μM (data not shown). However, when titrations were performed up to 250 nM, no inhibition of transcription was seen with control vector pMLP (Fig. 1*B*, control lanes). The extent of nonspecific transcription inhibition seen with this PNA was greater than seen previously with other homopyrimidine PNA sequences (9) and could reflect the binding of factors in the cell extract to the PNA. Nonspecific inhibition for this PNA was not seen in our other assays, such as inhibition of reverse transcription (data not shown).

$(TC)_5T_5$-Lys gave a dose-dependent inhibition of transcription elongation with both pN5 and pN3 (Fig. 1*B*). The extent of inhibition was similar at each dose, which indicated that

this PNA could bind to its target duplex regardless of the orientation. Slightly more inhibition was observed for pN5, in which the PNA would bind in the $NH_2 \rightarrow 5'$ orientation, than pN3 (58% vs. 33%, respectively, at 250 nM PNA). This slight preference for the $NH_2 \rightarrow 5'$ orientation may reflect the actual stabilities of the two PNA·DNA complexes, or the results may be influenced by the position of the lysine with respect to the polymerase during transcription elongation. In the $NH_2 \rightarrow 5'$ orientation, the polymerase would encounter the lysine first as it tracks along the DNA.

Association and Dissociation of PNAs with DNA. To evaluate the rate of PNA complex formation and dissociation with DNA, we used the transcription-termination assay. Because of the nonspecific inhibition seen using $(TC)_5T_5$-Lys, which prevents us from obtaining a high percentage of inhibition, we used an SV40 T-antigen antisense 15-mer PNA, $CTTCAT_6CTTC$-Lys (9). To examine PNA strand-invasion, the PNA·DNA complex was formed for various times in a low-salt buffer solution, followed by *in vitro* transcription. We have determined that formation of the PNA·DNA complex does not occur in the presence of the transcription buffer (Fig. 2*A*, lane 2, and data not shown). Under low-salt conditions, rapid formation of the PNA·DNA complex was observed, as indicated by loss of the 448-base transcript and formation of the 388-base transcript (Fig. 2*A*, lanes 3–13). Formation of the complex was essentially complete in 10 min and was 50% complete in <4 min. No inhibition of the pMLP transcript was seen, even after a 1-hr preincubation with the PNA (Fig. 2*A*, lane 15).

To monitor dissociation of the PNA from the DNA, the D-loop was formed for 1 hr in low salt, followed by the addition of transcription buffer and a 25-fold excess of oligodeoxynucleotide complementary to the PNA (5′-TATGCTCTCGAGAAGAAAAAATGAAGAAAAACA). Transcription was initiated at various times after addition of the buffer and oligodeoxynucleotide. Fig. 2*B* (lanes 4–8) reveals a slow re-emergence of the full-length 448-base transcript (≈50% between 5 and 20 hr). Because the off-rate was slow, control experiments were done to ensure that the PNA was not re-associating with the DNA during incubation. Without preincubation of PNA and DNA in the low-salt buffer, no strand-invasion (transcription inhibition) was seen, even after a 20-hr incubation in the transcription buffer before initiation of transcription (Fig. 2*B*, lanes 9 and 10).

PNA Strand-Invasion Is Inhibited by NaCl. To examine the dependence of PNA strand-invasion on ionic strength, we used a gel-mobility-shift assay. After D-loop formation, plasmid DNA was digested with a restriction enzyme(s), and the fragments were analyzed by PAGE. A T_{10}-Lys PNA was used in this assay because essentially all plasmid molecules containing an A_{10} sequence can form a D-loop (compared with 80% for the SV40 15-mer PNA at similar doses).

After preincubation of T_{10}-Lys with an A_{10}-containing plasmid (pCXAB) in various concentrations of NaCl, the complex was digested with *Bss*HII and *Pvu* II and then analyzed by gel electrophoresis. *Bss*HII and *Pvu* II digestion of pCXAB generated four small restriction fragments (217, 181, 107, and 94 bp) (Fig. 3*A*). The 217-bp fragment contained the A_{10} target for T_{10}-Lys. Fig. 3*B* shows that the 217-bp fragment migration was shifted (arrow) in the presence of T_{10}-Lys (compare the no-PNA lane with that of the 0-NaCl lane). The migration of the other fragments did not change after incubation with T_{10}-Lys, which shows the specificity of strand-invasion with this assay. As the NaCl concentration was increased during preincubation of T_{10}-Lys and pCXAB, the extent of D-loop formation (band-shifted product) decreased (Fig. 3*B*, lanes 10–100 mM NaCl). Under these conditions and in the pol II termination assay, strand-invasion was ≈50% at 50 mM NaCl.

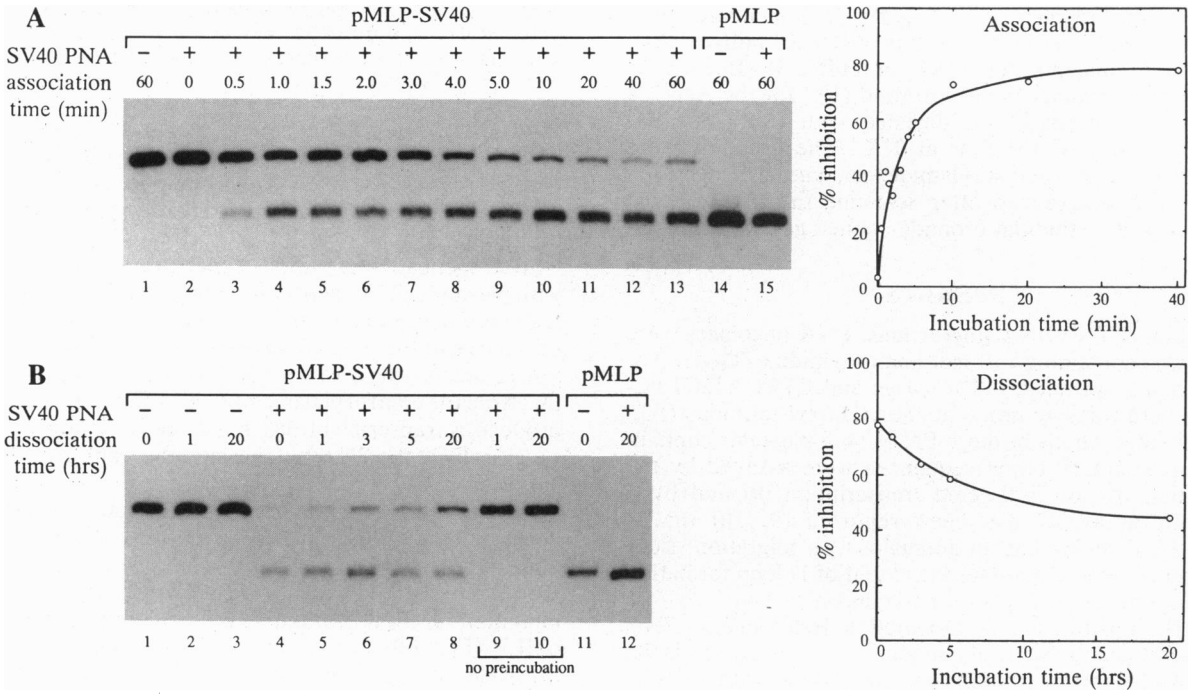


FIG. 2. Kinetics of DNA strand-invasion by a PNA. (*A*) Association. Supercoiled plasmid pMLP-SV40 (lanes 1–13) or pMLP (lanes 14 and 15) was incubated with (lanes 2–13 and 15) or without (lanes 1 and 14) PNA SV40-Lys at 25°C for 0–60 min. Transcription was initiated by the addition of buffer, HeLa cell extract, and NTPs. (*B*) Dissociation. Supercoiled plasmid pMLP-SV40 (lanes 1–10) or pMLP (lanes 11 and 12) was incubated with (lanes 4–10 and 12) or without (lanes 1–3 and 11) PNA SV40-Lys at 25°C. Lanes 9 and 10 had no incubation at low ionic strength. After addition of transcription buffer and an oligomer complementary to the PNA, reactions were incubated at 25°C for 0, 1, 3, 5, or 20 hr to allow PNA dissociation. Transcription was initiated by the addition of HeLa cell extract and NTPs. Association and dissociation curves of strand-invasion (site-specific transcription inhibition) by the PNA represent quantitation of the gels shown in *A* and *B*. Additional experiments have yielded similar results.

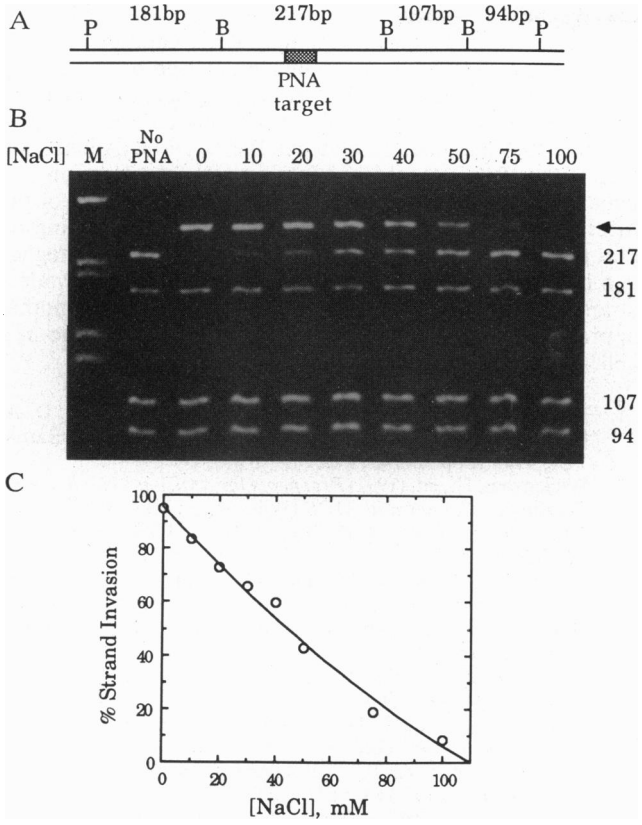


FIG. 3. Salt dependence of PNA strand-invasion. An observed band shift upon PNA binding to DNA was used to determine the extent of strand-invasion. PNA T_{10}-Lys was preincubated with pCXAB to allow strand-invasion. (*A*) The complex was digested with *Bss*HII (B) and *Pvu* II (P) to generate fragments of 217, 181, 107, and 94 bp (and larger fragments). (*B*) Agarose gel electrophoresis was done. The 217-bp fragment contains the A_{10}-binding site for T_{10}-Lys. Lane M contains markers of 298, 220, 201, 154, and 134 bp. The arrow indicates position of the 217-bp fragment that has bound T_{10}-Lys. (*C*) The graph represents quantitation of the gel of *B*. Additional experiments have yielded similar results.

D-Loop Formation Bends or Kinks DNA. The decrease in gel migration due to PNA strand-invasion could be from the increased mass and/or a change in charge of the complex (PNAs have a net positive charge). However, the D-loop structure could bend or kink the DNA helix. It is a well-characterized phenomenon that bent DNA molecules migrate more slowly in polyacrylamide gels than would be predicted on the basis of their size (17–21). Bending of DNA has been described for a number of DNA sequences (periodically spaced runs of adenine), for the interaction of many proteins with DNA, for bulges in DNA, for the binding of ligands (such as spermine), and even for damaged DNA, such as thymidine dimers (17–23). A method commonly used to determine whether the DNA helix is bent at a particular site is to position the suggested bend at various positions relative to the DNA ends. A more pronounced gel retardation is seen when a bend is in the middle of the DNA molecule, instead of near one end (as migration of DNA through a polyacrylamide gel depends on end-to-end distance of the DNA) (19–21).

To examine PNA-specific DNA bending, we used pCXAB, in which the PNA-binding site could be positioned at various distances from the end of restriction fragments, each of which maintained the same overall size (217 bp) (Fig. 4*A*). After strand-invasion by T_{10}-Lys on the pCXAB plasmid, the complex was digested with *Cla* I, *Xho* I, Asp-718, or *Bss*HII, and the fragments were resolved by gel electrophoresis. As

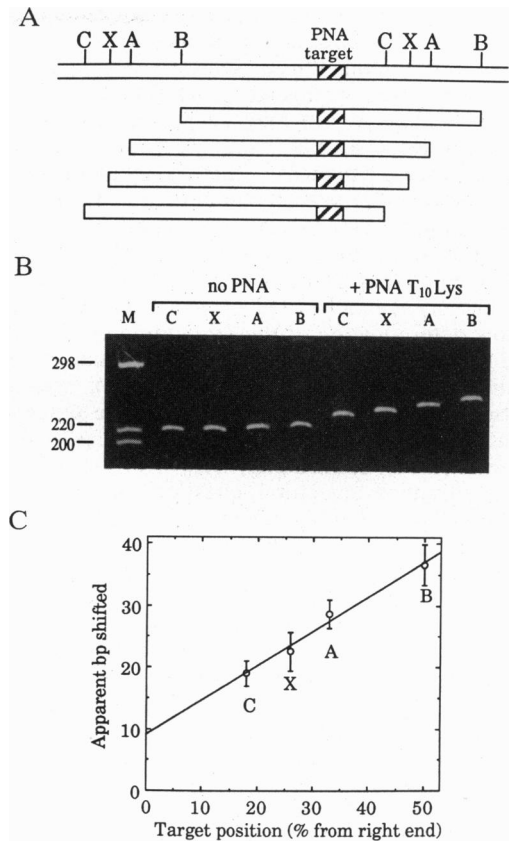


FIG. 4. DNA bending induced by PNA strand-invasion. (*A*) Schematic of pCXAB, indicating positions of restriction sites *Cla* I (C), *Xho* I (X), Asp-718 (A), and *Bss*HII (B) relative to the A_{10} target site for PNA T_{10}-Lys strand-invasion. Digestion of pCXAB with *Cla* I, *Xho* I, Asp-718, or *Bss*HII creates a fragment of 217 bp with the middle of the A_{10} target site 40 bp (18%), 56 bp (26%), 71 bp (33%), and 108 bp (50%) from the right end, respectively. (*B*) Agarose gel of pCXAB digested with *Cla* I, *Xho* I, Asp-718, or *Bss*HII with or without prior strand-invasion with T_{10}-Lys. Lane M contains marker fragments of the indicated sizes. (*C*) Apparent size increase upon T_{10}-Lys binding as a function of DNA-fragment position. Size was determined by measuring migration distance of each restriction fragment with and without PNA T_{10}-Lys bound. Points represent means of four separate experiments (three for Asp-718).

shown in Fig. 4*B*, the restriction fragments all migrated essentially the same in the absence of PNA (however, the *Bss*HII fragment consistently appeared ≈5 bp larger than the other fragments). In the presence of T_{10}-Lys, each fragment exhibited a decrease in gel mobility (Fig. 4*B*, + T_{10}-Lys lanes). The *Cla* I fragment, where the PNA would bind 40 bp (18%) from one end, appeared to increase in size by only 19 bp. In contrast, for the *Bss*HII fragment, where the PNA site was 50% from each end, the apparent size increase was 37 bp. We observed a correlation between the apparent increase in fragment size and the position of the PNA site (Fig. 4*C*), which would be expected were the D-loop to bend the DNA helix.

DISCUSSION

We have shown that PNAs can invade a strand of duplex DNA, with little or no preference for either binding polarity. Although there have been several reports that DNA oligomers can be forced to associate in a parallel fashion (24–26), the antiparallel form is clearly more stable. In parallel DNA, the bases pair via reverse Watson–Crick hydrogen bonds (24) instead of the normal Watson–Crick scheme, and the stability of parallel DNA varies significantly with the sequence. The

failure of the PNA to exhibit a strong orientation preference likely reflects the achiral nature of the polyamide backbone, which does not contain the configuration and conformational restraint about the glycosidic bond found in DNA. The orientation independence may be problematic when using PNAs as antigene agents, as it theoretically doubles the number of target sequences within the human genome.

Egholm *et al.* (8) reported that orientation did not influence PNA binding to single-stranded oligodeoxynucleotides, based on melting temperature (T_m) studies. However, it should be noted that the PNA sequence used in these studies was essentially symmetrical (T_4CT_5-Lys) and, therefore, may not allow determination of the orientation of PNA binding. Changing sequence orientation may not produce the apparent 1-bp mismatch in the middle of their duplex (which would decrease $T_m > 10°C$) (8) because shifting the sequence by 1 bp creates a perfect alignment of the sequences (9 for 9) with a 1-base overhang at each end.

Many intriguing structural questions remain to be answered concerning the binding of a PNA to DNA, including the hydrogen-bonding scheme for each orientation, the type and direction of each helix (A-, B-, or Z-like), and the effects of sequence and mismatches. Others have shown that the T_m of a PNA·DNA heteroduplex is reduced by ≈12°C for each mismatch introduced into the 10-mer oligodeoxynucleotide (6–8). We have extended these findings to address questions relating to PNA target specificity during the strand-invasion of duplex DNA, as measured by pol II termination. By using this approach we conclude that the efficiency of PNA strand-invasion is sensitive to mismatches but that PNA·DNA stability depends on the position and the type of mismatches in the target sequence (data not shown).

With the SV40 15-mer PNA, the rate of strand-invasion was approximately two to three times faster than we and others (6, 9) had reported for T_{10}-Lys. In our earlier studies, we showed that complete strand-invasion of a linear DNA fragment by T_{10}-Lys required 20 min. We suggest that this difference can be attributed to the state of the DNA template (supercoiled vs. linear). We have speculated that the DNA must breathe for the PNA·DNA complex (D-loop) to form. If DNA breathing (formation of a bubble of *X*-bp size in the DNA) is rate limiting, then the target size and sequence and the flanking sequences would probably alter the association rate. Although on-rates varied, off-rates were found to be slow for all PNAs tested to date. This PNA attribute will likely prove beneficial when we begin to analyze the *in vivo* consequences of PNA strand-invasion of a target DNA sequence.

The inhibition of strand-invasion by moderate amounts of NaCl implies that DNA breathing is essential for D-loop formation. In support of this hypothesis, we have observed ≈50% PNA strand-invasion in 100 mM NaCl when the preincubation period is extended to 20 hr. Our observed salt effect is similar to that reported by Nielsen *et al.* (10), in which they observed a decrease in binding of PNA to DNA (monitored by inhibition of restriction enzyme cleavage of the DNA) at higher NaCl concentrations. They also observed that 1 mM Mg^{2+} did not inhibit strand-invasion. However, we observed complete inhibition of strand-invasion in the presence of 15 mM $MgCl_2$ and 1 mM spermine (data not shown). In addition, whereas 44 mM KCl inhibited strand-invasion by ≈50% (similar to NaCl), the addition of 6 mM $MgCl_2$ to the KCl solution completely blocked strand-invasion (data not shown).

The gel retardation we observed upon PAGE with restriction fragments containing the PNA-induced D-loop suggested that this structure bends the DNA helix. There are many examples of events, such as the binding of proteins, that bend DNA. Although several DNA–protein interactions have been implicated in DNA bending, a bulge in the DNA, caused by one strand containing one or more "extra" unpaired bases (27–29), has also been shown to bend the DNA. A D-loop, although not analogous to a bulge, does contain unpaired bases in one strand. The bend or kink caused by the D-loop may result from differential base stacking between the three strands or the PNA·DNA helix itself being curved. It is also possible that the junction between the DNA·DNA helix and that of the PNA·DNA helix might serve as the location of a bend or might act as a hinge in the axis. Regardless of the mechanism, if we can next demonstrate DNA bending on chromatin-containing DNA *in vitro* and *in vivo* (assuming we can devise a chemical means of allowing PNAs to invade a strand of DNA in physiologic salt), this could provide another approach for blocking transcription of a specific gene in a cell.

1. Neckers, L., Whitesell, L., Rosolen, A. & Geselowitz, D. A. (1992) *Critical Reviews in Oncogenesis* (CRC, Boca Raton, FL), Vol. 3, pp. 175–231.
2. Wickstrom, E., ed. (1991) *Prospects for Antisense Nucleic Acid Therapy of Cancer and AIDS* (Wiley–Liss, New York).
3. Maher, L. J., Wold, B. & Dervan, P. B. (1989) *Science* **245**, 725–730.
4. Francois, J.-C., Saison-Behmoaras, T., Thuong, N. T. & Helene, C. (1989) *Biochemistry* **28**, 9617–9619.
5. Hanvey, J. C., Shimizu, M. & Wells, R. D. (1990) *Nucleic Acids Res.* **18**, 157–161.
6. Nielsen, P. E., Egholm, M., Berg, R. H. & Buchardt, O. (1991) *Science* **254**, 1497–1500.
7. Egholm, M., Buchardt, O., Nielsen, P. E. & Berg, R. H. (1992) *J. Am. Chem. Soc.* **114**, 1895–1897.
8. Egholm, M., Nielsen, P. E., Buchardt, O. & Berg, R. H. (1992) *J. Am. Chem. Soc.* **114**, 9677–9678.
9. Hanvey, J. C., Peffer, N. J., Bisi, J. E., Thomson, S. A., Cadilla, R., Josey, J. A., Ricca, D. J., Hassman, C. F., Bonham, M. A., Au, K. G., Carter, S. G., Bruckenstein, D. A., Boyd, A. L., Noble, S. A. & Babiss, L. E. (1992) *Science* **258**, 1481–1485.
10. Nielsen, P. E., Egholm, M., Berg, R. H. & Buchardt, O. (1993) *Nucleic Acids Res.* **21**, 197–200.
11. Hoogsteen, K. (1959) *Acta Crystallogr.* **12**, 822.
12. Maher, L. J., Dervan, P. B. & Wold, B. (1990) *Biochemistry* **29**, 8820–8826.
13. Hanvey, J. C., Williams, E. M. & Besterman, J. M. (1991) *Antisense Res. Dev.* **1**, 307–317.
14. Young, S. L., Krawczyk, S. H., Matteucci, M. D. & Toole, J. J. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 10023–10026.
15. Koh, J.-S. & Dervan, P. B. (1992) *J. Am. Chem. Soc.* **114**, 1470–1478.
16. Sawadogo, M. & Roeder, R. G. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 4394–4398.
17. Hagerman, P. J. (1990) *Annu. Rev. Biochem.* **59**, 755–781.
18. Crothers, D. M., Haran, T. E. & Nadeau, J. G. (1990) *J. Biol. Chem.* **265**, 7093–7096.
19. Crothers, D. M., Gartenberg, M. R. & Shrader, T. E. (1991) *Methods Enzymol.* **208**, 118–146.
20. Crothers, D. M. & Drak, J. (1992) *Methods Enzymol.* **212**, 46–71.
21. Lane, D., Prentki, P. & Chandler, M. (1992) *Microbiol. Rev.* **56**, 509–528.
22. Feuerstein, B. G., Pattabiraman, N. & Marton, L. J. (1990) *Nucleic Acids Res.* **18**, 1271–1282.
23. Husain, I., Griffith, J. & Sancar, A. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 2558–2562.
24. van de Sande, J. H., Ramsing, N. B., Germann, M. W., Elhorst, W., Kalisch, B. W., Kitzing, E. V., Pon, R. T., Clegg, R. C. & Jovin, T. M. (1988) *Science* **241**, 551–557.
25. Germann, M. W., Kalisch, B. W., Pon, R. T. & van de Sande, J. H. (1990) *Biochemistry* **29**, 9426–9432.
26. Klysik, J., Rippe, K. & Jovin, T. M. (1990) *Biochemistry* **29**, 9831–9839.
27. Rice, J. A. & Crothers, D. M. (1989) *Biochemistry* **28**, 4512–4516.
28. Hsieh, C.-H. & Griffith, J. D. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 4833–4837.
29. Bhattacharyya, A. & Lilley, D. M. J. (1989) *Nucleic Acids Res.* **17**, 6821–6840.