Helical Periodicity of GA-Alternating Triple-Stranded DNA†

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ABSTRACT: Homopurine·homopyrimidine tracts (18 or 28 bp) containing predominantly GA-alternating sequences were inserted between two bent DNA loci composed of six $(dA)_6$·$(dT)_6$ repeats. For each homopurine tract, the DNA length between the bent DNA loci was varied by one base pair over a full helical turn. The two series of bent DNA fragments were electrophoresed in 5% polyacrylamide gel to measure the gel mobilities, which reflected the overall extent of DNA bending of each DNA fragment. By comparing the gel mobilities between the two series of bent DNA fragments, the helical periodicity of the GA-alternating duplex was determined to be 10.4 bp/turn. The two series of bent DNA fragments were also electrophoresed upon formation of the intermolecular triplex at the homopurine·homopyrimidine tracts. Comparision of the gel mobilities between the two series of DNA fragments showed that the helical periodicity of triplexed DNA of GA-alternating sequence was 11.2 bp/turn. Triplexed DNA with homopurine and homopyrimidine oligodeoxyribonucleotides had the same helical periodicity, while hybrid triplex DNA associated with homopyrimidine oligoribonucleotide had a slightly more wound structure with 11.1 bp/turn.

The formation of triple-stranded DNA has been demonstrated *in vivo* (Kohwi *et al.*, 1992) as well as *in vitro.* A triple-helix structure between repeated DNA sequences has been suggested to promote homologous recombination in *Escherichia coli* cells (Kohwi & Panchenko, 1993). Transcription initiation (Cooney *et al.*, 1988; Maher, 1992; Maher *et al.*, 1992; Mayfield *et al.*, 1994) and HIV DNA integration (Mouscadet *et al.*, 1994) have been inhibited *in vitro* by means of intermolecular triplex formation. These successes of *in vitro* systems led to applications of triplex formation as a tool to regulate various cellular processes occurring on DNA. Deoxyoligonucleotides with potentially reactive groups attached at one end can be induced to act as highly site-specific endonucleases upon the formation of triple helices with a complementary DNA duplex (Perrouault *et al.*, 1990; Beal & Dervan, 1991).

Extensive studies have been carried out on the structure and formation conditions of triple helices using spectroscopic, chemical, and enzymatic detection methods. X-ray diffraction, nuclear magnetic resonance (Macaya *et al.*, 1992; Radhakrishnan *et al.*, 1993), and vibrational spectroscopy (Ouali *et al.*, 1993) combined with theoretical calculations (Raghunathan *et al.*, 1993) revealed detailed local structures of triple helices. Chemical and enzymatic methods targeted toward single-stranded or triplexed regions were used to study nucleotide-level structures and conditions favoring triplex formation (Htun & Dahlberg, 1988, 1989; Voloshin *et al.*, 1988; Collier & Wells, 1990; Lyamichev *et al.*, 1991; Panyutin & Wells, 1992; Stonehouse & Fox, 1994). The homopurine·homopyrimidine sequences $(dGdA)_n$·$(dCdT)_n$ and $(dG)_n$·$(dC)_n$ can form $(py{\cdot}pu{\cdot}py)_n$-type triple helices under acidic conditions. The $(dGdA)_n$·$(dCdT)_n$ sequence also forms a $(pu{\cdot}pu{\cdot}py)_n$-type triplex in the presence of zinc ions or spermidine (Beltrán *et al.*, 1993; Kohwi & Kohmi-Shigematsu, 1993; Martínez-Balbás & Azorín, 1993), while the same type of triplex is formed by $(dG)_n$·$(dC)_n$ in the presence of magnesium ions (Kohwi & Kohwi-Shigematsu, 1988). The third strand is positioned in the major groove of Watson−Crick duplex, and it forms Hoogsteen (or reverse Hoogsteen) base pairs with purines of Watson−Crick duplex. The third strand runs parallel and antiparallel to the purine strand of Watson−Crick duplex in $(py{\cdot}pu{\cdot}py)_n$ and $(pu{\cdot}pu{\cdot}py)_n$, respectively. Intramolecular triplex formation is stimulated by negative DNA supercoiling through strand separation of the DNA duplex (Kohwi-Shigematsu & Kohwi, 1985; Voloshin *et al.*, 1988; Collier & Wells, 1990).

In this study, we determined the helical periodicity of a triplex DNA by using the fact that the overall bend angle of a DNA fragment having two bent DNA loci depends on the helical phasing between the two bends (Zinkel & Crothers, 1987; Salvo & Grindley, 1987; Tang & Draper, 1990; Drak & Crothers, 1991; Niederweis *et al.*, 1992). The relative magnitude of an overall bend angle was measured by determining the electrophoretic mobility of a DNA fragment in a polyacrylamide gel. The helical periodicity of triplex DNA of poly(dT)·poly(dA)·poly(dT) was determined to be 11.5 bp/turn by NMR (Radhakrishnan & Patel, 1994) and 12 bp/turn by X-ray fiber diffraction (Arnott & Selsing 1974). The physical measurement in this study suggested the helical periodicity to be 11.2 bp/turn for the triplex of GA-alternating sequence. The helical periodicity of a triple helix will be essential information in applying triple helices to the regulation of cellular processes where several factors binding to nearby DNA regions interact with each other.

MATERIALS AND METHODS

Oligonucleotide Synthesis. The following oligonucleotides were used in either cloning or intermolecular triplex formation. The letters "H", "D", "R", "Y", and "U" stand for

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homopurine·homopyrimidine duplex, oligodeoxyribonucleotide, oligoribonucleotide, pyrimidine, and purine, respectively. Numbers in the names indicate the number of nucleotides in the sequences.

H21	TAGAGAGAAGGAGAGAGAGCT (5'→3')
	TCGAATCTCTCTTCCTCTCTC
H22	TGAGAGAGAAGGAGAGAGAGCT
	TCGAACTCTCTCTTCCTCTCTC
H31	TAGAGAGAGAGAGAAGGAGAGAGAGAGAGCT
	TCGAATCTCTCTCTCTCTTCCTCTCTCTCTC
H32	TGAGAGAGAGAGAGAAGGAGAGAGAGAGAGCT
	TCGAACTCTCTCTCTCTCTTCCTCTCTCTCTC
DY28	TCTCTCTCTCTCTTCCTCTCTCTCTCTC (5'→3')
DY18	TCTCTCTTCCTCTCTCTC
DY16	TCTCTCTTCCTCTCTC
RY18	UCUCUCUUCCUCUCUCUC
DU16	GAGAGAGGAAGAGAGA

Some of the oligodeoxyribonucleotides were synthesized using an Applied Biosystems DNA synthesizer at Yale University, and others were custom-synthesized by Bio-Synthesis Inc. (Texas) or by Korea Biotechnology Inc. (Korea). The oligoribonucleotide RY18 was made at the oligonucleotide synthesis facility in the medical school of Yale University. The duplexed oligonucleotides H21, H22, H31, and H32 were used in cloning without an extensive purification. The DNA sequences inserted in plasmid DNAs were determined by Sanger's dideoxynucleotide method using T7 sequenase (Stratagene). The remaining oligonucleotides which were used as the third strand in intermolecular triplex formation were purified by denaturing polyacrylamide gel electrophoresis followed by electroelution.

Construction of Plasmid DNAs. The duplexed deoxyoligonucleotides H21, H22, H31, and H32 were phosphorylated at the 5′-ends using T4 polynucleotide kinase and ATP. The plasmid LB series constructed by Drak and Crothers (1991) were linearized at the *Sac*I site, dephosphorylated using calf intestine phosphatase, and then ligated with the duplexed oligonucleotides using T4 DNA ligase. The plasmid LB series consisted of six plasmid DNAs differing in by 2 bp DNA length between two bent DNA loci from the next plasmid in the series. Each bent DNA locus contained six $(dA)_6$·$(dT)_6$ repeats at an average distance of 10.5 bp, and one locus had two d(AATT) sequences contributing additional DNA bends at the circumference. The plasmids that had H21 or H22 sequences inserted in the *Sac*I site of LB series plasmids were named MB series plasmids. Similarly, the plasmids that had H31 or H32 sequences in the same site were named NB series plasmids. The potentially triplex-forming sequences were located between the two bent DNA sites in the MB and NB series plasmid DNAs, as illustrated in Figure 1. The orientations of insertion were determined by cleavage at the *Sac*I site, which was regenerated at only one junction between the inserts and LB plasmid DNAs. Only those plasmid DNAs that had the homopurine strand located in the same strand as thymine tracts of the bent DNA loci were used in this study.

Intermolecular Triplex Formation. Plasmid DNAs of the MB and NB series were digested with *Pvu*II and *Rsa*I simultaneously, extracted with phenol, and then precipitated in ethanol solution. The digested DNA products of each plasmid DNA (150 ng) were mixed with 0.5 μg of the single-stranded oligonucleotides above in 10 μL of the buffer solution (90 mM Tris·acetate, pH 5.0). The reaction mixture was incubated at 37 °C for 1 h and then at 16 °C for another 1 h.

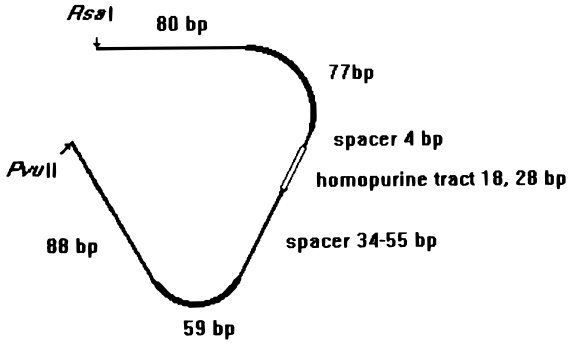

FIGURE 1: Schematic diagram of the bent DNA fragments produced by *Pvu*II−*Rsa*I digestion of the MB and NB series plasmid DNAs. The MB and NB series plasmid DNAs have the 18 and 28 bp long homopurine·homopyrimidine tracts, respectively. The DNA length between the two bent DNA loci was varied by 1 bp for a full helical turn in each plasmid series. Each of the two bent DNA loci contained six $(dA)_6$·$(dT)_6$ repeats separated by the average distance of 10.5 bp, and the 77 bp long bent DNA locus had additional DNA bends contributed by the d(AATT)·d(TTAA) sequence located at both sides of the locus.

Gel Electrophoresis. The DNA fragments incubated for triplex formation were electrophoresed in 5% polyacrylamide gel (acrylamide:bisacrylamide, 29:1) at a voltage gradient of 7.5 V/cm for 15−20 h in the cold room at 4 °C. The electrophoresis buffer was the same as the incubation buffer for triplex formation and was recirculated between the two chambers of opposite electrodes during electrophoresis. The DNA bands in the gels were visualized by silver staining.

RESULTS

In order to measure the helical periodicity of triplex DNA, two homopurine·homopyrimidine sequences with a potential for triplex formation were inserted to LB plasmid series, as illustrated in Figure 1. The six LB series plasmid constructed by Drak and Crothers (1991) had 2 bp differences in the length of spacer DNA between the two bent DNA loci. Insertion of the duplexed oligonucleotides H21 and H22 at the *Sac*I site of the six LB plasmids produced 12 plasmid DNAs, named the MB series. These MB series plasmids contained the 18 bp long homopurine·homopyrimidine tract and had increments of 1 bp in the spacer DNA length between the two bent DNA loci instead of 2 bp as in the LB series. Using the duplexed oligonucleotides H31 and H32, NB series plasmids containing the 28 bp long homopurine·homopyrimidine tract were constructed in the same way as for the MB series plasmids. In the middle of the 18 and 28 bp long homopurine tracts consisting of mostly GA-alternating sequence, an AAGG sequence was deliberately positioned to prevent a third strand from sliding during intermolecular triplex formation. These plasmid DNAs were named as MB1 to MB12 and NB1 to NB12 in the increasing order of the spacer DNA length in each series.

Upon digestion of the MB and NB plasmid DNAs with *Pvu*II and *Rsa*I restriction enzymes, three DNA fragments of 405, 676, and 1545 bp and a bent DNA segment

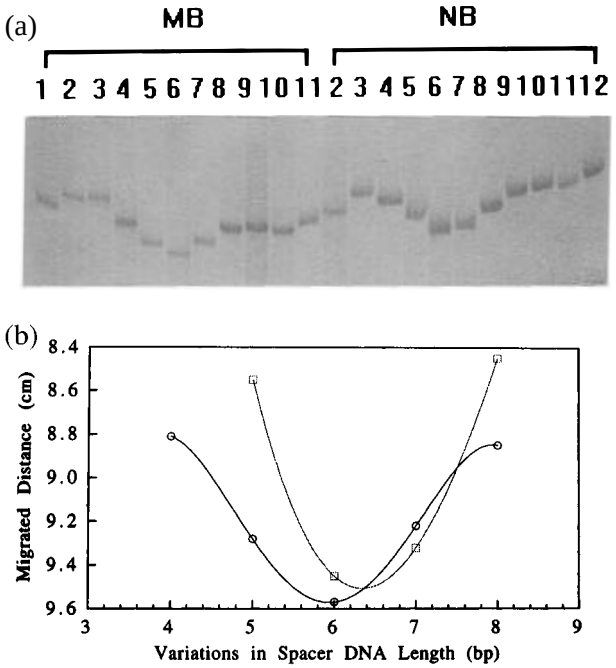

FIGURE 2: Gel mobilities of the bent DNA fragments of the MB and NB series plasmd DNAs. (a) The *Pvu*II−*Rsa*I DNA fragments of MB and NB series plasmids were electrophoresed in 5% polyacrylamide gel (acrylamide:bisacrylamide, 29:1) in the buffer solution (90 mM Tris·acetate, pH 5.0) at 4 °C. The numbers indicate increments in the length between the two bent DNA loci in each plasmid series. (b) Gel mobility data around the maximum mobility point in each series of panel a were fitted to a polynomial function to estimate the exact incremental DNA length at the maximum mobility. Open circles and rectangles are data points of the MB and NB series plasmids, respectively. Deviations from a helical repeat of 10.0 bp/turn are reflected in the shift of the NB minimum relative to the MB minimum.

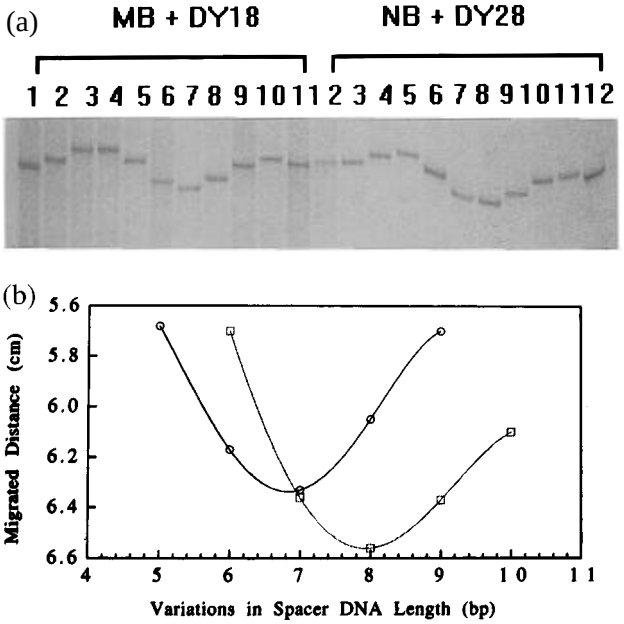

FIGURE 3: Gel mobilities of the bent DNA fragments of the MB and NB series plasmid DNAs upon intermolecular triplex formation. (a) The *Pvu*II−*Rsa*I DNA fragments of MB and NB series plasmids were incubated with DY18 and DY28 deoxynucleotides, respectively, in the buffer solution (90 mM Tris·acetate, pH 5.0). The incubated DNA mixtures were electrophoresed at the same conditions as described in Figure 2. (b) Gel mobility data around the maximum mobility point in each series of panel a were fitted to a polynomial function. Open circles and rectangles are data points of the MB and NB series plasmids, respectively.

diagrammed in Figure 1 were produced for each plasmid DNA. The DNA fragments were electrophoresed in 5% polyacrylamide gel (Tris·acetate 90 mM, pH 5.0) at 4 °C to measure the effects of the spacer DNA length on gel mobilities of the bent DNA fragments, as shown in Figure 2a. The bent DNA fragments with lengths ranging from 360 to 381 bp had retarded gel mobilities, falling between the 405 and 1545 bp long DNA fragments in 5% polyacrylamide gel (data not shown). For both of the MB and NB series plasmids, gel mobilities of the bent DNA fragments showed quasi-sinusoidal curves with respect to the DNA lengths between the two bent DNA loci. Earlier work (Zinkel & Crothers, 1987; Salvo & Grindley, 1987) demonstrated that the gel mobilities of bent DNA fragments reflect the helical phasing between the two bend loci. The gel mobility is minimized due to a maximized overall bending, when two bend centers are separated by an integral multiple of the helical periodicity of the DNA segment between the two bend centers. However, a distance between the two bend centers that is equal to a half-integral multiple of the helical periodicity results in a maximum mobility. Because the gel mobility curves with respect to the incremental DNA length in Figure 2a were not exactly sinusoidal, the points near a mobility maximum were used to compare the two gel mobility curves of the MB and NB series. The maximum mobility point in each series was estimated by fitting 4−5 flanking data points to a polynomial function. The incremental DNA length between the two bent DNA loci at a maximum gel mobility was 5.9 for the MB series and 6.3 for the NB series, as illustrated in Figure 2b. The bent DNA fragments of the NB series have GA-alternating homopurine·homopyrimidine tracts that are 10 bp longer than those of the MB series. The shift of 0.4 bp in the DNA length at the maximum mobility point for the NB series suggested that the helical periodicity of the GA-alternating DNA duplex is 10.4 bp/turn. In order to examine whether the measurement is dependent on the percentage of polyacrylamide gel, the experiments in Figure 2 were carried out in 8% polyacrylamide gel. The positions of maximum gel mobility were changed to 4.8 and 5.3 for the MB and NB series, respectively, suggesting a helical periodicity of 10.5 bp/turn (data not shown). However, in higher percentage polyacrylamide gels, gel mobilities of the bent DNA molecules were affected by superhelical chirality as well as overall bend angle (Drak & Crothers, 1991). Therefore, the value of 10.4 bp/turn obtained in lower percentage polyacrylamide gel is thought to be more accurate for the helical periodicity of GA-alternating duplex.

In order to measure the helical periodicity of a triplexed GA-alternating sequence, an intermolecular triplex was formed at the homopurine·homopyrimidine tracts of MB and NB plasmid DNAs. The DNA fragments generated by *Pvu*II−*Rsa*I restriction digestion of MB and NB plasmid DNAs were incubated with the 18 bp long homopyrimidine deoxynucleotide DY18 and the 28 bp long homopyrimidine deoxyoligonucleotide DY28, respectively, in the buffer solution (Tris·acetate 90 mM, pH 5.0). After the incubation, the DNA mixtures were electrophoresed in 5% polyacrylamide gel at the same electrophoresis conditions as described in Figure 2. The incremental DNA lengths at maximum mobilities were determined to be 6.8 for the MB series and 8.0 bp for the NB series, as shown in Figure 3. Therefore,

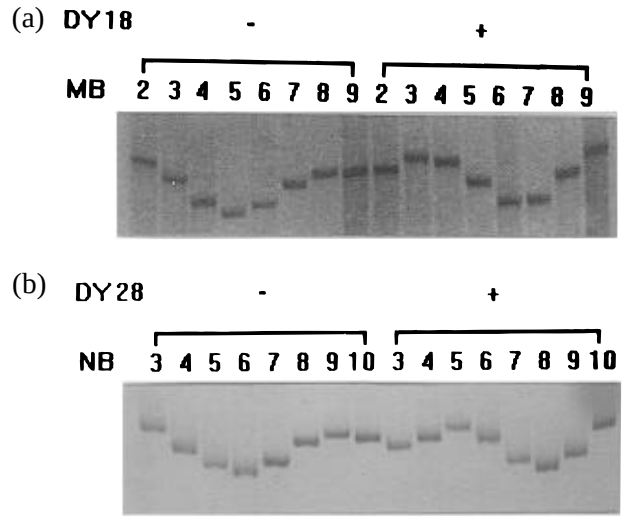

FIGURE 4: Changes in the gel mobilities of the bent DNA fragments of MB and NB series plasmid DNAs by intermolecular triplex formation. The electrophoresis conditions were the same as in Figure 2. (a) The *Pvu*II−*Rsa* I DNA fragments of MB series plasmids were incubated with or without DY18 oligodeoxyonucleotides in the buffer solution (90 mM Tris·acetate, pH 5.0). (b) The *Pvu*II−*Rsa*I DNA fragments of NB series plasmids were incubated in the buffer solution (90 mM Tris·acetate, pH 5.0) in the presence or absence of DY28 oligodeoxynucleotide.

the 1.2 bp shift in the DNA length suggested the helical periodicity of GA-alternating sequence associated with the homopyrimidine oligodeoxynucleotide to be 11.2 bp/turn.

The results in Figure 3 showed the effects of increasing the triplexed region from 18 to 28 bp on gel mobilitties of the bent DNA fragments, thus eliminating the influence of any possible end effects occurring at the boundaries of a triplexed region in the bent DNA fragments. The results in Figures 2 and 3 showed an increase of 0.8 bp/turn in the helical periodicity when the GA-alternating DNA duplex was changed to a triplex. To check for any end effects occurring at the junction between an intermolecular triplex DNA and its juxtaposing duplex DNA, the bent DNA fragments were electrophoresed after incubation in the presence and absence of third strands. A 1.3 bp shift was observed in the DNA length with a maximum mobility on 18 bp long triplex formation, as shown in the 5% polyacrylamide gel of Figure 4a. A 2.1 bp shift was observed in Figure 4b, when the 28 bp long duplex was transformed to a triplex. If the end effects are assumed to be nonexistent, then 1.3 and 2.0 bp shifts were expected for the 18 and 28 bp long triplex formation, respectively, on the basis of the helical repeats determined from Figures 2 and 3. Therefore, the end effects due to a possible structural distortion at the junction between the contiguous duplex and triplex DNAs seemed to be negligible. The positions of maximum mobility in Figure 4 did not exactly match those of the corresponding samples in Figures 2 and 3. Although the positions varied slightly in each experiment, the net shifts between two positions in one gel were consistent.

The DNA length at maximum gel mobility was shortened by 0.2 bp when the 18 bp long homopyrimidine deoxyribonucleotide DY18 was replaced by the homopyrimidine ribonucleotide RY18 in the intermolecular triplex formation of the MB series, as shown in Figure 5. Thus, a slightly more wound triplex with a helical screw of 11.1 bp/turn was formed when the third strand was a oligoribonucleotide. To

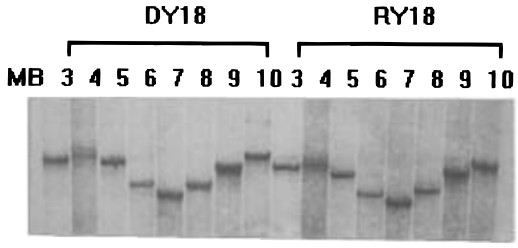

FIGURE 5: Dependence of the gel mobilities of the bent DNA fragments of MB series plasmid DNAs on the third strand of the intermolecular triplex. The *Pvu*II−*Rsa*I DNA fragments of MB series plasmids were incubated in the buffer solution (90 mM Tris·Acetate, pH 5.0) with DY18 oligodeoxyribonucleotide or RY18 oligoribonucleotide. Electrophoresis was carried out as described in Figure 2.

examine whether triplex with a homopurine third strand has a different helical periodicity, DNA fragments prepared by *Pvu*II−*Rsa*I digestion of the MB series plasmid DNAs were incubated with either the homopyrimidine deoxynucleotide DY16 or the homopurine deoxynucleotide DU16 in identical buffer solutions (90 mM Tris·acetate, pH 6.0, 10 mM zinc acetate). The DNA samples were electrophoresed in 5% polyacrylamide gel with the same buffer solution, in which zinc ions were needed to stabilize the triplex with a purine third strand. No difference was found in the positions of maximum gel mobility between the two series of bent DNA fragments, suggesting that a triplex with a homopurine third strand has the same helical periodicity as the triplex with a homopyrimidine third strand (data not shown). When the experiment in Figure 4 with and without the 18 bp long deoxynucleotide was repeated in the buffer condition of pH 6.0, with and without 10 mM magnesium chloride, no distinguishable changes in the net shift of the spacer DNA length with a maximum mobility were observed. The helical periodicity of a triplex DNA did not seem to be significantly affected by pH change or by presence of magnesium ions.

DISCUSSION

The gel mobilities were measured for the two series of DNA fragments having 18 or 28 bp long homopurine·homopyrimidine tracts with varied lengths of spacer DNA between two bent DNA loci. The helical periodicity of the duplexed form of GA-alternating sequence was determined to be 10.4 bp/turn in Figure 2. This estimate is very close to the value 10.4−10.6 bp/turn for a random sequence DNA obtained by DNase I (Rhodes & Klug, 1980) or hydroxy radical reaction (Tullius & Dombroski, 1985) of DNA precipitated on a solid surface, and the gel mobility shift method of plasmid DNA topoisomers (Wang, 1979). Among the homopurine·homopyrimidine sequences, poly(dA)·poly(dT) was found to have a helical periodicity of 10.0 bp/turn (Peck & Wang, 1981; Rhodes & Klug, 1981). Using the same approach as used in this study, the helical periodicity of poly(dG)·poly(dC) was determined to be 11.1 bp/turn (Biburger *et al.*, 1994). It was not unexpected for the GA-alternating sequence to have a helical periodicity between the values of the two homopolynucleotides.

The helical periodicity of a triplex form of GA-alternating sequence with a complementary third DNA strand was determined to be 11.2 bp/turn, as shown in Figure 3. This value is smaller than 11.5 bp/turn based on NMR data (Radhakrishnan & Patel, 1994) or 12.0 bp/turn obtained by

X-ray diffraction of poly(dA)·poly(dT)·poly(dT) (Arnott & Selsing, 1974). However, Ouali *et al.* (1993) proposed a smaller helical periodicity of 11.04 bp/turn for the triplex of GA-alternating sequence by molecular modeling. Triplex DNAs of GA-alternating sequence with homopyrimidine and homopurine DNA third strands had the same helical periodicity. However, the hybrid triplex associated with a homopyrimidine RNA third strand was more wound by 0.1 bp/turn than the homogeneous DNA triplex. This observation may be related to the fact that a triplex with an RNA third strand is thermodynamically more stable than the homogeneous DNA triplex (Roberts & Crothers, 1992; Escudé *et al.*, 1993). Changing the pH to 6.0 or adding magnesium ions did not affect the helical periodicity of triplex DNA. Since magnesium ions wind duplex DNA slightly, triplex DNA may be more wound in the presence of magnesium ions, to an extent not detectable in our measurement. The fact that intramolecular triplex formation is promoted by negative DNA supercoiling could be partly due to the unwound structure of triplex DNA relative to duplex DNA (White *et al.*, 1988).

On the basis of a gel mobility assay and theoretical modeling (Chomilier *et al.*, 1992) it has been proposed that DNA bending occurs at the junction of duplex and triplex regions in a DNA fragment. However, the work of Maher *et al.* (1992) argues against bending using the same gel mobility assay. Our preliminary results (data not shown) support the absence of bending at the junction. Ouali *et al.* (1993), using spectroscopic and molecular modeling methods, suggested that triplex DNA probably has a B-like rather than an A-like conformation. Therefore, the bending expected at the junction between contiguous A-DNA and B-DNA regions is not likely to occur at the junction between duplex and triplex DNAs. Thus, the net shifts in the spacer DNA lengths with maximum mobility in Figures 3 and 4 were not likely to be complicated by the possibility of bending at the junction.

Although the helical periodicity of a triplex DNA has been reported by NMR (Radhakrishnan & Patel, 1994) and by molecular modeling (Ouali *et al.*, 1993), the more direct physical measurement used here is likely to produce a more accurate value. This information will be valuable in the application of triplex formation, especially in promoter modulation. Since there are several DNA sequences acting in transcription initiation, the helical phasing between these DNA sequences with bound proteins are important in the control of transcription initiation.

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