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Helical Periodicity of $(dT)_n \cdot (dA)_n \cdot (dT)_n$ Triple-Stranded DNA

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Abstract : The helical periodicity of the triple-stranded $(dT)_n \cdot (dA)_n \cdot (dT)_n$ sequence was determined by measuring gel-mobilities of bent DNA fragments containing the sequence. In the bent DNA fragments, a $GA_{22}G \cdot CT_{22}C$ sequence was located between two bent DNA loci composed of six $A_6 \cdot T_6$ repeats, and the DNA length between the bent DNA loci was varied by 1 base pair over a full helical turn. The gel mobility of each bent DNA fragment reflected the overall extent of DNA bending and varied with the DNA length between the two bent loci. Mobilities of the bent DNA fragments in 5% polyacrylamide gel were measured after preincubating the DNA fragments both in the presence and absence of $CT_{22}C$ oligonucleotide. By comparing the bent DNA fragments containing an intermolecular triplex structure with those of a genuine duplex structure in the gel mobilities, the helical periodicity of the $T_n \cdot A_n \cdot T_n$ triplex DNA was determined to be 11.5 (±0.3) bp/turn.
Key words : DNA bending, gel mobility, helical screw, triplex DNA

A triplex structure of homopurine·homopyrimidine sequence generally inhibits various biological processes occurring on DNA by preventing proteins from binding to the sequence or by blocking the movement of DNA-tracking enzymes (Lu and Ferl, 1993). Transcription initiation (Cooney *et al.*, 1988; Maher, 1992; Maher *et al.*, 1992; Mayfield *et al.*, 1994; Tu *et al.*, 1995), HIV DNA integration (Mouscadet *et al.*, 1994), and movement of DNA and RNA polymerases (Young *et al.*, 1991; Sarkar and Brahmachari, 1992; Duval-Valentin *et al.*, 1992; Samadashwily *et al.*, 1993) have all been inhibited by a triplex DNA structure. In relatively rare cases, the triple helix structure has been implicated in promoting such cellular reactions as homologous recombination (Kohwi and Panchenko, 1993; Rooney and Moore, 1995) and transcription of the human *decorin* gene (Santra *et al.*, 1994). A triplex structure can be also applied to chemical-modification and cleavage of specific DNA sites using oligonucleotides with potentially reactive groups (Perrouault *et al.*, 1990; Beal and 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 (Arnott and Selsing, 1974), nuclear magnetic resonance (Macaya *et al.*, 1992; Radhakrishnan and Patel, 1994a,b), 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 and Dahlberg, 1988; Voloshin *et al.*, 1988; Htun and Dahlberg, 1989; Collier and Wells, 1990; Lyamichev *et al.*, 1991; Panyutin and Wells, 1992; Stonehouse and Fox, 1994). Under proper conditions, a homopurine·homopyrimidine sequence can form triple helix of $(py \cdot pu \cdot py)_n$ type or $(pu \cdot pu \cdot py)_n$ type (Beltrán *et al.*, 1993; Kohwi and Kohmi-Shigematsu, 1993; Martínez-Balbás and Azorín, 1993). The third strand forms Hoogsteen-base pairs in the major groove of the Watson-Crick duplex and runs parallel to the purine strand of the Watson-Crick duplex in $(py \cdot \cdot pu \cdot py)_n$, while it forms reverse Hoogsteen-base pairs and runs antiparall in $(pu \cdot pu \cdot py)_n$ (Frank-Kamenetskii and Mirkin, 1995).

Previously, we determined the helical periodicity of GA-alternating triplex DNA (Shin and Koo, 1996) 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 DNA bends (Salvo and Grindley, 1987; Zinkel and Crothers, 1987; Tang and Draper, 1990; Drak and Crothers, 1991; Niederweis *et al.*, 1992). The magnitude of an overall bend angle was measured by the electrophoretic mobility of a DNA fragment in polyacrylamide gel. Using the same method in this study, the helical periodicity of $(dT)_n \cdot (dA)_n \cdot (dT)_n$ triplex was determined to be 11.5(±0.3) bp/turn.

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Materials and Methods

Oligodeoxyribonucleotide synthesis

The following oligodeoxyribonucleotides were synthesized by KOMA Biotech or by Bioneer, Inc. in Korea. Duplexed oligodeoxyribonucleotides A_{22}-28 and A_{22}-29, of which the last two digits in each name indicate the total number of nucleotides, were used in cloning without an extensive purification. $CT_{22}C$ oligodeoxyribonucleotide was purified by electrophoresis in a 20% denaturing polyacrylamide gel followed by electroelution.

A_{22}-28 G(A_{22})GAGCT (5' → 3')
TCGAC(T_{22})C

A_{22}-29 GG(A_{22})GAGCT (5' → 3')
TCGACC(T_{22})C

$CT_{22}C$ $CT_{22}C$

Construction of plasmid DNAs

The duplexed oligodeoxyribonucleotides A_{22}-28 and A_{22}-29 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 the DNA length between two bent DNA loci by 2 bp from the next plasmid in the series. Each bent DNA locus contained six $A_6 \cdot T_6$ repeats at an average distance of 10.5 bp. The plasmids which had A_{22}-28 or A_{22}-29 sequences inserted at the *Sac*I site of LB series plasmids were named as UB series plasmids. The triplex-forming sequence was located between the two bent DNA sites in the UB series plasmid DNAs, as illustrated in Fig. 1. Insertional orientations of the A_{22} oligodeoxynucleotides in the UB series plasmid DNAs were determined by mapping 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.

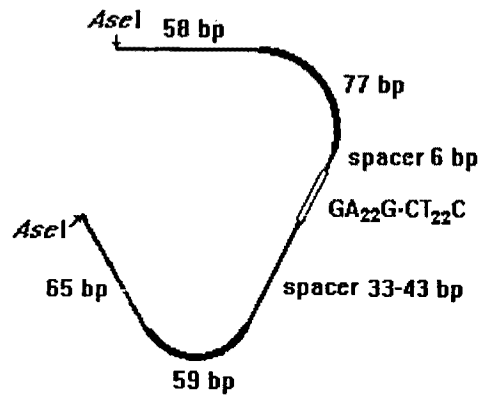

Fig. 1. Schematic diagram of the bent DNA fragments produced by *Ase*I digestion of the UB series plasmid DNAs. The DNA length between the two bent DNA loci varied by 1 bp for a full helical turn in the plasmid series. Each of the two bent DNA loci contained six $(dA)_6 \cdot (dT)_6$ repeats separated by an 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. The actual helical phasing between the two bent loci changed as the DNA length between the two bent loci varied.

Intermolecular triplex formation

Plasmid DNAs of the UB series were digested with *Ase*I restriction enzyme, extracted with phenol, and then precipitated in an ethanol solution. The digested DNA products of each plasmid DNA (40 ng) were mixed with 0.5 μg of $CT_{22}C$ oligodeoxyribonucleotide in 10 μl of the buffer solution (90 mM Tris·acetate, pH 6.0, 50 mM $MgCl_2$) (Pilch *et al.*, 1990). The reaction mixture was incubated at 4°C for 3 h.

Gel electrophoresis

After being preincubated in both the presence and absence of $CT_{22}C$ oligodeoxyribonucleotide, the bent DNA fragments were electrophoresed in 5% polyacrylamide gels (acrylamide:bisacrylamide, 29:1) at a voltage gradient of 5 volt/cm for 48 h in a cold room of 4°C. The electrophoresis buffer was the same as the preincubation buffer for triplex formation and was recirculated between the two chambers of opposite electrodes during electrophoresis. DNA fragments in the gels were visualized by silver staining.

Results and Discussion

In order to measure the helical periodicity of $T_n \cdot A_n \cdot T_n$ triplex DNA, a $GA_{22}G \cdot CT_{22}C$ sequence with a potential of triplex formation was inserted into the LB plasmid series, as illustrated in Fig. 1. Six LB series plasmids constructed by Drak and Crothers (1991) had 2 bp differences in the length of spacer DNA between the two bent DNA loci. By inserting the duplexed oligonucleotides A_{22}-28 and A_{22}-29 at the *Sac*I site of LB plasmids, 11 plasmids named as UB series were constructed. These plasmids had increments of one base pair in the spacer DNA length between the two bent DNA loci and were named as UB1 to UB11 in the increasing order of the spacer DNA length.

Upon digesting each of the UB plasmid DNAs with *Ase*I restriction enzyme, three DNA fragments of 23,

1,235, and 1,477 bp and a bent DNA fragment diagrammed in Fig. 1 were produced. The DNA fragments were electrophoresed in a 5% polyacrylamide gel (Tris·acetate 90 mM, pH 6.0, 50 mM Mg^{2+}) at 4°C to measure the gel mobilities, as shown in Fig. 2(a). The bent DNA fragments with lengths ranging from 322 bp to 332 bp had retarded gel mobilities corresponding to about 730 bp long DNA fragments in the 5% polyacrylamide gel. Gel mobilities of the bent DNA fragments showed a quasi-sinusoidal curve with respect to the DNA lengths between the two bent DNA loci. Earlier work (Salvo and Grindley, 1987; Zinkel and Crothers, 1987) demonstrated that the gel mobility of a bent DNA fragment reflects the helical phasing between two bent loci in a bent DNA fragment. 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 equal to a half-integral multiple of the helical periodicity results in a maximum mobility. The bent DNA fragment produced from the UB3 plasmid DNA showed a minimum gel mobility in Fig. 2(a) and was thought to have the two DNA bends composed of repeated $A_6 \cdot T_6$ sequences at cis orientation. The two bent DNA fragments produced from the UB6 and UB7 plasmid DNAs showed gel mobilities close to a maximum in Fig. 2(a), and the relative orientation between the two DNA bends in each of these DNA fragments could be approximated at *trans*.

In order to form an intermolecular triplex at the $GA_{22}G \cdot CT_{22}C$ sequence of the bent DNA fragments, the bent DNA fragments were preincubated with $CT_{22}C$ oligodeoxynucleotide in the same buffer solution as used in Fig. 2(a). When the bent DNA fragments were electrophoresed after the incubation, a quasi-sinusoidal dependence of the gel mobilities on the DNA length between the two bent DNA loci was also observed (Fig. 2(b)). Triplex formation at the sequence did not occur either when T_{22} oligodeoxynucleotide was used in the place of $CT_{22}C$ oligodeoxynucleotide or when Mg^{2+} concentration was lowered from 50 mM to 30 mM. The overall dependence of the gel mobilities on the DNA length between the two bent DNA loci was lessened in Fig. 2(b) compared to that in Fig. 2(a). This phemomenon can be partially ascribed to a possible momentary perturbation in the barely stabilized intermolecular triplex structure.

Because the gel mobility curves with respect to the incremental DNA length in Fig. 2 were not exactly sinusoidal, the points near either a mobility minimum or a mobility maximum were used to compare the two gel mobility patterns in Fig. 2. Several bent DNA fragments near the minimum and maximum mobility points were electrophoresed again in the same polyacrylamide gel of Fig. 3(a), some with and some without the intermolecular triplex formation. The gel mobilities were plotted against the incremental DNA length, and 4 data points near a minimum or maximum mobility were fitted to a polynomial function using the Kaleidagraph program (Abelbeck software). The incremental DNA length between the two bent DNA loci at a minimum gel mobility was 3.1 bp before the triplex formation and then shifted to 5.8 bp with the triplex formation as illustrated in Fig. 3(b). The corresponding shift at the maximum mobility point was from 6.4 bp to 10.0 bp. The net shifts in the incremental DNA length at the minimum and maximum mobility points were +2.7 and +3.6 bp, respectively. The disagreement between the two net shifts reflected the fact that the two quasi-sinusoidal mobility patterns in Fig. 2 could not be fitted to an identical function. Therefore, the shift between the two gel mobility curves was calculated to be +3.2 bp upon the triplex formation by taking an average value between the minimum and maximum mobility points.

Based on the shift of +3.2 bp between the two gel mobility curves in Fig. 2(b), the average helical periodicity of the $GA_{22}G \cdot CT_{22}C$ sequence was calculated to have increased by 1.5 bp/turn upon the triplex formation. The calculated value of 1.5 bp/turn has an er-

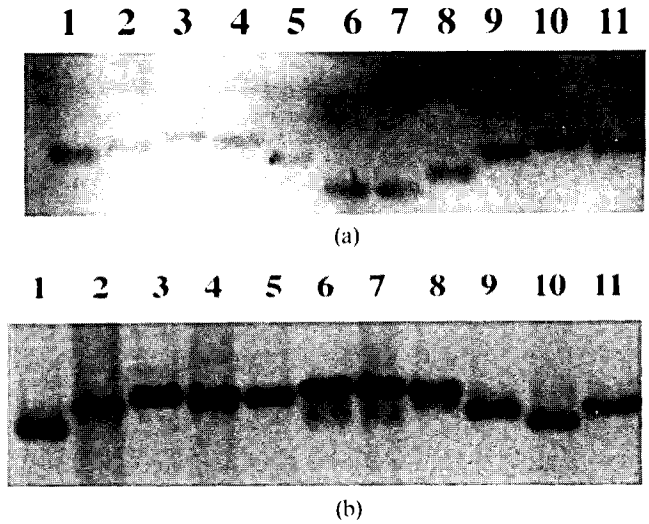

Fig. 2. Gel mobilities of the bent DNA fragments produced from the UB series plasmid DNAs. The *Asel-Asel* DNA fragments of the UB series plasmids were electrophoresed in a 5% polyacrylamide gel (acrylamide:bisacrylamide, 29:1) in the buffer solution (90 mM Tris·acetate, pH 6.0, 50 mM $MgCl_2$) at 4°C in Panel (a). The numbers indicate increments in the length between the two bent DNA loci in the plasmid series. In Panel (b), the Asel-Asel DNA fragments were preincubated with $CT_{22}C$ oligodeoxynucleotide in the buffer solution (90 mM Tris·acetate, pH 6.0, 50 mM $MgCl_2$) at 4°C for 3 h, and then electrophoresed as described in Panel (a).

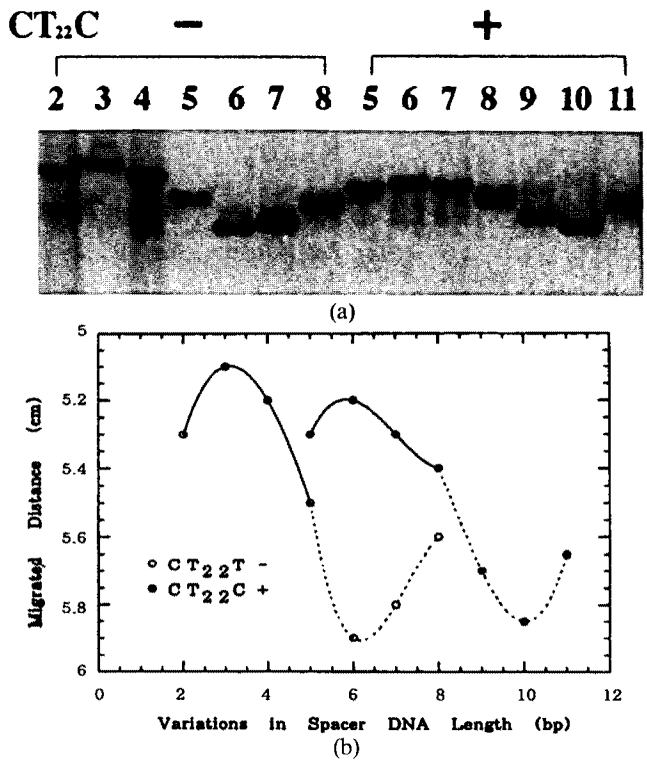

Fig. 3. Changes in the gel mobilities of the bent DNA fragments of the UB series plasmid DNAs by intermolecular triplex formation. (a) The *Ase*I-*Ase*I DNA fragments of the UB series plasmids were incubated both with and without $CT_{22}C$ oligodeoxynucleotide in the buffer solution (90 mM Tris·acetate, pH 6.0, 50 mM $MgCl_2$). The electrophoresis conditions were the same as in Fig. 2. The numbers indicate increments in the length between the two bent DNA loci in the plasmid series. (b) Gel mobility data around the minimum and maximum mobility points in Panel (a) were fitted to a polynomial function. Closed circles and open circles are data points obtained with and without $CT_{22}C$ oligodeoxynucleotide, respectively.

ror range of ±0.3 bp/turn, considering the difference in the shifts at the minimum and maximum mobility points. The helical periodicity of the GA-alternating sequence was determined to be 10.5 bp/turn in the duplexed form and 11.2 bp/turn in the triplexed form using the same method as used here (Shin and Koo, 1996). The change in the helical periodicity of the GA-alternating sequence with pu·pu·py triplex formation was +0.7 bp/turn, which is much smaller than the +1.5 bp/turn obtained here for the $GA_{22}G{\cdot}CT_{22}C$ sequence. In order to calculate the helical periodicity of the $T_n{\cdot}A_n{\cdot}T_n$ triplex, it is necessary to know a change in the helical periodicity of the $A_n{\cdot}T_n$ sequence with the triplex formation. If we assume that the helical periodicity at the G·C base pairs of the $GA_{22}G{\cdot}CT_{22}C$ sequence was increased by the same magnitude as in the GA-alternating sequence, the change in the helical periodicity at the $A_{22}{\cdot}T_{22}$ sequence is deduced to be 1.52 bp/turn. Since this deduced value is within the error range of the calculated value of 1.5 bp/turn, we can treat 1.5 bp/turn as an increase in the helical periodicity of the $A_n{\cdot}T_n$ sequence with the triplex formation. The helical periodicity of $A_n{\cdot}T_n$ was determined to be 10.0 bp/turn by distributional analyses of plasmid DNA topoisomers (Peck and Wang, 1981) and DNase I digestion of DNA on a solid surface (Rhodes and Klug, 1981). Therefore, the helical periodicity of the $T_n{\cdot}A_n{\cdot}T_n$ triplex can be calculated to be 11.5 (±0.3) bp/turn.

Before drawing our conclusion on the helical periodicity of the $T_n{\cdot}A_n{\cdot}T_n$ triplex, a few factors which may affect the helical periodicity will be considered. One is a possible DNA bending at the $A_{22}{\cdot}T_{22}$ sequence. The $A_{22}{\cdot}T_{22}$ sequence in the bent DNA fragments has 21 ApA dinucleotide steps corresponding to two helical turns plus one extra dinucleotide step. Since DNA bending at each ApA dinucleotide step is cancelled out by DNA bending in the opposite direction located five base pairs away, the overall extent of DNA bending at the $A_{22}{\cdot}T_{22}$ sequence is negligible compared to that at the two bent loci, where each is composed of six $A_6{\cdot}T_6$. A structural distortion at the junction between an intermolecular triplex DNA and its juxtaposing duplex DNA has been reported (Kane *et al.*, 1995). However, the possibility that a transitional structure at the junction may affect the gel mobilities was excluded in the previous report (Shin and Koo, 1996).

The helical periodicities of triplex DNAs have been determined by different methods. The helical periodicity of the $T_n{\cdot}A_n{\cdot}T_n$ triplex was determined to be 12.0 bp/turn or 13.0 bp/turn by X-ray diffraction (Arnott and Selsing, 1974; Liu *et al.*, 1996). However, helical periodicities were determined to be 13.0 bp/turn for a triplex composed of C·G·C and T·A·T base triplets and 12.2 bp/turn for the $G_n{\cdot}G_n{\cdot}C_n$ triplex (Liu *et al.*, 1996; Vlieghe *et al.*, 1996). By NMR spectroscopy, the helical screw was shown to vary at each dinucleotide step in triplex DNAs (Radhakrishnan and Patel, 1994b). Using the same method as used in this work, our previous report showed that the helical periodicity of GA-alternating triplex DNA was 11.2 bp/turn (Shin and Koo, 1996). In the present report, the helical periodicity of the $T_n{\cdot}A_n{\cdot}T_n$ triplex was determined to be 11.5 bp/turn. In duplexed DNA, the $A_n{\cdot}T_n$ sequence is more tightly wound than the AT-alternating, $G_n{\cdot}C_n$, and random sequences (Peck and Wang, 1981; Rhodes and Klug, 1981). However, in triplexed DNA, the $T_n{\cdot}A_n{\cdot}T_n$ sequence is less tightly wound than the $(CT)_n{\cdot}(GA)_n{\cdot}(CT)_n$ and random sequences (Liu *et al.*, 1996; Shin and Koo, 1996). The triplexed $T_n{\cdot}A_n{\cdot}T_n$ sequence may be less tightly wound because the triplex structure was merely stabilized at a relatively high concentration of magnesium

ion. It is noted that the helical periodicities (bp/turn) of triplex DNAs determined by our method have smaller values than those by X-ray diffraction (Arnott and Selsing, 1974; Liu *et al.*, 1996), but are similar to those by NMR spectroscopy (Radhakrishnan and Patel, 1994a,b).

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