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Helical Periodicity of DNA, Poly(dA) · Poly(dT) and Poly(dA-dT) · Poly(dA-dT) in Solution

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Helical periodicity of DNA, poly(dA) · poly(dT) and poly(dA-dT) · poly(dA-dT) has been measured in solution by using the band shift method of Wang [Wang, J. (1979) *Proc. Natl Acad. Sci. USA*, *76*, 200–203]. The method makes use of the effect, on the superhelicity of closed circular DNA molecules, of the insertion of specific nucleotide sequences of known length. The method was applied to a variety of recombinant plasmid DNAs which were constructed by inserting DNA, poly(dA) · poly(dT) or poly(dA-dT) · poly(dA-dT) into pBR322 DNA. When compared to DNA, poly(dA) · poly(dT) was found to have a smaller pitch (by about 0.5 base pair/turn), whereas poly(dA-dT) · poly(dA-dT) has a slightly larger pitch (by 0.1 base pair/turn). These features correlate well with the known ability of the alternating copolymer to reconstitute nucleosomes upon incubation with histones, in contrast to the non-alternating one which fails to do so. Finally, a detailed analysis of the principles underlying the methods developed by Wang [reference quoted above and Wang, J. (1978) *Cold Spring Harb. Symp. Quant. Biol. 42*, 29–33] leads to an increase in the estimate of the helical periodicity of DNA of 0.15 base pair/turn, over the reported value of 10. 4 base pairs/turn (references quoted above). This essentially accounts for the discrepancy observed with the value of 10.6 base pairs/turn obtained by nuclease digestion of DNA immobilized on a surface [Rhodes, D. & Klug, A. (1980) *Nature (Lond.) 286*, 573–578].

The polymorphism of the DNA double helix upon variation in the nucleotide sequence is now a widely accepted idea. This property will probably be of key importance for the understanding of some of the biological functions of DNA, such as its interaction with specific proteins in the course of the regulation of gene activity. The idea of the polymorphism of DNA has been substantiated by the study of synthetic polymers, where particular features of the DNA are repeated and therefore similarly amplified. Such studies involved a variety of techniques including infrared dichroism on gels [1,2] and X-ray diffraction on fibers [3,4]. More specific data, obtained by X-ray diffraction on single crystals of oligonucleotides of defined sequences [5,6], confirmed and extended the idea.

What has been found with DNA in gels, fibers and crystals is likely to apply to DNA in solution. In particular, the question may be asked whether a polymorphism of the double helix in solution could be revealed by the measurement of some average property such as its helical periodicity over a few tens or a few hundreds of base pairs. This question has been partly answered by Wang [7,8], who found that the sequence dependence of the helical periodicity of DNA in solution is small, if any. Recently, however, Wang referred to the case of poly(dA) · poly(dT) which has a pitch of 9.9 base pairs/turn [9], that is about 0.5 base pair/turn less than the value for DNA [7,8].

In this paper, helical periodicity of DNA, poly(dA) · poly(dT) and poly(dA-dT) · poly(dA-dT) was measured in solution using the band shift method of Wang [8]. We confirm the absence of a sequence dependence of the helical periodicity of DNA, and show that the copolymers have helical periodicities in solution different from that of DNA. In agreement with Wang [9], poly(dA) · poly(dT) was found to have a smaller pitch (by about 0.5 base pair/turn). In contrast, poly(dA-dT) · poly(dA-dT) has a slightly larger pitch (by about 0.1 base pair/turn). These features are discussed in the light of the different ability of these copolymers to reconstitute nucleosomes upon incubation with histones [10–12].

A detailed analysis of the principles underlying the methods developed by Wang [7,8] for the measurement of the helical periodicity of DNA in solution is also presented. This analysis leads to an increase in the estimate of the helical periodicity of 0.15 base pair/turn over the reported value of 10.4 base pairs/turn [8]. This essentially accounts for the discrepancy observed with the value of 10.6 base pairs/turn obtained by nuclease digestion of DNA immobilized on a surface [13].

Abbreviations. Polynucleotides are abbreviated according to the recommendations of the IUPAC-IUB Commission on Biochemical Nomenclature [*Eur. J. Biochem. 15*, 203–208 (1970)]: poly(dA) · poly(dT) consists of a chain of poly(deoxyadenylate) hydrogen-bonded to a chain of poly(thymidylate); poly(dA-dT) · poly(dA-dT) consists of two hydrogen-bonded chains of alternating dA and dT residues. SV 40, simian virus 40.

Enzymes. Terminal deoxyribonucleotidyl transferase (EC 2.7.7.31); polynucleotide kinase (EC 2.7.1.78); alkaline phosphatase (EC 3.1.3.1); DNA ligase (EC 6.5.1.1); DNase I (EC 3.1.21.1); S1 nuclease (EC 3.1.30.1); DNA polymerase I (EC 2.7.7.7); restriction endonucleases: *Sau*3A (EC 3.1.23.–), *Bam*HI (EC 3.1.23.6), *Hinc*II (EC 3.1.23.20), *Pst*I (EC 3.1.23.31), *Hind*III (EC 3.1.23.21), *Hae*III (EC 3.1.23.17), *Hha*I (EC 3.1.23.19), *Alu*I (EC 3.1.23.1), *Hpa*II (EC 3.1.23.24), *Eco*RI (EC 3.1.23.13).

MATERIALS AND METHODS

Materials and DNA Preparation

Restriction endonucleases and T4 polynucleotide kinase were purchased from New England Biolabs. Terminal deoxynucleotidyl transferase was from PL Biochemicals. T4 DNA

ligase, large fragment DNA polymerase I and bacterial alkaline phosphatase were from Bethesda Research Laboratories. S1 nuclease and DNase I were from Sigma. Enzymes were used, except were otherwise stated, according to specifications from the manufacturers. Unlabelled and ^{3}H-labelled deoxynucleoside triphosphates were from Boehringer and Amersham, respectively. [γ-^{32}P]ATP was also from Amersham.

pBR322 [14], PMC 1 [15] and recombinant plasmid DNAs constructed here were purified by ethidium bromide/CsCl banding [16,17].

Construction of Recombinant Plasmid DNAs

Plasmids with DNA Inserts. Four recombinant plasmid DNAs (p60, p96, p104 and p372) were prepared by inserting *Sau*3A fragments 60, 96, 104 and 372 nucleotides long, respectively, into *Bam*HI-cleaved pBR322 DNA, using T4 DNA ligase. *Sau*3A 60-nucleotide-long fragment was purified from a *Sau*3A digest of wild-type SV 40 DNA [18] by preparative gel electrophoresis as described [19]. *Sau*3A fragments 96, 104 and 372 nucleotides long were purified as follows. *Escherichia coli* lac DNA-containing plasmid PMCI DNA [15] was digested with *Hin*cII, and the two lac fragments 789 and 935 base pairs long were purified by gel electrophoresis. The sequences of both these fragments are known (Maxam, A., Gilbert, W., Chapman, N., Copenhaver, G., Donis-Keller, H., Herr, W., and Rosenthal, W., unpublished data) [20]. Upon *Sau*3A digestion, the 789-base-pair fragment gives only one *Sau*3A-ended fragment, 96 nucleotides long; the 935-base-pair fragment gives two *Sau*3A-ended fragments, 104 and 372 nucleotides long, respectively. These fragments were finally purified by gel electrophoresis.

Poly(dA) · poly(dT)-Containing Plasmid DNAs. Approximately 400 dAMP or dTMP residues were added to 3′ termini of *Pst*I-cleaved pBR322 DNA, using terminal deoxynucleotidyl transferase as described [21], with $CoCl_2$ as an activator and the appropriate deoxynucleoside triphosphates. Poly(dA)-tailed and poly(dT)-tailed pBR322 DNAs were mixed at 37 °C in 10 mM Tris/HCl, pH 8.0, 1 mM EDTA and 0.1 M NaCl and allowed to anneal at the same temperature for 4 h.

Poly(dA-dT) · poly(dA-dT)-Containing Plasmid DNAs. These DNAs were constructed by inserting poly(dA-dT) · poly-(dA-dT) into *Hin*dIII-cleaved pBR322 DNA, using T4 DNA ligase. Single-stranded ends of restricted plasmid DNA had previously been filled as follows: 10 μg of DNA, in 200 μl of 20 mM Tris/HCl, pH 7.5, 10 mM $MgCl_2$ and 1 mM dithiothreitol, were incubated with 33 μM each of the four deoxynucleoside triphosphates and with 10 units of large fragment *E. coli* DNA polymerase I, for 5 min at 15 °C. Blunt-ended poly(dA-dT) · poly(dA-dT) fragments, of average length about 200 base pairs, were prepared by digestion of 10 μg of the high-molecular-weight copolymer (a gift of J. Brahms) with 20 units of S1 nuclease, for 30 min at 37 °C, in 0.1 ml of 30 mM sodium acetate, pH 4.6, and 0.5 mM $ZnCl_2$.

Transformation and Identification of Recombinants

Ligated or annealed products were introduced into *E. coli* HB 101, using standard procedures [22]. Strains carrying recombinant plasmids were screened for ampicillin-sensitivity/tetracycline-resistance phenotype, for poly(dA) · poly(dT)-containing plasmids, or ampicillin-resistance/tetracycline-sensitivity phenotype for plasmids with DNA and poly(dA-dT) · poly(dA-dT) inserts.

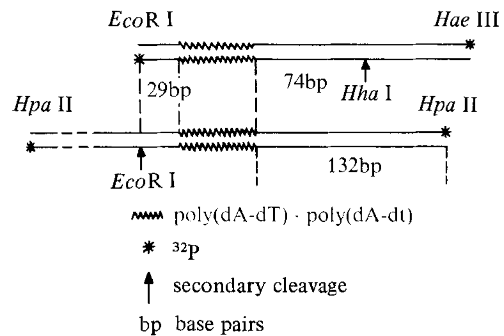

Fig. 1. *Diagram of the sequencing strategy used for poly(dA-dT) · poly-(dA-dT)-containing recombinant DNAs.* Insertion is located at the *Hin*dIII site of pBR322 [23]. Secondary cleavages with *Hha*I and *Eco*RI were performed on the labelled *EcoR*I + *Hae*III and *Hpa*II fragments, respectively. The resulting singly end-labelled fragments were sequenced as described in Materials and Methods

Characterization of Recombinant Plasmids

After propagation in *E. coli* and purification, all recombinant DNAs were digested with *Hae*III and compared to a similar digest of pBR322 DNA by gel electrophoresis. Only the expected fragments [23] (one in each recombinant DNA pattern) were found to be shifted.

Plasmids with DNA Inserts. After excision from the recombinant DNAs by *Sau*3A digestion, inserts appear to have lengths identical to those of the respective *Sau*3A fragments used for their construction (see above), as shown by gel electrophoresis. Moreover, restriction mapping of the inserts showed that specific restriction sites were present at their expected location: p372 insert has a *Hae*III site at about its middle; p104 and p96 inserts have a *Hha*I and an *Alu*I site, respectively, at about 20 and 15 base pairs from their ends. No mapping of p60 insert was performed.

Poly(dA) · poly(dT)-Containing Plasmids. Inserts and surrounding regions of two plasmids, pAA24 and pAA82, were sequenced using procedures described by Maxam and Gilbert [24]. DNAs were first cleaved with *Hae*III and submitted to electrophoresis in a preparative polyacrylamide gel. Insert-containing fragments were purified, digested with *Hpa*II, dephosphorylated with bacterial alkaline phosphatase and labelled with [γ-^{32}P]ATP and T4 polynucleotide kinase. Labelled fragments were then submitted to an electrophoresis in a DNA strand separation gel, and insert-containing single strands, which showed a large separation, eluted from the gel. Sequence determinations of the 5′-^{32}P-labelled single strands were carried out as described [24]. Sequencing gels 1 m long were used in the case of pAA82.

Poly(dA-dT) · poly(dA-dT)-Containing Plasmids. In contrast to the preceding case, complementary strands of insert-containing fragments could not be separated. The two ends of these fragments were therefore segregated by secondary restriction endonuclease cleavage as depicted in Fig. 1. Three DNAs (pAT29, pAT44 and pAT73) were separately digested with *Eco*RI + *Hae*III and with *Hpa*II, and insert-containing fragments purified by gel electrophoresis. Fragments were then dephosphorylated and end-labelled with ^{32}P as indicated above. Labelled fragments were cleaved (see Fig. 1) with *Hha*I and *Eco*RI, respectively, and the six singly end-labelled insert-containing double-stranded fragments were purified by gel electrophoresis. Sequence determinations were performed as described [24], using 1 m-long sequencing gels in the case of pAT73.

Relaxation and Closure of Plasmid DNAs. Gel Electrophoresis and Densitometric Tracing

Supercoiled DNAs were relaxed by introducing nicks with DNaseI [25]. In this procedure, 10 μg of DNA, in 600 μl of 4 mM Tris/HCl, pH 7.5, 125 mM NaCl, 10 mM $MgCl_2$ and 0.3 mg/ml of ethidium bromide (Sigma), were incubated with 28 units of DNaseI for 15 min at 30 °C. Digestion was terminated by adding EDTA to 20 mM. The digest was made 2% in sodium dodecylsulfate and 1 M in NaCl, shaken with 1 vol. of chloroform/isoamyl alcohol (24:1; v/v) and centrifuged. After precipitation with ethanol, DNA was dissolved in a ligase buffer containing 60 mM Tris/HCl, pH 7.5, 7 mM $MgCl_2$, 0.2 mM ATP, 10 mM dithiothreitol and 50 μg/ml bovine serum albumin. No ethidium bromide remained in the sample at this step, as checked by ultraviolet illumination. Nicked plasmid DNAs were closed by incubation with T4 DNA ligase, at 6 °C, in the buffer described above. Incubation mixtures were extracted with 1 vol. of water-saturated phenol, and phenol traces removed by shaking with 5 vol. of ether.

Electrophoresis of plasmid DNAs was performed in 1.4% agarose slab gels (0.15 × 14 × 16 cm), in 40 mM Tris/acetate, 20 mM sodium acetate and 2 mM Na_2 EDTA, pH 7.8, at room temperature (about 23 °C). Buffer was recirculated, and electrophoresis was performed at 70 V for 13 h.

Negatives (Polaroid type 665) were traced with a Joyce-Loebl microdensitometer. The film response was calibrated by electrophoresis of several dilutions of closed circular plasmid DNAs in the above described gels. Using controlled conditions of ethidium bromide staining and exposure, heights of the peaks in the tracing were found to be approximately proportional to the amount of DNA in the peaks. No correction for any possible differential binding of ethidium bromide to the different topoisomers was performed [26].

PRINCIPLE OF THE MEASUREMENT

Both the Gaussian center and the band shift methods developed by Wang [7,8] make use of the known topological properties of closed circular DNA molecules. An open circular DNA molecule which has been closed with ligase can be characterized by three parameters, L, T and W. These parameters are related by the equation $L = T + W$. L, the linking number, measures the number of times one strand goes around the other one, and is an integer. W, the writhing number, depends on the path followed by the axis of the double helix and T, the twist, measures the winding about this axis. Both W and T are in general fractional numbers (see Crick [27] for a full description of these terms). Upon insertion of n base pairs into the molecule and its resealing with ligase, L, T and W are incremented by ΔL, ΔT and ΔW. These increments are related by the equation $\Delta L = \Delta T + \Delta W$. ΔT is equal to n/h, where h is the pitch of the inserted sequence. ΔL is the closest integer to the fractional number n/h. ΔW is then the residual of n/h. ΔW is equal to zero only when n is an integral multiple of h. Replacing ΔT, one obtains

$$h = \frac{n}{\Delta L - \Delta W} \quad (1)$$

which allows one to calculate h from ΔW. The integer ΔL can easily be estimated when an approximate value of h is available. It is important to note that Eqn (1) does not depend on environmental conditions of the DNAs. When both starting

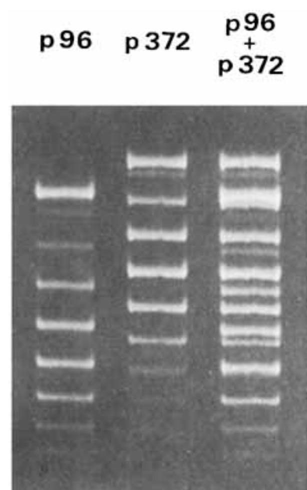

Fig. 2. *Fractionation of topological isomers by gel electrophoresis.* 0.4 μg each of ligase-closed p96 and p372 DNAs were loaded separated and mixed in a 1.4% agarose slab gel. Gel dimensions and electrophoresis conditions were as described in Materials and Methods. About 0.06 μg of the open circular form, as obtained by nicking with DNase I, was added to each ligated DNA. The figure shows a photograph of the gel after staining with ethidium bromide

and 'inserted' closed DNAs are transferred from ligation to gel electrophoresis, h and ΔW change but still obey Eqn (1).

Due to conformational fluctuations of the double helix, ligation results in a distribution of molecules with different superhelicities, called topological isomers (topoisomers). Such a population, as a whole, can be characterized by the average parameters $\bar{L}$, $\bar{T}$ and $\bar{W}$, which are also related by the equation $\bar{L} = \bar{T} + \bar{W}$. Upon transfer from ligation to gel electrophoresis, the double helix unwinds and $\bar{T}$ is incremented by $\Delta\bar{T}$ ($\Delta\bar{T} < 0$). The correlative change $\Delta\bar{W}$ of $\bar{W}$ is: $\Delta\bar{W} = -\Delta\bar{T} = \bar{W}$ (in gel electrophoresis) $- \bar{W}$ (in ligation). $\bar{W}$ in ligation conditions is zero (the average topoisomer is relaxed). $\bar{W}$, in gel electrophoresis, is positive ($\bar{W} = -\Delta\bar{T}$) and appears to be proportional to the number of base pairs, N. This results from the fact that each base pair contributes by a small rotation angle to the total unwinding $\Delta\bar{T}$. The ratio $\bar{W}/N$, which is proportional to the superhelix density, is therefore equal in both reference and 'inserted' DNAs. In contrast, Wang [7,8] implicity assumed that $\bar{W}$ is equal in both DNAs. Such a discrepancy has consequences which are described below.

Details of the measurement are given below in the particular case of a pair of recombinant plasmids with DNA inserts: p372 (index I) and p96 (index R) DNAs. They were chosen for their large length difference (276 base pairs). (p96 DNA was used as a reference instead of pBR322 DNA because topological isomers of p372 and pBR322 DNAs are not well resolved from each other by gel electrophoresis.)

Fig. 2 shows the band patterns obtained upon gel electrophoresis of the ligase-closed DNAs just described, which were run separately and mixed. A densitometer tracing of the 'mixed' pattern is shown in Fig. 3A. Adjacent bands of each DNA pattern correspond to topoisomer species differing by one in L [28] and also in W, as long as W remains too small to generate constraints which would alter T. Bands of each pattern are enveloped by a Gaussian curve [26] whose center corresponds to the average parameters $\bar{L}$, $\bar{T}$ and $\bar{W}$ described above. The pitch of inserted DNA, h, can be measured from the band pattern of Fig. 3A using either one of the two methods of Wang [7,8], as follows.

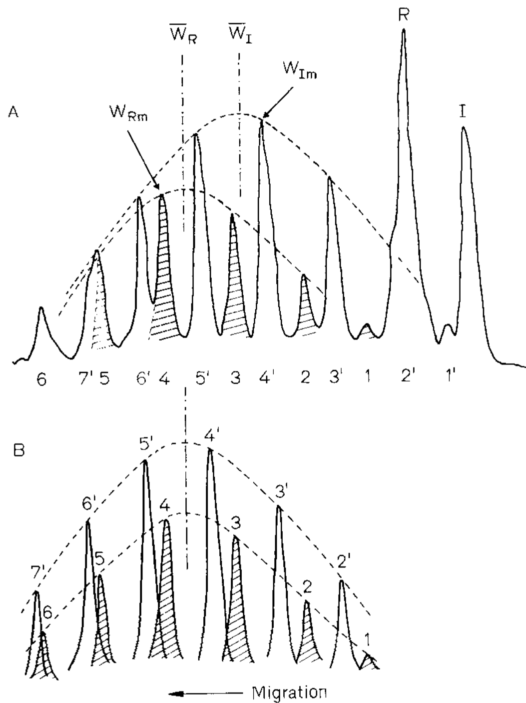

Fig. 3. *Densitometric tracing of a topological isomer distribution obtained by gel electrophoresis.* (A) A pattern corresponding to a mixture of p96 and p372 DNAs, similar to the one shown in Fig. 2, was traced with a Joyce-Loebl microdensitometer. In this case, however, open circular DNAs were mixed before ligation in order to ensure identical closure conditions for both DNAs. Open circular p96 and p372 DNAs (peaks R and I, respectively) were also added to the ligated sample. Index R and bands 1 to 6 refer to p96 DNA. Index I and bands 1′ to 7′ refer to p732 DNA. (B) Band pattern obtained from A upon shifting of the whole p372 DNA distribution (bands 1′ to 7′) in the migration direction so that centers of Gaussian envelopes coincide. Peaks 2′, 5 and 7′, which are poorly resolved in A, were estimated from adjacent lanes where the DNAs were run separately

The Gaussian Center Method

In this method, positions of topoisomers of each series are measured relative to the center of their respective Gaussian envelope. If W_{Rm} and W_{Im} are the writhing numbers of the most abundant topoisomers of p96 and p372 DNAs, respectively (see Fig. 3A), the increment upon insertion, ΔW of Eqn (1), is: $W_{Im} - W_{Rm}$. ΔW is also equal to $(W_{Im} - \overline{W_I}) - (W_{Rm} - \overline{W_R}) + (\overline{W_I} - \overline{W_R})$, where $\overline{W_R}$ and $\overline{W_I}$ are the average writhing numbers of the two populations, respectively. Wang [7] implicitly assumed that $\overline{W_I}$ is equal to $\overline{W_R}$. ΔW then becomes $\Delta\omega$. Thus

$$\Delta\omega = (W_{Im} - \overline{W_I}) - (W_{Rm} - \overline{W_R}) . \qquad (2)$$

In fact, we know (see above) that $\overline{W_R}/N = \overline{W_I}/(N+n)$, where N is the number of base pairs in the reference DNA, and n the number inserted. Thus: $\overline{W_I} = \overline{W_R} + n\overline{W_R}/N$

and

$$\Delta W = \Delta\omega + n\overline{W_R}/N . \qquad (3)$$

Replacement of ΔW in Eqn (1) by $\Delta\omega$ [from Eqn (2)] or ΔW [from Eqn (3)] gives two values of the pitch, h_ω and h_w, respectively. h_w shows, when compared to h_ω, a positive increment Δh. Thus $\Delta h = h_w - h_\omega = h_w \cdot h_\omega \cdot \overline{W_R}/N$ with $h_w \cdot h_\omega \approx 110$. Δh does not depend on insertion length but only on the average superhelix density of the reference DNA. With $\overline{W_R} = 6$ (see below), h_w is higher than h_ω by 0.15 base pair/turn. Table 1 gives the values of $\Delta\omega$, h_ω, ΔW and h_w measured from Fig. 3A. As indicated by Wang [7], h_ω is the pitch of the inserted DNA in ligation conditions. [This results from the fact that $\overline{W_R}$ is then equal to zero, and that Eqn (3) gives $\Delta W = \Delta\omega$.] In contrast, h_w is the pitch in the conditions of the electrophoresis.

Table 1. *Writhing number increments and helical periodicities (h) from measurement of DNA pair p372/p96 using the Gaussian center method* $|W_{Rm} - \overline{W_R}|$ was calculated from Fig. 3A by dividing the distance between band 4 and the center of the envelope by the distance between bands 3 and 4. $|W_{Im} - \overline{W_I}|$ was calculated using the same procedure. $\Delta\omega$ was calculated using Eqn (2) and ΔW from Eqn (3), with $\overline{W_R} = 6$, $n = 276$ base pairs and $N = 4458$ base pairs. h_ω and h_W were calculated from $\Delta\omega$ and ΔW, respectively, using Eqn (1), with $\Delta L = 26$. Eqns (1), (2) and (3) are discussed in the section on Principle of the Measurement

Parameter	Value
$W_{Rm} - \overline{W_R}$	+ 0.30
$W_{Im} - \overline{W_I}$	− 0.34
$\Delta\omega$	− 0.64
ΔW	− 0.27
h_ω	10.36 base pairs/turn
h_w	10.51 base pairs/turn

The Band Shift Method

Here, positions of topoisomers of each series are measured relative to one another. A correction for the length dependence of topoisomer mobility is therefore required. If the two DNAs had the same length, centers of the two envelopes would coincide. The correction for the length difference between the two DNAs is therefore obtained by shifting, in the migration direction, the entire band pattern of the 'inserted' DNA, so that centers of the envelopes coincide, as shown in Fig. 3B. As seen in Fig. 3A, displacements of the centers of the envelopes and of the nicked circular forms are equal. This suggests that the length dependence of topoisomer mobility does not depend on its superhelicity. This observation was confirmed as follows. The experiment shown in Fig. 3 was repeated using different ligation temperatures (0−15 °C). This causes the average superhelix density of the population to change within the region of interest. Again, centers of the envelopes and nicked circular forms were found to be equally shifted (data not shown). This shows, as assumed by Wang [8], that the correction for the length dependence of the mobility of supercoiled DNA molecules is obtained from the shift observed between their open circular forms. In the following measurements of DNA pairs, this shift is obtained using mixtures of the open circular forms (as obtained from nicking with DNase I) before ligation. In the cases of the smaller insertions, DNAs are not well resolved from each other. Open circular p372 DNA is then added to each DNA of the pairs and used as an internal marker.

As seen above, centers of the envelopes correspond to the same average superhelix density. Therefore, two topoisomers, one in each series, which have the same mobility in Fig. 3B also have the same superhelix density. This differs from the implicit assumption of Wang [8] that they have the same absolute number of superhelix turns. In the general case, however, topoisomers of the two series do not have the same

Table 2. *Writhing number increments and helical periodicities (h) from measurement of DNA pair p372/p96 using the band shift method*
$\Delta\omega$ was calculated from Fig. 3 B, using Eqn (4), by linear interpolation between pairs of bands, as indicated in the section on Principle of the Measurement. Writhing number of topoisomer number i of reference DNA, W_{Ri}, is taken equal to $W_{R1} + (i-1)$, with $W_{R1} = 3.2$ (see Principle of the Measurement section). W_R, relative to band pair i and $i+1$, is calculated from Eqn (4): $W_R = W_{Ri} + \Delta\omega$. n and N are the same as in Table 1. ΔW was calculated from Eqn (5). h_ω and h_w were calculated from $\Delta\omega$ and ΔW, respectively, using Eqn (1), with $\Delta L = 27$. Eqns (1), (4) and (5) are discussed in the Principle of the Measurement section

Band pair	$\Delta\omega$	$n W_R/N$	ΔW	h_ω	h_w
				base pairs/turn	
1–2	0.48	0.23	0.71	10.41	10.50
2–3	0.40	0.28	0.68	10.38	10.49
3–4	0.37	0.34	0.71	10.36	10.50
4–5	0.31	0.40	0.71	10.34	10.50
5–6	0.14	0.45	0.59	10.28	10.45
Average pitch				10.35	10.49

mobility. Let us consider in Fig. 3 B bands 2 and 3, for example. They have writhing numbers respectively equal to W_{R2} and W_{R3}. ($W_{R3} = W_{R2} + 1$; see above.) Let us call W_I the writhing number of topoisomer 3′ and W_R the writhing number of a topoisomer of the reference DNA which would comigrate with topoisomer 3′. (W_R can be calculated from W_{R2} and W_{R3} by linear interpolation between bands 2 and 3.) The increment, ΔW, of the writhing number upon insertion is: $\Delta W = W_I - W_{R2}$. Again Wang [8] implicity assumed that $W_I = W_R$, which leads to a writhing number increment $\Delta\omega$. In contrast, with equal superhelix densities, one has: $W_R/N = W_I/(N+n)$. Thus

$$\Delta\omega = W_R - W_{R2} \tag{4}$$

$$\Delta W = \Delta\omega + n W_R/N\,. \tag{5}$$

As above, $\Delta\omega$ and ΔW can be used in Eqn (1) to calculate the two values of the pitch, h_ω and h_w. Eqn (5) is identical to Eqn (3). This shows that h_w is also increased by $\Delta h \approx 110\ W_R/N \approx 0.15$ base pair/turn over h_ω. h_ω has been reported [8] to be the pitch of the inserted DNA in electrophoresis conditions. The above analysis indicates that h_ω is instead the pitch in ligation conditions, as in the Gaussian center method (see above). The pitch in electrophoresis is therefore measured by h_w.

Table 2 shows the values of $\Delta\omega$, ΔW, h_ω and h_w obtained with consecutive pairs of adjacent bands of p96 DNA in Fig. 3 B. Average values of h_ω and h_w, measured on all pairs of bands, are also shown in the table. As seen in Table 2, $\Delta\omega$ depends on which pair of bands is used, and shows a regular trend which results from the different periodicities of the two band patterns (see Fig. 3 B). (The shorter inter-band spacing of p372 DNA, as compared to p96 DNA, is intuitively expected from the fact that p372 DNA is larger and migrates less than p96 DNA.) Interestingly, the correction term nW_R/N compensates for the trend of $\Delta\omega$ so that ΔW is approximately constant.

Estimation of the Average Number of Superhelical Turns

The average number of superhelical turns of p96 DNA ($\overline{W}_R$; see Fig. 3 A) can be estimated to be close to $W_{R1}+3$, with W_{R1} being the number of superhelical turns of the first resolved topoisomer (see Fig. 3). Upon gel electrophoresis of the same DNA in gel A system of Shure and Vinograd [29] (2% agarose, in 40 mM Tris, 30 mM NaH_2PO_4, 1 mM EDTA, pH 7.8; room temperature) the number of bands observed between the first resolved one and the center of the envelope is the same as in Fig. 3 (not shown). Since $\overline{W}_R$ is about the same in both gel systems (ionic strengths of both electrophoresis buffers are similar), W_{R1} is also the same. The first resolved SV 40 topoisomer has been assigned four superhelical turns in gel A system [29]. Assuming that both first-resolved p96 and SV 40 topoisomers have the same superhelix density, W_{R1} is equal to 3.2. This gives a figure of about 6 for $\overline{W}_R$.

Alternatively, $\overline{W}_R$ can be estimated from the known contributions, on DNA twisting, of changes in temperature [26] and ion concentrations [29–31] when DNA is transferred from ligation to gel electrophoresis. (This takes into account that $\overline{W}_R$ in ligation conditions is zero; see above.) The detailed calculations, which will not be described here, lead to a value of $\overline{W}_R$ of 6.5, in good agreement with the one estimated above.

RESULTS

Sequence Determinations

Sequences were determined as described in Materials and Methods. Sequences of inserts and adjacent vector DNA are shown in Fig. 4 and the actual insert lengths are listed in Table 3. Fig. 4 shows that pAA24, pAT44 and pAT73 DNAs have the expected structure. In contrast pAA82 and pAT29 DNAs show deletions of pBR322 sequences adjacent to the insertion sites of 6 and 16 base pairs respectively. In both cases deletion boundaries are dA-dT clusters (see Fig. 4), suggesting that they are the result of recombination events.

Measurement of the Helical Periodicity

The band shift method was used as described above. Pairs of DNAs consisted of the recombinant plasmid DNAs constructed here and of pBR322 DNA as a common reference. In contrast to the other DNAs, p60, p372, pAT29 and pAT73 topoisomers are not well separated from pBR322 topoisomers by gel electrophoresis. The comparison with pBR322 DNA was made using a secondary reference as follows. Each one of the two DNAs in a pair was mixed with an appropriate third DNA (the secondary reference) and submitted to electrophoresis in two separate lanes of the same gel. The third DNA was chosen so as to be resolved from each of the first two. Band patterns of the first two DNAs can then be aligned with the help of the third one and compared. The secondary reference DNA used with pairs p60/pBR322, p372/pBR322 and pAT73/pBR322 was p96. With the pair pAT29/pBR322 it was p104.

When performed on a pair of DNAs, the method gives the average helical periodicity, h_w, of the nucleotide sequence which is in excess in the larger DNA. Table 3 shows that the length of inserted dA + dT sequences is either smaller or larger than the total insertion length. A correction is therefore required to obtain the helical periodicity of poly(dA) · poly(dT) (h_{AA}) and poly(dA-dT) · poly(dA-dT) (h_{AT}) from the average periodicity, h_w, of the insertion. Knowing the structure of inserted sequences (see Table 3), the following equations can be derived for different DNA pairs:

pAA24/pBR322 $\quad 24/h_{w1} = 20/h_{AA} + 4/h_0$

pAA82/pBR322 $\quad 82/h_{w2} = 84/h_{AA} - 2/h_0$

Table 3. *Characterization of dA + dT-containing recombinant plasmid DNAs*
Data in the table were obtained from the sequences of inserts and adjacent vector DNA shown in Fig. 4. Recombinant plasmid DNAs were constructed by inserting poly(dA) · poly(dT) (pAA24 and pAA82) and poly(dA-dT) · poly(dA-dT) (pAT29, pAT44 and pAT73) in the *Pst*I and *Hin*dIII sites, respectively, of pBR322 DNA [23], as described in Materials and Methods

Character	Number of base pairs in plasmid DNA				
	pAA24	pAA82	pAT29	pAT44	pAT73
Length difference with pBR322	24	82	29	44	73
Length of poly(dA) · poly(dT)	20	84			
Length of poly(dA-dT) · poly(dA-dT)			41	40	69

pAA82/pAA24	$58/h_{w3} = 64/h_{AA} - 6/h_0$
pAT29/pBR322	$29/h_{w4} = 41/h_{AT} - 12/h_0$
pAT44/pBR322	$44/h_{w5} = 40/h_{AT} + 4/h_0$
pAT73/pBR322	$73/h_{w6} = 69/h_{AT} + 4/h_0$.

In these equations, added or deleted sequences of pBR322 DNA are assumed to have the same pitch h_0. h_0 was taken equal to the average pitch obtained with the four DNA inserts, i.e. 10.56 base pairs/turn (see below).

Table 4 shows values of h_w obtained with all pairs of DNA, along with values of h_{AA} and h_{AT} calculated from the above equations. Values of h_w were averaged on all pairs of bands, as shown in Table 2.

In the comparison of p96, p104 and p372 DNAs to pBR322 DNA, h_w does not depend significantly on the pair of bands used for the measurement. This is also observed with DNA pair p372/p96 (see Table 2). This is in contrast, however, to what is found when DNAs containing poly(dA) · poly(dT) and poly(dA-dT) · poly(dA-dT) are compared to pBR322 DNA. In these cases, $\Delta\omega$ increases with the band

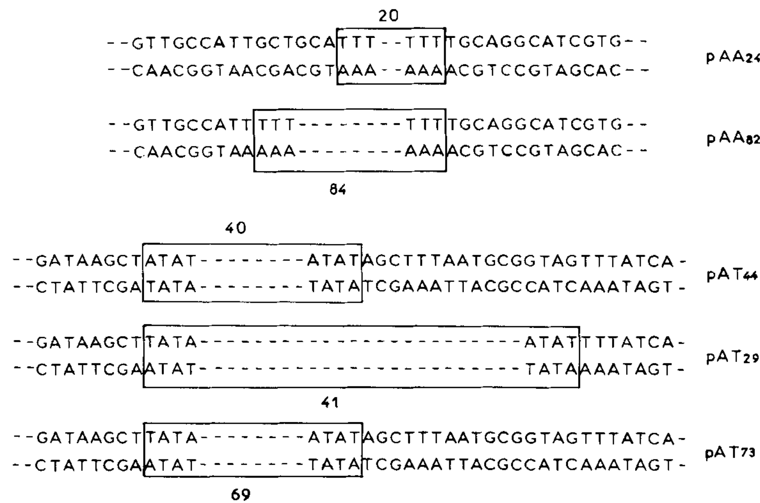

Fig. 4. *Nucleotide sequences of inserts and surrounding regions of recombinant DNAs.* Numbers about the boxes indicate the number of inserted nucleotides consisting of pure alternating and non-alternating dA and dT. pAA24 and pAA82 inserts are located at the *Pst*I site of pBR322 DNA [23]. pAT44, pAT29 and pAT73 inserts are located at the *Hin*dIII site of pBR322 DNA [23]. Sequences of surrounding regions were found to be identical to those published by Sutcliffe [32]. These sequences are aligned to show more clearly the deletions which occur in pAA82 and pAT29 DNAs

Table 4. *Helical periodicities (h) from measurements of DNA pairs using the band shift method*
The band shift method is described in the section on Principle of the Measurement. Construction and structure of the various plasmid DNAs are described in Materials and Methods and in Table 3 and Fig. 4. *h* values were rounded off to the first decimal. dG + dC content of inserts is taken from their sequences. Only part of the sequence of p96 DNA insert is known (Maxam, A. et al.; unpublished). Its length is however known from the amino acid sequence of the corresponding protein. h_{AA} and h_{AT} were calculated from h_w using the appropriate equations (see Results)

DNA pair	Δ Length	h_w	h_{AA}	h_{AT}	dG + dC
	base pairs	base pairs/turn			%
p60/pBR322	60	$10.5_5 \pm 0.1$			43
p96/pBR322	96	10.6 ± 0.1			–
p104/pBR322	104	10.6 ± 0.1			62
p372/pBR322	372	10.5 ± 0.1			52
pAA24/pBR322	24	$10.1_5 \pm 0.1$	10.1 ± 0.1		
pAA82/pBR322	82	10.1 ± 0.1	10.1 ± 0.1		
pAA82/pAA24	58	$10.0_5 \pm 0.1$	10.1 ± 0.1		
pAT29/pBR322	29	10.7 ± 0.1		$10.6_5 \pm 0.1$	
pAT44/pBR322	44	10.7 ± 0.1		10.7 ± 0.1	
pAT73/pBR322	73	$10.6_5 \pm 0.1$		$10.6_5 \pm 0.1$	

pair number, instead of decreasing as observed in Table 2. This effect is not specific for dA + dT inserts since it is also observed with the DNA pair p60/pBR322. As a result, h_w increases by about 0.1 base pair/turn (larger insertions) to about 0.3 base pair/turn (smaller insertions) from the first band pair to the fifth one. This implies that averaged values of h_w, which are listed in Table 4, depend on the average superhelix density of the DNAs. All measurements were therefore carried out in identical conditions in order for all DNAs to have identical superhelix densities. Despite these variations, for which we have no explanation, data for any DNA pair are very reproducible from one gel to another (h_w values stay within $\pm$ 0.02 base pair/turn). However, given the uncertainties which remain in the method, we assign a standard deviation of 0.1 base pair/turn to all data.

Table 4 shows that helical periodicity of DNA varies between 10.5 and 10.6 base pairs/turn, with an average, calculated from the four inserts, of 10.56. These variations do not appear, however, to be correlated to the insert length or dG + dC content. Table 4 also shows that, when compared to DNA, poly(dA) · poly(dT) has a smaller helical periodicity (by about 0.4−0.5 base pair/turn). This is in contrast to the case of poly(dA-dT) · poly(dA-dT), whose pitch (the average of the data in Table 4 is 10.67 base pairs/turn) is slightly larger (by about 0.1 base pair/turn).

DISCUSSION

The analysis of the principles underlying the methods of Wang [7, 8] for the measurement of the helical periodicity of DNA in solution, which has been performed here, has led to the introduction of two quantities, h_ω and h_w. h_ω measures the pitch of DNA in ligation conditions whereas h_w measures it in gel electrophoresis conditions. In the present ligation conditions (see Materials and Methods), h_w is increased by about 0.15 base pair/turn over h_ω. The same increment of h_w over h_ω is expected in the conditions of Wang [8], where DNAs are relaxed and closed with calf thymus topoisomerase. This is shown by estimating the average superhelicity of the DNAs from the electrophoretic patterns displayed in [8], as described in the section on Principle of the Measurement: the same number of bands as that in Fig. 3 is found between the first resolved topoisomer and the center of the Gaussian envelope. From the measurement of eight DNA pairs with length differences ranging from 11 to 58 base pairs, Wang [8] found a value of h_ω which varied between 10.4 and 10.5 base pairs/turn, with an average of 10.45. This therefore gives an average value of h_w of 10.6 base pairs/turn, in good agreement with the present average estimate of 10.56 measured from the four DNA inserts (see Table 4).

These estimates of h_w are close to the value of the helical periodicity measured by nuclease digestion of mixed sequence DNA immobilized on a surface (10.6 base pairs/turn [13]). This experiment makes use of an observation of Liu and Wang [33] that DNA adsorbed on a surface is cut by DNase I only on its exposed side, whereas the other side is protected by the surface. Periodicity of exposure of each DNA strand therefore reflects the twisting of the double helix. The question must be asked, however, whether these helical periodicities can be directly compared. For this to be true, DNA molecules should have the same environment in both experiments. This is not the case. DNA was adsorbed onto the surface at room temperature, but was digested at 37 °C [13]. In contrast, gel electrophoresis of topoisomers was performed here at room temperature. Divalent cations (Mg^{2+} or Ca^{2+}) were present in the digestion experiment [13], although the exact amount which remains available to react with DNA is unknown. In contrast, they are absent in the gel electrophoresis buffer used here. Divalent cations, if present to 1 mM or more [26, 30], are expected to overwind the double helix and to decrease its pitch by about 0.1 base pair/turn. In contrast, an unwinding, resulting in an increase of the pitch of 0.05 base pair/turn, is expected when temperature is raised from about 23 °C to 37 °C [26], assuming that bound DNA is free to rotate. Altogether, helical periodicity in the digestion experiment is expected to be 0.05−0.1 base pair/turn smaller than the one found here. Understanding why the two estimates are instead found to be about equal may require investigating the dependence of the helical periodicity of bound DNA on experimental conditions. For example, binding and digestion could be performed at the same temperature to eliminate the uncertainty about the rotation of bound DNA (see above). DNA could also be adsorbed to prewashed magnesium or calcium phosphate crystals, in order to remove free Mg^{2+} or Ca^{2+} ions. The overwinding effect of these divalent cations would, however, remain uncertain because they would have to be added back to activate the enzyme. In conclusion, given these uncertainties, helical periodicities estimated from the two methods appear to be in rather good agreement.

Helical periodicity of poly(dA) · poly(dT) is found to be smaller than that of DNA (by 0.4−0.5 base pair/turn; see Table 4). This result offers an explanation for the failure of this polymer to reconstitute nucleosomes upon incubation with histones in appropriate conditions [10, 12]. The ability of this polymer to form triple-stranded structures [34] which would be too stiff to wrap around the histone octamer may not be responsible for this failure. This result may bear on the function of the poly(dA) · poly(dT) sequences found in the eukaryotic genome. Published sequences of genes and surrounding regions show however that such sequences are usually not larger than 10 base pairs. The question of interest is obviously how long a run of dA or dT has to be to prevent the formation of nucleosomes *in vivo*.

In contrast, poly(dA-dT) · poly(dA-dT), whose helical periodicity is only slightly larger than that of DNA (by about 0.1 base pair/turn; see Table 4) can reconstitute nucleosomes upon incubation with histones [10−12]. This indicates that DNA-histone interactions in a nucleosome are flexible enough to permit limited changes in the structure of the double helix. This is in keeping with the natural occurrence of nucleosomes in a large variety of sequences, from (dA + dT)-rich spacers of histone genes [35] to (dG + dC)-rich satellite DNAs.

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