High Resolution Proton Nuclear Magnetic Resonance Investigation of the Structural and Dynamic Properties of $d(C_{15}A_{15})\cdot d(T_{15}G_{15})$†

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ABSTRACT: The high resolution proton nuclear magnetic resonance (NMR) spectra of the synthetic DNA block polymer $d(C_{15}A_{15})\cdot d(T_{15}G_{15})$ were studied in order to more completely understand telestability in DNA, and to provide fundamental NMR data on DNA helices and random coils. Spectra were measured in the spectral region from 0 to 15 ppm downfield from the usual standard, sodium 4,4-dimethyl-4-silapentane-1-sulfonate (DSS), at various temperatures (24–98 °C) in solutions containing either moderate or high ionic strength. The effect of actinomycin binding to the block polymer also was studied. The major conclusions derived from this study are as follows: (1) The majority of base pairs in the AT helix of the block polymer have the same conformation as in $d(A)_n\cdot d(T)_{25}$ and $d(A)_{21}\cdot d(T)_{21}$. (2) The conformation of the GC helix in the block polymer is different from the AT helix and this perturbs the conformation of three or four A·T base pairs at the junction of the AT–GC helix. (3) The conformation of the AT helix is unaffected by salt over the range examined (~0.04–~2 M), but the conformation of the GC helix changes. (4) There are subtle changes in the conformation of the AT helix as the temperature is increased and resonances characteristic of the random coil and the double-helical state can be simultaneously observed. (5) Binding of actinomycin, which is specific for the GC helix, induces quite large (over 1 ppm) upfield shifts of the resonances from the GC base pairs. This is consistent with an intercalation model in which actinomycin D (Am) is assymetrically sandwiched between two GC base pairs in such a manner that overlap with the guanosine residues is greater than with the neighboring cytidines. (6) The presence of the drug may also perturb A·T base pairs located near the AT–GC junction, but it has no effect on the majority of the AT pairs. However, as expected, Am elevated the T_m of the AT helix, even though it binds to the other end of the DNA.

Previous studies (Burd et al., 1975a,b) have demonstrated the existence of telestability in DNA; one region of a DNA can influence the properties of a remote but contiguous region of the helix. This conclusion was derived from the following studies on the three duplex block polymers $d(C_{15}A_{15})\cdot d(T_{15}G_{15})$, $d(C_{20}A_{15})\cdot d(T_{15}G_{20})$, and $d(C_{20}A_{10})\cdot d(T_{10}G_{20})$: UV[1] absorbance–temperature studies over a 100-fold range of salt concentration, helix–coil transition studies in the presence of the base-pair-specific drugs actinomycin D (Am) or netropsin, and *Escherichia coli* exonuclease I susceptibility studies in the absence and presence of the specific drugs. It has been shown (Wartell and Burd, 1976) that telestabilization over the distance found in these systems was unanticipated. These studies suggested a model for gene regulation over a distance.

The long-range effects noted above could be due to propagation of static conformational change down the DNA helix. Alternatively, telestability could be attributed to alteration in the ability of the DNA to undergo transient changes, such as localized breathing or other conformational changes. Without being more specific, we shall refer to these latter properties as dynamic properties.

In addition to these studies, previous chemical, physical, enzymatic, and biological studies (Wells and Wartell, 1974; Grant et al., 1972; Wells et al., 1970; Selsing et al., 1975, and papers cited therein) on twelve high-molecular-weight duplex DNAs with defined repeating nucleotide sequences demonstrated that different DNAs have different properties and conformations in solution. Since high resolution ^1H NMR is a sensitive tool for probing the properties of polynucleotides (Ts'o et al., 1969; Kroon et al., 1974; Patel and Hilbers, 1975; Borer et al., 1975; Kan et al., 1975; Patel 1975; Sander and Ts'o, 1969; McDonald et al., 1967; Cross and Crothers, 1971) and tRNA (Kearns and Shulman, 1974; Kearns, 1976) in solution, it was logical that these techniques be extended to higher molecular weight synthetic and natural DNAs. In light of the work mentioned above, $d(C_{15}A_{15})\cdot d(T_{15}G_{15})$ was an attractive molecule for such a study because (1) it was previously investigated (Burd et al., 1975a) by several physical and enzymatic techniques, (2) it has an appropriate length (shorter molecules lack conformational features of larger DNAs and very large DNA may not tumble rapidly enough to give good spectral resolution (McDonald et al., 1967)), and (3) it was sufficiently thermostable to permit study over a wide temperature range. In the present paper, we have used NMR to investigate the following questions. Do the AT and the GC portions of the block polymer have conformations which are identical with $d(A)_n\cdot d(T)_n$ and $d(G)_n\cdot d(C)_n$, respectively? Are the conformations of the AT and GC portions of the block polymer identical and, if not, how many base pairs are involved in the transition from one conformation to the other? How does the AT helix affect the dynamic properties of the GC helix (and vice versa), and how do the NMR results correlate with the UV helix–coil transition studies (Burd et al., 1975a,b)? What influence does actinomycin have on the dynamic and static properties of each end of $d(C_{15}A_{15})\cdot d(T_{15}G_{15})$? Am binds only to the GC parts of this molecule (Burd et al., 1975b).

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[1] Abbreviations used are: NMR, nuclear magnetic resonance; DSS, sodium 4,4-dimethyl-4-silapentane-1-sulfonate; Am; actinomycin D; UV, ultraviolet; EDTA, (ethylenedinitrilo)tetraacetic acid.

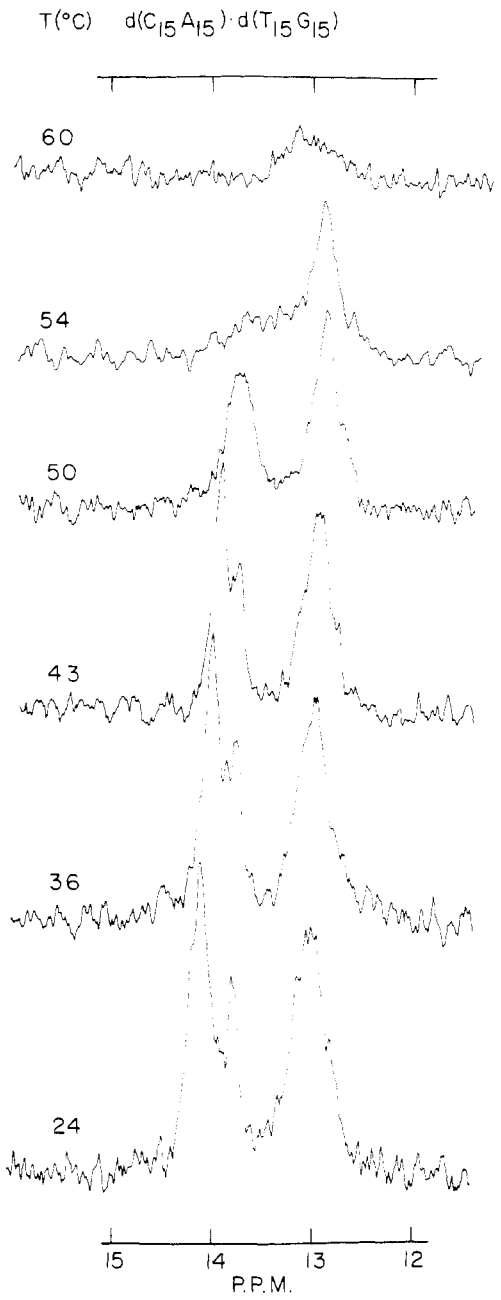

FIGURE 1: The 300 MHz low-field NMR spectrum of $d(C_{15}A_{15})$·$d(T_{15}G_{15})$ in a solution containing approximately 40 mM sodium ions, ~5 mM Na_2HPO_4 at pH 7, and 10^{-4} M EDTA. Spectra were signal averaged for approximately 1–2 h.

How does ionic strength affect the NMR properties of each end of the block polymer?

Materials and Methods

$d(C_{15}A_{15})$·$(T_{15}G_{15})$ was prepared and characterized as described (Burd and Wells, 1974). For the NMR measurements, 50 A_{260} units of the polymer in 2.0 ml was dialyzed into 0.5×10^{-3} M Na_2HPO_4–10^{-6} M EDTA and then concentrated tenfold. Due to uncertainties in ion concentration at these low molarities, the final sodium ion concentrations must be considered approximate; however, it is probably close to 40 mM in sodium ion. To facilitate examination of the resonances in the aromatic and methyl regions, D_2O was used in place of H_2O. D_2O samples were obtained by drying the sample in the NMR tube and D_2O was added in place of H_2O. Oligomer samples in the presence of high salt and actinomycin D (Am) were prepared by evaporating an NMR sample to dryness and redissolving them in the appropriate solution.

NMR spectra were obtained with a Varian Associates HR 300 NMR spectrometer operated in the field-sweep mode. All spectra were averaged (typically, 1–2 h) using a Nicolet, Model 1020A, signal averager to improve the signal-to-noise. Temperature was controlled to ±1 °C and special Wilmad microcells were used. Resonance positions are given in parts per million (ppm) downfield relative to the standard DSS (sodium 4,4-dimethyl-4-silapetane-1-sulfonate).

Ring-current shifts were calculated using a point dipole approximation to Giessner-Prettre and Pullman's calculations (1970). The magnitudes of the dipoles were scaled to fit the Giessner-Prettre and Pullman's results in a plane 3.4 Å above the plane of the base. For pyrimidines, a single dipole, centered in the middle of the six-membered ring, was used, but, for the purines, two dipoles, centered in the middle of the five and six-membered rings, respectively, were used. After the analytical expressions were properly scaled to fit the Giessner-Prettre and Pullman (1970) results at 3.4 Å, they were then used to compute ring-current shifts for any point in space. Comparison of our calculated shift maps with those presented by Giessner-Prettre and Pullman (3.4 Å plane) and more recent calculations by Kroon et al. (1974) for a plane located 6.8 Å away showed that the agreement was better than 0.04 ppm. We conclude that the simplified dipolar expression which we have used is an adequate representation of the more detailed theoretical expression. A more detailed account of this work will be presented elsewhere (Early and Kearns, manuscript in preparation).

All other materials and methods were reported earlier (Burd et al., 1975b).

Results

Assignment of Resonances of $d(C_{15}A_{15})$·$d(T_{15}G_{15})$. The ^1H NMR spectrum of the block polymer was measured in the low field extending from 16 to 11 ppm (Figure 1), the aromatic and ribose regions located between 8.5 and 5.5 ppm (Figure 2), and in the high-field region from 3 to 1.5 ppm (Figure 3). Many resonances observed in these different spectral regions can easily be assigned by comparison with the appropriate model systems (see Table I).

Low Temperature Spectra of the Block Polymer. The lowest field resonance in the block polymer is located at 14.2 ppm. This is identical with the lowest field resonance observed with $d(A)_n$·$d(T)_n$ (see Figure 4) and can, therefore, be assigned to the ring nitrogen proton of T-N_3. The 13.0 ppm resonance of the block polymer coincides almost exactly with the lowest field peak observed in poly(dG)·poly(dC) (Figure 4) and can, therefore, be assigned to the ring nitrogen proton of G-N_1 in a G·C base pair. The anomalous satellite peak at 13.8 ppm is also assigned to A·T base pairs for the following reasons. First, according to present estimates, a resonance of a G·C base pair is never expected to occur below 13.6 ppm (Kearns and Shulman, 1974, Patel and Tonelli, 1974) and because of ring current shifts from nearest-neighbor bases is usually expected to be somewhat higher field than 13.5 ppm. Second, integration of the intensity of the low-field spectrum shows there is nearly equal intensity between 14.4 and 13.6 and from 13.6 to 12.5 ppm. Since the block polymer contains equal numbers of A, G, T, and C (Burd and Wells, 1974), it follows that the 13.8 peak should be assigned to AT base pairs. We will discuss evidence that these resonances eminate from three or four A·T's

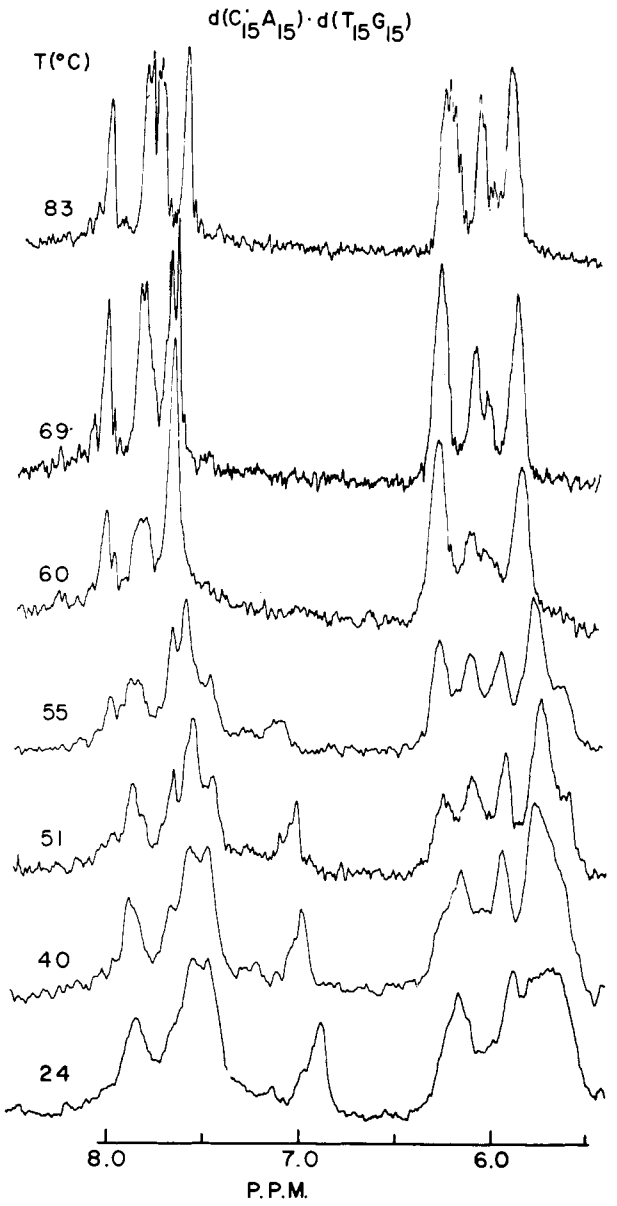

FIGURE 2: The ^1H NMR spectrum of $d(C_{15}A_{15})\cdot d(T_{15}G_{15})$ in the aromatic region in D_2O. For other experimental conditions, see Figure 1.

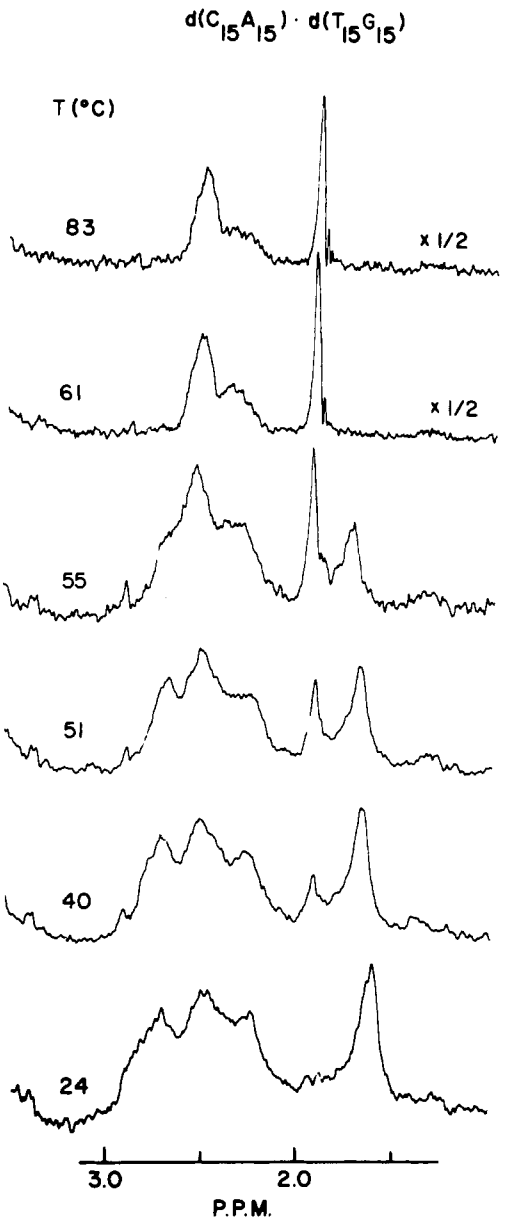

FIGURE 3: The ^1H NMR spectrum of $d(C_{15}A_{15})\cdot d(T_{15}G_{15})$ in the high-field region in D_2O. For other experimental conditions, see Figure 1.

at the junction between the AT and GC blocks in the molecule rather than at the free AT end.

The model systems are again useful in assigning resonances in the aromatic region (Figure 2). Resonances at 7.85 and 6.85 ppm can clearly be assigned to the H_8 and H_2 protons of A, respectively, since these are identical with resonances observed in $d(A)_n\cdot d(T)_{21}$ (see Table I), and the H_8 assignment was confirmed independently by exchange with D_2O (Early and Kearns, unpublished results). Comparison of the intensity at 6.9 ppm with the total intensity in the 6 to 8 ppm region shows that the 6.9 peak in the block polymer accounts for only 11 to 12 of the expected 15 resonances. The remainder of the missing intensity, corresponding to about three to four base pairs, is located in the region between 7.4 and 7.1 ppm and apparently results from three to four A·T base pairs which are in a conformation or environment which is different from the average A·T conformation.

Resonances between 7.4 and 7.7 ppm overlap somewhat; nevertheless, it is possible to assign a peak in the block polymer at 7.48 ppm to the H_6 proton of T, since an analogous resonance is observed at 7.40 ppm in $d)a)_n\cdot d(T)_{21}$. The two remaining resonances, which are located between 7.5 and 7.6 ppm, are, therefore, assigned to the H_8 proton of G and the H_6 proton of C, but they overlap so much that we are unable to distinguish between the two. Assignments of resonances in the ribose region are tentative, since they overlap badly, and we have not been able to follow their melting behavior as well as with the aromatic resonances. Fortunately, none of the conclusions drawn in this paper depend upon these assignments. We made no attempt to assign the resonances from the ribose protons which occur between 2.2 and 3.0 ppm. The resonance at 1.63 ppm in Figure 3 is unambiguously assigned to the methyl group of T, since an analogous resonance is observed at the same position in the spectrum of $d(A)_n\cdot d(T)_n$, and the intensity of this peak relative to the collection of ribose peaks is $\frac{3}{8}$, as expected.

Melting Behavior of the Block Polymer. The effect of the temperature on the block polymer spectra is shown in Figures 1-3. Lowfield: Between 24 and 50 °C the peak assigned to the

TABLE I: Assignments of Resonances in $d(C_{15}A_{15})\cdot d(T_{15}G_{15})$.

Proton	Assignment	Chemical Shifts δ_{Block} (ppm)[c]	Chemical Shifts δ_{Model} (ppm)[c]	Model System[d]
		Random Coil		
A	2	7.81	7.77	$(dA)_n$
	8	8.02	8.02[a]	$(dA)_n$
	C_1'	5.93	5.93	$(dA)_n$
T	6	7.63	7.60	$(dA)_n\cdot(dT)_{21}$
	Me	1.91	1.90	$(dA)_n\cdot(dT)_{21}$
	C_1'	6.25	6.25	$(dA)_n\cdot(dT)_{21}$
G	8	7.83	7.86	$(dG)_n\cdot(dC)_n$
	C_1'	5.93	5.94	$(dG)_n\cdot(dC)_n$
C	5	6.25	6.30 (6.25)	$(dG)_n\cdot(dC)_n((dI)_n\cdot(dC)_n)$
	6	7.76	7.79 (7.74)	$(dG)_n\cdot(dC)_n((dI)_n\cdot(dC)_n)$
	C_1'	6.09	6.11 (6.05)	$(dG)_n\cdot(dC)_n((dI)_n\cdot(dC)_n)$
		Double Helix		
A	2	6.86	6.88	$(dA)_n\cdot(dT)_{21}$
	8	7.85	7.80[a]	$(dA)_n\cdot(dT)_{21}$
	C_1'	5.7	5.74	$(dA)_n\cdot(dT)_{21}$
T	6	7.48	7.40	$(dA)_n\cdot(dT)_{21}$
	Me	1.63	1.66	$(dA)_n\cdot(dT)_{21}$
	C_1'	6.2	6.23	$(dA)_n\cdot(dT)_{21}$
G	8	7.5–7.6	7.61	$(dG)_n\cdot(dC)_n$
	C_1'			
C	5	5.8–6.0[b]		
	6	7.5–7.6	7.61	$(dG)_n\cdot(dC)_n$
	C_1'			

[a] This assignment was confirmed by D_2O exchange experiments. [b] Assignment is based on following the behavior of the C_1' as sample is cooled from 83 → 24 °C, and on ring current shift calculations. [c] Chemical shift, in parts per million, downfield from DSS. [d] Early and Kearns, unpublished results.

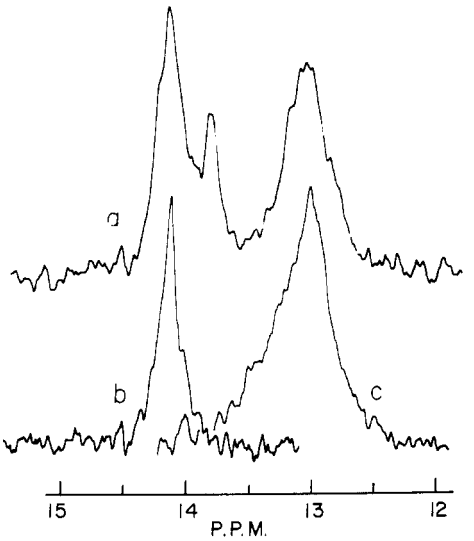

FIGURE 4: A comparison of the low-field NMR spectrum of $d(C_{15}A_{15})\cdot d(T_{15}G_{15})$, (a), with the corresponding spectra of $d(A_{21})\cdot d(T_{21})$, (b) and $d(C)_n\cdot d(G)_n$ (c). All spectra were obtained at 24 °C. The conditions for a are given in Figure 1. The spectra shown in b and c were obtained in solutions containing 0.1 M NaCl and phosphate buffer at pH 7.

TABLE II: Comparison of the Melting Behavior of $d(C_{15}A_{15})\cdot d(T_{15}G_{15})$ as Determined by NMR and Optical Methods.

Temp (°C)	Fraction of AT Base Pairs Melted, f_{AT}: NMR Low Field	NMR Methyl	Optical[a]	f_{GC}: NMR Low Field
24	0	0	~0.05	0.0
40		0.26	0.15	0.0
43	0.26		0.18	0.07
50	0.31	0.36	0.30 ± 0.05	0.11
54	0.60	0.55	0.35 ± 0.05	0.13

[a] These values are uncertain, since they represent interpolations between data obtained in 0.02 and 0.1 M NaCl.

G·C base pairs remains unchanged in intensity and position and the position of the satellite peak at 13.8 ppm is little affected by the increase in temperature. This is to be contrasted with the behavior of the main A·T peak which exhibits a significant upfield shift (14.2–13.8 ppm) and by 50 °C this resonance clearly loses some intensity (see Table II). At 54 °C, the main AT peak is greatly reduced in intensity and there is extensive line broadening ($\Delta\nu_{1/2} \sim 200$–300 Hz). The analysis of Crothers et al. (1974) and Patel and Hilbers (1975), and studies of the melting behavior of tRNA molecules and fragments (Kearns and Shulman, 1974), suggest the pronounced broadening which occurs at 54 °C can probably be attributed to a reduced lifetime of the ring nitrogen proton in the base-paired state. According to the uncertainty principle, $\Delta\nu_{1/2} = 1/\pi\tau$, so, a line width of 300 Hz corresponds to a lifetime of 10^{-3} s. A complete analysis of this and other possible cases has been presented elsewhere (Crothers et al., 1973, 1974) and used recently (Patel, 1976) in interpreting the melting behavior of short deoxyoligonucleotide duplexes.

The GC helix is clearly more stable than the AT helix, since the low-field GC peak (13.0 ppm) is only slightly decreased in intensity when the temperature is raised to 54 °C, and

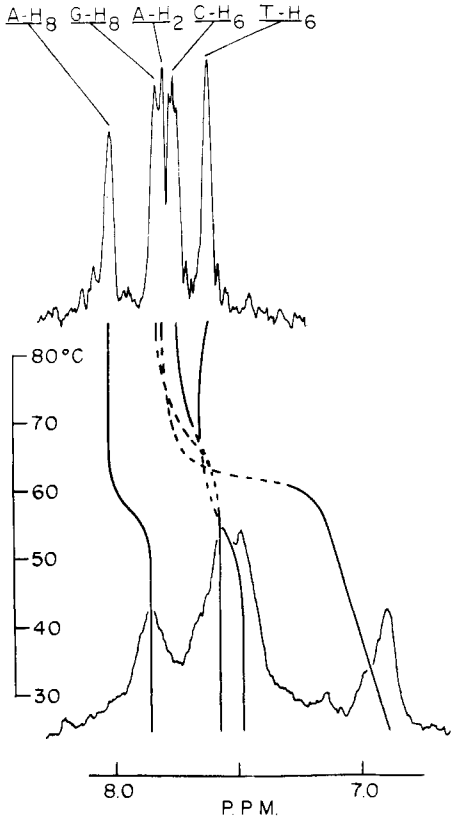

FIGURE 5: Correlation between the resonances observed in the high- and low-temperature spectra of the block polymer $d(C_{15}A_{15})\cdot d(T_{15}G_{15})$ in the aromatic region. Proposed assignments of the resonances are also indicated. In the intermediate temperature range, it is difficult to follow some resonances, and this is indicated by dashed lines.

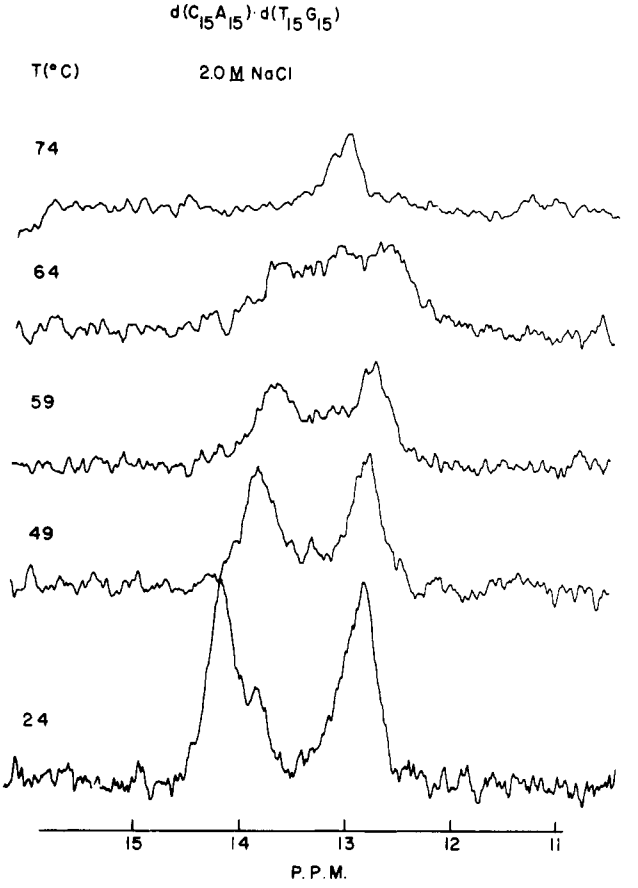

FIGURE 6: The low-field NMR spectrum of $d(C_{15}A_{15})\cdot d(T_{15}G_{15})$ in a solution containing 2 M NaCl, 5 mM Na_2HPO_4, and 10^{-5} M EDTA.

broadening of this resonance only becomes significant somewhere between 54 and 60 °C. Simply comparing the melting behavior of the low-field resonances, we conclude that the T_m of the GC helix is about 5 °C higher than that of the AT portion, in agreement with results derived from a consideration of the temperature dependence of resonances from nonexchangeable protons.

Aromatic Resonances. Between 24 and 55 °C, the resonances assigned to $A\text{-}H_2$ (6.9 ppm in the 24 °C spectrum) shifts downfield by about 0.25 ppm and gradually decreases in intensity. Correspondingly, there is an increase in the intensity at 7.8 ppm, the position characteristic of the H_2 resonance of A in the completely melted state. The melting behavior of the resonance assigned to T_6 is similar. This resonance starts at 7.5 ppm at 24 °C and as the temperature is increased it gradually diminishes in intensity and a new peak characteristic of the random coil (located at 7.7 ppm) grows in intensity. The spectra are not sufficiently well resolved to permit the intensity of the T_6 peaks to be accurately determined, but it appears that the 7.5 and 7.7 ppm peaks have equal intensity at 51 °C, indicating 50% melting of the AT helix at this temperature.

Resonances from H_8 of G and H_6 of C are both located at 7.5 to 7.6 ppm at low temperatures and remain there to 51 °C. This indicates that up to 51 °C there is virtually no change in the conformation of the GC helix. Between 51 and 55 °C, however, there is some slight downfield shift of these resonances from 7.55 to 7.65 ppm and by 69 °C the melting of the GC helix is nearly complete. Between 24 and 40 °C, the methyl resonances (initially at 1.6 ppm) shifts downfield slightly and then gradually diminishes in intensity. Concomitant with the decrease in intensity, a new peak appears at 1.9 ppm, the position characteristic of the random-coil state. The fact that resolved resonances characteristic of *both* the double-helical state and the random coil are simultaneously present in the spectrum indicates that the rate of interconversion between the double helix and the random coil is significantly slower than $(\pi\Delta\nu)$, where $\Delta\nu$ is the chemical-shift difference (in Hz) between the two resonance positions. Therefore, by comparing the relative intensities of the two thymine methyl resonances, the fraction, f_{AT}, of A·T base pairs which have melted at any temperature can be computed. These results are shown in Table II, along with values deduced from optical measurements and measurements on the low-field resonances. Judging from the behavior of the methyl resonances, we conclude that the AT portion of the block polymer is 50% melted at about 55 °C. The total half-width at 55 °C is 30 Hz, so the rate of disruption of the AT helix must be smaller than 100 s^{-1}, since much of the observed line broadening is probably due to slow tumbling of the molecule. When the block polymer is heated above 70 °C, it melts and by 80 °C it is single stranded. In this condition, the resonances in the high-temperature spectrum of the block polymer coincide with resonances observed in the high-temperature spectra of poly(dG)·poly(dC) and $d(A)_n\cdot d(T)_{21}$, and these results are summarized in Table I. The correlation between resonances observed in the high- and low-temperature spectra in the aromatic region is given in Figure 5, along with the proposed assignments.

Effect of High Salt. Spectra of the block-polymer spectrum in high salt (2 M NaCl) are shown in Figures 6 and 7. Relative to spectra obtained in low salt there are several changes which we believe are significant. First, the position of the resonance

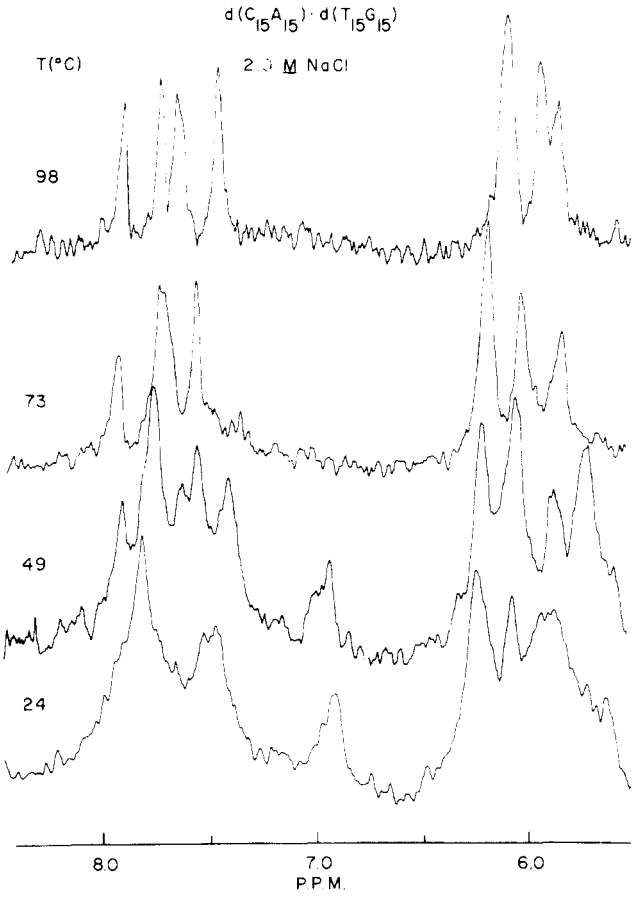

FIGURE 7: The NMR spectrum of $d(C_{15}A_{15})$·$d(T_{15}G_{15})$ in the aromatic region in a D_2O solution containing 2 M NaCl. All other conditions are the same as specified in Figure 1.

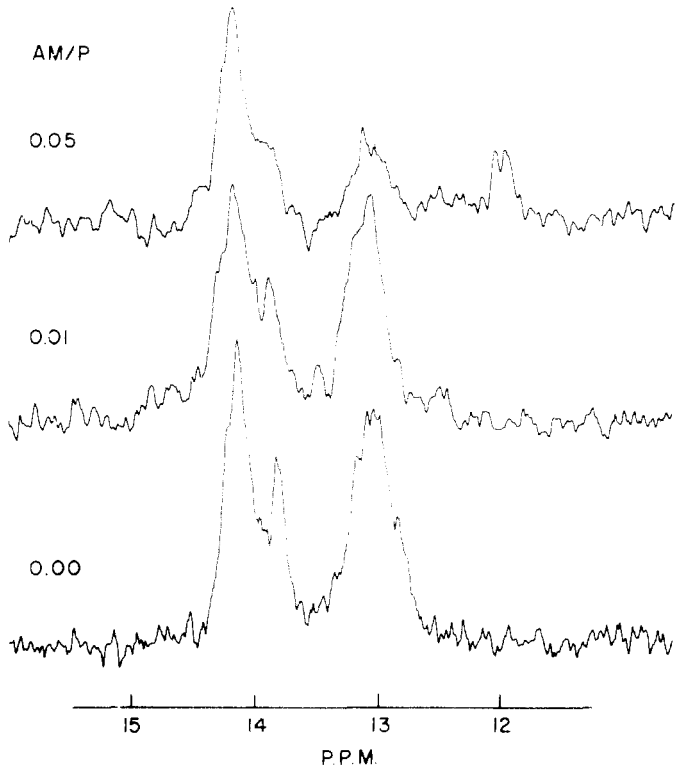

FIGURE 8: The effect of actinomycin (Am) binding on the low-field spectrum of $d(C_{15}A_{15})$·$d(T_{15}G_{15})$. All experimental conditions are the same as those specified in Figure 1. The amount of Am added (specified in terms of Am molecules per phosphate group) is indicated at the left of the figure.

from G·C base pairs shifts from 13.0 to 12.8 ppm and there is a redistribution of intensity in the aromatic region corresponding to a shift of a resonance from 7.6 to 7.75 ppm. Significantly, there is *no* change in the position of the low-field resonance assigned to the A·T base pair (at 14.2 ppm), nor in the resonance at 7.0 ppm which is assigned to H_2 of A. We, therefore, attribute all of the salt-induced changes in the spectrum to changes in the GC helix of the block polymer. The addition of salt also increases the melting temperature of both the AT and the GC helices of the block polymer, and this is evident in both the low-field and aromatic region of the spectrum. That the extent of the T_m elevation (estimated to be 10–15 °C) is less than predicted (Burd et al., 1975a,b) may be due to the approximation of the absolute salt concentration.

Actinomycin Binding. When low levels of Am are added to the block polymer (0.01 Am/nucleotide), there are relatively few changes in the low-field spectrum, except for a slight loss of resolution (compare Figure 1 and 8). At higher levels (0.05 Am/nucleotide), the effects on the G·C resonance are quite pronounced. The peak at 13.0 ppm is significantly reduced in intensity, and a new resonance appears at 12.1 ppm along with a new intensity, which appears distributed throughout the 12 to 13 ppm region. The satellite resonances on the high-field side of the main A·T peak also appear to be broadened by the binding of Am, but, significantly, the main A·T peak does not appear to have been affected.

Because of complications associated with converting back and forth between H_2O and D_2O, the effects of Am on the aromatic and ribose regions of the spectrum were examined only after the addition of 0.05 Am/nucleotide and these results are shown in Figure 9 for various temperatures. As with the low-field spectra, the principal Am effects are on resonances assigned to G and C. It is difficult to follow the melting behavior quantitatively but by monitoring the behavior of the resonance from H_2 of A (located at 7.0 ppm) the effect of Am on the melting behavior of the AT helix can be studied. Comparing spectra obtained with and without Am, it appears that the degree of melting of the AT helix is about the same at 59 °C with Am as it was at 55 °C in the absence of Am (compare Figures 2 and 9).

Discussion

X-ray diffraction (Selsing et al., 1975; Arnott and Selsing, 1974a,b; Davies and Baldwin, 1963; Arnott et al., 1974; Bram and Tougard, 1972;), circular dichroism (Brunner and Maestre, 1974), and other studies (Wells et al., 1970) indicate that the conformation of DNA depends upon the DNA sequence. However, most DNAs in solutions of moderate ionic strength, including very GC-rich DNAs, are believed to have a conformation which is close to the standard B form (Arnott, 1970). On the other hand, $d(A)_n$·$d(T)_n$ is believed to have a different conformation (B′) (Arnott and Selsing, 1974a,b). Various optical studies (Brunner and Maestre, 1974; Pohl and Jovin, 1972; Ivanov et al., 1973; Arnott and Selsing, 1974a,b) also show that there are very substantial ionic-strength effects on DNA conformation and that the extent of the salt-induced changes depend upon the DNA sequence. Because $d(G)_n$·$d(C)_n$ and $d(A)_n$·$d(T)_n$ exhibit such different properties, the block polymer $d(C_{15}A_{15})$·$d(T_{15}G_{15})$ provides an interesting system for study.

The studies described herein confirm and extend the previous work (Burd et al., 1975a,b), which showed that one end

TABLE III: A Comparison of the Computed Ring Current Shifts for Different DNA Geometries with Those Observed Experimentally in $d(C_{15}A_{15})\cdot d(T_{15}G_{15})$.

Proton	Computed Ring Current Shifts[a] (Conformation) A	B	C	D	B′	Obsd Shift (36 °C) (ppm)	Reference Spectrum Used to Cald Obsd Shift
TN-H	0.75	0.55	0.49	0.52	0.72	0.5	poly(dT)
T-Me	0.17	0.03	0.02	0.04	0.09	0.2	
$A-H_2$	1.12	1.18	1.04	1.51	1.62	1.1	poly(dA-dT)
$A-H_8$	0.76	0.36	0.21	0.15	0.28	0.35	
GN-H	0.67	0.50	0.46	0.47	0.64	0.6	poly(d(dG-dT))
$G-H_8$	0.46	0.22	0.14	0.09	0.17	0.2-0.3	

[a] The geometries of the different DNA conformations are indicated below. These helical parameters are identical to those used by Studdert and Davis (1974), except the radius is measured from the helix axis to the twist axis.

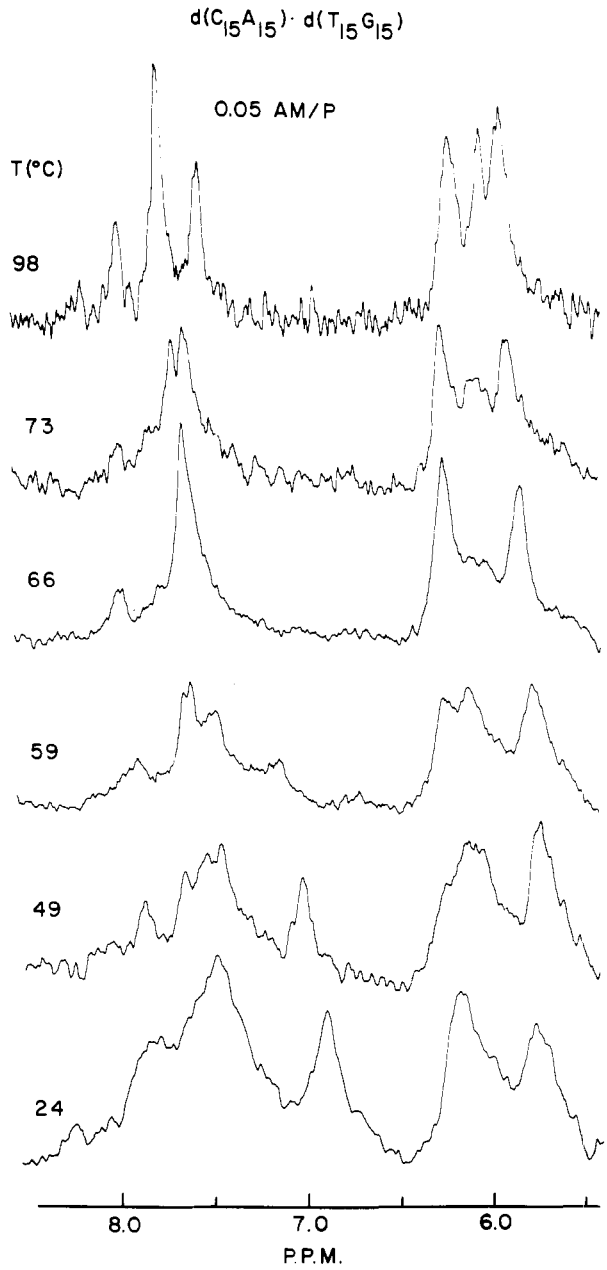

FIGURE 9: The effect of actinomycin on the aromatic NMR spectrum of $d(C_{15}A_{15})\cdot d(T_{15}G_{15})$ in the aromatic region in D_2O. The salt and pH conditions are specified in Figure 1.

of $d(C_{15}A_{15})\cdot d(T_{15}G_{15})$ influences its other end. Specifically, we find the following. (1) The presence of 15 A·T pairs had a pronounced influence on the melting characteristics of the GC block and vice versa. However, in contrast to the UV-absorbance measurements, NMR studies detect a melting of the AT end of the block polymer slightly before the GC end; as discussed below, this is due to the fact the two techniques monitor different aspects of the melting process. (2) Am binds only to the GC end of the polymer but thermostabilizes both the GC and AT ends. (3) The majority of base pairs in the AT helix have a conformation which is identical with that found for $d(A)_{21}\cdot d(T)_{21}$. Hence, telestabilization of the AT helix by the GC helix is not due to a general conformational change in the AT helix (induced by the coupling to $d(G)_{15}\cdot d(C)_{15}$). Rather, it should be interpreted in terms of a change in the dynamics of the AT helix to coil transition.

Conformation of the AT and GC Helices in the Block Polymer. Since the principal resonances found in the block polymer have positions which are identical with those observed in either $d(A)_n\cdot d(T)_{21}$ or $d(G)_n\cdot d(C)_n$ (see Table I), we conclude that the conformations of the AT and GC helices in the block polymer are identical with those of $d(A)_n\cdot d(T)_{21}$ and $d(G)_n\cdot d(C)_n$, respectively. We have computed the effect which ring currents have on the resonance positions of DNA in the four major DNA conformations (A, B, C, and D) and for the special conformation (B′) which Arnott and Selsing (1974) recently reported for $d(A)_n\cdot d(T)_n$ and these results are shown in Tables III and IV together with the experimental shift values. In comparing calculated and "experimental" values, it should be kept in mind that the observed shifts may include contributions from a number of effects, whereas the computed values are only for the ring currents. As Borer et al. (1975) have pointed out, the observed chemical shifts could contain contributions from (1) changes in the electronic structure due to base-pair formation (2) changes in local solvation, (3) changes in the shielding of protons by the phosphate group, and (4) changes in the binding of cations. Although most of these effects are expected to be small (usually less than 0.1 ppm), their contribution to the relative chemical shifts may be important when the ring current shifts are small. This is especially true for the H6 protons of C and T which are expected to be most sensitive to shielding effects from the phosphate group, and in the block polymer are predicted to have very small ring current shifts for all DNA geometries examined. In view of these considerations, we have focused our attention on other resonances where the observed shifts are considerably larger.

TABLE IV: Computed Ring Current Shifts of Low Field Resonances.

Base Pair and Location	Computed Ring Current Shift	
	B Conformation	C Conformation
Terminal T·A	0.14	0.33
$T{\cdot}A_2$	0.38	0.40
$T{\cdot}A_{3-13}$	0.54	0.49
$T{\cdot}A_{14}$	0.56	0.43
Junction $T{\cdot}A_{15}$	0.89	0.49
Junction $G{\cdot}C_{16}$	1.40	0.78
$G{\cdot}C_{17}$	0.62	0.49
$G{\cdot}C_{18-28}$	0.51	0.45
$G{\cdot}C_{29}$	0.41	0.38
Terminal $G{\cdot}C_{30}$	0.18	0.27

Within this limitation, we find that a reasonable fit of all the shift data can be obtained assuming either a B or C conformation, although a slightly better fit is obtained with the B conformation. Significantly, the B′ structure reported for $d(A)_n{\cdot}d(T)_n$ (Arnott and Selsing, 1974) gives a much poorer fit of the data. Specifically, the ring current shift predicted for H_2 of A is much too large and the shifts on the ring nitrogen protons of G and T are also too high. With the A-DNA conformation, the predicted shifts are too large for the ring nitrogen protons of both A·T and G·C, and for A_8 and G_8 protons. With the D conformer, the predicted shift for A_2 is much too large. Thus, while our data do not allow determination of a unique structure, three of the five possible structures which have been observed for DNA fibers are seen to give poorer accounts of the data.

Influence of the GC Helix on the Conformation of A·T Base Pairs at the AT–GC junction. While the majority of the base pairs in the AT helix have a conformation which is identical with that observed with $d(A)_{21}{\cdot}d(T)_{21}$ (Figure 2), our NMR data provide evidence that some of the A·T base pairs in the block polymer are in an unusual conformation or environment. The following considerations indicate that the low-field satellite peak at 13.8 ppm (Figure 2) is due to A·T base pairs located at the junction of the AT and GC helices, rather than at the free end of the 30-mer duplex.

Comparison with $d(A)_{21}{\cdot}d(T)_{21}$. From a comparison of the low-field NMR spectra of the block polymer, $d(A)_{21}{\cdot}d(T_{21})$ and $d(G)_n{\cdot}d(C)_n$ in (Figure 2), it is clear that the 13.8 ppm satellite peak has no counterpart in the spectra of $d(A)_{21}{\cdot}d(T)_{21}$. This provides strong evidence that the AT satellite peak is due to A·T base pairs located at or near the AT–GC junction. Theoretically, some sort of end effect associated with the free ends of the block polymer might be expected because terminal base pairs lack ring current shifts from neighboring bases on one side (Patel and Hilbers, 1975; Kan et al., 1975; Kearns and Shulman, 1974) but experimentally this seems to be rather small in the case of $d(A)_{21}{\cdot}d(T)_{21}$ (see Figure 4).

Temperature Effects. The temperature dependence of the low-field spectrum provides additional evidence that the 13.8 ppm satellite peak is not due to terminal A·T base pairs. Melting of the block polymer should initiate at the free ends of the molecule (Patel and Hilbers, 1975) and, therefore, resonances from terminal base pairs should be more susceptible to temperature effects. In the case of the low-field resonances, "fraying" of the free end could produce a significant upfield shift of resonances from A·T base pairs located at or near the free end of the AT helix (Patel and Hilbers, 1975). This effect, already observed in the NMR spectra of several short deoxyoligonucleotide duplexes (Patel, 1976; Patel and Hilbers, 1975; Kan et al., 1975; Crothers et al., 1973; Wong and Kearns, 1974) might then account for the 13.8 ppm satellite peak. However, if this were the explanation, the 13.8 ppm resonance should be *more* temperature sensitive than the resonances from the main AT helix. Experimentally, the *opposite* behavior is observed (see Figure 1). It is the *main* AT peak (at 14.2 ppm), rather than the 13.8 satellite peak, which shifts upfield when the temperature is raised. There is relatively little shift in the low-field resonance from the G·C base pairs. These results suggest that the average conformation of the AT segment changes with temperature, but the conformation of the GC helix and adjacent A·T base pairs are much less affected.

TABLE V: Derivatives of the Ring Current Shifts (δ) on the Ring Nitrogen Proton of Thymine and the H_2 Proton of Adenine with Respect to Changes in the Helical Parameters of the B Conformer of DNA.

Parameter (P)	$\frac{\partial\delta}{\partial P}$ T–NH	$\frac{\partial\delta}{\partial P}$ A–H_2
Radius	0.14 ppm/Å	0.04
Stack	−0.04 ppm/Å	−0.65
Pitch	0.35 ppm/rad	0.78
Tilt	−0.89 ppm/rad	−0.53
Twist	−0.36 ppm/rad	−2.22

Satellite Peaks on the A–H_2 Resonance. If part of the AT helix is in an unusual conformation, this should also be manifested in several spectral regions. The A–H_2 resonance is especially sensitive to the conformation of the AT helix, since it receives a very large ring current shift from neighboring A residues. While satellite peaks are not evident on the 6.9 ppm peak, integration of the aromatic spectrum (Figure 2) shows that the main A–H_2 peak is missing about 3 to 4 resonances and that the missing intensity appears as satellite on the low-field side between 7.0 to 7.5 ppm (24 °C spectrum). No satellite resonances are observed in the 7.1 to 7.4 ppm region of the spectrum of $d(A)_{21}{\cdot}d(T)_{21}$ (Early and Kearns, unpublished results).

Am Binding. Am binds specifically to the GC region of the block polymer, and when 3 Ams/block polymer are added, there are pronounced changes in the low-field spectrum in resonances associated with G·C base pairs and the 13.8 satellite peaks are broadened (Figure 4). The principal A·T peak at 14.2 ppm remains well resolved, however.

The above set of observations, taken collectively, indicate that the satellite peaks on the main T–N_3H and A–H_2 resonances are due to A·T base pairs located at or near the junction of the AT and GC helices. This immediately raises the question as to what is responsible for the perturbation of the A·T resonances. Clearly, A·T and G·C base pairs at the junction of the two helices could receive ring current shifts which are different from A·T and G·C base pairs in the interior of the two helices. Using the ring current shift theory, we calculated the ring current shifts on base pairs which are at, or adjacent to, the junction of the two helices and the results are shown in Table V. Several interesting conclusions emerge from these results. First, if the AT and the GC helices were both in a B conformation, then we could not account for the A·T satellite peaks, since only one A·T resonance would be significantly upfield shifted from the main A·T peak. Moreover, theory predicts that the low-field resonance associated with the G·C base pair at the AT–GC junction should be shifted upfield from the main GC resonance (at 13.0 ppm) by *0.9 ppm.* Examination of the low-field spectra presented in Figure 1 reveals no evidence for

TABLE VI: Geometries of DNA Conformations.

Conformation	Radius (Å)	Stack (Å)	Pitch (deg)	Tilt (deg)	Twist (deg)
A	4.6	2.56	32.7	20	−4
B	−0.1	3.33	36	−2.1	4.9
C	−2	3.32	38.6	−6	5
D	−1.8	3.03	45	−16	4
B′	0.1	3.29	36	−7.9	−1

such a peak, and, in fact, the most upfield-shifted GC peak is displaced less than 0.25 ppm upfield from the main GC peak. We therefore conclude that the AT and GC base pairs in the junction region are not in a standard B conformation. The A, D, and B′ conformations also predict a high-field satellite on the GC peak. Of the five geometries examined, only the C conformational type predicts a relatively small upfield displacement of the resonance from the main GC peak. However, if both the AT and GC helices were in a C conformation then we would be unable to account for the collection of 3 to 4 AT satellite peaks which are observed in the spectrum. Furthermore, because of the rapid decrease in the magnitude of ring current fields with distance, this mechanism could only account for one specially shifted resonance, the one from the AT base pair at the junction. Therefore, within the group of five possible geometries examined, we are unable to arrive at a *common* geometry for both the AT and the GC helices, which will simultaneously account for all our observations on the satellite peaks. Alternative explanations must be considered and one possibility suggested by previous studies of sequence effects on DNA conformation is that the AT and the GC helices in the block polymer have different conformations. In this case, some base pairs near the junction region would be in a perturbed conformation because of the transition from one geometry to another, and in the transition region the ring current shifts could be quite different from those in the main AT helix.

To obtain some estimate of the conformational changes which would simultaneously upfield shift T-N_3H resonances from 14.2 to 13.8 and downfield shift A-H_2 resonances from 6.9 to 7.3 to 7.5 ppm, we computed derivatives of the ring current fields with respect to changes in the usual helical parameters, and these results are shown in Table VI. Starting from a normal DNA B conformation, we find that all distortions which would cause an upfield shift of T-N_3H would also cause an upfield shift of A-H_2 and, therefore, at least two helical parameters must be changed to account for an *upfield* shift of T-N_3H and a *downfield* shift of the A-H_2 resonance. While there does not appear to be a unique solution to the precise conformation changes required, we note that a 5 to 8° increase in twist angle would lead to a 0.2 to 0.3 ppm reduction in the shift of the H_2 resonance of A and that a decrease of 10 to 15° in the tilt angle would produce an upfield shift of 0.2 to 0.3 ppm on the ring nitrogen proton.

Comparison of the NMR and Optical Melting of $d(C_{15}A_{15})\cdot d(T_{15}G_{15})$. UV absorption-temperature studies have demonstrated that the AT helix of the block polymer melts at a higher temperature than would be expected in the absence of telestabilization (the T_m of $d(A)_{15}\cdot d(T)_{15}$ is 17 °C and even for $d(A)_{30}\cdot d(T)_{30}$ the T_m is 32 °C at the approximate salt concentration used in the NMR experiments). Correspondingly, the T_m of the GC helix was lowered in the block polymer relative to T_m of $d(G)_{15}\cdot d(C)_{15}$ (Burd et al. (1975a). The UV absorbance-temperature studies also indicated that the AT and GC helices of the block polymer melt simultaneously (Wartell and Burd, 1976).

The NMR measurements provide a slightly different picture of the melting behavior of the block polymer. Three different resonances, the methyl resonance at 1.6 ppm, the H_2 resonance of A at 6.9 ppm, and the low-field A·T resonance at 14.2 ppm, can be used to follow different aspects of the melting of the AT helix. As the temperature increases from 24 to 60 °C, the methyl peak characteristic of the double-helical state (1.6 ppm) gradually diminishes in intensity and the new peak (1.9 ppm) characteristic of the random coil grows in intensity (Figure 8). Since both peaks are reasonably well resolved, this seems to indicate that $k_{h\rightarrow c}$, the rate of conversion from the helical (h) to random coil (c) state, is significantly less than 100 s^{-1}, at least up to 55 °C where the AT portion of the molecule is over half melted. Since the interconversion between the helical and random coil states is less than 100 s^{-1} at this temperature, the small downfield shift of the methyl resonance observed on warming from 24 to 55 °C is attributed to a change in the average conformation of the molecule, rather than rapid interconversion between the two states. The fraction of A·T base pairs which have melted out at any temperature can be determined by measuring the relative intensities of the resonances characteristic of the random coil and the double-helical state (see Figure 3), and the results given in Table II show that there is reasonable agreement with values determined from optical measurements and from measurements in the low-field region.

The behavior of the A-H_2 resonance parallels that of the methyl resonance in that it gradually diminishes in intensity and shifts downfield as the temperature increases (Figure 5). The shift with temperature is much larger for the A-H_2 resonance than the methyl resonance, consistent with the fact that the helix-coil chemical-shift differences are much larger for A-H_2 than for the methyl resonances. Again, the shift from 6.9 to 7.1 ppm between 24 to 55 °C indicates a change in conformation with temperature.

The low-field resonance exhibits a somewhat different melting behavior (Figure 1). Upon heating from 24 to 60 °C, the resonance at 14.2 ppm first shifts upfield by about 0.4 ppm, broadens and then disappears. From the relation between the line width ($\Delta\nu_{1/2}$) and the lifetime, τ, of a proton in the base-paired state ($\Delta\nu_{1/2} = 1/\pi\tau$), we conclude that the lifetime of an A·T base pair is reduced to less than 1 ms at 54 °C. The different behavior of the low-field and the A-H_2 resonance can be attributed to the fact the low-field peak monitors disruption of the hydrogen bonds in the A·T base pairs and this can occur without unstacking of the bases in the two strands. However, the A-H_2 resonance is sensitive to the transition to the completely melted state, and, therefore, melting of the low-field resonance must precede melting of the A-H_2 resonance.

Resonances from the ring nitrogen proton of G provides a convenient monitor of the melting of the GC helix (Figure 1). At 54 °C, the intensity of the 13.0 ppm resonance is only decreased by 13% (compared to 60% reduction in the intensity of the AT peak) and this resonance is still observed at 60 °C, even though the resonance from the A·T base pairs has dis-

appeared. At 54 °C, the line width of the 13.0 ppm resonance is ~100 Hz, indicating that the lifetime of a proton in a G·C base pair is on the order of 10 ms. From the NMR measurements, it is clear that the AT helix melts at lower temperature than the GC helix, but at a temperature above that expected in the absence of telestabilization.

Effect of Salt on the Conformation of the Block Polymer. The conformation of DNA in fibers is sensitive to the salt concentration and it was therefore of interest to see how changes in the salt concentration affected the conformation of the block polymer. Spectra of the block polymer obtained in high salt are shown in Figures 6 and 7. By comparing spectra obtained in low salt (Figure 1 and 2) with spectra obtained in high salt, we find there is a salt-induced shift of the resonance from N_1-H of G from 13.0 to 12.8 ppm, as well as shifts of other resonances in the aromatic region which can be assigned to G and C.

Interestingly, none of the resonances unambiguously assigned to the main AT helix (at 14.2, 6.9, and 1.6 ppm) are affected by the increase in salt. We think it particularly significant that the main A_2 peak, which is very much upfield shifted by nearest-neighbor ring current effects and, therefore, very sensitive to changes in geometry, appears at the same position (6.9 ppm at 24 °C) in both the high- and low-salt samples. If high salt induced a change in the conformation of the AT helix, this resonance would undoubtedly have shifted. The spectra are not sufficiently well resolved to determine whether there is a salt effect on the AT satellite peak. The fact that there are changes in all resonances associated with the GC helix (including resonances in the low-field aromatic and, perhaps, the ribose region) indicates that salt induces a change in the conformation of the GC helix but not the AT helix. Pohl and Jovin (1972) have previously presented evidence for a salt-induced change in the conformation of $(dG\text{-}dC)_n \cdot (dG\text{-}dC)_n$. Previous studies (Selsing et al., 1975; Burd et al., 1975a) demonstrated that AT- and GC-rich DNAs respond differently to the addition of high salt and our NMR results provide additional support for this notion. Since the conformation of GC is salt dependent whereas the conformation of AT is independent of salt over the same range, it follows that there may only be a limited range of salt concentrations (at a specific temperature) where the GC and AT segments of the block polymer have identical conformations. This provides additional support for our conclusion that the conformations of the AT and GC helices are different from each other in 40 mM NaCl.

While increased NaCl affects the conformation of only the GC portion of the molecule, it clearly stabilizes both the AT and GC helices with respect to thermal denaturation by over 10 to 15 °C (compare Figures 1 and 7).

Influence of Actinomycin on $d(C_{15}A_{15})\cdot d(T_{15}G_{15})$. NMR studies on the block polymer in the presence of Am were of interest, since Am binds only to the G·C pairs of this molecule (Wells and Larson, 1970; Burd et al., 1975b). Also, it was shown by thermal denaturation and by *E. coli* exonuclease I susceptibility studies (Burd et al., 1975b) to influence both the AT and GC portions, even though it does not bind to the AT portion. Therefore, one goal of the Am binding study was to find out whether structural perturbations introduced in the GC portion of the molecule were transmitted into the AT helix.

The low-field NMR results agree with the conclusion (Burd et al., 1975a,b) that Am binds specifically to the GC portion of the block polymer. There does not appear to be any effect on the conformation of the main AT helix, although A·T base pairs located near the AT-GC junction are perturbed (Figure 8). Resonances from the G·C base pairs are, however, significantly upfield shifted (from 13.0 to up to 12.0 ppm) when Am binds. The fact that a range of shift values were observed can be attributed the lack of symmetry of the Am (Patel, 1974) and the different effect which Am has on the two adjacent base pairs (in one case a relatively small ring current shift from a C is replaced by a large Am ring current shift, whereas in the other case the relatively large shift from a neighboring G is replaced by Am). Changes in GC resonances are also expected because the Am binding introduces structural perturbation in the DNA helix which might not be damped out for 3 to 4 base pairs (Wartell et al., 1975; Patel, 1976). Because of a perturbed geometry, the ring current shifts on bases located even three sites away from the Am binding site could be affected. As a result of these factors, we expect at least 6 of the 15 resonances from GC base pairs to be perturbed by the Am binding and, experimentally, we find a new resonance at 12.1 ppm (intensity of 3 to 4 protons) and a broad collection of resonances located between 12.2 and 12.8 ppm (intensity of 3 to 4 protons) which appear with the binding of Am (see Figure 8). The intensity remaining at 13.0 ppm, the position assigned to unperturbed G·C pairs, corresponds to approximately 7 base pairs, assuming that the collection of resonances located between 14.4 and 13.5 ppm correspond to 15 protons. We conclude that the rather large distortions which occur in the GC portion of the molecule upon Am binding are "damped" out in a short distance, as suggested earlier (Wartell et al., 1975), and have relatively little affect on the low-temperature conformation of the AT helix.

The rather large (~1 ppm) upfield shift on the ring N protons of the G·C base pairs is consistent with the ring current calculations of Giessner-Prettre and Pullman (1970) and the intercalation model proposed by Sobell and Jain (1972) to account for the binding of Am to DNA.

The binding of Am clearly produces significant changes in the aromatic region of the spectrum (Figure 9), but there is no evidence for changes in any resonances associated with the AT helix. Specifically, the resonance from H_2 of A remains unchanged at 6.9 ppm (compare Figures 2 and 9). The high-field region of the spectrum is greatly complicated by resonances from Am itself, but the T-CH_3 peak at 1.6 ppm was still evident. (A couple of resonances from Am are also expected in the aromatic region, but these are apparently obscured by the more intense block polymer peaks.)

Although Am only affects the *conformation* of the GC helix, it affects the thermal stability of both the GC and the AT helix and this is manifested in several ways. First, comparison of the behavior of the A-H_2 resonances in the presence and the absence of the Am (see Figures 2 and 9) suggests the T_m of the AT helix is raised by about 5 °C by Am. The low-field resonances, which monitor "breathing" of the AT and GC base pairs, also indicate that the A·T base pairs are stabilized by Am. In samples containing Am, a vestige of the 14.0 ppm resonance from A·T base pairs can be seen at 60 °C (data not shown), whereas in the Am-free samples, this resonance has all but disappeared by 54 °C.

The Nature of Telestabilization. Earlier studies clearly indicate that the properties of $d(A_{15})\cdot d(T_{15})$ are altered when this helix is joined with $d(G_{15})\cdot d(C_{15})$ (Burd et al., 1975a,b; Burd and Wells, 1974). This includes altered melting properties (monitored optically), sensitivity to enzymatic digestion, and other effects. The actinomycin binding studies also demonstrated that binding of the drug to the GC helix affects the properties of the AT helix in $d(C_{15}A_{15})\cdot d(T_{15}G_{15})$. These various long-range stabilizing effects, termed telestabilization

(Burd et al., 1975a), could be interpreted in terms of a long-range propagation of a conformational change in which the static conformation of most, or all, of the AT helix is changed by coupling with the GC helix. Alternatively, telestabilization may result from a change in the dynamic properties of the AT helix induced by the GC helix. Our NMR results clearly show there is a difference in the conformations of the main AT and GC helices, but they also show that the structural perturbation only extends by about 3 to 4 base pairs into the AT helix, and the conformation of the main AT helix is unchanged when joined with the GC helix. We therefore attribute most of the telestabilization of the AT helix to a change in the dynamic properties, rather than the conformation of the AT helix.

Biological Implications. The characteristics of telestability and the implications of this property of DNA for the regulation of gene expression were discussed previously (Burd et al., 1975b). The NMR studies described herein confirm and extend the previous work with $d(C_{15}A_{15})\cdot d(T_{15}G_{15})$. The GC block stabilizes the AT block and the AT block destabilizes the GC block. In addition, the binding of Am to the GC block telestabilizes the AT block. These NMR studies indicate that this telestabilization is due to a dynamic property, rather than to a change in the conformation of the AT end induced by the ligand binding to the GC end.

Two most important new observations are revealed by the NMR studies. First, the conformation of 3 to 4 AT pairs at the junction of the AT–GC blocks is different from the conformation of the majority of the A·T pairs. Hence, if this conformational anomaly is found in chromosomal DNA, the presence of contiguous blocks of AT and GC pairs would provide a mechanism for generating a novel DNA conformation which could serve as a recognition site for regulatory proteins. Several examples of these types of sequences already have been revealed in certain regulatory sequences (reviewed in Wells, Blakesley, Burd, Chan, Dodgson, Hardies, Horn, Jensen, Larson, Nes, Panayotatos, Selsing, Tamblyn, and Wartell, manuscript in press). This data also provides the first evidence, to our knowledge that two DNA conformations can exist simultaneously in a DNA. Second, these studies demonstrate an influence of high-salt concentrations on the conformation of the GC, but not on the AT helix. Thus, the conformation of a GC-rich region of DNA might easily be changed into other conformations by environmental influences within the cell. These environmental variances could include protein binding to DNA, changes in ionic conditions, etc. For example, different promoter sequences could be turned on or off by environmental alterations depending on the base sequence of that particular promoter. Studies currently in progress on the isolation of large amounts of defined genetic regulatory sites and on chemical, physical, and biological investigations on these sites may determine the validity of these assertions (Hardies, Horn, and Wells, unpublished work).

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