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Base pairing structure in the poly d(G-T) double helix: wobble base pairs

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ABSTRACT

High resolution nuclear magnetic resonance (NMR) and ethidium bromide binding studies are used to demonstrate that poly d(G-T) forms an ordered double helical structure at low temperatures (below 24°C in 0.3 M NaCl) in which G and T are hydrogen bonded together in a wobble base pair hydrogen bonding scheme as proposed earlier by Lezius and Domin[1]. Alternative hydrogen bonding schemes involving the tautomeric form of either T or G, such as have been proposed to account for mutation rates in DNA synthesis, are eliminated.

INTRODUCTION

Base pairing between G and U (T) was first proposed by Crick[2] to account for the degeneracy in the genetic code (see Fig. 1a). G•T base pairs have recently been proposed by Topal and Fresco[3] to account for spontaneous mutation in the translation of DNA. While there have been many attempts to experimentally demonstrate the formation of wobble base pairs, the results to date have been conflicting. Chan et al.,[4] for example, found no evidence for formation of G•U wobble base pairs in DMSO-water systems, but suggest instead that U pairs with a tautomeric form of (G) in the manner indicated in Fig. 1b. Gray et al.,[5] found no CD evidence for base pairing in poly r(G-U), but under slightly different experimental conditions (higher salt, lower temperature), Lezius and Domin[1] and, more recently, Gray and Ratliff[6] found CD evidence for the formation of an ordered (and presumably base paired) structure with poly d(G-T). NMR studies of the base pairing between dinucleotides gave no evidence for self pairing of d(pGpT) under conditions where d(pGpC) self pairs[7]. G•U oppositions frequently occur in the secondary structure of tRNA molecules[8], and x-ray diffraction data on yeast $tRNA^{Phe}$ are consistent with formation of G•U wobble base pairs[9,10,11]. Certain resonances in the NMR spectra of tRNA molecules (10-11 ppm) have also been attributed to G•U pairs[12] but these assignments have been questioned[13]. Because of this uncertain experimental situation and the possible biological importance of G•U and G•T pairs, we have

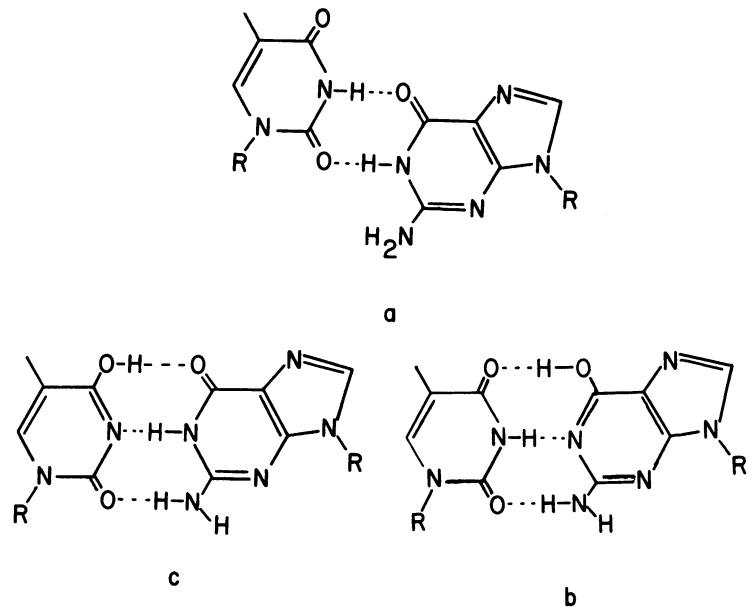

Figure 1. A summary of possible hydrogen bonding schemes for G and T. (a) The Crick wobble base pair, (b) Base pair involving the tautomeric form of G (G*) with T and (c) Base pairing involving the tautomeric form of T (T*) with G.

re-examined the behavior of poly d(G-T) since this is the one system where the circumstantial evidence for formation of wobble base pairs is strongest. In our study two different experimental techniques, NMR and ethidium bromide binding, are used to investigate the structure of poly d(G-T) in aqueous solution. High resolution NMR was used to provide information about the hydrogen bonding scheme. Ethidium bromide was used because its fluorescence is significantly enhanced on binding to double helical RNA and DNA. These studies demonstrate that poly d(G-T) forms a stable double helical structure, in which most (if not all) Gs and Ts are base paired according to the wobble base pairing scheme proposed by Crick[2] and Lezius and Domin[1]. The formation of base pairs involving the tautomeric states of G and T is eliminated, as are other possible pairing schemes.

MATERIALS AND METHODS

Ethidium bromide (Cal Biochem) showed only a single spot on TLC (1:1 butanol-acetic acid-water) and was used without further purification. The poly d(G-T) was prepared using methods described elsewhere[14]. Gel electrophoresis of poly d(G-T) using polyoma DNA restriction fragments as markers indicated that the average molecular weight size of the poly d(G-T) polymers

was 385 residues (see Fig. 2). For most of the NMR experiment the poly d(G-T) (approximately 30 mg/ml) in a solution containing ∿ 0.3 M NaCl and 10 mM cacodylate at pH 7.0 was used. The exact salt concentration is uncertain because this sample was prepared by lyophylization of a more dilute solution. The poly A, poly G and poly U samples used in the ethidium bromide binding experiments were obtained from Miles Laboratories.

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

Most experiments on the binding of ethidium bromide to poly d(G-T) were carried out on optical quantities of the polymer (OD_{260} = 0.65, nucleotide concentration = 6.2 x 10^{-5} M), buffered at pH = 7.0 with 0.01 M cacodylate, 0.1 M NaCl (except where otherwise noted), and with 1 mM $MgCl_2$ to minimize nonintercalative, electrostatic binding. Fluorescence titration curves were obtained by successive addition of small (5-30 µl) aliquots of concentrated ethidium bromide to solutions (3 ml) of polymer solution contained in a

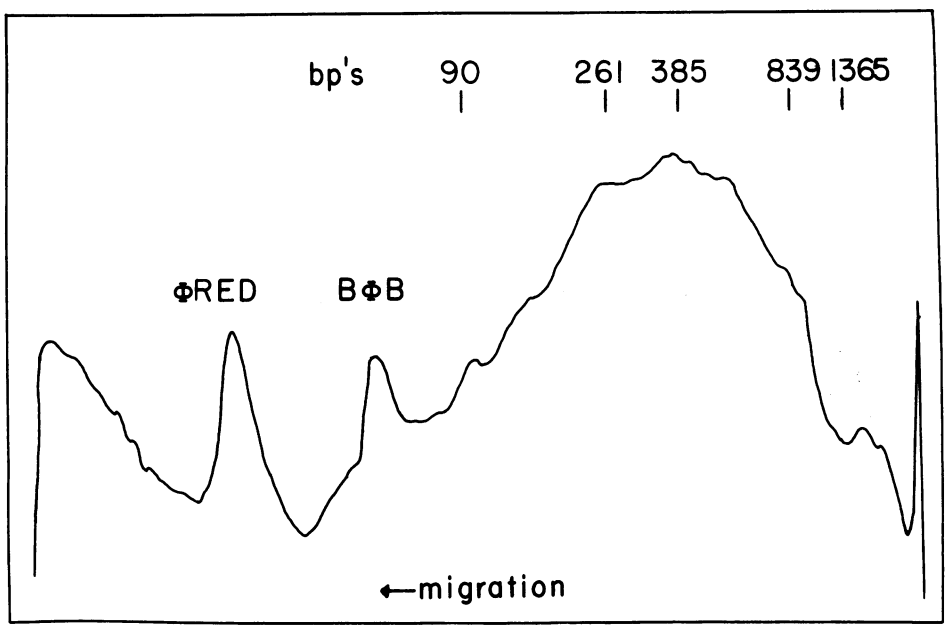

Figure 2. The electrophoretic mobility of poly d(G-T) in a 4% polyacrylamide gel. The approximate base pair numbers indicated on the figure were derived from an HpaII restriction enzyme digest of polyoma DNA run on an identical gel.

fluorescence cell. Fluorescence was excited by 546 nm light from a 200-watt Hg lamp passed through a Bausch and Lomb high intensity monochromator, and emission was viewed at 90° to excitation through a Corning 2-58 filter by an RCA 7265 photomultiplier tube. Fluorescence melting curves of polymer solutions containing bound ethidium bromide were obtained using this same apparatus. The fluorescence cell was positioned in a metal block through which water was circulated from a Haake constant-temperature bath. Because relatively high sample concentrations were used in the NMR experiments, some additional ethidium bromide binding studies were carried out on the same sample used in the NMR experiments. For comparison, analogous binding studies were also carried out on concentrated samples of poly(A)·poly(U), poly(A), poly(G) and on calf thymus DNA. All samples were prepared to have the same ethidium bromide concentration (1.3×10^{-5} M) although the polynucleotide concentrations varied between 25 to 100 mM in P. Fluorescence decay curves for ethidium bromide bound to poly d(G-T) were measured by single-photon counting and were computer-fitted as sums of two exponentials by the method of moments as previously described[15,16].

RESULTS

NMR Spectra: Assignment of Resonances of poly d(G-T). The proton NMR spectra of poly d(G-T) were measured in H_2O in the region downfield from the water resonance (16 to 6 ppm downfield from the internal reference DSS) with the results shown in Figs. 3 and 4. Five resonances were observed in this region with chemical shifts (relative intensities to ± 15%) of 6.05 (2.0), 6.45 (2.0), 7.35 (1.0), 8.0 (1.0) and 11.0 (2.0) ppm at 24°C. Each resonance in the polymer spectrum can easily be assigned by comparison with the corresponding dinucleotide spectra (see Table I).

The resonance at 6.05 ppm is assigned on the basis of position and relative intensity (2.0) to the C_1 protons of T and G. The resonance at 6.45 ppm (intensity of two protons) is from the free amino group of G. Ordinarily, resonances from amino groups involved in base pairing (hydrogen bonding) shift to lower fields (approx. 8.5 ppm in G·C base pairs[17]). Since the resonances from the amino protons of G in poly d(G-T) exhibit no such downfield shift, we conclude that they are not involved in base pair hydrogen bonds. The resonances at 7.35 and 8.0 ppm (intensity corresponding to one proton each) are easily assigned on the basis of dinucleotide spectra to T-H_6 and G-H_8, respectively. Comparison of the intensities of the peaks located at 8.0 ppm (relative intensity 1.0) and 7.35 ppm (relative intensity 1.0) proves that the sample contains equal amounts of G and T (within 10%). Furthermore,

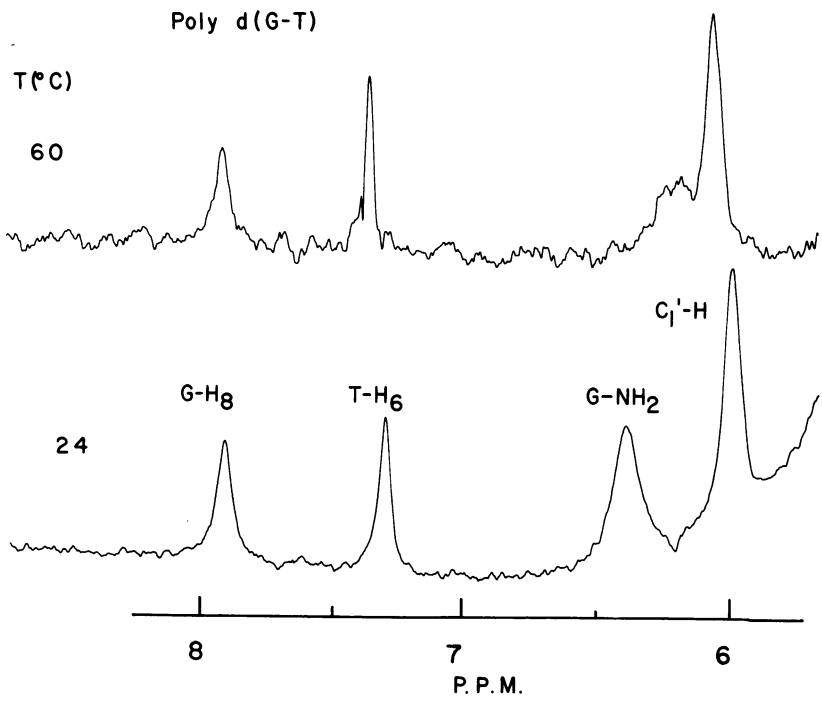

Figure 3. The 300 MHz proton NMR spectra of poly d(G-T) in H_2O in the aromatic region (8.5-6.0 ppm). Assignments are indicated. For other experimental conditions see text.

the positions of the resonances are consistent with an alternating structure in which the resonance of T-H_6 is slightly shifted by neighboring Gs but in which there is little or no shift of G-H_8. By way of contrast, the G-H_8 resonance in poly d(G) is shifted upfield to 7.82 ppm due to shifts from adjacent G residues[18]. The resonance from T-H_6, at 7.4 ppm, is close to that observed in single stranded poly d(A-T) (7.35 ppm)[19], but upfield from that observed with poly d(T) [7.6 ppm (T.A. Early and D.R. Kearns, unpublished results)]. Comparison with spectra of other samples we have examined [(d($C_{15}A_{15}$)·d($T_{15}G_{15}$), poly d(G)·poly d(C), poly dI·poly dC, poly d(A-T), tRNA] indicates that the total intensity observed in poly d(G-T) corresponds (± 10%) to that expected from a sample containing 31 mg/ml. This rules out the possibility that we are observing resonances from only the low molecular weight material in the sample.

The integrated intensity of the resonance at 11 ppm (Fig. 3) corresponds to two protons per G·T pair at 9°C, assuming the resonances at 7.35 and 8.0 ppm each correspond to one proton per residue. At 18°, the intensity at 11 ppm decreased to 1.4 protons per G·T pair, and by 24° the resonance has almost

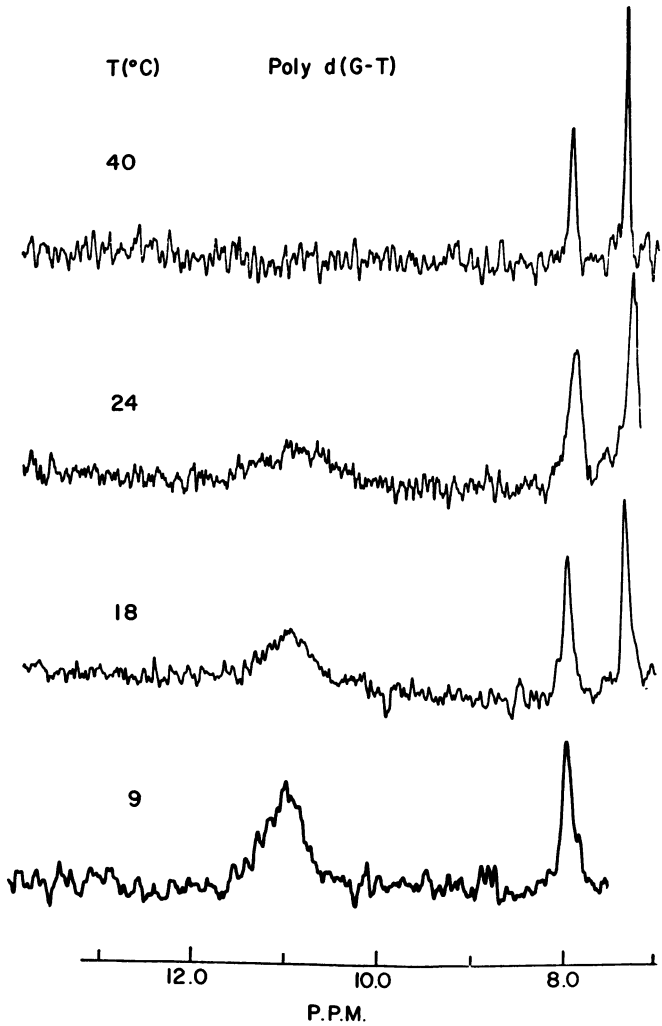

Figure 4. The lowfield spectrum of poly d(G-T) in H_2O as a function of temperature. No other resonances were observed at fields lower than 11.0 ppm.

broadened beyond detection. In D_2O, this resonance is absent, even at the lowest temperatures. Because of position, and the fact that they are only observed at temperatures below 24° (the chemical shift remains the same between 2-18°),the two resonances at 11 ppm are assigned to the ring nitrogen protons of G and T residues. Alternative possible assignments are eliminated since resonances from all other exchangeable protons (e.g., amino protons) have been identified elsewhere in the spectrum. In no case are resonances observed from the imino protons of G or T(U) in H_2O unless they are hydrogen bonded to other

TABLE I

Assignments of the Resonances in Poly d(G-T)

	Chemical Shifts, δ		
Proton Assignment	δ Poly d(G-T) ppm	δ Model ppm[a]	Model System
G-NH_1···U	11.0		
U-NH_3···G	11.0		
G-H_8	8.0	8.0	pdG-dT
T-H_6	7.35	7.5	pdG-dT
G-NH_2	6.45	6.4	pdG-dT
GT-$C_{1'}$	6.05	6.2	pdG-dT in D_2O

a) T.R. Krugh and M.A. Young, Biochem. Biophys. Res. Comm. 62, 1025 (1975). δ model corrected for reference to DSS.

bases (either to ring nitrogen atoms or to carbonyl oxygens). For example, in aqueous solutions of mono- or dinucleotides, the ring nitrogen protons of G and T exchange too rapidly to be observed in the NMR[20], even at low temperature. However, when these ring nitrogen protons are involved in hydrogen bonding interactions with other bases, they give rise to resonances in the low field spectrum in the region below 10 ppm. A low field resonance can be observed at 11.35 ppm in the spectrum of poly U (P.H. Bolton and D.R. Kearns, unpublished results) at 1°C (intensity corresponding to ∿ 1/2 proton, $\Delta\nu_{1/2}$ ∿ 80 Hz), due to the formation of hairpins containing U·U base pairs[21]. By 6°C, this resonance is considerably decreased in intensity and broadened to over 300 Hz, and at higher temperatures it is not detected. Therefore, the fact that resonances from the imino protons of G and T are observed in poly d(G-T) up to 24°C indicates that both bases are involved in some sort of base pairing interaction. The integrations show that at low temperature, the pairing is nearly 100%.

Temperature Effects on NMR Spectra. Fig. 4 shows the temperature dependence of the spectrum in the 13.0 to 7.5 ppm region. Because of the high rf fields and rapid sweep rates used to obtain these spectra, the chemical shifts are accurate to only ± 0.1 ppm. A more careful measurement of the temperature

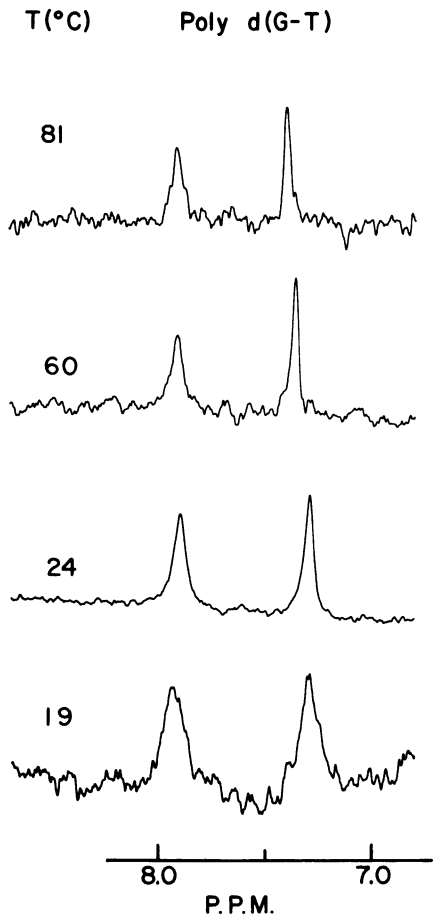

Figure 5. The temperature dependence of the resonances of G-H_8 and T-H_6 in D_2O. For assignments see Fig. 3.

dependence of the chemical shifts of the aromatic protons is shown in Fig. 5. The major changes in the spectra of poly d(G-T) on heating from ∿ 20° to 60° are as follows: (1) the aromatic resonances sharpen and the resonance from T-H_6 shifts downfield by ∿ 0.15, (2) the 11.0 ppm resonance broadens and disappears just above 24°C, and (3) the resonance from the amino protons in G shift upfield from 6.4 to 6.2 ppm. (The behavior of the amino protons of free GMP and poly C in aqueous solutions is entirely similar.)

Ethidium Bromide Binding Studies. When a dilute solution of poly d(G-T) is titrated with ethidium bromide at 5°C, the onset of binding with fluorescence enhancement is immediate (Fig. 6). At 25°C very little fluorescence enhancement is detected until the ethidium bromide/base ratio exceeds 1/5, after which the fluorescence signal increases rapidly and nearly linearly with

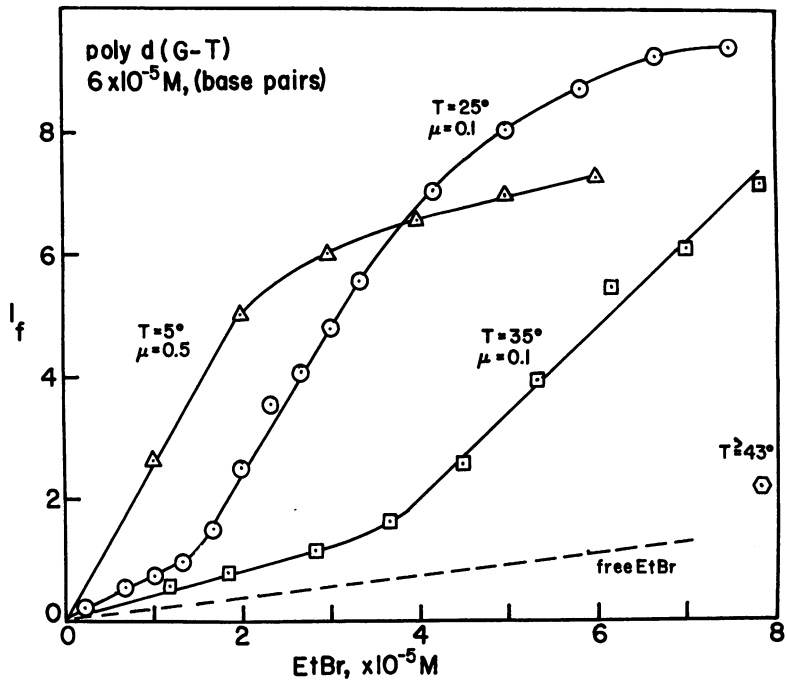

Figure 6. Fluorescence titrations of poly d(G-T) with ethidium bromide at various temperatures and ionic strengths. The intensity scale is in arbitrary units, but intensities under different conditions are measured relative to the same standard. Dashed line shows the fluorescence behavior of ethidium bromide in nucleic acid-free aqueous solution. The single hexagonal point shows residual fluorescence intensity of poly d(G-T) titrated to 8 x 10^{-5} M ethidium bromide at 25° and then heated until fully melted.

added ethidium bromide, up to one dye per two base pairs (see Fig. 6). Above this point the fluorescence increases more gradually. The titration curve is sensitive both to temperature and salt concentrations as Fig. 6 demonstrates. At 35°C significant fluorescence enhancement sets in at higher levels of ethidium bromide, increases less rapidly, and does not reach as high a limiting value. Above 40°C, no evidence for strong binding is observed even at dye/base ratios in excess of 1 to 1. The fluorescence intensity of poly d(G-T) - ethidium bromide solutions is nonetheless measurably higher at these higher temperatures than is the intensity from a free ethidium bromide solution of the same dye concentration. A similar small, but measurable, enhancement of fluorescence intensity is observed at low dye/base ratios (see Fig. 6).

Ethidium bromide lifetime determinations (summarized in Table II) are in accord with the fluorescence intensity titration curves. A strongly fluorescence-enhanced binding is indicated by the long lifetime component of 18.3 ns,

TABLE II

Fluorescence Lifetimes of Ethidium Bromide-Poly d(G-T) Complexes in Solution

Dye/Base[a]	Temperature	NaCl (M)	f_1[b]	τ_1(ns)	f_2[b]	τ_2(ns)
0.8	25°C	0.1	0.43	2.5	0.57	18.3
1.25	25°C	0.1	0.47	2.4	0.53	18.3
1.25	40°C	0.1	0.91	2.1	0.09	13.1
1.25	25°C	0.5	0.66	2.8	0.34	17.8
1.25	25°C	1.0	0.95	2.3	0.05	17.1

a) Polymer concentration = 6×10^{-5} M (bases) in 0.01 M cacodylate, 1 mM Mg^{++}, pH = 7.0.

b) Fractional population displaying this lifetime, obtained from pre-exponential coefficients of the best two exponential fit to the data.

this lifetime being slightly reduced (to 17.1 ns), and the number of sites being strikingly reduced (f_2, the fraction of long-lived emitters, falls from 0.53 to 0.05) as the ionic strength is increased to 1.0 M. Increasing the temperature to 40°C eliminates almost all of the binding sites and markedly reduces the lifetime of those few dye molecules which remain bound. The shorter lifetime component, 2.4 ± 0.4 ns, indicates there is some fluorescence enhancement even in the absence of strong binding, since the lifetime of free ethidium bromide in the absence of polymer is 1.8 ns[15,22].

By combining lifetime and intensity data for poly d(G-T) and DNA under conditions where ethidium bromide is completely bound, it is possible to compute the amount of dye bound to the synthetic polymer in the presence of excess dye. The result is that at 25°C the bound dye/base ratio is 1.25, or roughly 2 bound dye molecules for every 3 base pairs. Poly d(G-T) thus seems able to accommodate significantly more dye than can DNA, which, according to our fluorescence intensity measurements, binds only one molecule in a highly fluorescent site for every three base pairs under these same concentrations and buffering conditions.

The effect of ionic strength on the fluorescence melting curves for dilute solutions of poly d(G-T) in the presence of excess of ethidium bromide (dye/base = 1.25) are shown in Fig. 7A. Because of the presence of excess ethidium

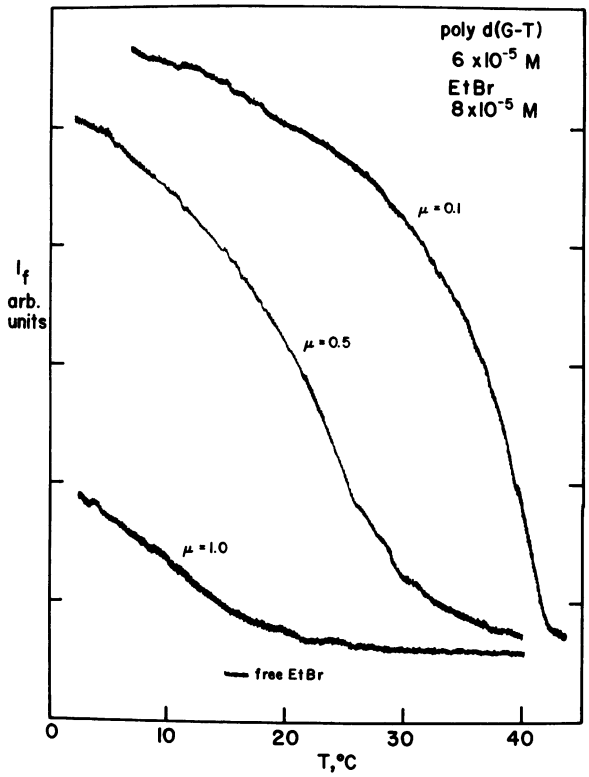

Figure 7A. Fluorescence melting curves of poly d(G-T) in the presence of excess ethidium bromide (D/N = 1.25).

bromide, the G•T helix is stabilized; nevertheless, increasing the ionic strength greatly decreases its binding to the poly d(G-T). At 0.1 M NaCl, the major loss in fluorescence intensity occurs over the 25-40°C temperature range, whereas at 0.5 M the loss occurs between 10° and 30°. Gray and Ratliff[6] reported a $T_m \sim 5°$ for free poly d(G-T) (determined from hypochromicity measurements at 0.1 M NaCl) so it is clear that ethidium bromide binding distinctly stabilizes secondary structure in 0.1 M NaCl. The optical measurements also show that the ordered poly d(G-T) structure is stabilized by high salt, but our experiments show that this has adverse effects on the ethidium bromide binding.

Since the NMR experiments indicated an unexpectedly high melting temperature ($\sim$ 24°C), additional ethidium bromide binding experiments were carried out using the concentrated NMR sample. Comparative studies were also carried out on concentrated samples of poly(A), poly(G), poly(A)•poly(U) and on calf thymus DNA, and these results are shown in Fig. 7B. Under these conditions

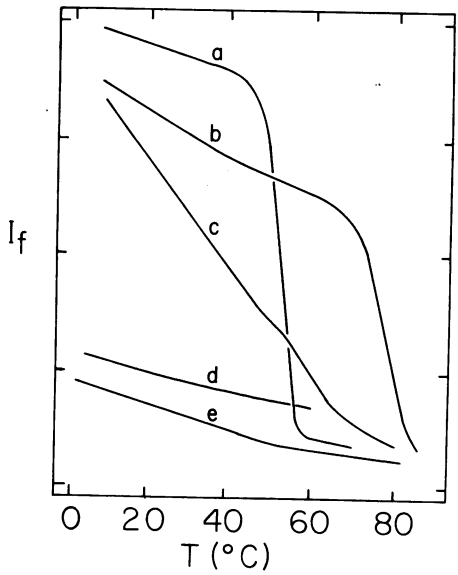

Figure 7B. The effect of temperature on the fluorescence of ethidium bromide bound to various polynucleotides. All samples contained 1.3 x 10^{-5} M ethidium bromide and a polymer concentration which ranged from 25-100 mM in P, pH 7.5 ± 0.5. The polynucleotides present in the different solutions were as follows: (a) 1:1 mixture of poly rA and poly rU, (b) calf thymus DNA which has been partially denatured by heating at 100°C for several minutes, followed by cooling to room temperature, (c) poly d(G-T), (d) poly rG, and (e) poly rA. The temperature dependence of the free ethidium bromide fluorescence was found to be identical with that obtained in the presence of poly rA (curve e).

we find that substantial enhancement of the ethidium bromide fluorescence is still evident in the poly d(G-T) sample to about 60°C. Evidently the higher polymer concentration has increased the stability of the ordered poly d(G-T) structure, at least judged by the fluorescence enhancements. By contrast, there is little fluorescence enhancement when ethidium bromide is added to concentrated solutions of poly(A) or poly(G) (see Fig. 7B).

DISCUSSION

The earlier CD studies of Lezius and Domin[1] clearly demonstrated that poly d(G-T) forms an ordered structure at low temperatures which was attributed to formation of a wobble base pair double helix. However, since it is well known that other polymers (e.g., poly A)[23] also form ordered, single stranded structures at low temperature, definitive evidence for formation of a poly d(G-T) double helical structure was still lacking. In the present study we have used the enhancement of the ethidium bromide fluorescence and proton

NMR to provide information about the nature of the ordered low temperature structure of poly d(G-T).

Ethidium bromide is widely used to probe DNA and RNA structures since its fluorescence is enhanced (factor of 10-20) when it binds to double helical DNA or RNA[24,25]. The interaction of ethidium bromide with low concentrations of poly d(G-T) can be summarized as follows: above a certain dye concentration, the value of which depends on temperature and ionic strength, there occurs a cooperative binding leading to a substantial enhancement of the ethidium bromode fluorescence and stabilization of the secondary structure of the polymer (Fig. 6). The degree of fluorescence enhancement, as measured by lifetime change, is 10-fold, and this is slightly less than the 13-fold enhancement observed (see Fig. 7B) when ethidium bromide binds intercalatively to DNA[26]. Binding of ethidium bromide with strong fluorescence enhancement is a phenomenon which has been shown to be specific for base-paired double helical polymers like DNA and poly rI·poly rC[24] or for base-paired regions of polymers like denatured DNA and RNA[26]. There is no evidence for any significant enhancement of ethidium bromide fluorescence quantum yield on binding to single stranded polynucleotides. In particular, our results show that there is relatively little enhancement on binding to either poly(G) or poly(A) (see Fig. 7B) even though poly(A) is known to adopt an ordered single stranded structure at low temperatures[23]. Therefore, the observation that the ethidium bromide fluorescence is enhanced by a factor of $\sim$ 10 on binding to poly d(G-T) is strong experimental evidence that poly d(G-T) forms a base-paired double helix (two stranded or hairpin double helix) under appropriate experimental conditions.

At room temperature (dilute solutions) the poly d(G-T) helix is in a random coil state, but a cooperative conformational change leading to double helix formation can be induced by addition of high levels of ethidium bromide. The analogous behavior has been observed with poly d(G-C) where CD studies indicate the formation of some ordered structure and ethidium bromide is readily intercalated with stabilization of the secondary structure[27]. Two observations indicate that the poly d(G-T) double helical structure is more "open" (and perhaps more flexible) than are other DNA helices. First, poly d(G-T) accommodates higher levels of ethidium bromide than does DNA. Secondly, the fluorescence enhancement upon binding is reduced relative to DNA, implying greater accessibility of the dye to solvent[15].

The effect of increasing salt concentration on the stability of the dye-polymer complex is attributed to a reduction in the stability of the ethidium bromide-poly d(G-T) complex as observed in other studies[28].

To obtain information regarding the base pairing scheme in the G-T double helix, we examined the NMR spectrum of poly d(G-T) in aqueous solution. Only three plausible base pairing schemes need to be considered (four including reverse wobble base pairs) and these are indicated in Fig. 1. Structures 1b and 1c involve tautomeric forms of T or G (T·G* or G·T*, respectively) in which only one ring nitrogen proton is involved in an N-H···N hydrogen bond[3]. Another proton is involved in an O-H···O hydrogen bond, and in both cases the amino group of G is also hydrogen bonded to the keto group of T. Therefore, if either of these hydrogen bonding schemes were involved in the G·T pair, we would expect one lowfield resonance from the N-H···N proton somewhere below 12.5 ppm and one (or two) resonance(s) from the amino proton(s) shifted downfield to about 8.0 ± 0.5 ppm. In the wobble base pairing scheme (1a), however, there is no N-H···N hydrogen bond since the ring nitrogen protons from both G and T are involved in N-H···O hydrogen bonds, and these are expected to give rise to two resonances somewhere between 10-12 ppm[13]. (In the double helical state, the low field resonance from the U·U base pair is located at 11.35 ppm.) Since the amino group of G is not involved in base pairing in the wobble base pair, the two resonances from this amino group would be expected to behave much as they do in a free nucleotide (see Table II).

Integration of the 9° NMR spectrum indicates there are <u>two</u> resonances per G·T pair at 11 ppm, and two resonances from the <u>amino</u> group of G located at very nearly the position they are observed in free GMP. These observations are incompatible with the hydrogen bonding schemes depicted in Fig. 1b or 1c, but they are as expected for the wobble base pair. This indicates that poly d(G-T) in its ordered low temperature conformation forms a double helix with G and T $\sim$ 100% base paired in a wobble base pairing scheme. (The reverse wobble base pairing scheme cannot be eliminated.) This could be either a two-stranded double helix or a hairpin double helix involving just one strand. Because the precise conformation of the poly d(G-T) helix is unknown, it is not possible to accurately predict the upfield ring current shift that is exerted on the protons in the ring N-H···O hydrogen bonds. However, taking 1.0 ppm as a reasonable upper limit, we would conclude that the intrinsic, unshifted position of the resonances is around 12.0-11.5 ppm.

At 18°C, the intensity at 11 ppm is reduced to 1.4 protons and by 24° this resonance is significantly broadened due to a reduction of lifetime of the protons in the base paired state. Because of differences in techniques, we do not expect to find a one-to-one correlation between the NMR melting data and the optical studies reported earlier. Nevertheless, it is interesting to note

that the loss of the lowfield resonances in our high salt NMR sample occurs at a temperature that is somewhat higher than would be indicated by the optical measurements[1,6]. A high melting temperature for the concentrated NMR sample of poly d(G-T) is also indicated by the ethidium bromide binding studies. These experiments demonstrated that ethidium bromide, when added to the NMR samples of poly d(G-T), shows enhanced fluorescence even at temperatures above 40°C, indicating that in these samples poly d(G-T) is in an ordered helical state well above 20°C. Evidently the high concentration of polynucleotide present in the NMR sample stabilized the system against melting.

The effect of temperature on the T-H_6 and G-H_8 resonances is rather small. Upon heating 19°C to 80°C, there is a small ($\sim$ 0.15 ppm) downfield shift and sharpening of the resonance from T-H_6 (Fig. 5) due to disruption of the stacking interactions in the double helix. The small change in the position of the T-H_6 resonances is due to the fact that T-H_6 is located at the periphery of the helix where ring current shifts from G are small[18]. The analogous resonance in poly d(A-T) shifts only $\sim$ 0.2 ppm on melting[19]. The G-H_8 proton receives shifts only from neighboring Ts, and these are expected to be even smaller for regular helical conformations[18].

It might seem surprising that the poly d(G-T) spectra are so well resolved despite the high molecular weight (a substantial amount of the material is between 100-400 base pairs long). Comparison of the spectra obtained with poly d(G-T) and other DNA and RNA samples clearly indicates we are observing an intensity commensurate with that expected from a sample containing $\sim$ 30 mg/ml. Evidently, the poly d(G-T) double helix is more flexible than is, say, native DNA of comparable molecular weight. This could be due to the formation of bulges which propagate down the helix and impart to it a higher degree of flexibility. Other alternating, self complementary polymers are believed to exhibit an analogous behavior[19].

In summary, the ethidium bromide binding studies and the NMR measurements indicate that poly d(G-T) forms a double helical structure with a hydrogen bonding scheme that is consistent only with the wobble (or reversed wobble) base pairing as proposed earlier by Lezius and Domin[1].

The fact that T and G form a wobble base pair in poly d(G-T) does not prove that the same base pairing will occur when a G•T or G•U base opposition occurs in the middle of a double helix involving regular Watson-Crick base pairs, as in many tRNA. Conceivably, the steric constraints might prevent this from occurring. It will be interesting to investigate this matter further, and such studies are in progress.

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