
Evidence for sequence-specific conformational changes in DNA from the melting temperatures of DNA phosphorothioate derivatives

J. William Suggs and Donna A. Taylor

Department of Chemistry, Brown University, Providence, RI 02912, USA

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ABSTRACT

Analogs of alternating purine-pyrimidine DNA polymers such as poly(dA-dT)-poly(dA-dT) can be made with phosphorothioate groups in the DNA backbone. A phosphorothioate diester at the 5'-purine-pyrimidine-3' step causes a significant lowering of the polymer's melting temperature compared to a phosphorothioate diester at the 5'-pyrimidine-purine-3' step. This may occur because sulfur substitution increases anionic charge density in the DNA minor groove and 5'-purine-pyrimidine-3' steps narrow the minor groove. The ability to modulate charge density in the DNA backbone via sulfur substitution should prove useful in studies of sequence-dependent conformational changes in DNA.

INTRODUCTION

Alternating purine-pyrimidine DNA polymers can adopt alternating, non-B DNA conformations. The Z DNA helices adopted by poly(dG-dC)-poly(dG-dC) and poly(dG-dT)-poly(dA-dC) sequences are the most extensively studied members of this family,¹ but there is evidence to suggest that the sequence poly(dA-dT)-poly(dA-dT) also can form an alternating structure.² For example, DNase I cleavage of poly(dA-dT)-poly(dA-dT) selectively produces fragments with 5'pT ends while digestion of random sequence DNA does not show pronounced preferences for d(ApT) phosphodiester bonds.³⁻⁵ The ³¹P NMR spectrum of poly(dA-dT)-poly(dA-dT) shows two resonances which have been interpreted as arising from different conformations of the d(ApT) and d(TpA) phosphates.⁶ Similarly, the ¹³C NMR spectrum of poly(dA-dT)-poly(dA-dT) suggests the purine sugars have a slightly different conformation than that of the pyrimidine sugars.⁷

The alternating structure of poly(dA-dT)-poly(dA-dT) which these biochemical and spectroscopic probes detect is not known with certainty. An alternating B structure with mixed sugar puckers was proposed based on the crystal structure of a d(ATAT)⁸ salt, however, a 2D NOE ¹H NMR study of poly(dA-dT)-poly(dA-dT) was not consistent with this model and instead was

interpreted as supporting a right-handed B conformation.⁹ Raman studies also indicate predominately C2'-endo sugar pucker in this polymer.¹⁰ From fiber diffraction studies a wrinkled B DNA structure was put forward in which the purine-pyrimidine steps of alternating purine-pyrimidine DNAs were similar to B DNA but the pyrimidine-purine steps were in an altered conformation, particularly the torsion angle around the C3'-O3' bond.¹¹ Finally, using ¹H NMR NOE measurements on poly(dA-dT)-poly(dA-dT) under conditions slightly different from the other ¹H NOE experiment, poly(dA-dT)-poly(dA-dT) was assigned a structure consisting of 30% right-handed and 70% left-handed B DNA units.¹² However, this evidence for left-handed structures could arise from spin diffusion during the NOE experiment. The helical periodicity of poly(dA-dT) segments measured using the band shift method is inconsistent with poly(dA-dT)-poly(dA-dT) having a left-handed structure.¹³

In this paper we describe how substitution of phosphorothioate for phosphate groups in the DNA backbone can be used to modulate the charge distribution along the DNA backbone. The resulting changes in melting temperature can be correlated with alternating, sequence-dependent conformations in poly(dA-dT)-poly(dA-dT).

MATERIALS AND METHODS

The hemithiopolymers poly d(AsT) and poly d(TsA) were prepared as described earlier,¹⁴ except hemithiopolymers were used as templates in the polymerization reactions. ³¹P NMR spectra were as expected for these polymers. Sonicated DNA fragments (100-300 bp) were passed through a Bio-Gel P-6DG column with distilled water, lyophilized and dissolved in 25mM tris-HCl, pH 7.4 buffer with added alkali metal cations at the indicated molarity. Thermal denaturation of the DNA was monitored in a Perkin-Elmer 552A UV spectrometer with a solid state temperature controller. The DNA solutions of 1 A₂₆₀ unit/ml were heated in 2-5°C increments and the A₂₆₀ was measured when the readings stabilized. The binding constant of dipyrandium was measured following the procedure of Saucier in 16mM sodium phosphate, pH 7.0, 1mM EDTA, at 25°C.¹⁵

Abbreviations: poly d(AsT), the alternating duplex copolymer of dAMP and thymidine 5'-O-phosphorothioate; poly d(TsA), the alternating copolymer of dTMP and 2'-deoxyadenosine 5'-O-phosphorothioate; T_m, midpoint of the optical DNA melting curve measured at 260 nm; dipyrandium, 3β,17β-dipyrrolidin-1'y1-5α-androstane dimethiodide.

RESULTS AND DISCUSSION

The alternating hemithiopolymers poly d(TsA) and poly d(AsT), being made enzymatically, have only the R_p configuration at the phosphorothioate diester linkage.¹⁴ As a result, the sulfur atom points into the DNA major groove and the oxygen atom points into the DNA minor groove, as illustrated in figure 1.¹⁶ Given the electronegativity difference between sulfur and oxygen (2.5 vs. 3.5 on the Pauling scale,¹⁷ the same magnitude as the difference between carbon and oxygen) phosphorothioate DNA polymers should have increased charge density in the minor groove compared to natural DNA. This can be demonstrated by comparing the binding constant of the steroidal diamine dipyrandium (an organic dication) to poly(dA-dT)-poly(dA-dT) with the steroid's binding constant to poly d(AsT) and poly d(TsA). For poly(dA-dT)-poly(dA-dT) the binding constant of dipyrandium is 5.4×10^4 while with poly d(TsA) it is 10.9×10^4 and with poly d(AsT) it is 9.3×10^4 (figure 2). At higher ionic strength (35mM sodium phosphate) the binding constant of dipyrandium to all three polymers is essentially the same. ¹H NMR studies have shown that dipyrandium binds across the minor groove with the positively charged ends of the steroid interacting with the negatively charged DNA backbone.¹⁸⁻²⁰

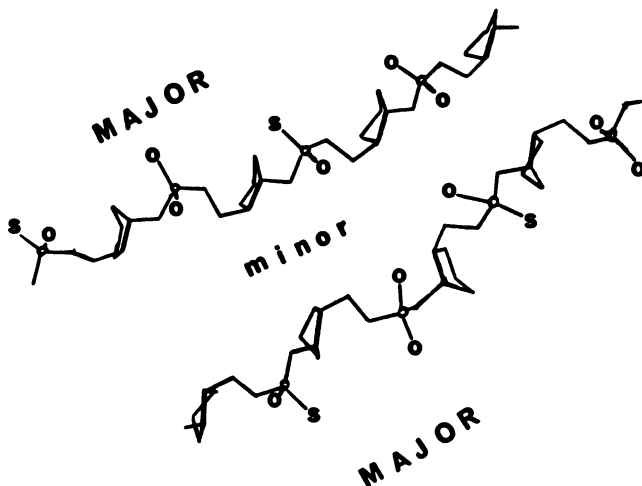


Figure 1. An unrolled projection of a DNA backbone showing R_p phosphorothioate diesters with the major and minor groove labeled.

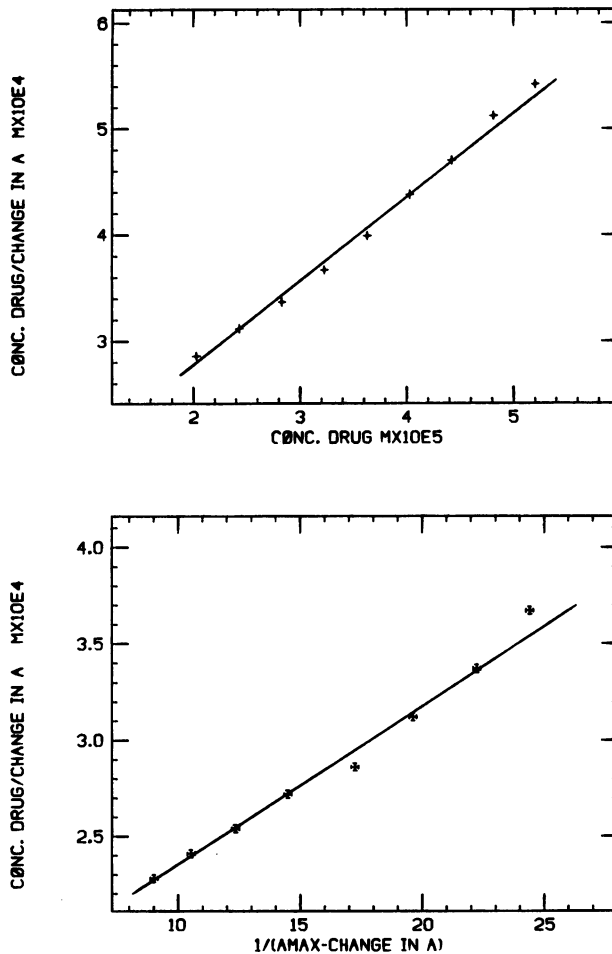


Figure 2. Representative binding curves¹⁵ for the binding of dipyrandium to poly d(TsA) in 16mM sodium phosphate pH 7.0, 1mM EDTA, 25°, indicating a binding constant of $1.1 \times 10^5 \text{ M}^{-1}$. Reported binding constants are the result of three independent measurements.

The increased binding constant of dipyrandium to the hemithiopolymers at low ionic strength demonstrates that the poly d(AsT) and poly d(TsA) DNAs have increased negative charge density in the minor groove compared to poly(dA-dT)-poly(dA-dT).

The solution structures of poly(dA-dT)-poly(dA-dT) and the two hemithiopolymers are apparently quite similar. The ³¹P NMR chemical shifts of the phosphate groups in poly(dA-dT)-poly(dA-dT) and the corresponding phosphates

in poly d(AsT) and poly d(TsA) are the same, implying the phosphodiester torsion angles are the same.^{14,21} What does differ among the polymers is the T_m (Table 1, figure 3). For all the salts and concentrations tested, poly(dA-dT)-poly(dA-dT) melts slightly above poly d(TsA) and both are significantly higher melting than poly d(AsT). Since there are no unusual ³¹P NMR or UV features of the random coil melted hemithiopolymers, we assume the variation in T_m reflect different stabilities of the duplexes.

One can explain the melting behavior of these polymers by considering the effect of sulfur on charge distribution along the minor groove together with the ideas on sequence-dependent conformations of base pair steps developed by Dickerson's group²² and Calladine.²³ According to these workers, bases in DNA propeller twist in order to improve intrastrand base stacking.²⁴ A 5'-purine-pyrimidine-3' base pair step can normally exhibit a substantial propeller twist since this brings the purine bases together across the major groove, where there is enough room to avoid a purine-purine inter-strand clash. However, a 5'-pyrimidine-purine-3' base pair step will have a lessened propeller twist since for this step the purines close into the minor groove where they quickly experience steric hindrance.

Table 1. T_m values for phosphate and hemithio DNA polymers at indicated cation concentrations.

Na^+	25mM	100mM	500mM
poly d(AT)	54	61	71
poly d(AsT)	42	49	59
poly d(TsA)	52	61	70
K^+			
poly d(AT)	54	61	71
poly d(AsT)	41	48	57
poly d(TsA)	50	57	67
Cs^+			
poly d(AT)	56	63	74
poly d(AsT)	42	50	59
poly d(TsA)	51	59	69
Mg^{+2}			
poly d(AT)	67	67	--
poly d(AsT)	55	55	--
poly d(AsT)	64	64	--

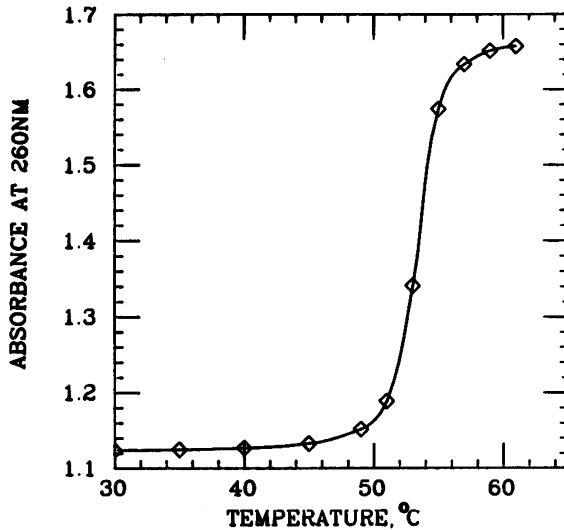


Figure 3. A typical melting curve, poly d(TsA), 50 mM KCl.

A consequence of positive propeller twist is the narrowing of the minor groove. This arises since propeller twist produces a torque on the C1' carbon on each strand which operates in the direction to bring the strands closer together along the minor groove. For example, a propeller twist of 15° narrows the minor groove by 3\AA .²⁵ Propeller twist alone cannot be the deciding feature in producing an alternating poly(dA-dT)-poly(dA-dT) structure since each dA residue is part of a d(TpA) step and a d(ApT) step. A way to accommodate propeller twist and produce an alternating backbone is to decrease the local helical twist angle at d(TpA) steps. This is not necessary for d(ApT) steps since propeller twisting causes no significant dA-dA minor groove steric clashes. The crystal structure of the A DNA helix d(GGTATACC) clearly showed an alternation in the helix angles with the d(TpA) steps at 30° and the d(ApT) step at 34° .²⁶ To a certain extent the unwinding of the d(TpA) steps will give them the character of the relatively unwound base pair steps in A DNA and as a result the phosphate will be drawn toward an A DNA conformation, one of whose characteristics is a wide minor groove.²⁷ In this model then, d(ApT) steps tend to close the DNA minor groove while d(TpA) steps do not.

For the hemithiopolymers, first consider poly d(TsA). The d(TsA) phosphorothioate step in this polymer will not tend to close the minor groove but the d(ApT) phosphate step will. As this closure occurs at the d(ApT)

step, charge can leak from the oxygen pointing into the minor groove to the oxygen pointing into the major groove, decreasing anion-anion repulsion in the minor groove.²⁸ In the case of poly d(AsT), the d(AsT) phosphorothioate step will attempt to close the minor groove, but the charge cannot easily transfer into the major groove due to the less electronegative sulfur atom there. As a result the poly d(AsT) polymer cannot relax to its lowest energy conformation analogous to poly(dA-dT)-poly(dA-dT) and therefore is less stable. This sequence-dependent narrowing of the minor groove correctly predicts that poly(dA-dT)-poly(dA-dT) and poly d(TsA) should have similar T_m 's and both should melt at a higher temperature than poly d(AsT). The slightly lowered T_m for poly d(TsA) must reflect, at least in part, the charge localization in a phosphorothioate diester bond relative to a phosphodiester bond.

The melting temperatures of poly(dG-dC)-poly(dG-dC), poly d(CsG), and poly d(GsC) follow the same trend as the d(AT) polymers, with the 5'-purine-pyrimidine-3' hemithiopolymer melting at a lower temperature than the 5'-pyrimidine-purine-3' hemithiopolymer, which in turn melts slightly below the unmodified DNA polymer.³⁰ However, while the difference in T_m between poly(dA-dT)-poly(dA-dT) and poly d(AsT) is 12°, the difference in melting point between poly(dG-dC)-poly(dG-dC) and poly d(GsC) is only 8°. This is to be expected, since dG containing steps propeller twist less than dA containing steps. In the crystallized B DNA helices the average propeller twist of d(AT) base pairs is 17° while the d(GC) base pairs twist an average of 11.5°. Thus, there is less of a tendency for dG-containing polymers to close the minor groove than there is for dA-containing polymers. Also consistent with our analysis is the melting behavior of the RNA duplexes poly r(A-U) and the analog in which all the backbone phosphates have been synthesized as phosphorothioates, poly r(sA-sU).³¹ These two polymers show identical T_m s at several ionic strengths. Being RNAs, both polymers are expected to adopt an A helix conformation, with a narrow, major groove and a wide minor groove. Thus, any effect which operates across the minor groove should be diminished in a A helix vs. a B family helix.

A final interesting observation is the melting behavior of an equimolar mixture of poly d(TsA) and poly d(AsT). Figure 4 shows the melting curve of such a sample after three previous meltings and slow coolings (a subsequent melting curve after annealing twelve hours was identical to fig. 4). One would perhaps have expected to see three melting transitions (in the intensity ratio 1:1:2) due to duplex poly d(AsT), duplex poly d(TsA) and the hetero duplex poly d(AsT)-poly d(TsA). However, a transition due to the hetero

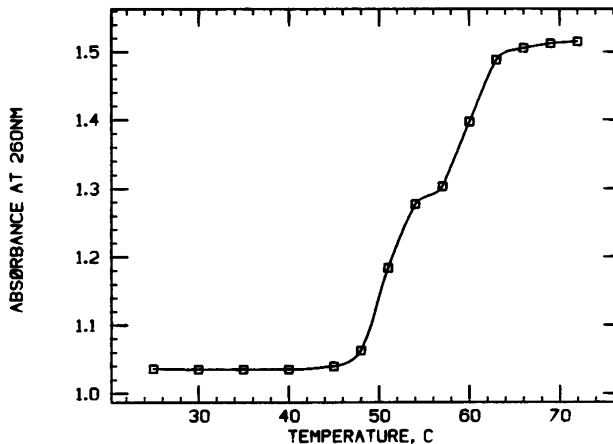


Figure 4. Melting curve for 0.5 OD poly d(AsT) and 0.5 OD poly d(TsA) in 1 ml of 0.1 M NaCl, tris buffer pH 7.5. The sample had been subjected to three heating (80°) and cooling cycles prior to this measurement.

duplex is absent. Even if the hetero duplex converted to one-chain hairpin helices prior to melting one would still have expected to see a premelting transition.⁴ One explanation for the result shown in fig. 4 is that the hetero duplex poly d(AsT)-poly d(TsA) is much less stable than the homoduplexes poly d(AsT) and poly d(TsA). The hetero duplex is unusual in that the base pair pseudodyad axis is absent due to a phosphodiester linkage on one strand and a phosphorothioate diester linkage on the other. Perhaps this added asymmetry in some way destabilizes the hetero duplex.

The picture that results for the structure of poly(dA-dT)-poly(dA-dT) itself, applying the sequence-dependent structural principles put forward by Dickerson's group and Calladine, is a polymer with a wrinkled backbone. The phosphates of the d(ApT) steps are pulled into the minor groove relative to the d(TpA) phosphates. This would explain the two ³¹P resonances seen for poly(dA-dT)-poly(dA-dT). Furthermore, the preference for DNase I cleavage at d(ApT) steps in the polymer arises because the d(TpA) steps have a decreased local twist angle, which decreases the rate of DNase I catalyzed hydrolysis at that bond.³

The principles of sequence effects on DNA structure which we have used here were developed from an analysis of relatively short oligonucleotides in molecular crystals. Nevertheless, they can be used to explain the properties of high molecular weight DNA polymers in solution. It seems likely

the sequence-dependent structural variations observed in the oligonucleotide crystals reflect intrinsic properties of DNA duplexes and are little affected by crystal packing forces or end effects.

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