

Use of phosphorothioate analogs of poly(dA-dT)·poly(dAdT) to study steroidal-diamine induced conformational change in poly(dA-dT)·poly(dA-dT)

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Introduction of phosphorothioate groups into the backbone of poly(dA-dT) allows one to label the d(ApT) and d(TpA) phosphate resonances in the ^{31}P NMR spectrum. Upon binding the steroidal diamine dipyrandenium to poly d(AsT) and poly d(TsA), ^{31}P NMR shows that it is the d(ApT) phosphodiester bond which is most perturbed. Other work has shown that 2 M Cs^+ causes the same ^{31}P shift. The DNA conformational change induced by both cations probably involves a narrowing of the minor groove.

^{31}P NMR DNA phosphorothioate Steroidal diamine DNA conformation

1. INTRODUCTION

There is extensive evidence that the self-complementary DNA duplex poly(dA-dT) can exist in solution in a dinucleotide repeat conformation [1]. The evidence comes from studies using DNase I digestion [2], ^{31}P NMR [3] and ^{13}C NMR [4]. However, there is also evidence that is consistent with poly(dA-dT) being a B DNA helix such as a 2D ^1H NOE investigation of poly(dA-dT) which concluded that the polymer existed in a B DNA helix conformation [5]. Raman studies at room temperature and moderate salt concentration indicated predominately C2'-endo sugar pucker in poly(dA-dT) [6] rather than a mixed sugar pucker as predicted by some models of poly(dA-dT) [7].

Abbreviations: poly d(AsT), the duplex DNA polymer from dATP and dTTP α S; poly d(TsA), the duplex DNA polymer from dTTP and dATP α S; dipyrandenium, 3 α ,17 β -dipyrrolidin-1-yl-5 β -(Δ 9,11)-androstene dimethiodide; dipyrandium, 3 β ,17 β -dipyrrolidin-1-yl-5 α -androstane dimethiodide

Also, the helical repeat of poly(dA-dT) tracts in plasmids was found to be within 0.1 base pair/turn of random sequence DNA [8]. Thus, the differences between this alternating form adopted by poly(dA-dT) and B DNA are much less than those between B DNA and the left-handed dinucleotide repeat structure of Z DNA.

Conditions exist which seem to enhance the dinucleotide repeat of poly(dA-dT) in solution, including addition of tetramethylammonium cations, steroidal diamines [9] and Cs^+ (but not Na^+) [10]. This new conformation induced by Cs^+ has been termed X DNA [11] and its exact structure is presently unknown. Recently, Eckstein and Jovin have shown how phosphorothioates can be used to distinguish between d(ApT) and d(TpA) phosphodiester bonds in the ^{31}P spectrum of poly(dA-dT) [12]. Using their technique we can show that both Cs^+ and the bulky steroidal diamine dipyrandenium (fig.1) are in fact inducing the same conformational transition in poly(dA-dT). We will also present evidence that shows the transition involves a narrowing of the minor groove.

2. MATERIALS AND METHODS

The thiophosphates dATP α S and dTTP α S were either synthesized as the S_p diastereomers as described for ATP α S or obtained as a mixture of R_p and S_p diastereomers from Pharmacia P-L Biochemicals. A small scale polymerization and isolation using *M. luteus* DNA polymerase (Pharmacia P-L Biochemicals) was carried out with a poly(dA-dT) template as described [12] to obtain the hemithiopolymers poly d(AsT) and poly d(TsA). In preparative reactions the hemithiopolymers were used as templates and the *M. luteus* DNA polymerase solution contained 1 mg/ml of bovine serum albumin.

The dipyrandium was synthesized following the published procedure [14]. The dipyrandium was prepared by reductive amination of 5 β -3,17-androstene-(Δ 9,11)-dione with pyrrolidine (10 eq.) in formic acid (20 eq.) at reflux for 10 h followed by neutralization and flash chromatography over silica gel (CH₂Cl₂-CH₃OH 3:1) to give 3 α , 17 β -dipyrrolidin-1'-yl-5 β -(Δ 9,11)-androstene (¹H NMR, CDCl₃, δ 5.3 (m, C-11), 1.07 (s, C-19 CH₃), 0.75 (s, C-18, CH₃)) m.p. 113–115.7. The dithiodide salt, dipyrandium, was made by heating 0.1 mmol of the diamine in 1.5 ml EtOH with 0.3 mmol CH₃I at 75°C in a sealed screw-top vial for 2 h. Upon evaporation of solvent the salt was recrystallized from water [¹H NMR, D₂O, δ 5.3 (m, C-11), 3.0 (s, CH₃-N(3)), 2.8 (s, CH₃-N(17)), 0.95 (s, C-18 CH₃), 1.11 (s, C-19 CH₃)]. It decomposed upon heating above 200°C.

The ³²P NMR studies were performed on polymers that had been sonicated in 10 mM Tris-HCl, 10 mM EDTA, pH 8, for 1 h at 5°C. Agarose gel electrophoresis indicated a size of 200–2000 base pairs. ³¹P NMR spectra were taken at 44°C with a Bruker WM250 spectrometer operating at 101.2 MHz with 20 A₂₆₀ units of DNA in 0.35 ml of sonication buffer with external aqueous trimethyl phosphate as reference. Low power broad band decoupling was used. Linewidths varied from 15 Hz in the absence of amine to 50 Hz.

3. RESULTS AND DISCUSSION

Eckstein and Jovin have established that the low field ³¹P resonance in poly(dA-dT) is due to the

d(TpA) phosphate and the high field resonance is due to the d(ApT) phosphate [12]. As shown in table 1, dipyrandium causes both ³¹P resonances to shift to a higher field, but this effect is greater for the d(ApT) step. Since changes in the phosphodiester torsion angles result in changes in ³¹P chemical shifts [15], the steroidal diamine causes the greatest change around the d(ApT) linkage. The phosphodiester resonances in the hemithiopolymers do not shift at all from their position in poly(dA-dT). This similarity of chemical shift changes indicates that the structures of the sulfur containing polymers are essentially unchanged from that in native DNA [16]. The dipyrandium results exactly parallel the effects of 2 M Cs⁺ on these polymers [12]. However, Cs⁺ causes an even greater perturbation of the d(ApT) resonance. Analogous shifts for the 2 cations indicate that these shifts do not arise from changes in the ionic strength of the medium, but must reflect the ability of large cations to bind to and change the conformation of poly(dA-dT).

Dipyrandium has been shown by solution calorimetry to bind to DNA as a monocation [17]. The *N*-methyl resonance in the ¹H NMR of dipyrandium at δ 3.0 does not change upon binding to DNA which indicates it is the other N-CH₃ group at δ 2.8 which interacts with the phosphate backbone [9]. By preparing the steroidal monoamines at C-3 and C-17 we have assigned the methyl resonance at δ 2.8 to C-17. Thus, only the ammonium ion at C-17 is interacting with the DNA backbone. Since the 2 ammonium groups are on opposite sides of the steroid ring, it is not surprising that only one ammonium group can bind to DNA. We can use the effect of sulfur substitution in the DNA backbone to show that this ionic binding occurs across the DNA minor groove. Since the phosphorothioate polymers are made enzymatically, all the phosphorothioate diester linkages are of the R_p configuration [12,18]. This means that the oxygen of the phosphorothioate is pointing toward the minor groove and is better able to ion-pair with cations in the minor groove while the sulfur is pointing toward the major groove. Given the greater electronegativity of oxygen relative to sulfur [19] there should be more negative charge density in the minor groove of phosphorothioate polymers than in natural DNAs where the negative charge is

Table 1

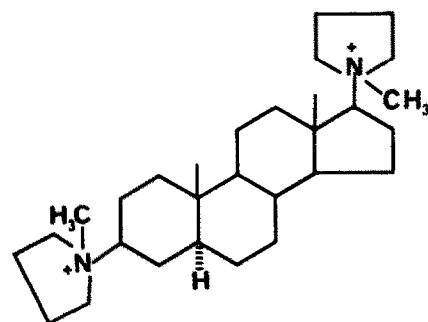
^{31}P chemical shifts of poly(dA-dT), poly d(AsT), and poly d(TsA) in the presence and absence of drug (dipyrandenium, nucleotide/drug = 5:1)

Polymer	^{31}P chemical shift (ppm)	
	d(TpA) step	d(ApT) step
Poly (dA-dT)	-3.9	-4.0
Poly (dA-dT) + drug	-4.0	-4.2
Poly d(AsT)	-3.9	52.5
Poly d(AsT) + drug	-4.0	52.2
Poly d(TsA)	52.3	-4.0
Poly d(TsA) + drug	51.6	-4.3

delocalized equally between the 2 oxygens. Thus, minor groove binding cations should bind more strongly to phosphorothioate DNAs than natural DNAs. This we find to be the case.

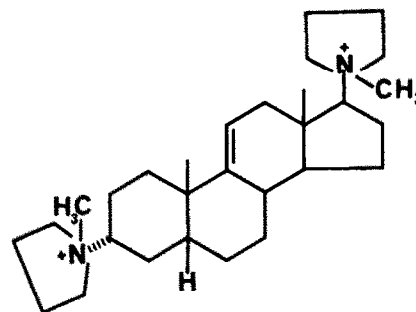
While binding of dipyrandenium to DNA has no effect on its UV spectrum, the binding of the closely related steroidal diamine dipyrandium (3 β ,17 β -dipyrrolidin-1'-yl-5 α -androstane dimethiodide, see fig.1) can be measured optically [20,21]. Using the procedure described by Saucier [20], we find that at pH 7.0 in a 16 mM sodium phosphate buffer the binding constant for dipyrandium to poly(dA-dT) is 5.4×10^4 , while it increases to 10.9×10^4 for poly d(AsT) and 9.3×10^4 for poly d(TsA). These results are consistent with increased charge density in the minor groove of DNA phosphorothioate polymers and with ^1H NMR studies that had suggested steroidal diamines such as dipyrandium and dipyrandenium bind in the minor groove of DNA [9,22,23]. Since Cs^+ and dipyrandenium cause the same NMR shift, it follows that Cs^+ must also be exerting its effects by binding in the minor groove [24].

Any model for the conformational transition in poly(dA-dT) caused by these large cations must explain why Cs^+ but not Na^+ is effective in promoting this conformational transition and also why the greatest changes occur around the d(ApT) step. The outlines of a model may be provided in recent work on sequence-induced structural variations in right-handed DNA which are based on X-ray crystal studies of oligonucleotides. In B DNA the minor groove width is 12 Å [25]. Taking the radius of the phosphate group to be 2.9 Å [26]



3 β ,17 β -dipyrrolidin-1'-yl-5 α -androstane dimethiodide

(Dipyrandium)



3 α ,17 β -dipyrrolidin-1'-yl-5 β -(Δ 9,11)-androstene dimethiodide

(Dipyrandenium)

Fig.1. Structures of the steroidal amines.

leaves 6.2 Å of available space between the strands. However, a minor conformational change, such as a tilt of the mean planes of the base pairs of -10° (i.e. the ends of base pairs rotate counterclockwise so that the normals to the mean base pair planes make an angle of -10° to the overall helix axis) closes the minor groove to 9 Å, which, taking the phosphate radius into account gives a width of 3.2 Å [27]. Since the ionic diameter of Cs^+ is 3.34 Å [19] a Cs^+ ion should bind snugly in the minor groove and promote such a narrowing. The ionic diameter of Na^+ is only 1.94 Å [19] so it cannot bind as snugly in the minor groove. It has also been observed that d(ApT) steps will tend to close the minor groove while d(TpA) steps will not since the latter suffer steric clashes between dA rings on opposite strands when the minor groove narrows, due to base pair propeller twist [28]. A Cs^+ or other large cation can thus, through some combination of base pair twist,

propeller twist, roll and slide [29], induce one of the d(ApT) phosphate oxygens to rotate into the minor groove causing a narrowing of the minor groove and a relatively greater change in the ^{31}P resonance of the d(ApT) phosphate. The ability of large cations, particularly the hydrophobic dipyrandinium, to disrupt the ordered water structure in the minor groove (which stabilizes the B DNA conformation) may also play a role in the B to X transition [30].

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