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Biochemistry

Right-handed alternating DNA conformation: Poly(dA-dT) adopts the same dinucleotide repeat with cesium, tetraalkylammonium, and 3α,5β,17β-dipyrrolidinium steroid dimethiodide cations in aqueous solution

(conformational equilibrium/salt- and ligand-induced transition/glycosidic torsion angle/phosphodiester linkage/telestability)

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ABSTRACT We demonstrate that poly(dA-dT) can adopt two conformations in solution, with the relative proportions dependent on the nature and concentration of the counter ion and cationic ligands. The synthetic DNA exhibits a dinucleotide repeat conformation on addition of CsF and Me_4NCl at molar concentrations, with the NMR spectral changes reflecting a common conformational change at one glycosidic torsion angle and one phosphodiester linkage. We also observe the same dinucleotide repeat in the neighbor-exclusion 3α,17β-dipyrrolidin-1′-yl-5β-$\Delta^{9,11}$-androstene dimethiodide (3α,5β,17β-dipyrandenium) complex, with the steroid diammonium ligand binding in the groove of the stacked poly(dA-dT) duplex and the complex stabilized through the interaction of one of the charged ends with the backbone phosphate. We demonstrate further that 3α,5β,17β-dipyrandenium bound to poly(dA-dT) at low binding ratios induces a switch to the dinucleotide repeat conformation at adjacent steroid-free duplex regions. This observation contrasts with a previous demonstration that the diastereoisomeric 3β,5α,17β-dipyrandium binds to poly(dA-dT) by partial insertion between unstacked tilted base pairs. The NMR parameters rule out a left-handed alternating DNA structure (Z DNA) for the observed poly(dA-dT) dinucleotide repeat conformation, but right-handed alternating DNA models are under consideration. The facile interconversion of poly(dA-dT) between two conformations, one of which exhibits a dinucleotide repeat and can be induced by ligand binding, may provide a mechanism for the recognition of specific nucleic acid sequences by DNA-binding proteins.

Structural aspects of synthetic DNAs and RNAs with an alternating purine-pyrimidine sequence can be readily investigated by high-resolution NMR spectroscopy in solution (1, 2). Thus, proton and phosphorus NMR studies demonstrated a dinucleotide repeat (3) for the $(dG\text{-}dC)_n$ duplex in concentrated salt solution (4), and this conclusion was independently established by the observation of the left-handed alternating DNA helix (Z DNA) for $(dG\text{-}dC)_n$ oligonucleotides in the crystalline state (5–7) and poly(dG-dC) in the fiber state (8).

The x-ray structure of the ammonium salt of pdA-dT-dA-dT, solved by Viswamitra, Kennard, and their collaborators, exhibits different glycosidic torsion angles and sugar puckers at the adenosine and thymidine residues and different phosphodiester torsion angles linking the dA(3′-5′)dT and dT(3′-5′)dA residues (9). This led Klug and coworkers to propose a dinucleotide repeat for poly(dA-dT) as defined by a right-handed "alternating B-DNA" conformation (10) which incorporates structural features observed in the tetranucleotide crystal (9).

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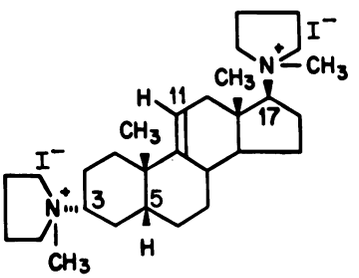

FIG. 1. Chemical structure of 3α,17β-dipyrrolidin-1′-yl-5β-$\Delta^{9,11}$-androstene dimethiodide (designated 3α,5β,17β-dipyrandenium).

We compare below the NMR parameters at the exchangeable base protons, nonexchangeable base and sugar protons, and the phosphate backbone for poly(dA-dT) in 10 mM buffer in the presence of high concentrations of NaCl, CsF, and Me_4NCl as well as in a neighbor-exclusion 3α,5β,17β-dipyrrolidinium steroid dimethiodide (Fig. 1) complex. We shall demonstrate that poly(dA-dT) adopts the same conformation with a dinucleotide repeat in the presence of molar concentrations of Cs^+ and Me_4N^+ counterions and mM concentrations of the steroid diammonium cation.

RESULTS

Poly(dA-dT) conformation in CsF solution

Vorlicková and coworkers (11) recently reported on a noncooperative inversion of the long-wavelength circular dichroism spectrum of poly(dA-dT) at high concentrations of CsF solution that was not observed for the corresponding homopolymer duplex poly(dA)·poly(dT) under the same conditions. These workers proposed that poly(dA-dT) adopts a conformation with different geometries at the purine and pyrimidine residues in 6 M CsF solution (11).

We shall first describe and compare the NMR parameters for poly(dA-dT) in the presence of 6 M NaCl and 6 M CsF with those in the absence of added salt in 10 mM buffer solution.

Hydrogen Bonding. The base-paired duplex state in poly(dA-dT) can be readily characterized by monitoring the thymidine H-3 imino exchangeable proton in H_2O solution (1, 12). This proton is readily observable at 13.12 ppm for poly(dA-dT) in 10 mM sodium phosphate buffer, at 13.07 ppm in the presence of 6 M NaCl, and at 13.01 ppm in the presence of 6 M CsF solution at 25°C. This demonstrates that the base pairs are intact in the synthetic DNA in the absence and presence of high NaCl and CsF concentrations. The temperature dependence of the thymidine H-3 exchangeable proton chemical shift in the synthetic DNA in the absence and presence of salt is plotted in Fig. 2 for the premelting transition region.

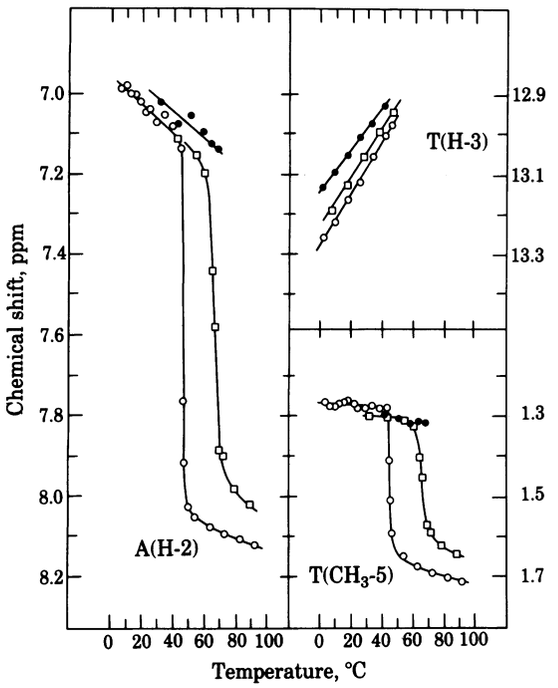

FIG. 2. Temperature dependence of the base proton chemical shifts (relative to 2,2-dimethyl-2-silapentane-5-sulfonate) of poly(dA-dT) in 10 mM phosphate/1 mM EDTA/H_2O solution in the absence of added salt (○), in the presence of 6 M NaCl (□), and in the presence of 6 M CsF (●). The nonexchangeable adenosine H-2 and thymidine CH_3-5 proton data were collected in ^2H_2O solution. The exchangeable thymidine H-3 data in 4:1 (vol/vol) $H_2O/^2H_2O$ solutions were recorded at pH 6.68 (no added salt), pH 8.35 (6 mM NaCl), and pH 7.95 (6 M CsF) solutions.

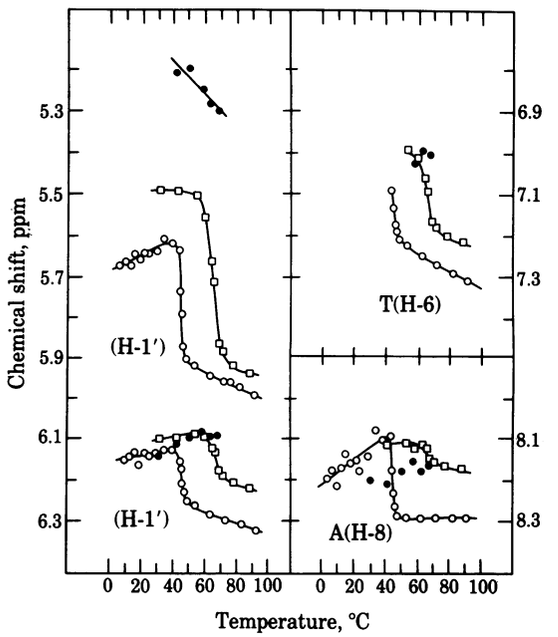

FIG. 3. Temperature dependence of the nonexchangeable thymidine H-6, adenosine H-8, and the sugar H-1′ chemical shifts of poly(dA-dT) in 10 mM phosphate/1 mM EDTA/^2H_2O solution in the absence of added salt (○), in the presence of 6 M NaCl (□), and in the presence of 6 M CsF (●). The solutions were at pH 7.15 (no added salt), pH 8.40 (6 M NaCl), and pH 7.80 (6 M CsF).

Base Pair Overlap. The thymidine H-3 exchangeable proton, the thymidine CH_3-5, and adenosine H-2 nonexchangeable protons are sensitive indicators of base pair overlap geometry in the duplex state. The temperature-dependent duplex-to-strand transition can be readily monitored for poly(dA-dT) in the absence and presence of 6 M NaCl but sample precipitation results at temperatures above 70°C in 6 M CsF solution resulting in the measurement of NMR parameters of the synthetic DNA only in the duplex state in CsF solution.

We observe temperature-dependent chemical shifts for the thymidine H-3, thymidine CH_3-5, and adenosine H-2 protons of poly(dA-dT) in 6 M NaCl and 6 M CsF solutions in the duplex state similar to those observed in no added salt solution (Fig. 2). This implies that there is essentially no change in the base pair overlap geometry of poly(dA-dT) on addition of high concentrations of salt or that there are changes in the base pair overlaps that result in no net changes in the upfield ring current contributions (13, 14).

Base–Phosphate Interaction. The adenosine H-2 and thymidine H-6 resonances are superimposable in the poly(dA-dT) duplex state, with the observed chemical shift reflecting the position of the narrower adenosine H-2 proton resonance. The thymidine H-6 proton resonates at approximately 7.05 ± 0.05 ppm between 0°C and 30°C in the absence of added salt. This duplex chemical shift remains unperturbed at 7.0 ppm on addition of either 6 M NaCl or 6 M CsF solution (Fig. 3). By contrast, the adenosine H-8 resonance is observed at 8.1 ppm in the duplex state of poly(dA-dT) in the absence and presence of 6 M NaCl but shifts to 8.2 ppm in 6 M CsF solution (Fig. 3).

The adenosine H-8 and thymidine H-6 protons are directed towards the sugar-phosphate backbone in the *anti* conformation about the glycosidic bond. Their chemical shifts reflect contributions from base pair overlaps, the magnitude of the glycosidic torsion angle, and the conformation of the sugar-phosphate group. The observed change in the adenosine H-8 resonance of poly(dA-dT) on addition of 6 M CsF suggests a conformational change in one or all of these conformational parameters on addition of salt at a high concentration.

Glycosidic Torsion Angle. We have monitored the sugar H-1′ resonances of poly(dA-dT) on addition of a high concentration of salt. The sugar H-1′ resonance at ≈6.1 ppm in the duplex remains unperturbed on addition of either 6 M NaCl or 6 M CsF (Fig. 3). By contrast, the sugar H-1′ resonance at ≈5.6 ppm in the duplex shifts to ≈5.5 ppm on addition of 6 M NaCl and ≈5.2 ppm on addition of 6 M CsF (Fig. 3).

The sugar H-1′ chemical shifts are sensitive to changes in the glycosidic torsion angle (15) but do not monitor the base pair overlap geometry. The selective 0.4-ppm upfield shift of the 5.6-ppm sugar H-1′ proton of poly(dA-dT) to 5.2 ppm in 6 M CsF solution (Fig. 3) demonstrates a change in the glycosidic torsion angle of this residue. We are unable to unambiguously assign the sugar H-1′ resonances at this time and hence we cannot determine whether the selective glycosidic torsion angle change occurs at the adenosine or thymidine residue.

Phosphodiester Backbone. The proton-noise-decoupled ^{31}P NMR spectrum of the poly(dA-dT) duplex exhibits two partially resolved resonances in the absence of added salt. The observed chemical shift separation of ≈0.13 ppm in $(dA\text{-}dT)_n$ at the polynucleotide level compares favorably with an earlier observation of ≈0.22 ppm in 145-base-pair $(dA\text{-}dT)_n$ (16) in 10 mM buffer at ≈30°C (transition midpoint = 42.5°C). The increase in the transition midpoint with salt concentration permits spectra in the duplex state to be recorded at higher temperature in the presence of high salt concentrations. We observe two resolved resonances in the ^{31}P spectra of poly(dA-dT) in concentrated NaCl and CsCl solution at 61°C, with an approximately constant chemical shift difference of 0.3 ± 0.05 ppm above 2 M salt solution (Table 1). This demonstrates that the dT-dA and dA-dT

Table 1. ^{31}P chemical shift differences between the two phosphodiester resonances of poly(dA-dT) in concentrated salt solutions at 61°C

Salt	Chemical shift differences ($\Delta\delta$), ppm*		
	2 M	4 M	6 M
NaCl†	0.25	0.28	0.31
CsCl‡	0.31	0.37	0.35
CsF†	0.34	0.49	0.67

* Chemical shifts are upfield from trimethylphosphate.
† Buffer: 10 mM sodium phosphate/1 mM EDTA/^2H_2O.
‡ Buffer: 10 mM sodium cacodylate/1 mM EDTA/^2H_2O.

phosphodiester can be resolved at the polynucleotide level in concentrated NaCl and CsCl solutions.

^{31}P spectra of poly(dA-dT) in 2 M, 4 M, and 6 M CsF at 61°C are presented in Fig. 4. We observe resolved dT-dA and dA-dT phosphodiester resonances with one of the resonances remaining constant at ≈4.195 ppm with added salt while the other resonance at higher field shifts upfield as an average peak with increasing CsF concentration. The chemical shift differences between the two phosphodiesters is 0.34 ppm in 2 M CsF solution and increases to 0.67 ppm in 6 M CsF solution (Table 1), demonstrating a conformational change at one of the phosphodiester linkages of poly(dA-dT) with increasing CsF concentration. We are unable to distinguish between the dT-dA and dA-dT phosphodiesters at this time for the two resolved peaks in Fig. 4.

3α,5β,17β-Dipyrandenium·poly(dA-dT) complex

We report below on NMR studies of the neighbor-exclusion poly(dA-dT) complex with a 3α,5β,17β-dipyrrolidinium androstene dimethiodide containing a 9,11 double bond (Fig. 1).

Hydrogen Bonding. The thymidine H-3 resonance at 13.12 ppm in the poly(dA-dT) duplex shifts to 12.94 ppm in the 3α,5β,17β-dipyrandenium complex at a nucleotide-to-drug molar ratio (Nuc/D) of 5 in 10 mM phosphate solution, pH ≈7 at 25°C. The imino protons in the synthetic DNA and in the 3α,5β,17β-dipyrandenium complex exhibit similar temperature-dependent chemical shifts of ≈0.006 ppm/°C, and the broadening of the resonances coincide with the onset of the melting transition at neutral pH. The linewidth of the imino proton resonance in poly(dA-dT) and in the 3α,5β,17β-dipyrandenium complex in 10 mM phosphate is independent of the pH between 7 and 8.7.

The absence of any significant sensitivity to temperature or pH of the thymidine H-3 chemical shift and line width in the premelting region of the neighbor-exclusion 3α,5β,17β-dipyrandenium·poly(dA-dT) complex requires that the base pairs be shielded from solvent in the same manner as base pairs in the interior of DNA duplexes.

Groove Binding. We observe downfield shifts at olefinic H-11 and the two ring CH_3 groups of the 3α,5β,17β-dipyrandenium on complex formation with poly(dA-dT) (Fig. 5). This implies that these markers on the steroid ring are roughly in the plane of the base pairs and experience downfield in-plane ring current contributions (13) consistent with the 3α,5β,17β-dipyrandenium binding in the groove of the synthetic DNA.

We note that one NCH_3 group shifts 0.25 ppm to high field while the other NCH_3 group is essentially unperturbed when 3α,5β,17β-dipyrandenium binds to poly(dA-dT) in 10 mM phosphate solution (Fig. 5). These results demonstrate that only one charged end of the dipyrrolidium steroid dimethiodide interacts with the sugar-phosphate backbone of the synthetic DNA.

Glycosidic Torsion Angle. The downfield sugar H-1′ at 6.12 ppm remains unperturbed, while the sugar H-1′ at 5.62 ppm

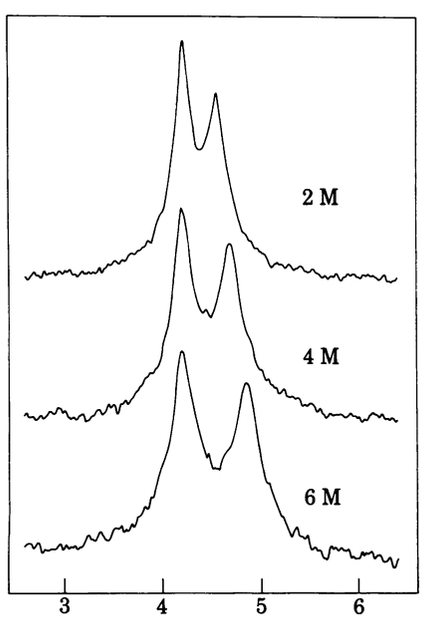

FIG. 4. Proton-noise-decoupled 80.995-MHz ^{31}P spectra of poly(dA-dT) in 10 mM phosphate/1 mM EDTA/^2H_2O solution at 61°C, in the presence of 2 M, 4 M, and 6 M CsF. The decoupler was turned on during the data acquisition (1 sec) but turned off during the delay (2 sec) between successive pulses. The chemical shifts are upfield from standard trimethylphosphate.

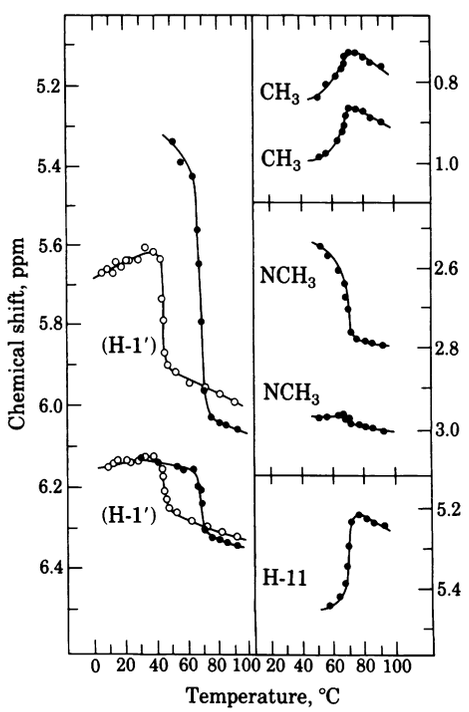

FIG. 5. Temperature dependence of selective proton chemical shifts of poly(dA-dT) (○) and the 3α,5β,17β-dipyrandenium·poly(dA-dT) complex, Nuc/D = 5 (●), in 10 mM phosphate/1 mM EDTA/^2H_2O solution, at pH 7.15 and pH 8.0, respectively. The left panel contains the sugar H-1′ proton data of the nucleic acid in the absence and presence of the 3α,5β,17β-dipyrandenium, while the right panels contain data for the resolvable 3α,5β,17β-dipyrandenium protons in the complex as a function of temperature.

shifts upfield to 5.3 ppm on formation of the neighbor-exclusion 3α,5β,17β-dipyrandenium·poly(dA-dT) complex in 10 mM phosphate solution (Fig. 5). This result strongly requires that 3α,5β,17β-dipyrandenium induces a change in one glycosidic torsion angle of poly(dA-dT) on complex formation.

Phosphodiester Linkage. Two resolved phosphorus resonances are observed in the Nuc/D = 5 3α,5β,17β-dipyrandenium·poly(dA-dT) complex in 10 mM phosphate with a chemical shift separation of 0.45 ppm at 69.5°C (Fig. 6, spectrum A) and 0.66 ppm at 44.5°C. The large shift difference is consistent with the dT-dA and dA-dT phosphodiesters adopting different conformations in the 3α,5β,17β-dipyrandenium complex with the alternating purine-pyrimidine synthetic DNA.

The dT-dA and dA-dT phosphodiesters are also resolved in the ^{31}P spectrum of poly(dA-dT) in 4 M Me_4NCl with a chemical shift separation of 0.43 ppm at 69.5°C (Fig. 6, spectrum B) and 0.61 ppm at 31.5°C.

Diastereoisomeric Steroid Diammonium·DNA Complexes. Waring and Henley (17) and Gabbay and Glaser (18) have demonstrated that the DNA binding properties of the 3,17-dipyrrolidinium-substituted androstane dimethiodides are dramatically dependent on the relative stereochemistry at the 3, 5, and 17 positions.

We reported previously that 3β,5α,17β-dipyrandium binds to poly(dA-dT) by partial insertion between unstacked tilted base pairs (19) similar to proposed models for 3β,5α,17β-steroid diammonium·DNA complexes in the literature (20, 21).

By contrast, the 3α,5β,17β-dipyrandenium, which has its charged ends on different faces of the steroid, binds through only one of its charged ends to poly(dA-dT) with the nonpolar segment of the steroid residing in the groove of the DNA. A conformational transition is observed in the alternating purine-pyrimidine duplex on complex formation, with the most pronounced change localized at one glycosidic torsion angle and one phosphodiester linkage.

Telestability. We have recorded the ^{31}P spectra of poly(dA-dT) and its 3α,5β,17β-dipyrandenium complexes as a function of Nuc/D ratio in 0.1 NaCl/10 mM buffer at 55°C. The partially resolved phosphodiesters are separated by 0.18 ppm in the synthetic DNA (Fig. 7, spectrum A), with the separation increasing to 0.36 ppm in the complex containing 1 3α,5β,17β-dipyrandenium per 25 base pairs in the Nuc/D = 50 complex (Fig. 7, spectrum B). The separation between the dT-dA and dA-dT phosphodiesters levels off at 0.46 ppm in the Nuc/D = 18 (Fig. 7, spectrum C) and Nuc/D = 12.5 (Fig. 7, spectrum D) complexes.

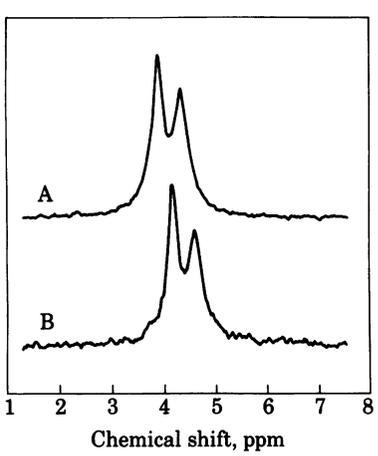

FIG. 6. Proton-noise-decoupled 80.995-MHz ^{31}P spectra of the Nuc/D = 5 3α,17β-dipyrandenium complex in 10 mM phosphate/1 mM EDTA/^2H_2O solution, pH 8.0 at 65.5°C (A) and poly(dA-dT) in 4 M Me_4NCl/10 mM phosphate/1 mM EDTA/^2H_2O, pH 7.95 at 69.5°C (B). The pulse repetition time was 4 sec and the decoupler was turned on continuously during acquisition of spectrum A. The decoupler was turned on during data acquisition (1 sec) but turned off during the delay (4 sec) between successive pulses for spectrum B. The chemical shifts are upfield from internal standard trimethylphosphate.

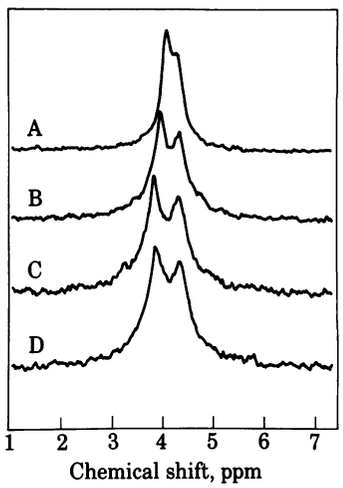

FIG. 7. Proton-noise-decoupled 80.995-MHz ^{31}P spectra of poly(dA-dT) (A) and its Nuc/D = 50 (B), Nuc/D = 18 (C), Nuc/D = 12.5 (D), 3α,5β,17β-dipyrandenium complexes in 0.1 M NaCl/10 mM cacodylate/^2H_2O, pH 8.05 at 52°C. The chemical shifts are upfield from internal standard trimethylphosphate.

The NMR data convincingly demonstrate that the binding of the 3α,5β,17β-dipyrandenium to poly(dA-dT) at low binding ratios switches the synthetic DNA to a dinucleotide repeat conformation at steroid-free base pair regions.

It has previously been shown that netropsin induces a conformational change in poly(dA-dT) that is propagated in either direction from its binding site (22), and a similar observation has been reported for the complexes of the related peptide antibiotic distamycin with certain DNAs in solution (23).

DISCUSSION

Dinucleotide Repeat. We observe an ≈0.6-ppm separation of the two phosphorus resonances of poly(dA-dT) on addition of molar concentrations of Me_4NCl (1, 24) and CsF and on formation of the neighbor-exclusion 3α,5β,17β-dipyrandenium complex (Figs. 4 and 6), demonstrating different conformations at the dT-dA and dA-dT phosphodiester linkages in the alternating purine-pyrimidine synthetic DNA under these solution conditions. The poly(dA-dT) upfield sugar H-1′ proton selectively shifts to high field on addition of the above cations (Figs. 3 and 5 and refs. 1 and 24) requiring a change in the glycosidic torsion angle at every other residue in the synthetic DNA.

These results demonstrate that poly(dA-dT) adopts a dinucleotide repeat in which every other glycosidic torsion angle and phosphodiester linkage exhibit a conformation different from standard B DNA in molar Me_4NCl (1, 24), in 6 M CsF, and in the 3α,5β,17β-dipyrandenium complex in solution.

Alternating Conformation. Two conformations incorporating the dinucleotide repeat in alternating purine-pyrimidine synthetic DNAs in solution are relevant to this discussion. The "alternating B-DNA" model is a right-handed duplex in which every purine glycosidic torsion angle and every purine(3′-5′)pyrimidine phosphodiester linkage differs from that in regular B DNA (10). This model was constructed for poly(dA-dT) on the basis of x-ray data for pdA-dT-dA-dT (9) and may be adopted by poly(dG-dC) as well. The "Z-DNA" conformation

of poly(dG-dC) is a left-handed alternating DNA duplex in which the guanosine residue is in the *syn* conformation and the dC(3'-5')dG and dG(3'-5')dC exhibit different phosphodiester conformations. This structure is based on the x-ray analysis of $(dC\text{-}dG)_n$(5–7) and has also been observed in fibers of poly(dA-s^4dT) and poly(dA-dC)·poly(dG-dT) (8).

The NMR parameters reflecting the dinucleotide repeat are strikingly different for $(dG\text{-}dC)_n$ in 4 M NaCl (3) on the one hand and poly(dA-dT) in molar Me_4NCl and CsF on the other. Specifically, we observed an ≈1.4-ppm *downfield* ^{31}P shift at one phosphodiester and an ≈0.4-ppm *downfield* shift at one sugar H-1′ proton on formation of the $(dG\text{-}dC)_n$ Z-DNA conformation (3). By contrast, we observe an ≈0.6 ppm *upfield* ^{31}P shift at one phosphodiester and an ≈0.3-ppm *upfield* shift at one sugar H-1′ on formation of the dinucleotide repeat conformation for poly(dA-dT) in solution.

These results demonstrate that the poly(dA-dT) dinucleotide repeat conformation in molar Me_4NCl and CsF solutions is *not* the left-handed alternating DNA (Z-DNA) conformation. It should be stressed that the above NMR markers for poly(dA-dT) in 4 M NaCl exhibit chemical shifts that are also in the opposite direction to poly(dG-dC) in 4 M NaCl.

Conformational Transition. The conversion of $(dG\text{-}dC)_n$ in high salt concentration to the Z-DNA conformation occurs in a cooperative manner (4) and the 22 kcal (92 kJ)/mol energy barrier (4) results in slow exchange between the two conformations on the NMR time scale (3). This is not surprising in light of the change from a right-handed duplex to a left-handed duplex (5–8) with addition of salt.

By contrast, the circular dichroism studies (11) and the ^{31}P NMR studies in Fig. 4 demonstrate that poly(dA-dT) undergoes a noncooperative structural transition on addition of molar amounts of CsF. The ^{31}P NMR resonance shifts as an average peak on gradual addition of CsF (Fig. 4), consistent with fast exchange on the NMR time scale. The lower barrier implies that the poly(dA-dT) conformational change on addition of CsF does not involve breaking of base pairs and reformation of the duplex but rather most likely reflects interconversion between right-handed duplexes, one of which contains a dinucleotide repeat.

Ligand-Induced Dinucleotide Repeat Conformation. Baldwin and coworkers (25), in a seminal investigation, demonstrated that pancreatic DNase I cleaves poly(dA-dT) specifically at the dA(3'-5')dT phosphodiester linkage, in contrast to cleavage at every base pair in natural DNA (26). Further, Klug *et al.* demonstrated that the cleavage at the purine(3'-5')pyrimidine linkage exhibits a 5-fold preference in poly(purine-pyrimidine) duplexes compared to poly(purine)·poly(pyrimidine) duplexes (10). This enzymatic cleavage specificity in poly(dA-dT) suggests either that this polynucleotide adopts a dinucleotide repeat in solution or that this conformation can be induced after binding to the DNase I enzyme (10).

Our NMR studies on the binding of the 3α,5β,17β-dipyrandenium in the groove of poly(dA-dT) bear directly on this question. We have demonstrated that 3α,5β,17β-dipyrandenium induces formation of a dinucleotide repeat conformation at the binding and adjacent nonbinding regions on poly(dA-dT).

Thus we have demonstrated that ligand binding in the presence of excess nucleic acid can induce conformational changes such as the formation of a dinucleotide repeat structure for an alternating purine-pyrimidine duplex under conditions of neutral pH and low ionic strength. Similar interconversions may provide a mechanism for the recognition of specific nucleic acid sequences by DNA binding proteins.

Further Studies. Our NMR studies to date demonstrate that poly(dA-dT) adopts a right-handed dinucleotide repeat duplex in the presence of various cations but are unable to specify the details of this conformation. We will have to differentiate between the adenosine and thymidine H-1′ resonances as well as assign the two resolved phosphodiester peaks to the dT-dA and dA-dT residues in order to deduce which glycosidic torsion angle and which phosphodiester linkage change on formation of the dinucleotide repeat conformation.

The 360-MHz correlation spectra of nucleic acid exchangeable protons in H_2O solution were recorded at the Mid-Atlantic Regional Facility at the University of Pennsylvania Medical School (funded by National Institutes of Health Grant RR542).

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