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Chemistry

Steroid diamine–nucleic acid interactions: Partial insertion of dipyrandium between unstacked base pairs of the poly(dA-dT) duplex in solution

[dipyrandium·poly(dA-dT) complex/nonintercalating drug/synthetic DNA/base-pair destacking/drug-induced flexibility]

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ABSTRACT **We report on an NMR investigation of steroid diamine·nucleic acid complexes as a function of phosphate-to-drug ratios in aqueous solution in order to evaluate the structural and kinetic aspects of the binding of a nonintercalative drug to a synthetic DNA in solution. The nonexchangeable proton chemical shift parameters for the dipyrandium·poly(dA-dT) complex demonstrate unstacking of base pairs and partial insertion of the steroid diamine at the complexation site. The chemical shifts and linewidths of the exchangeable protons as a function of pH demonstrate that the base pairs are intact but partially exposed to solvent at the steroid diamine binding site. The phosphorus chemical shifts suggest that the base pairs unstack upon complex formation without changes in the ω,ω' polynucleotide backbone torsion angles. The NMR line shape parameters require rapid exchange of the steroid diamine among potential binding sites and are consistent with greater segmental flexibility in the complex compared to the synthetic DNA in solution. The NMR experiments are discussed in relation to Sobell's proposed model [Sobell, H. M., Tsai, C. C., Gilbert, S. G., Jain, S. C. & Sakore, T. D. (1976) *Proc. Natl. Acad. Sci. USA* 73, 3068–3072] for the steroid diamine·DNA complex.**

Recent investigations into the structure and dynamics of nucleic acids suggest that the DNA helix undergoes conformational transitions as a function of salt (1) and solvent (2–4) and that this flexibility may be of fundamental importance in the specificity of interactions of small molecules and macromolecules with nucleic acids (5–7). This flexibility is further manifested in the folding of DNA around histones in chromatin and the packaging of nucleic acids into phage heads. It has been proposed that DNA either folds smoothly (8, 9) or its chain direction changes abruptly by the generation of kinks every few base pairs (10, 11).

Sobell and coworkers (11) recently proposed the kink as a key intermediate in the process of drug intercalation into DNA. They suggested further that steroid diamines bind to and stabilize kinked sites in DNA. The kink is generated in this model by partial unstacking and unwinding (−12°) of adjacent base pairs, a change in the glycosidic torsion angles and in the sugar pucker to a C3′*endo*(3′–5′)C2′*endo* configuration, and only minor adjustments in the backbone rotation angles. It is proposed that the steroid diamine binds to DNA through the minor groove at the kink site and that the complex is stabilized by electrostatic interactions with phosphates on partner strands (11).

Experimentally, the strong binding of steroid diamines to nucleic acids is saturated at one drug molecule per five nucleotides, corresponding to a neighbor-exclusion model for complex formation (12–14). Sedimentation studies demonstrate that the steroid diamine unwinds covalently circular superhelical DNA to half the extent on a molar basis as the ethidium bromide intercalative complex (15).

NMR spectroscopy can be used to probe the structural aspects of nonintercalative drug·nucleic acid complexes and hence studies were undertaken on the complex of a synthetic DNA [poly(dA-dT)] with the steroid diamine dipyrandium iodide (Fig. 1) as a function of the phosphate (P_i)-to-drug ratio in aqueous solution. Because NMR spectroscopy monitors proton markers distributed throughout the base pairs and sugar rings of the polynucleotide, as well as the steroid diamine, the components of the complex can be independently monitored through the temperature-dependent melting transition. The studies were undertaken in the absence of added salt in order to maximize the contributions of electrostatic interactions to the stability of the complex.

RESULTS

Ultraviolet absorbance melting studies

The thermal melting profiles in differentiated form (260 nm) of the dipyrandium·poly(dA-dT) complex (synthetic DNA concentration fixed at 0.15 mM) recorded at P_i/drug ratios ranging from 50:1 to 1:1 in 10 mM cacolylate buffer, pH 5.3, are presented in Fig. 2. The steroid diamine stabilized the thermal transition of the synthetic DNA.

We evaluated the increase in transition midpoint on addition of dipyrandium to a series of synthetic DNAs substituted with halogen atoms at the 5 position of the pyrimidine base. The values on formation of the P_i/drug = 5 complex in 10 mM cacodylate buffer were 21.5, 26, and 30°C for poly(dA-dU), poly(dA-br^{5}dU), and poly(dA-i^{5}dU), respectively, and 15 and 20.5°C for poly(dI-dC) and poly(dI-br^{5}dC), respectively.

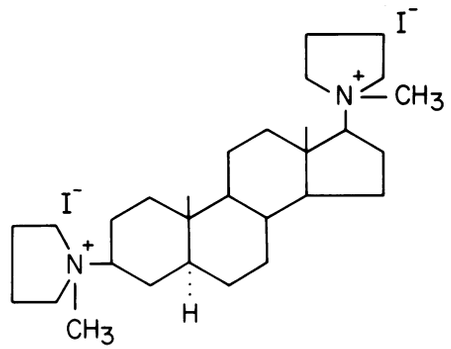

FIG. 1. Structure of dipyrandium iodide (3β,17β-dipyrrolidin-1′-yl-5α-androstane). The sample from May and Baker (Essex, UK), was forwarded to us by H. M. Sobell (University of Rochester, NY).

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Abbreviation: P_i, inorganic phosphate.

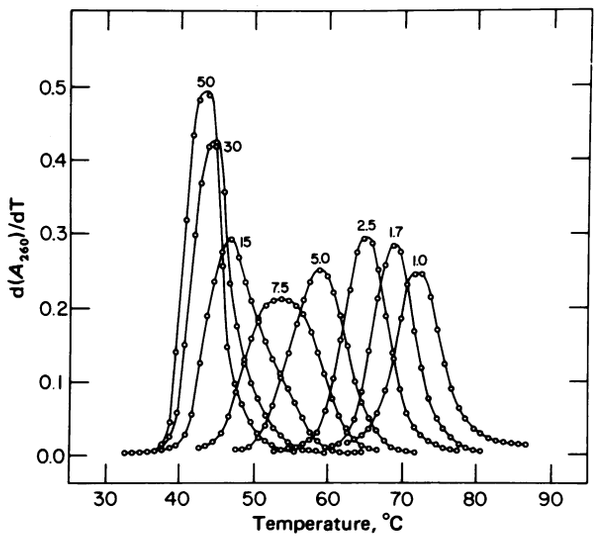

FIG. 2. Ultraviolet–visible absorbance (260 nm) melting curves (first heating cycle) in differentiated form of poly(dA-dT) and the dipyrandium·poly(dA-dT) complexes (P_i/drug = 50:1 to 1:1) in 10 mM cacodylate buffer/1 mM EDTA, pH 5.3. The poly(dA-dT) concentration was fixed at 0.15 mM. The melting curves were run on a Gilford 2400-2 spectrophotometer equipped with a thermoelectric device, a thermoprogrammer, and a reference compensator. The samples (0.25 ml) were heated at a constant rate of 1°C/min from 25 to 98°C. At the end of the run, the samples were cooled to 25°C and subjected to a second heating cycle. No differences were observed between heating cycles.

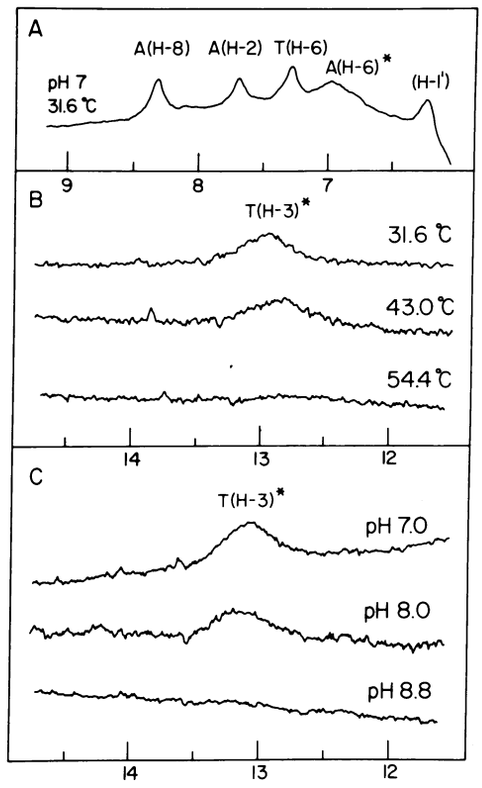

FIG. 3. Continuous-wave 360-MHz proton NMR spectra of the dipyrandium·poly(dA-dT) complex at P_i/drug = 5 in 25 mM cacodylate/0.25 mM EDTA, pH 7. The synthetic DNA concentration was 50.5 mM. The spectra are calibrated relative to standard TSP. Exchangeable protons are designated by *. (*A*) Spectral region 6–9 ppm at pH 7 and 31.6°C. (*B*) Spectral region 11.5–14.5 ppm at pH 7.0 as a function of temperature. (*C*) Spectral region 11.5–14.5 ppm at 20.3°C as a function of pH.

Watson–Crick protons

The 360-MHz continuous-wave proton NMR spectra of the dipyrandium·poly(dA-dT) complex at P_i/drug = 5 (synthetic DNA concentration, 50.5 mM) in 25 mM cacodylate buffer were recorded as a function of temperature and pH. The nonexchangeable adenosine H-8 and H-2, thymidine H-6, and a sugar H-1′, as well as the exchangeable adenosine H-6 amino protons, were well resolved in the spectrum of the complex between 6 and 9 ppm at pH 7.0 and 31.6°C (Fig. 3*A*). The thymidine H-3 Watson–Crick proton was observed at 12.97 ppm and exhibited a linewidth of 155 Hz (Fig. 3*B*) in contrast to the nonexchangeable protons which exhibited a linewidth of ~50 Hz (Fig. 3*A*) at 31.5°C. The thymidine H-3 resonance broadened with increasing temperature to ≈225 Hz at 43.0°C and was too broad to measure at 54.5°C (Fig. 3*B*).

The pH dependence of the thymidine H-3 resonance in the P_i/drug = 5 complex at 20.3°C is presented in Fig. 3*C*. This resonance exhibited a linewidth of ≈170 Hz at pH 7.0 and ≈195 Hz at pH 8.0, and it broadened out at pH 8.8.

Nucleic acid proton chemical shifts

The Fourier transform NMR spectra of the nonexchangeable base and sugar H-1′ nucleic acid protons in the dipyradium·poly(dA-dT) complex (synthetic DNA concentration, 17 mM) at P_i/drug = 11, 5, and 3.5 in 10 mM cacodylate/^2H_2O were recorded as a function of temperature. The experimental chemical shifts for the adenosine H-2 resonance in poly(dA-dT) and the steroid diamine complexes between 0 and 100°C are plotted in Fig. 4*A*. The chemical shift difference at the adenosine H-2 resonance associated with the melting transition of poly(dA-dT) and the P_i/drug = 5 complex were 0.960 and 0.246 ppm, respectively, at temperatures corresponding to their transition midpoints (after correction of the chemical shifts associated with the premelting and postmelting transitions). The chemical shifts in the duplex state of the remaining base and sugar resonances in the dipyrandium·poly(dA-dT) complex at P_i/drug = 5 also were downfield from their corresponding values in poly(dA-dT) (Fig. 5).

The transition midpoint of the adenosine H-2 resonance increased from 45.0°C in poly(dA-dT) to 74.5°C in the P_i/drug = 5 complex to 79.5°C in P_i/drug = 3.5 complex (Fig. 4*A*). The adenosine H-2 resonance in the P_i/drug = 11 complex exhibited biphasic behavior with transition midpoint values of ≈58°C and ≈66.5°C (Fig. 4*A*).

Steroid diamine proton chemical shifts

The temperature dependence of the most upfield single proton dipyrandium resonance is plotted in Fig. 4*B* for the P_i/drug = 11, 5, and 3.5 complexes and in the absence of poly(dA-dT) in 10 mM cacodylate solution. This steroid diamine resonance shifted upfield from 0.2 ppm at 95°C to −0.3 ppm at 60°C for the P_i/drug = 11 complex (Fig. 4*B*), conditions under which the nucleic acid is in excess and all the dipyrandium is complexed to the strong binding sites below the melting transition. Similar upfield complexation shifts were observed at several other protons and the two CH_3 groups; the shifts of the NCH_3 groups were smaller in magnitude for the dipyrandium·poly(dA-dT) complex at P_i/drug = 5 on decreasing the temperature from 100 to 40°C (Fig. 6).

Nucleic acid proton line widths

The temperature dependence of the adenosine H-8 linewidths for poly(dA-dT) and the P_i/drug = 5 dipyrandium·poly(dA-dT) complex in 10 mM cacodylate between 5 and 95°C are plotted in Fig. 7. We observed narrower linewidths in the premelting transition range [<40°C for poly(dA-dT) and <65°C for P_i/

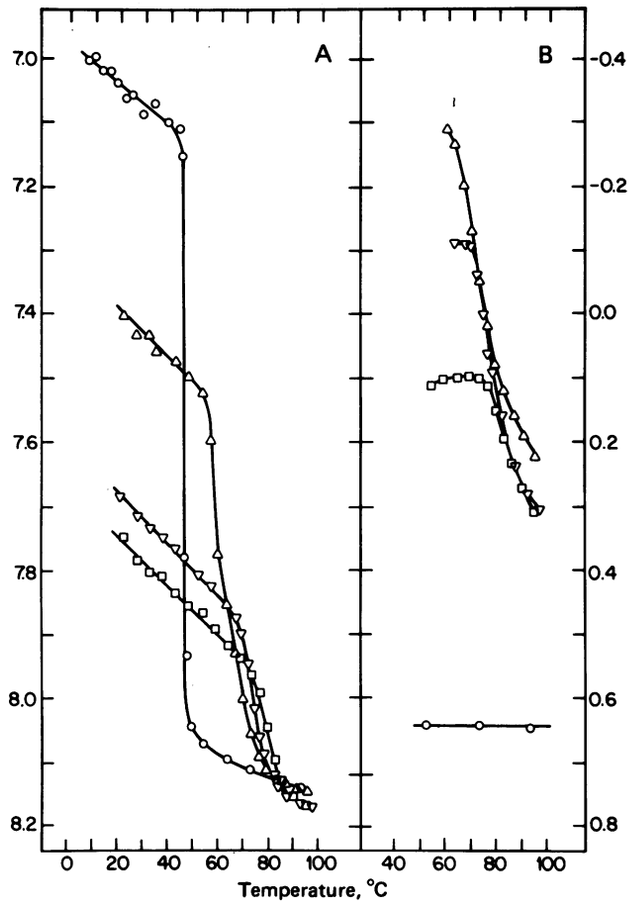

FIG. 4. Temperature dependence of the chemical shifts of resonances in the dipyrandium·poly(dA-dT) complex at P_i/drug = 11.5 (△), 5 (▽), and 3.5 (□) in 10 mM cacodylate buffer/0.1 mM EDTA/^2H_2O. The synthetic DNA concentration was fixed at 17 mM. (*A*) Adenosine H-2 resonance. The chemical shifts for poly(dA-dT) alone are represented by ○. (*B*) Highest field dipyrandium resonance. The chemical shifts of dipyrandium alone are represented by ○.

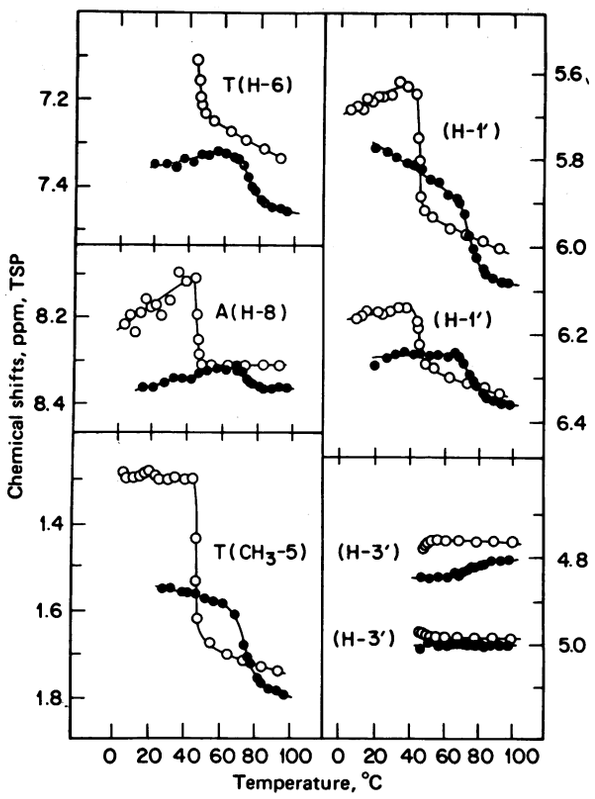

FIG. 5. Temperature dependence of the nucleic acid base and sugar H-1′ chemical shifts of poly(dA-dT) (○) and the dipyrandium·poly(dA-dT) complex at P_i/drug = 5 (●) in 10 mM cacodylate/0.1 mM EDTA/^2H_2O, at pH 7.15 and pH 7.80, respectively. The poly(dA-dT) sample concentration was fixed at 27 mM.

drug = 5 complex] for the steroid diamine· synthetic DNA complex.

Phosphorus chemical shifts

The 145.7-MHz proton noise decoupled ^{31}P Fourier transform NMR spectra of poly(dA-dT) in the absence and presence of dipyrandium (synthetic DNA concentration, 27 mM) in 10 mM cacodylate buffer were recorded as a function of temperature in the premelting transition range. The poly(dA-dT) spectrum exhibited a ^{31}P envelope centered at 4.26 ppm with a shoulder at 4.46 ppm [relative to internal standard $(CH_3O)_3PO$] at 29.5°C (Fig. 8 *upper*). The P_i/drug = 5 complex exhibited resonances at 4.23 and 4.42 ppm at 31°C. We observed that the ^{31}P resonances exhibited similar chemical shifts (<0.1 ppm) for the synthetic DNA in the absence and presence of dipyrandium at temperatures corresponding to the duplex state (Fig. 8 *lower*).

DISCUSSION

The NMR results presented above permit several conclusions to be drawn regarding structural and dynamic aspects of the dipyrandium·poly(dA-dT) complex in solution.

Hydrogen Bonding. The thymidine H-3 resonance was utilized as a probe to monitor the integrity of the Watson–Crick hydrogen bonds (16) in the steroid diamine·poly(dA-dT) complex. The observation of this resonance at 13.0 ppm in the P_i/drug = 5 complex confirms that the base pairs are intact at the steroid diamine binding site. The linewidth of this exchangeable resonance in the complex (≈170 Hz) was twice its value in poly(dA-dT) under the same conditions. Furthermore, the linewidth of the thymidine H-3 resonance of poly(dA-dT)

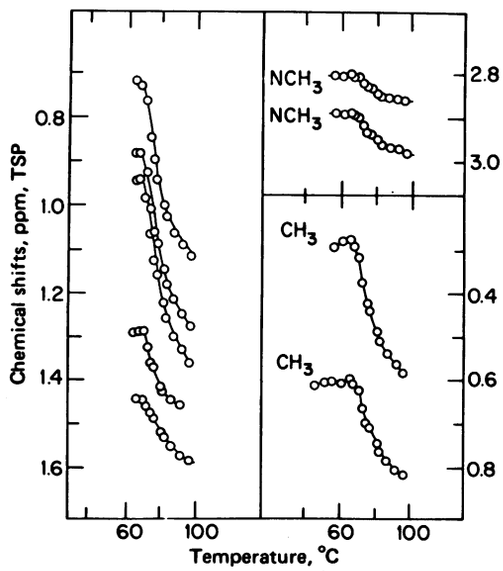

FIG. 6. Temperature dependence of several steroid diamine proton and methyl chemical shifts in the dipyrandium·poly(dA-dT) complex at P_i/drug = 5 in 10 mM cacodylate/0.1 mM EDTA/^2H_2O, pH 7.80. The synthetic DNA concentration was 27 mM. The steroid diamine protons broadened considerably below 60°C and could not be monitored below this temperature.

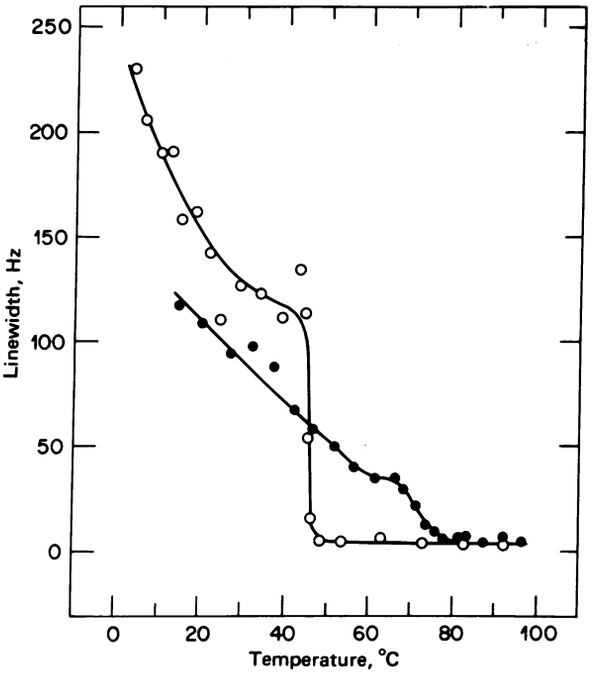

FIG. 7. Temperature dependence of the adenosine H-8 linewidths of poly(dA-dT) (○) and the dipyrandium·poly(dA-dT) complex at P_i/drug = 5 (●) in 10 mM cacodylate buffer/0.1 mM EDTA/^2H_2O at pH 7.15 and 7.80, respectively. The poly(dA-dT) concentration was 27 mM.

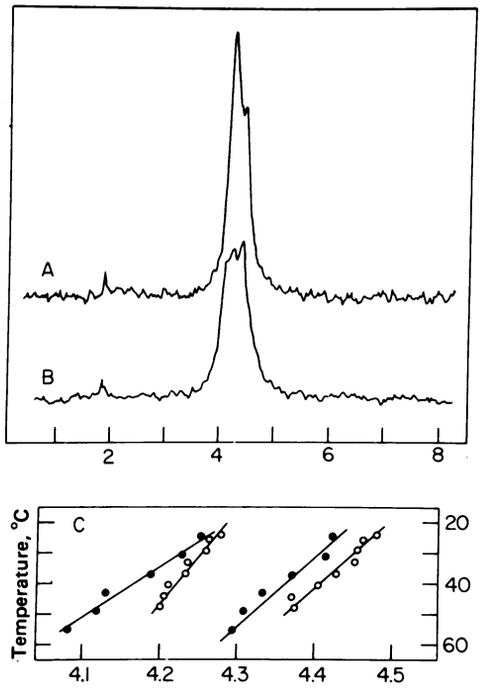

FIG. 8. (*Upper*) Proton noise decoupled 145.7-MHz ^{31}P Fourier transform NMR spectra [1–8 ppm upfield from internal standard $(CH_3O)_3PO$] of poly(dA-dT) (curve A) and dipyrandium·poly(dA-dT) complex at P_i/drug = 5 (curve B) in 10 mM cacodylate/0.1 mM EDTA/^2H_2O, pH 6.85, at 29.5 and 31°C, respectively. The synthetic DNA concentration was 27 mM. No additional resonances were observed in a spectral region extending from −5 to 0 ppm. (*Lower*) Temperature dependence of the two partially resolved ^{31}P chemical shifts in poly(dA-dT) (○) and P_i/drug = 5 complex (●) in the premelting transition region.

is insensitive to pH between 7 and 9, but this resonance broadens out for the P_i/drug = 5 complex when the pH is increased to 8.8 (Fig. 3*C*). The larger linewidths of the thymidine H-3 resonance and its susceptibility to base catalysis (17) suggest that the base pairs are partially exposed to solvent at the steroid diamine binding site.

The thymidine H-3 exchangeable proton linewidth broadens out at 54.5°C in the P_i/drug = 5 complex at neutral pH (Fig. 3*B*) even though a transition midpoint of 77°C is monitored by the nonexchangeable protons for the dissociation of the complex (Fig. 4). This demonstrates that the exchange of the Watson–Crick imino protons with H_2O in the steroid diamine·poly(dA-dT) complex must occur by transient breakage of the base pairs (18).

The thymidine H-3 resonance exhibits a similar chemical shift in poly(dA-dT) and the P_i/drug = 5 complex. This suggests a compensation of contributions arising from a decrease in hydrogen bond strength (upfield shifts) and a decrease in base pair overlaps (downfield shifts) at the steroid diamine binding site.

Base Pair Overlap Geometries. The magnitude of the upfield chemical shifts of the base protons of poly(dA-dT) on duplex formation (19) are dramatically decreased on complex formation with the steroid diamine (Figs. 4*A* and 5). These upfield shifts are associated with the distance-dependent ring current contributions of the nucleic acid bases (20). The NMR data require a reduction of stacking interactions between adjacent base pairs at the steroid diamine binding site.

Steroid Diamine–Base Pair Interactions. Several protons and the two CH_3 groups of the steroid diamine shift upfield upon complex formation with poly(dA-dT) with magnitudes ranging from 0.2 to 0.5 ppm when the temperature of the P_i/drug = 5 complex is decreased from 100°C to 60°C (Figs. 4*B* and 6). Actually, the magnitude of the complexation shifts are approximately twice these values if one compares the chemical shift of these resonances for the steroid diamine alone in solution with its value when complexed to duplex poly(dA-dT) in solution (Fig. 4*B*). By contrast, the dipyrandium NCH_3 protons shift only by 0.1 ppm on complex formation with poly(dA-dT) (Fig. 6). The NMR data require that those protons that exhibit large upfield shifts on complex formation must be located above the planes of the nucleic acid base pairs at the binding site and experience upfield ring current contributions of the adenosine and thymidine rings.

The maximal separation between parallel base pair planes is 6.8 Å and corresponds to an intercalation site. The steroid diamine is nonplanar and therefore unable to insert into such a site while spanning the backbone phosphates through its charged ends. It therefore appears that the steroid diamine must partially insert into a binding site generated by unstacked base pairs that are tilted relative to each other.

Because we are unable to assign the individual dipyrandium protons to specific positions on the steroid diamine ring at this time and have no knowledge of the structural details of the tilted base pairs at the binding site, it is premature to propose detailed models for the structure of the complex.

Sugar–Phosphate Backbone. It has been proposed that the chemical shifts of the ^{31}P resonances may reflect, in part, variations in the ω,ω' angles of the polynucleotide backbone (21–23). Thus, a few internucleotide phosphate resonances of tRNA are scattered over a 6.0-ppm chemical shift range (21), and complexation shifts of $\approx$2 ppm are observed for actinomycin D·nucleic acid interactions (22). The similarity in the internucleotide ^{31}P chemical shifts (<0.1 ppm variation) in the duplex state of poly(dA-dT) and the P_i/drug = 5 complex (Fig. 7) suggests that changes in the ω,ω' angles do not occur on generation of the steroid diamine binding site.

Steroid Diamine Exchange between Binding Sites. The nucleic acid and steroid diamine protons shift as average resonances during the melting transition of the dipyrandium·poly(dA-dT) complex at P_i/drug = 11, 5, and 3.5 in 10 mM cacodylate solution (Figs. 4–6) which suggests that the steroid diamine exchanges rapidly among potential nucleic acid binding sites on the NMR time scale at this ionic strength. This result is in contrast to the slow exchange of the groove binding agent netropsin among potential binding sites in the netropsin·poly(dA-dT) complex in solution (24). The differences in exchange rates between the steroid diamine and netropsin with poly(dA-dT) may reflect the additional stabilization arising from peptide·nucleotide hydrogen bonding in the latter complex.

A premelting transition is observed for the adenosine H-2 resonance in poly(dA-dT), which persists in the P_i/drug = 11, 5, and 3.5 complexes (Fig. 4*A*). The premelting transition probably reflects the increase in branch formation of poly(dA-dT) with increasing temperature (25), and it is noteworthy that this equilibrium is not perturbed on complex formation with steroid diamines.

Segmental Flexibility. The NMR linewidths of macromolecules are a measure of the overall tumbling rates in solution modulated by the contributions of local segmental motions. We observe narrower resonances for the base and sugar protons in the duplex state of the P_i/drug = 5 complex compared to the duplex state of the synthetic DNA (Fig. 7). This suggests greater segmental flexibility for the base pairs and sugar rings of the nucleic acid in the presence of the steroid diamine. By contrast, the nucleic acid base and sugar resonances are much broader for the poly(dA-dT) complexes with the intercalating agent ethidum bromide (26) and the groove binding agent netropsin (24) compared to their values in synthetic DNA.

Binding to the Minor Groove. The 260-nm absorbance melting curves for dipyrandium complexed to a series of synthetic DNAs demonstrates a somewhat greater stabilization on introduction of bulky substituents at the 5 position of the pyrimidine ring which faces the major groove. Saucier (14) has demonstrated that spermine (which binds to the minor groove) and steroid diamines bind competitively to DNA. The above results suggest that steroid diamines may bind to the minor groove of DNA.

Steroid Diamine Binding to the Strand State. There is an equilibrium between stacked and unstacked strands in the postmelting transition region which shifts towards the latter with increasing temperature. The NMR data demonstrate that the steroid diamine also binds to poly(dA-dT) in the postmelting transition region because the chemical shifts of the dipyrandium protons in the complex at high temperature after completion of the duplex-to-strand transition are upfield from the corresponding values of dipyrandium alone in solution (Fig. 4*B*). Complexation of the steroid diamine to the strand state is also manifested in the observed differences of the base and sugar chemical shifts of poly(dA-dT) and the P_i/drug = 5 complex at 95°C (Fig. 5).

Sobell's Proposed Model for the Steroid Diamine·DNA Complex. The nucleic acid and steroid diamine proton chemical shift changes during the melting transition of the P_i/drug = 5 complex are consistent with the general concepts put forward by Sobell (11) for the steroid diamine·DNA complex. This model proposes that the Watson–Crick hydrogen bonds are intact (verified by observation of the thymidine H-3 resonance in the NMR spectrum) in the neighbor-exclusion complex and that every other set of base pairs partially unstacks [verified by loss of ring currents from adjacent base pair(s) resulting in a downfield NMR shift in the duplex state] and the steroid diamine partially inserts at this site (verified by upfield NMR ring current shifts of steroid diamine protons from base pairs at the binding site).

The NMR nonexchangeable resonances exhibit linewidths of ≥50 Hz in the duplex state of the P_i/drug = 5 complex so that it is not possible to evaluate the proton coupling constants that are a measure of the polynucleotide backbone torsion angles and the sugar pucker geometries. We are thus unable to deduce whether torsion angle changes in the backbone, the glycosidic angles, or the sugar pucker, or any combination of these, are altered to generate the steroid diamine binding site. The above NMR studies would have to be extended to the oligonucleotide duplex level (narrower NMR resonances) to differentiate among these possibilities.

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