Effect of Tetramethylammonium Ion on the Helix-to-Coil Transition of Poly(deoxyadenylylthymidine): A Nuclear Magnetic Resonance and Calorimetric Investigation†

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ABSTRACT: Differential scanning calorimetry, temperature-dependent NMR, and UV spectroscopy are used to investigate the helix-to-coil transition of poly(deoxyadenylylthymidine) [poly(dA-dT)] in 1 M NaCl and 1 M Me_4NCl (tetramethylammonium chloride). All three methods reveal that the polymer has a melting temperature, t_m, that is approximately 6 °C higher in 1 M Me_4NCl than in 1 M NaCl. The NMR data show that this increased stability does not result from fundamental changes in base stacking or base pairing in going from 1 M NaCl to 1 M Me_4NCl. Consistent with this observation, the calorimetric measurements yield essentially equal enthalpies for the helix-to-coil transition under the two salt conditions (6.8 kcal per base pair in 1 M NaCl and 7.0 kcal per base pair in 1 M Me_4NCl). Analysis of the shapes of the calorimetric curves shows that the transition is more cooperative in Me_4NCl than in NaCl. Comparison of the calorimetric and van't Hoff enthalpies allows specification of the size of the cooperative unit: 27 base pairs in 1 M NaCl and 32 base pairs in 1 M Me_4NCl. The NMR data reveal that the major Me_4NCl-induced structural alterations (relative to NaCl) are a change in one glycosidic torsion angle and a partial resolution of the two phosphates. The calorimetric experiments indicate that in the absence of fortuitous compensation these conformational changes are not accompanied by a significant enthalpy effect. On the basis of these data, we suggest that in 1 M NaCl poly(dA-dT) assumes predominately a B-DNA-like conformation where the symmetry repeat occurs every base pair. By contrast, in 1 M Me_4NCl the predominate conformation exhibits a dinucleotide repeat consistent with a right-handed alternating B-DNA structure.

The effect of added salts on the stability of nucleic acid structures has been and continues to be the subject of numerous experimental and theoretical investigations [for recent comprehensive reviews, see Record et al. (1978) and Manning (1978)]. The thermal stability of oligonucleotides and polynucleotides has been studied in the presence of various cationic and anionic species as well as at different overall salt concentrations (Palecek, 1976; Brahms & Van Holde, 1976; Gennis & Cantor, 1972; Ivanov et al., 1974; Breslauer & Bodnar, 1979). Several theoretical treatments have been able to correlate a good deal of these data in terms of specific (Shchelkina et al., 1977) and nonspecific interactions (Manning, 1977; Record & Lohman, 1978; Anderson et al., 1978). However, despite continued activity in this area, experimental data concerning the details of "salt effects" remain incomplete. Tetramethylammonium ion (Me_4N^+) provides a particularly good example.

Me_4N^+ has been reported to have several unusual effects on the double to single strand transition of natural and synthetic deoxyribonucleic acid polymers (Shapiro et al., 1969; Melchior & von Hippel, 1973; Wang & Kallenbach, 1971). The Me_4N^+ cation is unique in its ability to prevent premelting conformational changes in DNA (DeMurcia et al., 1978). In the presence of Me_4N^+, the thermal stability of various DNA's, as monitored by their melting temperatures (t_m), decreases relative to their stability in Na^+. The magnitude of this destabilizing effect decreases with increasing AT content with poly(dA-dT)·poly(dT-dA), actually having a higher t_m in Me_4N^+ than in Na^+ (DeMurcia et al., 1978). These results, in conjunction with equilibrium dialysis data (Shapiro et al., 1969), have led to the suggestion that Me_4N^+ exerts its unique stabilizing influence through preferential binding to AT base pairs. However, little experimental evidence exists concerning either the nature of this specific interaction or its effect on the helical structure. Does the proposed binding perturb the base-stacking interaction? Is the backbone structure influenced? Are the energetics of the helix-to-coil transition altered? To address these questions, we have employed nuclear magnetic resonance (NMR), differential scanning calorimetry (DSC), and temperature-dependent UV spectroscopy to investigate the double to single strand transition of poly(deoxyadenylylthymidine)[poly(dA-dT)] in the presence of sodium chloride (NaCl) and tetramethylammonium chloride (Me_4NCl).

Experimental Section

Materials

Poly(dA-dT) obtained from P-L Biochemicals, Inc. (Milwaukee, WI) was further purified by dialysis against distilled water and obtained in dry form by lyophilization.

Me_4NCl obtained from Fisher (reagent grade) was purified by recrystallizing the salt twice from distilled water and then drying approximately 24 h at 100 °C. The dry solid was washed twice with purified diethyl ether and the ether removed by drying for 24 h in a vacuum desiccator (Bencowitz & Renshaw, 1926; Jones & Whalen, 1925).

Me_4NCl used for the NMR studies was obtained from Merck and used without further purification.

Solvents. Calorimetric and spectroscopic measurements were carried out in a buffer system consisting of 0.01 M sodium phosphate and 10^{-4} M sodium EDTA, in 1 M NaCl (or 1 M Me_4NCl) adjusted to pH 6.85.

The NMR measurements were carried out in H_2O or D_2O buffer systems containing 1 M NaCl (or 1 M Me_4NCl), 10 mM cacodylate (or 10 mM phosphate), and 1 mM EDTA at

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Table I: Melting Temperatures, t_m, for Poly(dA-dT) as Monitored by Different Techniques in 1 M NaCl and 1 M Me_4NCl

physical method	melting temperature (°C)	
	1 M NaCl	1 M Me_4NCl
NMR	72.5	79.0
calorimetry	74.5	81.0
UV optical	75.4	81.3

pH values near 7. The exact conditions are indicated in each figure legend.

Methods

UV Spectroscopy. The absorbance vs. temperature profiles reported here were measured at 260 nm by using a temperature-programmable, thermoelectrically controlled Perkin-Elmer 575 spectrophotometer interfaced to a Tektronix 4051 computer for data acquisition and analysis. The temperature was increased continuously from 20 to 95 °C at a rate of 0.5 °C/min. Repetitive runs revealed the melting temperature, t_m, to be reproducible to better than ±0.5 °C. The polymer concentration was spectrophotometrically determined to be 2.35×10^{-4} M by using an extinction coefficient of 6.65×10^3 M^{-1} cm^{-1} in phosphate (Inman & Baldwin, 1962).

Calorimetry. The differential scanning calorimetry was carried out on a Microcal-1 instrument similar to one previously described in detail (Jackson & Brandts, 1970). In a typical experiment, the reaction and the reference platinum cells are each filled with 0.9 mL of solution, and the temperature is scanned from 20 to 95 °C at a rate of 0.93 °C/min. For a thermally induced endothermic transition, the temperature of the reaction cell will lag behind that of the solvent reference cell. In a given experiment, one continuously measures the additional energy fed back to the reaction cell to maintain it at the same temperature as the solvent reference cell. The instrument is calibrated by measuring the area produced by a controlled electrical pulse. These data (along with the known concentration of the solute) permit the construction of an enthalpy vs. temperature curve as shown in Figure 5 and a heat capacity vs. temperature curve as shown in Figure 6. The data plotted in these figures are excess enthalpy and heat capacity values relative to buffer and represent the average of two runs for each salt system.

Solutions used for the calorimetric experiments were prepared by dissolving the polymer in 2 mL of buffer and dialyzing against buffer for 3 days at 4 °C. The concentration of polymer in these solutions was determined spectrophotometrically to be 1.30×10^{-2} M and 1.40×10^{-2} M in phosphate for the Na^+ and Me_4N^+ runs, respectively.

NMR. Proton NMR spectra in H_2O were recorded in the correlation mode while proton and phosphorus NMR spectra in D_2O were recorded in the Fourier-transform mode on Bruker HX-360 and WH-360 spectrometers. Proton chemical shifts are referenced relative to internal sodium 5,5-dimethyl-5-silapentane-2-sulfonate, $(CH_3)_3Si(CH_2)_3SO_3Na_2$, while phosphorus chemical shifts are referenced relative to internal trimethyl phosphate, $(CH_3O)_3PO$.

Results

NMR

Overall Stability. The cooperative helix-to-coil transition of poly(dA-dT) can be monitored in both 1 M Me_4N^+ and 1 M Na^+ by following the temperature-dependent chemical shifts of selected base and backbone proton resonances (see Figure 1). The NMR melting curves shown in Figure 1 have

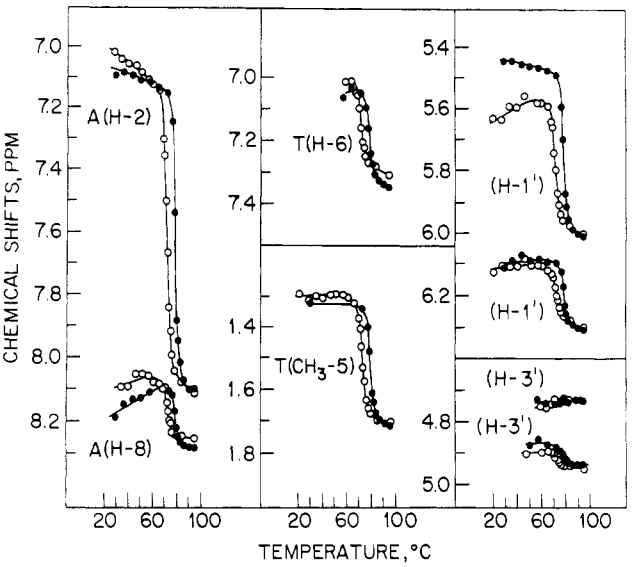

FIGURE 1: Temperature dependence of the base and sugar proton resonances of poly(dA-dT) in 1 M NaCl and 10 mM cacodylate solution (○) and in 1 M $(^2H_3C)_4NCl$ and 10 mM phosphate solution (●). $(^2H_3C)_4NCl$ was purchased from Merck and used without further purification.

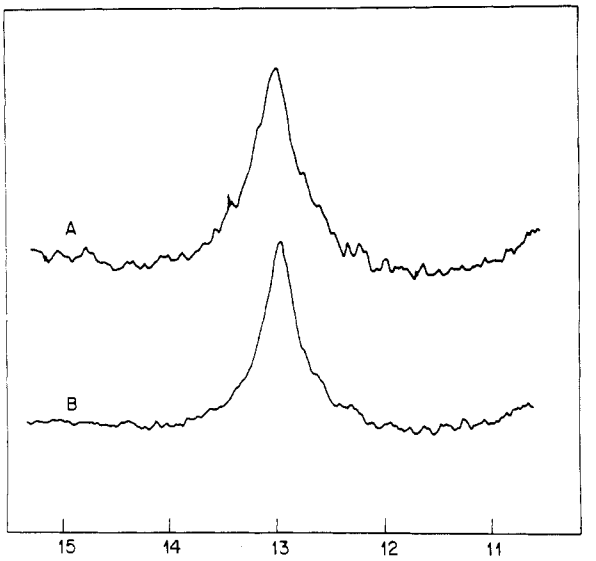

FIGURE 2: Correlation proton NMR spectra at 360 MHz of poly(dA-dT) in 1 M $(^2H_3C)_4NCl$, 10 mM phosphate, 1 mM EDTA, and 80% H_2O–20% D_2O at 37 °C. Spectrum A was recorded at pH 7.5 while spectrum B was recorded at pH 9.5.

transition midpoints of 72.5 °C in 1 M Na^+ solution and 79.0 °C in 1 M Me_4N^+ solution (see Table I). Thus, the poly(dA-dT) duplex melts over 6 °C higher in Me_4N^+ than in Na^+.

Hydrogen Bonding. As shown in Figure 2, the thymidine H-3 proton (which is normally involved in base pairing) can be monitored in 1 M Me_4N^+ solutions at 37 °C and pH values of 7.5 and 9.5. This demonstrates that the base pairs are intact in poly(dA-dT) and that their exchange is not catalyzed up to pH 9.5 at 37 °C. These results parallel observations on poly(dA-dT) with Na^+ as the counterion. Thus, one can conclude that the presence of Me_4N^+ ions does not disrupt the "normal" hydrogen-bonding interaction that exists in Na^+ solutions at pH 7.5 and 37 °C.

Base Stacking. The thymidine H-3 protons are located in the center of the base pairs and therefore provide a sensitive measure of base-pair overlap. Thus, the temperature dependence of the thymidine H-3 protons for poly(dA-dT) was monitored in both 1 M Na^+ and 1 M Me_4N^+ solutions. In-

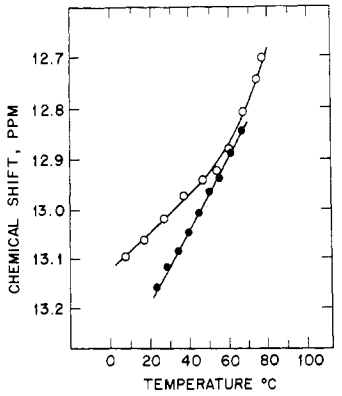

FIGURE 3: Comparison of the temperature dependence of the thymidine H-3 proton chemical shift of poly(dA-dT) in 1 M NaCl, 10 mM cacodylate, 0.1 mM EDTA, and H_2O, pH 6.53 (●) and in 1 M $(^2H_3C)_4NCl$, 10 mM phosphate, 1 mM EDTA, and H_2O, pH 7.5 (○).

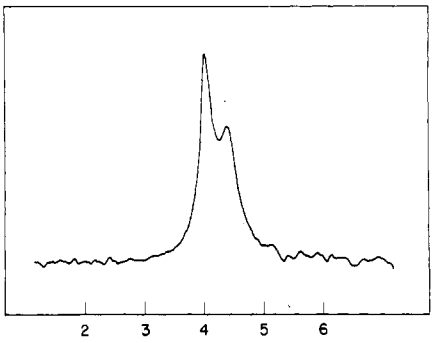

FIGURE 4: Proton noise decoupled 145.7-MHz ^{31}P NMR spectra of poly(dA-dT) in 1 M $(CH_3)_4NCl$, 10 mM cacodylate, 1 mM EDTA, and ^2H_2O, pH 7.95, at 67 °C. The chemical shifts are upfield from standard trimethyl phosphate.

spection of Figure 3 reveals that the chemical shifts are similar at high temperatures and differ by only 0.1 ppm at low temperatures. This suggests similar base-pair overlaps for poly(dA-dT) in 1 M Na^+ and 1 M Me_4N^+ as monitored at the thymidine H-3 proton.

The nonexchangeable protons (adenosine H-8 and H-2; thymidine H-6 and CH_3-5) also provide a means of monitoring base-pair overlap. The temperature-dependent chemical shifts of these protons are plotted in Figure 1. The parallel chemical shifts for the duplex in Na^+ and Me_4N^+ solutions suggest similar base-pair overlap geometries in the double helix.

Glycosidic Torsion Angle. The sugar H-1′ protons provide a sensitive probe of the glycosidic torsion angle. A comparison of the temperature dependence of the H-1′ and H-3′ protons reveals a selective upfield shift at the H-1′ resonance at higher field on proceeding from Na^+ to Me_4N^+ solutions (Figure 1). In addition, the direction (slope) of this premelting transition for the H-1′ proton is different under the two salt conditions. These results suggest that the Me_4N^+ ion induces a torsion angle change relative to the Na^+ conformation at either the adenosine or the thymidine glycosidic bond.

Phosphodiester Linkages. The dT*p*dA and dA*p*dT phosphodiester linkages do not give resolved ^{31}P spectra for poly(dA-dT) in 1 M NaCl solution (Patel, 1979). It is therefore striking that two partially resolved resonances are observed for this synthetic DNA in 1 M Me_4N^+ solution (Figure 4). The chemical shift separation is 0.42 ppm at 52 °C and 0.34 ppm at 67 °C. Partially resolved ^{31}P resonances have been reported for 150 base-pair $(dA\text{-}dT)_n$ in the absence of Me_4N^+, but the reported shift difference of ~0.25 ppm (Shindo et al., 1979) is smaller than the results reported here in Me_4N^+ solution. The two ^{31}P resonances should exhibit equal areas

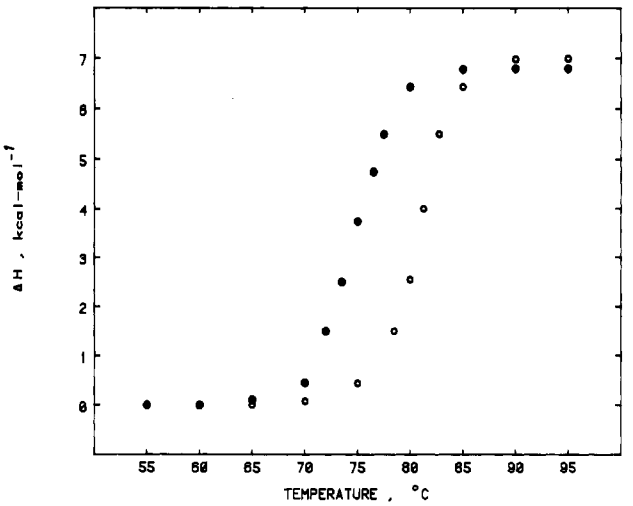

FIGURE 5: Calorimetric enthalpy vs. temperature curves for poly(dA-dT) at pH 6.85 in 0.01 M sodium phosphate, 10^{-4} M EDTA, and 1 M NaCl (●) or 1 M Me_4NCl (○). The enthalpy shown is the excess enthalpy relative to buffer.

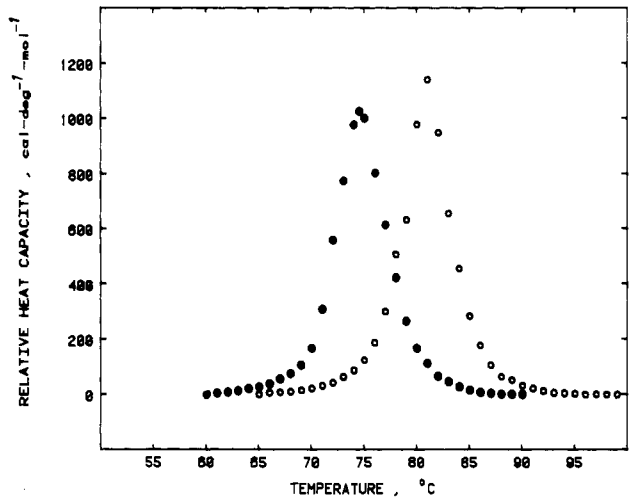

FIGURE 6: Calorimetric heat capacity vs. temperature curves for poly(dA-dT) at pH 6.85 in 0.01 M sodium phosphate and 10^{-4} M EDTA in 1 M NaCl (●) or 1 M Me_4NCl (○). The heat capacity shown is the excess heat capacity relative to buffer.

Table II: Calorimetrically Determined Enthalpy Changes for Poly(dA-dT) in 1 M NaCl and 1 M Me_4NCl

salt	ΔH (kcal/base pair)
1 M NaCl	6.8
1 M Me_4NCl	7.0

if one corresponds to the dT*p*dA and the other to the dA*p*dT phosphodiester linkages. We do not understand the origin of the unequal areas in the ^{31}P spectrum of poly(dA-dT) in Me_4N^+ solution with the relative ratios maintained in 2 and 4 M salt.

The ^{31}P NMR results reveal that in 1 M Me_4N^+ solution (in contrast to 1 M Na^+ solution), there are differences between the purine-(3′–5′)-pyrimidine and pyrimidine-(3′–5′)-purine phosphodiester linkages for poly(dA-dT). This reflects a dinucleotide repeat for poly(dA-dT) in 1 M Me_4NCl.

Calorimetry

Overall Stability. The calorimetric enthalpy vs. temperature and heat capacity vs. temperature melting curves for poly(dA–dT) in 1 M Na^+ and 1 M Me_4N^+ are shown in Figures 5 and 6. The transition midpoints are 74.5 °C in 1 M Na^+ and 81.0 °C in 1 M Me_4N^+. Thus, as observed from the

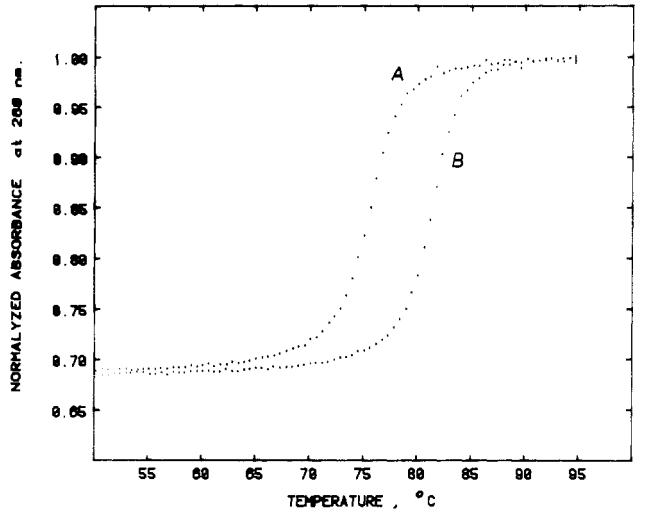

FIGURE 7: Absorbance vs. temperature profiles for poly(dA-dT) in 0.01 M sodium phosphate buffer and 10^{-4} M EDTA in 1 M NaCl (A) or 1 M Me_4NCl (B).

NMR results, the poly(dA-dT) duplex melts over 6 °C higher in Me_4N^+ than in Na^+.

Enthalpy Change. The calorimetric experiments provide a direct measure of the enthalpy change accompanying the double to single strand transition of poly(dA-dT) in 1 M Na^+ and 1 M Me_4N^+ solutions. Inspection of Table II reveals that the enthalpy change is not significantly effected by the two different salt conditions. In 1 M Na^+, the ΔH is 6.8 kcal per base pair while in 1 M Me_4N^+ the ΔH is 7.0 kcal per base pair. Thus, the stabilizing effect of Me_4N^+ does not substantially alter the enthalpy of the transition.

Nature of the Transition. Although the energy difference between the initial and final states of poly(dA-dT) is not strongly influenced by a change in cation from Na^+ to Me_4N^+ see Table II), the nature of the transition appears to be affected. Inspection of Figures 5 and 6 reveals that the transition is broader (less cooperative) in the presence of Na^+ than in the presence of Me_4N^+. Specifically, the transition half-width is 5.4 °C for Na^+ and 5.0 °C for Me_4N^+. Thus, Me_4N^+ causes the transition to occur in a more cooperative manner. A parallel observation for calf thymus DNA previously has been noted (Klump, 1977) with a reported transition width of 4.4 °C for DNA in 1 M Me_4NCl.

The Me_4N^+-induced increase in cooperativity observed here can be quantified by using the calorimetric data to calculate a van't Hoff enthalpy change (ΔH_{vH}) for the transition. Analysis of the shapes of the two curves in Figure 6 by using the method of Gralla & Crothers (1973) yields ΔH_{vH} values of 184 kcal "mol^{-1}" in 1 M Na^+ and 221 kcal "mol^{-1}" in 1 M Me_4N^+. By dividing each of these van't Hoff enthalpies by the corresponding calorimetric enthalpies, one obtains a measure of the size of the unit that is melting in a cooperative manner. Thus, in 1 M Na^+, 27 base pairs melt cooperatively while in 1 M Me_4N^+ 32 base pairs melt in a cooperative manner.

UV Spectroscopy

Overall Stability. The temperature dependence of the UV absorbance for poly(dA-dT) in 1 M Na^+ and 1 M Me_4N^+ is shown in Figure 7. The transition midpoints are 75.4 °C in 1 M Na^+ and 81.3 °C in 1 M Me_4N^+ (see Table I). Thus, as observed by NMR and calorimetry, the poly(dA-dT) duplex melts over 6 °C higher in Me_4N^+ than in Na^+.

van't Hoff Enthalpy Changes. The shapes of the UV melting curves can be analyzed as previously described to yield van't Hoff enthalpies of 212 kcal "mol^{-1}" in 1 M Na^+ and 242 kcal "mol^{-1}" in 1 M Me_4N^+. These values are similar to those obtained by analysis of the shapes of calorimetric transition curves.

Discussion

Inspection of Table I reveals that the three physical methods used to monitor the double to single strand transition of poly(dA-dT) provide nearly equivalent means for determining melting temperatures. Furthermore, all three methods show that when the cation is changed from Na^+ to Me_4N^+ the poly(dA-dT) duplex melts approximately 6 °C higher. The UV melting studies of Orosz & Wetmur (1977) on several DNA's in various alkylammonium salt solutions reveal that the t_m depends only on the nature of the cation and is independent of whether the anion is chloride or bromide. Thus, the t_m shifts reported here may be interpreted as cation induced.

The NMR data show that the hydrogen-bonding interactions and base-pair overlaps are essentially unaltered in going from 1 M Na^+ to 1 M Me_4N^+. This is consistent with the calorimetric result which reveals no significant change in enthalpy for the transition in Na^+ vs. the transition in Me_4N^+. Thus, the observed 6 °C stabilization induced by Me_4N^+ relative to Na^+ cannot be attributed to any substantial alteration of "normal" base-pairing or base-stacking interactions. The major effect of Me_4N^+ relative to Na^+ appears in the selective glycosidic torsion angle change and the partial resolution of the phosphodiester linkages. The calorimetry suggests that these salt-induced structural alterations are not accompanied by significant enthalpy changes (assuming no fortuitous compensation).

The 1 M NaCl calorimetric data reported here (t_m = 72.5 °C) can be compared with the calorimetric results of Scheffler & Sturtevant (1969), who report an average value of 7.6 kcal per base pair (t_m = 42.6 °C) for the helix-to-coil transition of poly(dA-dT) in 5 mM $NaClO_4$. No previous calorimetric studies of poly(dA-dT) in 1 M Me_4NCl have been published. However, Klump (1977) reports a calorimetrically determined value of 9.8 kcal per base pair for calf thymus DNA in 1 M Me_4NCl. With consideration of the base heterogeneity of this latter system, the meaning of its comparison to poly(dA-dT) is not immediately obvious.

Significantly, the calorimetric data reveal that Me_4N^+ causes the transition to occur in a more cooperative manner relative to Na^+. If the transition is broader (less cooperative) in Na^+ due to transient opening of base pairs at temperatures below the melting temperature, then the binding of Me_4N^+ might serve to stabilize the AT base pairs and thereby inhibit the premelting phenomenon. This is consistent with the circular dichroism studies of DeMurcia et al. (1978), who found the Me_4N^+ cation to be unique in its ability to prevent premelting in a variety of DNA's. In addition, DNA melting studies in alkylammonium salt solutions (Orosz & Wetmur, 1977) suggest a specific stereochemical interaction of Me_4N^+ with DNA involving the nitrogen and two of the methyl groups.

The exact location of the Me_4N^+ binding site remains uncertain. Based upon analogy with Na^+ binding to crystalline ApU and TpT (Seeman et al., 1976; Camerman et al., 1976), one might suggest the O-2 of thymine. Clearly, as more data become available, any proposed interaction of Me_4N^+ with AT base pairs will have to be consistent with the NMR and calorimetric results reported here.

In summary, the NMR data reveal that in 1 M Me_4NCl poly(dA-dT) adopts a conformation with a dinucleotide repeat

in which every other glycosidic torsion angle and phosphodiester linkage differ from the standard B-DNA conformation observed in 1 M NaCl where the symmetry repeat occurs every base pair. The calorimetric data indicate that these two conformations do not manifest significantly different enthalpies of transition. A reasonable candidate for the 1 M Me_4NCl dinucleotide repeat conformation is a right-handed, alternating B-DNA structure.

Acknowledgments

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Alkaline Gel Electrophoresis of Deoxyribonucleic Acid Photoreacted with Trimethylpsoralen: Rapid and Sensitive Detection of Interstrand Cross-Links†

Thomas R. Cech

ABSTRACT: Restriction fragments of phage λ and φX174 deoxyribonucleic acid (DNA) were photoreacted with 4,5′,8-trimethylpsoralen to various extents, and the amount of covalent cross-linking was determined by electron microscopy of the DNA under totally denaturing conditions. The DNA was then analyzed by electrophoresis in alkaline agarose gels. A single cross-link in a DNA molecule produced a large decrease in its electrophoretic mobility. With DNA fragments 0.3–4 kilobase pairs in size, the apparent M_r (molecular weight) of the cross-linked DNA was 2.0 ± 0.1 times the M_r of the unreacted, single-stranded DNA. A single cross-link in a larger DNA molecule resulted in an even greater increase in apparent M_r. Further cross-linking produced a decrease in the apparent M_r of the DNA, reaching a plateau at a value of 1.4 ± 0.1 times the M_r of the unreacted, single-stranded DNA over a large range of fragment sizes (0.6–10 kilobase pairs). The apparent M_r of the cross-linked DNA was weakly dependent on the percentage of agarose in the gel. Although highly sensitive to interstrand cross-links, the electrophoretic mobilities appeared to be unaffected by low levels of monoadducts (trimethylpsoralen covalently bound to one strand of the DNA). The DNA bandwidths increased by as much as 4-fold at low extents of cross-linking, presumably due to heterogeneity in the locations of the cross-links in the DNA molecules. The bands became sharp again at high levels of reaction. These observations form the basis of a new assay for interstrand DNA cross-links that is both more sensitive and more convenient than previous methods.

Psoralen and its derivatives undergo photochemical reactions with deoxyribonucleic acid (DNA).[1] The psoralen can react with a pyrimidine base on one strand of the DNA to form a covalent monoadduct. Subsequent photoreaction with a pyrimidine on the opposite strand of the DNA can form a stable

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[1] Abbreviations used: DNA, deoxyribonucleic acid; Me_3psoralen, 4,5′,8-trimethylpsoralen; EDTA, (ethylenedinitrilo)tetraacetic acid; φX174 RFII, nicked double-stranded replicative form of bacteriophage φX174 DNA; bp, base pair(s); kbp, kilobase pair(s); Tris, 2-amino-2-(hydroxymethyl)-1,3-propanediol.

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