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Poly(d2NH$_2$A-dT): Effect of 2-amino substituent on the B to Z transition

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The effect of 2-amino substitution in adenosine (which permits formation of three hydrogen bonds to T) on the B to Z transition was examined in synthetic DNA of alternating purine-pyrimidine sequence. Results of CD, IR, and ^{31}P NMR experiments show that $(d2NH_2A\text{-}dT)_n$ undergoes a cooperative transition to a different helical structure under the same conditions as the B to Z transition of $(dG\text{-}dC)_n$.

Many studies have shown that the novel left-handed Z-DNA helix is formed by certain polydeoxynucleotides at high ionic strength (1-11). An alternating purine-pyrimidine sequence, principally GC, is a requirement of the structure. Neither $(dI\text{-}dC)_n$ nor $(dA\text{-}dT)_n$ appears to form Z-DNA. A 5-methyl (5) or 5-halogen (3) substituent on dC facilitates formation of the Z form. Superhelical winding of closed circular DNA can force appropriate sequences into the left-handed Z form (10). Possible biological functions of this conformation have been suggested (10, 12).

Crystallographic study of a B-form DNA dodecamer, CGCGAATTCGCG, has identified a regular, two-layer hydration structure in the minor groove (13). This "spine of hydration" occurs only in the AT region of the structure (and only in B-form DNA) and is disrupted by the $2NH_2$ group of G. Dickerson et al. (13) proposed that this regular hydration structure is very largely responsible for stability of the B helix, and that in the absence of N2 amino groups in the minor groove the spine of hydration may provide just enough free energy to keep the B helix from slipping over into either the A or Z helix. They also suggest that 2-aminoadenine should behave like guanine in disrupting the hydration structure. Other studies have shown that 2-aminoadenine elevates the Tm of ribo and deoxyribo homopolymer duplexes but that the increment is much less in the deoxy (presumably B-form) then in the ribo (presumably A form) series (14-16). These results are consistent with two contrary effects of the 2-amino group: a stabilizing contribution from formation of a third

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hydrogen bond (14, 15) and a destabilizing one from disruption of the spine of hydration in the minor groove of the B form (13). Possible biological relevance of a 2-aminoadenine substitution is suggested by the discovery of cyanophage by Kirnos et al. (17, 18) in which all of the adenine residues of the DNA are replaced by 2-aminoadenine.

We have prepared the alternating helical duplex $(d2NH_2A\text{-}dT)_n$ to observe the effect of $2NH_2A$ substitution on possible B → Z conversion in high salt. Experiments employing CD, IR, and ^{31}P NMR are presented below. CD experiments on the polymer (19) and on a related hexanucleotide (20) have been reported by other groups. Portions of the present material were presented at the 16th Jerusalem Symposium in Quantum Chemistry and Biochemistry (21).

MATERIALS AND METHODS

$(d2NH_2A\text{-}dT)_n$ was synthesized with DNA polymerase I from E. coli, using $(dA\text{-}dT)_n$ as a primer-template (22, 23). Selective degradation of the $(dA\text{-}dT)_n$ primer-template by the 5'-endonuclease activity of the enzyme was achieved by stopping the reaction just after the minimum of the absorbance-time curve was observed. Both $(dA\text{-}dT)_n$ and $(d2NH_2A\text{-}dT)_n$ were degraded by the 5'-endonuclease activity, but the former reaction was faster. The product was shown to be free of $(dA\text{-}dT)_n$. Sequence was established by nearest-neighbor frequency analysis (24). Spectroscopic methods have been described previously (6, 15).

RESULTS

The CD spectrum of $(d2NH_2A\text{-}dT)_n$ is shown in Fig. 1. In 0.1 M NaCl, the spectrum has a positive band at 293 nm, assigned to exciton splitting of the B_{2u} transition of $2NH_2A$ (15, 16). The second component of the

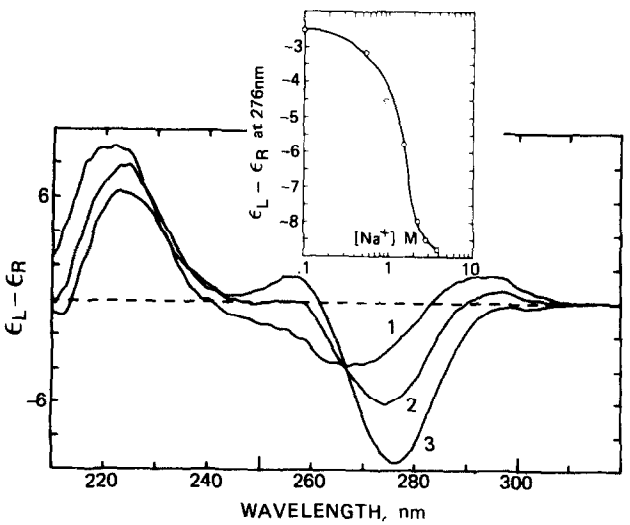

Fig. 1 CD spectra of $(d2NH_2A\text{-}dT)_n$ in 0.1 M NaCl (1), 1.55 M NaCl (2) and and 4 M NaCl (3). Sodium cacodylate buffer, 0.002 M, pH 7.0. Inset, dependence of $\Delta\epsilon$ on log $[Na^+]$.

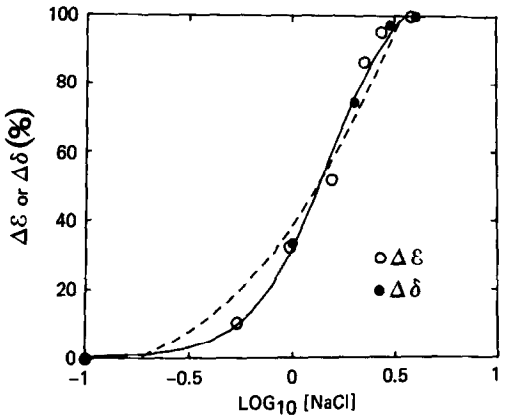

Fig. 2 CD data (Δε,0) and ^{31}P NMR chemical shift separation (Δε,●) for $(d2NH_2A\text{-}dT)_n$. The solid line is the fit to the equation with no constraints, the dashed line is the fit with n=1 (non-cooperative).

splitting contributes to the minimum at 270 nm. The B_{2u} transition of T also probably contributes to this minimum. Possible origins of the shoulder at ~ 255 nm and the peak at 220 nm are the B_{1u} and E_{1u} transitions of $2NH_2A$. In higher salt the spectrum exhibits sign inversions similar to but not identical with those observed in $(dG\text{-}dC)_n$ by Pohl and Jovin (3). Titration with NaCl is shown in Fig. 1. A similar pattern of change was observed with hexammine cobalt.

The spectral change was cooperative in $[Na^+]$ with a midpoint at 1.5 M (Figs. 1, 2), a value similar to that of 2.5 M observed for $(dG\text{-}dC)_n$ (3). The CD dependence is also cooperative in hexammine cobalt with the midpoint 4 x 10^{-5} M in $Co(NH_3)_6^{3+}$.

The infrared spectrum of (dG-dC)n in D_2O solution has carbonyl bands at 1686 and 1657 cm^{-1} in 0.1 M NaCl (Fig. 3). In 4 M NaCl these were replaced by a strong band at 1668 cm^{-1} and one of moderate intensity at

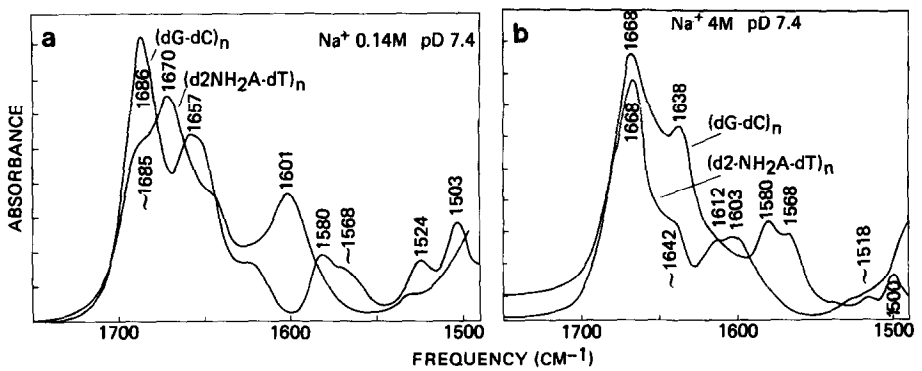

Fig. 3 Infrared spectra of $(d2NH_2A\text{-}dT)_n$ and $(dG\text{-}dC)_n$ in D_2O. (a) 0.1 M NaCl, 0.005 M Na_2HPO_4, pD 7.4. (b) 4 M NaCl, 0.02 M Na_2HPO_4, pD 7.4.

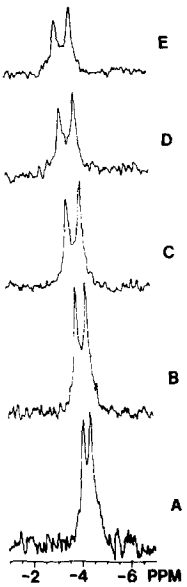

Fig. 4 Dependence of ^{31}P NMR spectra at 109.3 M Hz of $(d2NH_2A\text{-}dT)_n$ on NaCl concentration at 37°C; A, 0.1 M; B, 1 M; C, 2 M; D, 3 M; E, 4 M. Chemical shifts and are negative upfield from internal trimethyl phosphate.

1638 cm^{-1} (Fig. 3). A similar pattern was observed with $(d2NH_2A\text{-}dT)_n$ (Fig. 4). Here the spectrum in 0.1 M NaCl has carbonyl bands at 1686 cm^{-1} (sh) and 1670 cm^{-1} and a T ring vibration at 1646 cm^{-1} (sh). In 4 M NaCl the highest frequency band (1686 cm^{-1}), due to the 2C=O vibration, apparently shifted under the envelope of the intense band at 1668 cm^{-1}. Spectra of the two polymers in the carbonyl region above 1630 cm^{-1} are quite similar (Fig. 3). (For discussion of dependence of IR spectral changes on secondary structure, see ref. 25.)

The ^{31}P NMR spectrum of $(d2NH_2\text{-}dT)_n$ in low (0.1 M) salt consists of two resonances of approximately equal area (Fig. 4). These can be assigned by comparison with the similar spectrum of $(dA\text{-}dT)_n$ to $Tp2NH_2A$ and $2NH_2ApT$ as the lower and higher field components in tg^- and g^-g^- phosphodiester conformations, respectively (6, 26, 27). On increasing the salt concentration, the separation of the two components increased from 32 Hz to a maximal value of 71 Hz (Fig. 4). In order to quantitate the data for this transition, both the CD data ($\Delta\varepsilon$) and the ^{31}P NMR data ($\Delta\delta$) were expressed as % change and plotted against the $\log_{10}$ of the salt concentration. Both sets of data clearly defined the same transition (Fig. 2). The combined data were fitted with a modified Hill equation (28) of the form:

$$y = y_\infty + (y_0 - y_\infty)/(1 + (x/K)^n)$$

where y is the spectroscopic response, x is the concentration of added reagent, y_0 the response when x = 0, y_∞ the maximal response when x = ∞,

K is the concentration of x at the midpoint (equivalent to the equilibrium constant) and n is the degree of cooperativity. The results of this procedure showed that the transition is cooperative, with fitted parameters of pK = 0.17 ± 0.03 (K = 1.48 M) and n = 2.4 ± 0.3; the root mean square error for this fit was 4.0, while for the non-cooperative fit (n=1) it was 7.6 (Fig. 2).

DISCUSSION

$(d2NH_2A\text{-}dT)_n$ clearly undergoes a cooperative transition to a new helical conformation as the salt concentration (Na^+, hexammine cobalt) is increased. The CD and IR changes resemble those of $(dG\text{-}dC)_n$, and the final spectra observed in high salt resemble those of the Z form of this polymer, suggesting similar structures in the two polymers. The ^{31}P NMR results are also indicative of a cooperative transition (Fig. 2). The two resonances observed correspond to the two different phosphodiester conformations of the dinucleotide repeat structure (11, 26). The increase in the separation of the two resonances with increasing salt is superficially similar to the fast exchange process observed for the ^{31}P signals of $(dA\text{-}dT)_n$ (26). However, the maximal separation observed in $(dA\text{-}dT)_n$ ($\Delta\delta$ = 37 Hz in 4 M NaCl) was much less than observed for the 2-amino derivative (71 Hz), and the salt dependence of the signal separation in the former case was essentially linear (i.e., non-cooperative). The chemical shift of the downfield component (-3.35 ppm) for $(d2NH_2A\text{-}dT)n$ is also much closer to the values (-2.82 to -2.86 ppm) observed for $(dG\text{-}dC)_n$ and its 5-methyl derivative (4, 6, 11), although in these cases the transition was a slow but cooperative one on the NMR time scale (with $\Delta\delta$ = 160 and 139 Hz, respectively). None of these effects can be considered conclusive in themselves in terms of the conformation of the high salt form of $(d2NH_2A\text{-}dT)_n$. Nevertheless, the cooperative nature of the transition and the results of several spectroscopic probes support the conclusion that this is a left-handed or Z-type form. It thus appears that the presence of a third $2NH_2A\cdot T$ hydrogen bond in the minor groove of B-DNA favors the Z form in high concentrations of NaCl. This may be due to disruption of the spine of hydration by the $2NH_2$ group, as suggested by Dickerson et al. (13), though further work will be required to establish this point.

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