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# Z* DNA, the left-handed helical form of poly[d(G-C)] in $MgCl_2$-ethanol, is biologically active

**Johan H. van de Sande[1] and Thomas M. Jovin****

Abteilung Molekulare Biologie, Max Planck Institut für Biophysikalische Chemie, Postfach 968, D-3400 Göttingen, FRG

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**The interconversion between the right (R) and left (L) helical forms of poly[d(G-C)] occurs at low concentrations of $MgCl_2$ and EtOH, acting together in a highly synergistic manner. Thus, the cooperative R→L transition is induced by only 0.4 mM and 4 mM $MgCl_2$ in combination with 20% and 10% EtOH, respectively. The L form of poly[d(G-C)] formed under these conditions has the spectroscopic properties (absorption, circular dichroism) previously demonstrated under high salt conditions (Pohl and Jovin, 1972) and thought to correspond to the left-handed Z DNA structures recently established by X-ray crystallography (Wang *et al.*, 1979; Drew *et al.*, 1980). However, L DNA formed in $Mg^{2+}$-EtOH (which we designate as Z* DNA) has unique properties: a) it can be sedimented readily out of solution at low speed, indicative of condensation and intermolecular aggregation; b) it supports the binding of several intercalating (ethidium bromide, actinomycin D) and non-intercalating (mithramycin) drugs, although these interact preferentially with the R (i.e., B) form of DNA; and c) it functions as a template for *Escherichia coli* RNA polymerase. B and Z* DNAs can be generated under identical ionic conditions and compared in a number of biochemical systems. Our results suggest that left-handed DNA may form under physiological conditions and serve a biological function.**

*Key words:* Z DNA/transcription/intercalation/mithramycin/circular dichroism

## Introduction

The crystallographic elucidation of right-handed (Wing *et al.*, 1980) and left-handed (Wang *et al.*, 1979, 1981; Drew *et al.*, 1980; Drew and Dickerson, 1981a) DNA double helices has stimulated the consideration of DNA polymorphism and its biological significance. Oligonucleotides with the alternating purine-pyrimidine sequence $d(CG)_n$ exhibit a left-handed or Z conformation characterized by a repeating dinucleotide structural unit, a finding of particular interest because it provides an explanation for the highly cooperative salt-induced (Pohl and Jovin, 1972) and ethanol-induced (Pohl, 1976) so-called R-L conformational transition of poly[d(G-C)] in solution. The high-salt (>2.5 M NaCl) "L" conformation has been identified with the Z family of left-handed helices by a number of physical techniques: n.m.r. (Mitra, *et al.*, 1981; Patel *et al.*, 1982), X-ray fibre diffraction (Behe, *et al.*, 1981), laser Raman (Thamann *et al.*, 1981), and transient electric dichroism (Wu *et al.*, 1981). The B–Z transition can be promoted by solvent manipulations, certain ligands, topological stress, and modifications of the poly[d(G-C)] structure (e.g., Behe and Felsenfeld, 1981; Nordheim *et al.*, 1981, and references therein). However, the molecular mechanism(s) responsible for the inversion in helical sense are unknown.

The biological significance of Z DNA is the subject of much discussion (Wang *et al.*, 1979, 1981; Drew *et al.*, 1980; Behe and Felsenfeld, 1981; Klysik *et al.*, 1981; Kuhnlein *et al.*, 1980; Nordheim *et al.*, 1981). In general, it has been presumed that the left-handed configuration does not support biological functions directly but serves as a structural control element for processes involved in genetic expression. Its existence in nature appears established by the discovery of spontaneously generated anti-Z DNA antibodies (Lafer *et al.*, 1981) and their specific interactions with *Drosophila* polytene chromosomes (Nordheim *et al.*, 1981).

We have searched for conditions that would potentiate the B–Z transition in poly[d(G-C)] and possibly other naturally occurring sequences but remain compatible with *in vitro* tests of Z DNA as a substrate for binding and enzymatic reactions. Combinations of ethanol with simple salts proved successful, and we describe here the properties of "Z* DNA", formed in the presence of ethanol and $MgCl_2$.

## Results

### *B-Z* transition in $MgCl_2$-EtOH solutions*

$MgCl_2$ (Pohl and Jovin, 1972) and EtOH (Pohl, 1976) individually are able to induce the transition between the right-handed (R=B) and left-handed (L=Z) conformations of poly[d(G-C)] in solution. The transition midpoints from the absorbance assay are 0.66 M $MgCl_2$ and 48% EtOH (present study). The helical transformation also occurs in mixtures of the two reagents and define the B–Z* phase diagram depicted in Figure 1b. The concentration of $MgCl_2$ required at moderate concentrations of EtOH is markedly reduced, dropping more than three orders of magnitude to a value of 0.35 mM at 20% EtOH in standard buffer (3 mM $Na^+$, 10 mM Hepes, pH 7.3). The transitions determined by addition of $MgCl_2$ at fixed EtOH concentrations are highly cooperative (Figure 1a) with a Hill coefficient of 4 ± 1 in the range of 5–20% EtOH, a value less than that observed with $MgCl_2$ alone (12–18 for the data reported by Pohl and Jovin, 1972, and from the present study). The optical changes are closely paralleled by the sedimentation measure of intermolecular association, i.e., aggregation. Aggregation universally accompanies the R→L transformations of poly[d(G-C)] in the presence of $Mg^{2+}$ even without EtOH, although visible light scattering at the usual DNA concentrations (10–50 μM) is not observed except in the case of $Mg(ClO_4)_2$ (Hamori and Jovin, 1981). The aggregated state of poly[d(G-[$^{14}$C]C)] established in 10% EtOH, 10 mM $MgCl_2$ persists after dilution of the DNA and vortexing. In the centrifugation assay, 77% and 91% of the DNA sedimented at 0.5 μM and 100 μM, respectively. In contrast, the L forms generated in concentrated NaCl or 60% EtOH (and at <1 mM [DNA]) are not aggregated; there was no appreciable sedimentation in 10 min at $10^5$ *g*.

[1]Permanent address: Department of Medical Biochemistry, University of Calgary, Calgary, Alberta T2N 1N4, Canada.

**To whom reprint requests should be sent

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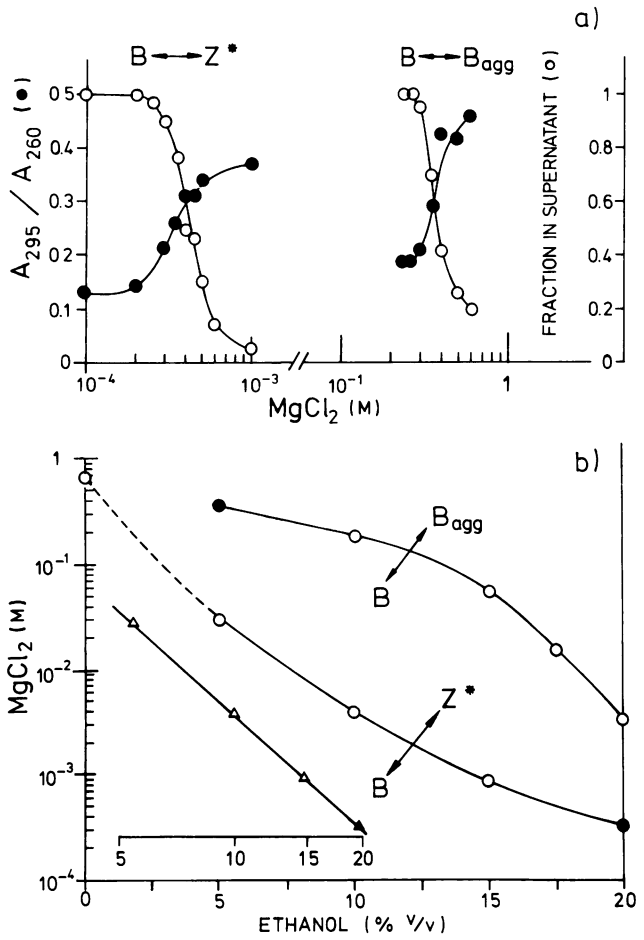


**Fig. 1.** Conformational states of poly[d(G-C)] in mixtures of $MgCl_2$ and EtOH. **a** transitions corresponding to the filled points in **b**, phase boundaries defined by the transition midpoints at 20–25°C. The values (mM $MgCl_2$, % EtOH in parenthesis) are: B-$B_{agg}$ [360(5), 190(10), 56(15), 16(17.5), 3.5(20)]; B–Z* [660(0), 33(5), 3.7(10), 0.7(15), 0.35(20)]. The B–Z* data are shown both with a linear (○) and logarithmic (△) scales of EtOH concentration.

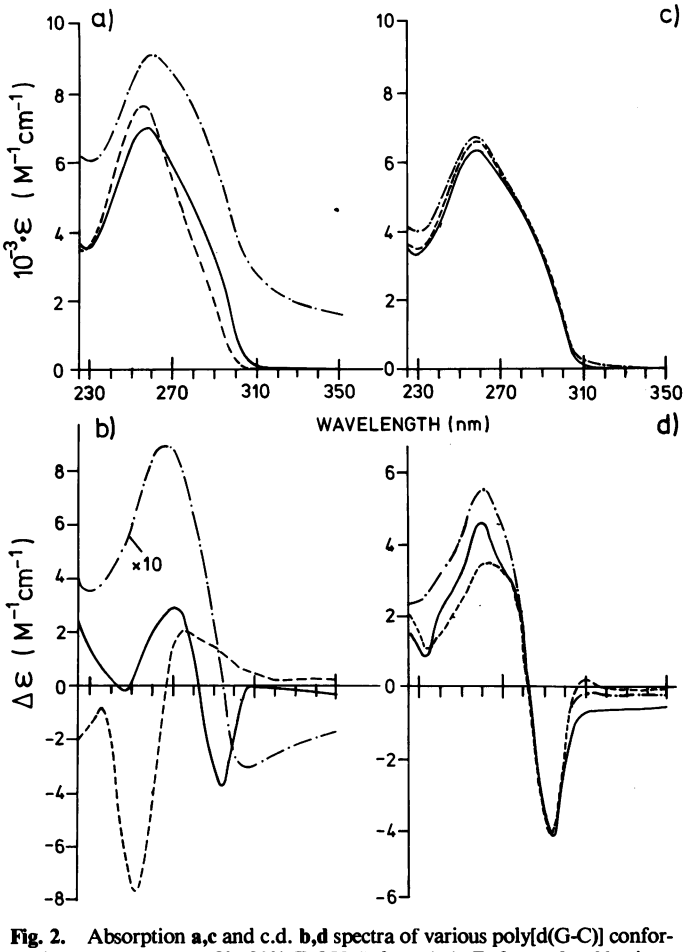


**Fig. 2.** Absorption **a,c** and c.d. **b,d** spectra of various poly[d(G-C)] conformations. **a,b** 1 mM $MgCl_2$, 20% EtOH: B form (---), Z* form after 30 min at 45°C (—); $B_{agg}$ in 20 mM $MgCl_2$, 20% EtOH (-·-·-). Values for $B_{agg}$ should be multiplied by 10. **c,d** Z and Z* forms in 60% EtOH (---); 0.96 M $MgCl_2$ (—); 10 mM $MgCl_2$, 10% EtOH (-·-·-).

### *Spectral properties of Z* DNA*

Characteristic absorption and c.d. spectra (Figure 2) were recorded for the initial B and final Z* forms of poly[d(G-C)] throughout the range given in Figure 1b. The $A_{295}/A_{260}$ ratio increased by the factor of 3.2 ± 0.3 (typically from 0.13 to 0.42) and the c.d. spectrum showed the inversion associated with the conversion to the L state at high salt or EtOH concentration (Figure 2; and Pohl and Jovin, 1972; Pohl, 1976). We conclude that Z* DNA is left-handed.

### *Temperature dependence and kinetics of the B–Z* transition*

The transition from the R to the L helical conformations of poly[d(G-C)] does not occur spontaneously at 20–25°C in the concentration domains of $MgCl_2$ and EtOH depicted in Figure 1. Thus, the B–Z* boundary in Figure 1 was established in the reverse direction by dilution of Z DNA generated in 60% EtOH. However, raising the temperature readily induces the kinetically resolved formation of Z* from B DNA (Figures 2 and 3). Subsequent cooling leads to the rapid restoration of the original B spectra to an extent dependent upon the concentrations of EtOH and $MgCl_2$ and in agreement with the equilibria established by dilution. The inset in Figure 3 depicts the shift in the B–Z* equilibrium in favor of the left-handed Z* form at the higher temperature and the hysteresis in the heating/cooling process.

The forward reaction kinetics at elevated temperature (44°C) were measured as a function of $MgCl_2$ concentration in 20% EtOH (Figure 4). The progress curves were complex and approached a first-order behavior only at the highest concentration of 1 mM $MgCl_2$, for which the half life was 79–93 s, as compared with values of ~10 s and 30 s for 60% EtOH and 1 M $MgCl_2$, respectively. The kinetics in 1 mM $MgCl_2$ were independent of DNA concentration in the range 10–200 μM. The reaction half life ($t_{1/2}$) increased from 25 s at 47.5°C to 1500 s at 36.5°C, and is shown in the form of an Arrhenius plot in Figure 4 (inset). The large apparent activation energy of 90 ± 15 kcal $mol^{-1}$ explains the failure to elicit an appreciable reaction at room temperature. The reverse Z*→B reaction induced by complexation of $Mg^{2+}$ with EDTA or dilution of either EtOH or $MgCl_2$ was too fast to be resolved from the mixing operation and thus can be regarded as kinetically unhindered.

### *The condensation-aggregation of B DNA in $MgCl_2$-EtOH solutions*

The addition of $MgCl_2$ to ethanolic solutions of poly[d(G-C)] at 20–25°C leads to a highly cooperative reaction characterized by hyperchromic shifts in the u.v. absorption spectra, turbidity evidenced by axial light loss at 350 nm and visible Tyndall scattering, and dramatic increases in the apparent c.d. intensities (Figures 1a, and 2a and b). Flocculation and precipitation ensue. We have established the phase diagram for this reaction as a function of $MgCl_2$ and EtOH concentrations (Figure 1b). Similar data for other DNA se-

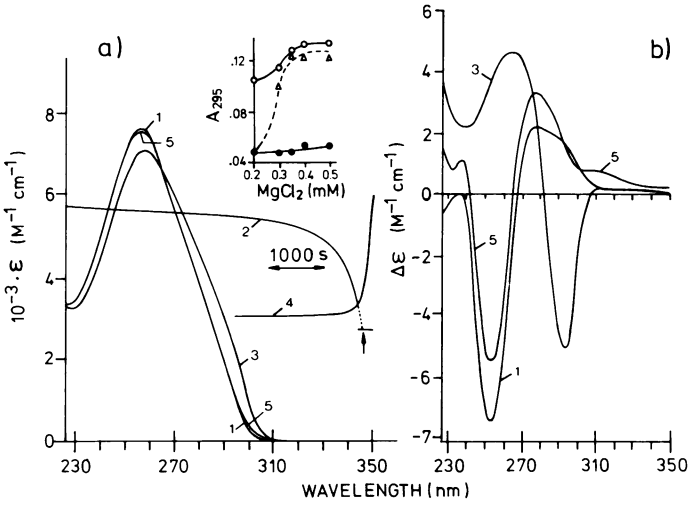


**Fig. 3.** Temperature dependence of B – Z* transition kinetics and equilibria. **a** absorption, **b** c.d. The solvent was 0.3 mM $MgCl_2$, 20% EtOH: 1) spectra (B form) at 21°C; 2) kinetics at 295 nm (2.5 x expanded) after shift to 45°C; 3) spectra (Z* form) at 45°C; 4) kinetics at 295 nm after shift to 24°C; 5) final spectra at 22°C. **Inset:** hysteresis in B – Z* equilibria in 20% EtOH progressing from 20°C (●) to 45°C (○) and back to 20°C (△).

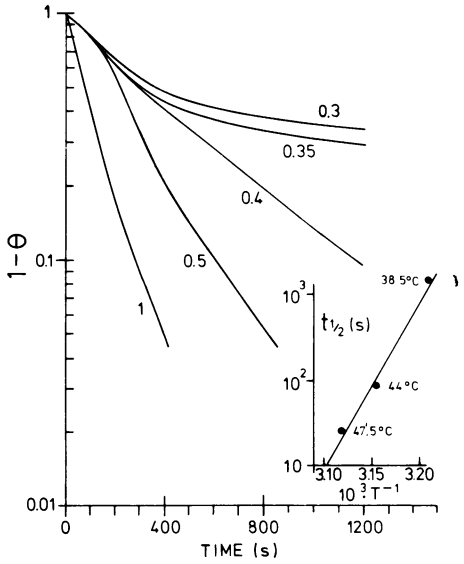


**Fig. 4.** Kinetics of B – Z* transition at 44°C. Curves at different $MgCl_2$ concentrations (mM) and 20% EtOH. The degree of transition $\theta$ was measured at 295 nm. **Inset:** Arrhenius plot using half lives ($t_{1/2}$) derived from first 80% of reaction at 1 mM $MgCl_2$.

quences will be reported elsewhere. Neither this condensed form of DNA designated $B_{agg}$ nor B DNA itself interconverts with the Z* conformation in the region above the B – Z* transition curve of Figure 1b, a feature which has been exploited in the functional studies described below.

*Drug binding to Z* DNA*

The binding capacities of right- and left-handed DNA for intercalating (ethidium bromide, actinomycin D) and nonintercalating (mithramycin, distamycin $A_3$) drugs were examined under the ionic conditions used to produce Z* DNA. Figure 5a shows the fluorescence titration of ethidium bromide with poly[d(G-C)] in the B or Z* conformations and under different conditions. The binding of ethidium bromide to B DNA is markedly reduced by the addition to standard buffer of $MgCl_2$ (10 mM) and even further by EtOH (10%). The corresponding dissociation constants estimated from Figure 5a, assuming an invariant quantum yield of the bound

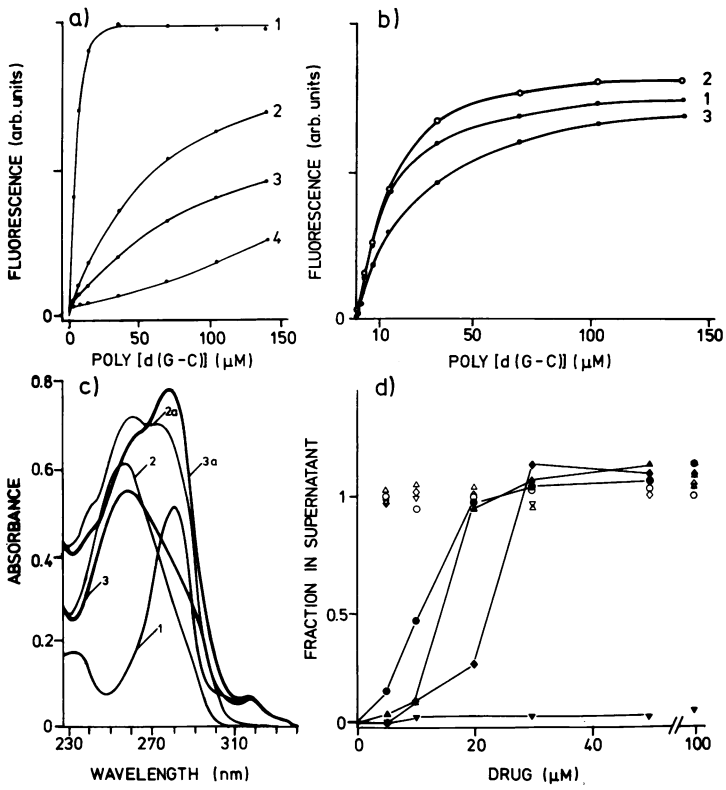


**Fig. 5.** Drug binding to B and Z* DNA's. **a** fluorescence titration of 2 μM ethidium bromide with B form in: 1) standard buffer; 2) (1) + 10 mM $MgCl_2$; 3) (2) + 10% EtOH; 4) with Z* form in 10 mM $MgCl_2$, 10% EtOH. **b** titration of 5 μM mithramycin with B form in: 1) standard buffer + 10 mM $MgCl_2$; 2) (1) + 10% EtOH; 3) with Z* form in 10 mM $MgCl_2$, 10% EtOH. **c** absorption spectra of 5 μM mithramycin (1) + 41 μM B form (2a) or Z* form (3a) in 10 mM $MgCl_2$, 10% EtOH. Corresponding DNA spectra: 2,3. **d** titration of 3.5 μM poly[d(G-[2-$^{14}$C]C)] in B form (open symbols) or Z* form (closed symbols) with actinomycin D (○), ethidium bromide (△), mithramycin (◇), and distamycin $A_3$ (▽). The sedimentable fraction was determined by centrifugation.

dye, were 0.7, 60, and 170 μM, respectively. In the case of $Mg^{2+}$-EtOH, the addition of Z* DNA (curve 4) led to an initial increment in fluorescence ~20% that obtained with the B form which yields a preliminary estimate of the dissociation constant of ~0.9 mM. After centrifugation of the samples from curves 3 and 4, the Z* DNA fluorescence remained with the pellet but with B DNA it dispersed in the supernatant.

The binding of mithramycin was easier to explore experimentally due to its requirement for $MgCl_2$ and GC-rich DNA (see Waring, 1970 and references therein). The dissociation constants of mithramycin for B and Z* DNA's calculated from the titration curves in $Mg^{2+}$-EtOH (Figure 5b) were 16 μM and 24 μM. Thus, only a moderate preference for the B form is observed. Absorption spectra determined under the same conditions of the titration clearly distinguished between the complexes formed with the two DNA's (Figure 5c).

The sedimentation assay for DNA conformation was used to assess the effects of high drug concentrations (Figure 5d). In this case, radioactive poly[d(G-C)] was placed in the B or Z* state in 10 mM $MgCl_2$, 10% EtOH, and increasing amounts of the drugs actinomycin D, ethidium bromide, mithramycin, or distamycin $A_3$ were added. The first three compounds reversed the sedimentability of Z* DNA with approximate transition midpoints of 12, 14, and 23 μM, respectively. Distamycin $A_3$, a compound specific for DNA containing A·T basepairs (Zimmer, 1975), had no effect. The solubility properties of preformed B DNA were also

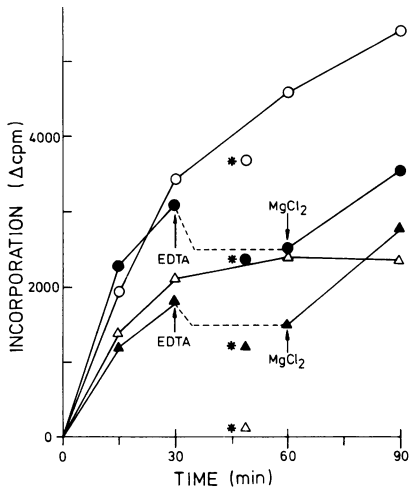


**Fig. 6.** Transcription of B and Z* DNA's. Incorporation of [5-$^3$H]CMP supported by template in B form (○) and Z* form (△). In parallel reactions (●,▲), 20 mM EDTA and $MgCl_2$ were added at the indicated times. An incorporation of 7000 c.p.m. represents an extent of RNA synthesis equivalent to the amount of DNA template. The starred points were determined from supernatants after centrifugation.

unaltered by any of the drugs. We interpret the observed transitions as indicative of a drug-induced reversal of the Z* to the B conformation on the basis of the following evidence. The addition of an excess of calf thymus DNA to the reaction end products, or throughout the titration in the case of ethidium bromide, competitively displaced the drugs from poly[d(G-C)] but did not restore the sedimentability property associated with the Z* conformation which, however, returned after heating. The spectroscopic properties (absorption, c.d.) of complexes formed at high concentrations of ethidium bromide and mithramycin were consistent with the substantial reversal of the Z* to the B conformation. The Z*→B transition elicited by ethidium bromide (Figure 5d) is rapid. The full effect was manifested within 10 s (the first point assayed) after addition of 33 μM drug. Finally, in a control experiment, up to 0.1 mM ethidium bromide failed to disaggregate $B_{agg}$ DNA formed in 50 mM $MgCl_2$, 20% EtOH.

*Transcription of Z* DNA*

The template functions of B and Z* DNA for the transcribing enzyme *Escherichia coli* RNA polymerase were compared in 10 mM $MgCl_2$, 10% EtOH (Figure 6). The Z* form was generated by dilution from 60% EtOH. The incorporation over the initial 30 min supported by the Z* template was 50−70% that with B DNA, but then reached a plateau. After 45 mins the labeled RNA product readily sedimented in the case of the Z* but not the B template. In parallel reactions, the addition of EDTA halted further incorporation and rendered the product non-sedimentable. Subsequent restoration of $MgCl_2$ led to the resumption of synthesis at equal rates in both reactions.

## Discussion

This study extends the original observations that poly[d(G-C)] in solution undergoes a cooperative conformational transition in the presence of high salt concentrations (Pohl and Jovin, 1972) or a nucleophilic solvent (EtOH: Pohl, 1976). We now find that the two agents have a synergistic effect on the transition. Thus, in 20% EtOH, only 0.4 mM $MgCl_2$ is required compared to $>10^3$ as much without alcohol. We identify the product, "Z* DNA", as a left-handed double helix due to the similarity in its absorption and c.d. spectra to those of the "high-salt" L form which corresponds to the Z structures elucidated by crystallographic analysis (see **Introduction**). However, Z* DNA has certain properties not previously observed under high salt conditions: a) it is condensed and/or aggregated to an extent detectable by simple sedimentation analysis but less evident optically. This characteristic is retained in the absence of EtOH (i.e., in high $MgCl_2$) but the inverse is not true; b) it does not arise directly at room temperature starting from the B conformation but can be generated by dilution of Z DNA preformed in 60% EtOH or by transient exposure to elevated temperature; c) its transition kinetics are not all-or-none; and d) it supports drug binding and transcription.

The simplest model which can accommodate the B−Z* transition consists of 3 sequential cooperative steps: a conformational isomerization (2) preceded by a nucleation (1) and followed by a condensation-aggregation (3).

$$\underset{}{B} \underset{1}{\longleftrightarrow} \underset{}{B'} \underset{2}{\longleftrightarrow} \underset{}{Z} \underset{3}{\longleftrightarrow} \underset{}{Z^*} \qquad (1)$$

To each step 1−3 we assign an equilibrium constant $K_i$. The overall equilibrium constant $K$ is defined by assuming that the spectroscopic changes score the formation of both Z and Z*.

$$K = K_1K_2(1 + K_3)/(1 + K_1) \cong K_1K_2K_3 \qquad (2)$$

where the approximation in Equation 2 derives from the assumption that the nucleation step is energetically unfavorable, i.e., $K_1 << 1$ (Pohl and Jovin, 1972; Schwarz, 1968) and that the aggregation (step 3) to form Z* is extensive, i.e., $K_3 >> 1$ (from the coincidence of the optical and sedimentation assays, Figure 1a). The equilibrium constant $K$ is a function of both the $MgCl_2$ and EtOH concentrations, $c_1$ (M) and $c_2$ (% v/v), respectively. An equation for a line corresponding to a constant value of $K$ in the concentration domain defined by $c_1$ and $c_2$ is obtained by setting the total derivative of $lnK$ to 0:

$$dlnc_1/dlnc_2|_K = -\frac{\partial lnK}{\partial lnc_2} / \frac{\partial lnK}{\partial lnc_1} = -\gamma \qquad (3)$$

From the linearity of the log-log plot in Figure 1b, $\gamma$ is constant with the value 3.1 in the range of 5−20% EtOH. The Hill coefficient for the titrations with $MgCl_2$ at constant EtOH is 4 ± 1 (**Results**) and equals the denominator in the right side of Equation 3, suggesting the functional form:

$$K = a\, c_1^{\alpha_1} c_2^{\alpha_2} \qquad (4)$$

where the exponents $\alpha_1$ and $\alpha_2 (= \alpha_1\gamma)$ have the values of ~4 and ~12, respectively, and represent the sum of the contributions made by each reactant to the various steps. Combining these results with the equilibrium data, we obtain the following approximate relationships ($c_t$ = transition midpoint):

| Reactants (+ 3 mM $Na^+$) | $K$ | $c_t$ |
|---|---|---|
| $MgCl_2$ alone (M) | $1.5 \times 10^2\,[Mg^{2+}]^{12}$ | 0.66 M |
| EtOH alone (%) | $2.9 \times 10^{-24}\,[EtOH]^{14}$ | 48% |
| $MgCl_2$ + EtOH | $5.3 \times 10^{-3}\,[Mg^{2+}]^4\,[EtOH]^{12}$ | Figure 1b |

(5)

The synergistic effects of $MgCl_2$ and EtOH derive from their combined yet unequal influences on the nucleation, propagation, and condensation-aggregation steps. EtOH is particularly complex since as a "soluble hydrocarbon" it perturbs the bulk water structure (Franks, 1975), as well as the hydration state of the ionic constituents (Friedman and Krishnan, 1973) and of the DNA (Texter, 1978). The latter differs greatly for

the various DNA conformations, as established by crystallographic studies of B DNA (Drew and Dickerson, 1981), Z DNA (Drew *et al.*, 1980, Wang *et al.*, 1981) and the right-handed A DNA (Conner *et al.*, 1982). EtOH progressively unwinds the oligo[d(G-C)] helix (Albergo and Turner, 1981), which should promote the nucleation step in Scheme 1. Yet the lowering of the dielectric constant tends to enhance the phosphate-phosphate repulsions in the DNA backbone that must be overcome to stabilize the Z conformation (Pohl and Jovin, 1972; Wang *et al.*, 1979; Drew *et al.*, 1980; Behe and Felsenfeld, 1981) although an opposing tendency would be an increase in counterion condensation and thus a lowering of the net charge density according to the "territorial" delocalized mechanism proposed by Manning (1978). The fact that the B→Z transition depends upon the nature and concentration of added salt (Pohl and Jovin, 1972; Hamori and Jovin, 1981; Behe and Felsenfeld, 1981) and the crystallographic demonstration of specific binding loci for $Mg^{2+}$, spermine, and anions (Drew *et al.*, 1980; Wang *et al.*, 1981) point to site-specific binding of counterions.

The effects of temperature on the B−Z* transition in $MgCl_2$-EtOH mixtures are complex. The forward reaction has a very large activation energy (90 kcal $mol^{-1}$ in 20% EtOH) and proceeds only at higher temperatures with progress curves which are generally not first-order. A hysteresis ensues upon subsequent cooling as manifested by a partial reversal of the transition to the corresponding equilibrium state. The back Z*→B reaction initiated by dilution or complexation of $Mg^{2+}$, or by cooling (Figure 3) is invariably rapid. Consequently, the equilibrium state is temperature dependent. Further the helical stability of the left-handed form is greater than that of B DNA (Widom and Baldwin, 1980; Patel *et al.*, 1982).

The kinetic features of the B−Z* transition (hindered forward reaction, rapid reversal) are reminiscent of those described for the DNA condensation induced by the trivalent ion hexaminecobalt $Co^{3+}(NH_3)_6$ (Widom and Baldwin, 1980). These authors proposed that intermolecular segment-segment interactions induced by the multivalent cations at high enough DNA concentrations (>1 μM) oppose the process of intramolecular condensation. Here, we report a generalized condensation-aggregation at room temperature preserving the B configuration (B→$B_{agg}$, Figure 1), the incipient phases of which should be manifest at the lower salt concentrations defining the B−Z* transition. Thus, a kinetic barrier would be created if sporadic cross-linking of intra- or intermolecular segments of B DNA by $Mg^{2+}$ reduces the highly chain-length dependent cooperativity of the helical transformation (Pohl and Jovin, 1972; Quadrifoglio *et al.*, 1981), particularly under the influence of diminished solvent power due to EtOH (Post and Zimm, 1980), possibly due to the modulation of the inter- and intrastrand phosphate-phosphate distances of the Z helix which are generally smaller than those of the B form (Wang *et al.*, 1981; Drew and Dickerson, 1981a). The coincidence of the sedimentation and optical transitions (Figure 1) argues against an extensive coaggregation of Z* and B DNAs.

The conditions required for the establishment of Z* DNA are compatible with many biochemical reactions of interest in nucleic acid metabolism. Our preliminary results attest to the potential biological activity of the left-handed helical form of DNA. For example, the binding of various drugs was monitored spectroscopically (fluorescence, absorption, c.d.) and by exploitation of the relative sedimentation properties of Z* and B DNA's under conditions compatible with both structures: 10% EtOH, 10 mM $MgCl_2$ (Figure 1). Fluorescence titrations with the two DNA's of ethidium bromide and mithramycin at low concentration led to the formation of complexes with enhanced emission. Ethidium bromide binds by intercalation (reviewed in LePecq, 1971; Berman and Young, 1981) with a preference for purine-pyrimidine sequences (Reinhardt and Krugh, 1978), whereas mithramycin interacts with DNA in the minor groove (K.F. Jorgenson and J.H. van de Sande, unpublished observations) by a non-intercalative mechanism requiring the presence of $Mg^{2+}$ and guanine in the sequence (Waring, 1970, and references therein). Our best estimate is that the affinity constant of Z* DNA for ethidium is ~1/5 that for B DNA; for mithramycin the corresponding factor 0.6 is much higher, indicating that this nonintercalating drug is relatively non-discriminatory with respect to helical sense. Further evidence that the dyes associate with Z* DNA was obtained by sedimentation (ethidium) and spectral analysis (mithramycin). However, the inverse titration of Z* DNA with the drugs, including the intercalative antitumor agent actinomycin D (Winkle and Krugh, 1981; Berman and Young, 1981, and references therein), led to a reversal of conformation to the B form according to sedimentation criteria as well as spectral analysis of the mithramycin complexes (data not shown). The A·T base pair specific antibacterial agent distamycin $A_3$ (Zimmer, 1975) had no effect in this assay. Altogether, the data suggest that the compositional dependence of drug binding is retained upon changing the helical sense of poly[d(G-C)]. The left-handed conformation in 4.4 M NaCl is reversed by ethidium bromide in an allosteric highly cooperative (Hill coefficient of 40) transition (Pohl *et al.*, 1972). The reactions described here were less cooperative presumably because the preferential binding to the B form is not so extreme as in NaCl. However, the drug concentration required in the case of ethidium was much below the estimated equilibrium constant, implying that only a few binding events per DNA helix induce the reverse helical transformation, possibly by interfering with the intersegmental interactions mediated by $Mg^{2+}$ and considered above as being crucial in maintaining the Z* state. The observation that intercalation can occur with left-handed poly[d(G-C)] is of particular interest because the Z crystal structures demonstrate the same conformations of the glycosidic bonds and sugar rings (*syn* and C-3′-*endo*; *anti* and C-2′-*endo*) seen as prominent features of the intercalation site (Arnott, *et al.*, 1980: Berman and Young, 1981), although Z DNA has been considered to be too stiff for intercalation (Patel *et al.*, 1982). The cooperative binding of actinomycin D to poly[d(G-C)] at moderate salt concentrations has also been reported (Winkle and Krugh, 1981), but the nature of the postulated allosteric transition in DNA structure is unclear since the starting configuration is presumably right-handed.

The role of DNA structure in gene regulation has been stressed repeatedly (e.g., Wells *et al.*, 1980; Jovin, 1976). Does left-handed DNA act as a conformational switch involved, for example, in the control of transcription (McKay and Steitz, 1981; Nordheim *et al.*, 1981; **Introduction**)? The unexpected finding reported here is that Z* DNA can function as a template for the *E. coli* RNA polymerase. In 10 mM $MgCl_2$, 10% EtOH the initial incorporation supported by Z* DNA was 0.5−0.7 that obtained with the B form of poly[d(G-C)]. The product RNA was sedimentable prior to the addition of EDTA which suppressed further catalysis and presumably reversed the template conformation from Z* to

B. Thus, we consider the following possibilities: a) RNA polymerase binds to Z* DNA and uses the template in a left-handed conformation; b) RNA polymerase binds but reverses the DNA conformation locally to B; or c) RNA polymerase binds to and transcribes from statistically infrequent sites with the right-handed helical sense. The observed rate and extent of synthesis argue against (c) as the exclusive mechanism. Furthermore, the fact that the product was sedimentable suggests that it remained associated with the DNA template which in turn must have preserved the aggregated Z* structure to a significant degree, unless the RNA itself acquired this characteristic due to the stereochemistry at the site of chain elongation. It remains to be established what relationship these findings have to the series of observed and postulated conformational changes in the enzyme and DNA (including a B to A transition) during transcription with templates in general (Wachsman and Anthony, 1980, and references).

What is the structure of Z* DNA? We are currently examining other DNA sequences, enzymes and ionic conditions. However, it is already evident that the Z family of left-handed helices is highly polymorphic (Wang *et al.*, 1981; Drew and Dickerson, 1981a; Behe *et al.*, 1981; Ivanov and Minyat, 1981; this work; see also **Introduction**). Alternative left-handed structures have also been proposed (Gupta *et al.*, 1980; Hopkins, 1981).

## Materials and methods

*Reagents*

Poly[d(G-C)] and poly[d(G-[2-$^{14}$C]C)] from P-L Biochemicals were dialyzed against standard buffer (10 mM Hepes, pH 7.3; [$Na^+$] = 3 mM) and generally sheared by sonication for 6 x 10 s in a Kontes micro-ultrasonic cell disruptor (3/4 maximal intensity). The size distribution by electrophoretic analysis was 300 – 1500 base pairs. Concentrations were determined with an $\epsilon$ of 7100 $M^{-1}$ $cm^{-1}$ at 255 nm (Pohl and Jovin, 1972). Actinomycin D and distamycin $A_3$ were obtained from Sigma, ethidium bromide from Serva, and mithramycin from Calbiochem. *E. coli* RNA polymerase (holoenzyme) was prepared according to Burgess (1976). Ethanol concentrations are expressed in percentage v/v where the total equals the sum of the individual volumes.

*Conformational transitions*

The conformational state of poly[d(G-C)] was assessed by: a) the absorption ratio $A_{295}/A_{260}$ (Pohl and Jovin, 1972), determined in a Zeiss PMQ II or Cary 118 spectrophotometer; and b) sedimentation analysis in an Eppendorf microcentrifuge operated for 6 min at 15 000 r.p.m. (12 000 *g*), an assay which scores species with a sedimentation constant greater than ~ $10^3$ S. Some experiments were performed in the Beckman Airfuge at $10^5$ *g*. The ratio of $A_{260}$ of the supernatant to the value prior to centrifugation was calculated. The fractional transition $\theta$ for each given ligand concentration c was used to construct a Hill plot: $\ln[\theta/(1-\theta)]$ versus lnc; the slope is the Hill coefficient. The equilibrium properties of poly[d(G-C)] solutions (40 – 50 $\mu$M) in mixtures of $MgCl_2$ (0 – 1 M) and EtOH (0 – 60%) were determined after 2 – 12 h incubation at 20 – 25°C. In the case of the B – Z* transition, appropriate dilutions were made of a concentrated (0.3 mM) solution of the Z form generated in 60% EtOH. Transition kinetics at 295 nm and spectra were measured in the thermostated Cary 118 spectrophotometer. C.d. spectra were obtained on a computerized Jobin-Yvon Mark IV Dichrograph with DNA concentrations of 0.04 – 0.2 mM. Ellipticities are expressed as $\Delta\epsilon = \epsilon_L - \epsilon_R$.

*Drug binding*

Fluorescence titrations were performed in an Aminco Bowman spectrofluorimeter, using excitation wavelengths of 525 nm and 430 nm, and emission wavelengths of 600 and 490 nm for ethidium bromide and mithramycin, respectively. In the sedimentation assay for the conformational state of the DNA as a function of dye concentration, incubation was for 0.25 – 3 h at ambient temperature prior to centrifugation and to (in control experiments) the addition of 0.3 mM calf thymus DNA. The radioactivity in the supernatant was used to calculate the fraction of rapidly sedimentable DNA.

*Transcription*

Reaction mixtures at 37°C contained 50 $\mu$M poly[d(G-C)] in either the B or Z* conformation, 10 mM $MgCl_2$, 10% EtOH, 10 mM Hepes, pH 7.3, 0.3 mM GTP, 0.3 mM [5-$^3$H]CTP (Amersham, 14 c.p.m. $pmol^{-1}$), and 60 nM RNA polymerase. Aliquots were spotted on Whatman DE 81 paper and washed.

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## References

Albergo,D.D., and Turner,D.H. (1981) *Biochemistry (Wash.),* **20**, 1413-1418.
Arnott,S., Bond,P.J., and Chandrasekaran,R. (1980) *Nature,* **287**, 561-563.
Behe,M., and Felsenfeld,G. (1981) *PNAS,* **78**, 1619- 1623.
Behe,M., Zimmerman,S., and Felsenfeld,G. (1981) *Nature,* **293**, 233-235.
Berman,H.M., and Young,P.R. (1981) *Annu. Rev. Biophys. Bioeng.,* **10**, 87-114.
Burgess,R.R. (1976) in Losick,R., and Chamberlin,M. (eds.), *RNA Polymerase,* Cold Spring Harbor Laboratory, CSH, NY, pp. 69-100.
Conner,B.N., Takano,T., Tanaka,S., Itakura,K., and Dickerson,R.E. (1982) *Nature,* in press.
Drew,H.R., and Dickerson,R.E. (1981) *J. Mol. Biol.,* **151**, 535-556.
Drew,H.R., and Dickerson,R.E. (1981a) *J. Mol. Biol.,* **152**, 723-736.
Drew,H., Takano,T., Tanaka,S., Itakura,K., and Dickerson,R.E. (1980) *Nature,* **286**, 567-573.
Franks,F. (1975) in Franks,F. (ed.), *Water, a Comprehensive Treatise,* **4**, Plenum Press, New York, USA, pp. 1-94.
Friedman,H.L., and Krishnan,C.V. (1973) in Franks,F. (ed.), *Water, a Comprehensive Treatise,* **3**, Plenum Press, New York, USA, pp. 1-118.
Gupta,G., Bansal,M., and Sasisekharan,V. (1980) *PNAS,* **77**, 6486-6490.
Hamori,E., and Jovin,T.M. (1981) *Biophys. J.,* **33**, 67a.
Hopkins,R.C. (1981) *Science (Wash.),* **211**, 289-291.
Ivanov,V.I., and Minyat,E.E. (1981) *Nucleic Acids Res.,* **9**, 4783-4798.
Jovin,T.M. (1976) *Annu. Rev. Biochem.,* **45**, 889-920.
Klysik,J., Stirdivant,S.M., Larson,J.E., Hart,P.A., and Wells,R.D. (1981) *Nature,* **290**, 672-677.
Kuhnlein,U., Tsang,S.S., and Edwards,J. (1980) *Nature,* **287**, 363-364.
Lafer,E.M., Möller,A., Nordheim,A., Stollar,B.D., and Rich,A. (1981) *PNAS,* **78**, 3546-3550.
LePecq,J.-B. (1971) *Methods Biochem. Anal.,* **20**, 41-86.
Manning,G. (1978) *Q. Rev. Biophys.,* **11**, 179-246.
McKay,D.B., and Steitz,T.A. (1981) *Nature,* **290**, 744-749.
Mitra,C.K., Sarma,M.H., and Sarma,R.H. (1981) *Biochemistry (Wash,),* **20**, 2036-2041.
Nordheim,A., Pardue,M.L., Lafer,E.M., Möller,A., Stollar,B.D., and Rich,A. (1981) *Nature,* **294**, 417-422.
Patel,D.J., Kozlowski,S.A., Nordheim,A., and Rich,A. (1982) *PNAS,* in press.
Pohl,F.M. (1976) *Nature,* **260**, 365-366.
Pohl,F.M., and Jovin,T.M. (1972) *J. Mol. Biol.,* **67**, 375-396.
Pohl,F.M., Jovin,T.M., Baehr,W., and Holbrook,J.J. (1972) *PNAS,* **69**, 3805-3809.
Post,C.B., and Zimm,B.H. (1980) *Biophys. J.,* **32**, 448-450.
Quadrifoglio,F., Manzini,G., Vasser,M., Dinkelspiel,K., and Crea,R. (1981) *Nucleic Acids Res.,* **9**, 2195-2206.
Reinhardt,C.G., and Krugh,T.R. (1978) *Biochemistry (Wash.),* **17**, 4845-4854.
Schwarz,G., (1968) *Biopolymers,* **6**, 873-897.
Texter,J., (1978) *Prog. Biophys. Mol. Biol.,* **33**, 83-97.
Thamann,T.J., Lord,R.C., Wang,A.H.-J., and Rich,A. (1981) *Nucleic Acids Res.,* **9**, 5443-5457.
Wachsman,W., and Anthony,D.D. (1980) *Biochemistry (Wash.),* **19**, 5981-5986.
Wang,A.H.-J., Quigley,G.J., Kolpak,F.J., Crawford,J.L., van Boom,J.H., van der Marel,G., and Rich,A. (1979) *Nature,* **282**, 680-686.
Wang,A.H.-J., Quigley,G.J., Kolpak,F.J., van der Marel,G., van Boom, J.H., and Rich,A. (1981) *Science (Wash.),* **211**, 171-176.
Waring,M. (1970) *J. Mol. Biol.,* **54**, 247-279.
Wells,R.D., Goodman,T.C., Hillen,W., Horn,G.T., Klein,R.D., Larson, J.E., Müller,U.R., Neuendorf,S.K., Panayotatos,N., and Stirdivant,S.M. (1980) *Prog. Nucleic Acid Res. Mol. Biol.,* **24**, 167-267.
Widom,J., and Baldwin,R.L. (1980) *J. Mol. Biol.,* **144**, 431-453.
Wing,R., Drew,H., Takano,T., Broka,C., Tanaka,S., Itakura,K., and Dickerson,R.E. (1980) *Nature,* **287**, 755-758.
Winkle,S.A., and Krugh,T.R. (1981) *Nucleic Acids Res.,* **9**, 3175-3186.
Wu,H.M., Dattagupta,N., and Crothers,D.M. (1981) *PNAS,* **78**, 6808-6811.
Zimmer,C. (1975) *Prog. Nucleic Acid Res. Mol. Biol.,* **15**, 285-318.