Vol. 115, No. 1, 1983 **BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS**
August 30, 1983 Pages 100-105

A THERMALLY DRIVEN INTERCONVERSION OF B AND Z-DNA

Kunal B. Roy and H. Todd Miles

Laboratory of Molecular Biology
National Institute of Arthritis, Diabetes,
and Digestive and Kidney Diseases
National Institutes of Health
Building 2, Room 201
Bethesda, Maryland 20205

Received July 14, 1983

SUMMARY: CD and IR studies show that poly(dG-dMeC) in dilute Mg++ and Na^+ solutions (e.g., 2 mM Mg^{++} and 55 mM Na^+) undergoes reversible thermal interconversion of B and Z forms, in contrast to previous reports that the transition is independent of temperature and has an enthalpy change of zero. The B conformation is stable under the above conditions at lower temperature (~5°) and the Z form above ~20°. The conversion is relatively slow in both directions, but is more rapid in the Z → B direction.

Single crystal analyses of short d(GC) oligomers have revealed the novel left-handed Z-DNA structure (1,2). Extensive studies of alternating d(GC) polymers and larger oligomers indicate that these molecules very probably form similar left-handed Z-DNA structures in dilute solution at high ionic strength (3-6). An important result of the solution studies has been the finding that the B - Z transition is independent of temperature (3,5) indicating that the enthalpy change for the process is small or zero (3). The latter property ($\Delta H = 0$) was listed as one of three requirements for any structural model of the transition by Pohl and Jovin (3). We have found, however, that under certain conditions a B - Z transition is thermally poised and can be driven completely to one form or the other by heating or cooling over a 10° range below ambient temperature. We confirm prior reports that under many of the conditions used to induce a B → Z conversion (e.g., high $[Na^+]$, high $[Mg^{++}]$, ethanol, hexammine cobalt) the transition is independent of temperature (7).

MATERIALS AND METHODS:

Poly(dG-dMeC) was obtained from P. L. Biochemicals. IR spectra were measured with a Perkin Elmer 580B spectrometer inferfaced to an LDACS

0006-291X/83 $1.50

distributed computer system (8). CD spectra were measured with a JASCO J-500A spectropolarimeter. Solutions of the polymer used for CD were checked in the UV at longer wavelengths. No scattering was observed between 220 and 400 nm. Solutions used for infrared measurement had no visible precipitate.

RESULTS:

The B and Z forms of poly(dG-dMeC) have quite different infrared spectra in the double bond region (Fig. 1, ref. 9). In 0.1 M Na^+ (helix in B-form) carbonyl bands occur at 1690 and 1656 cm^{-1}; in 2 M NaCl or 0.004 M Mg++ (Z-form) the corresponding bands are observed at 1675, and 1646 cm^{-1} (Fig. 1). When a solution at 25° or 45° is cooled to 3° the spectrum changes from one characteristic of the Z-form to that characteristic of the B-form (Fig. 1). The change is fully reversible. An isosbestic point at 1682 cm^{-1} suggests that only two species are present at significant concentrations during the interconversion, but does not rule out low concentrations of other intermediate species.

The thermal transition was studied by CD spectroscopy at much lower polymer concentration (10^{-4} M rather than 10^{-2} M) in 2 x 10^{-3} M Mg++ and 55 mM Na^+. Spectra like those reported by Behe and Felsenfeld (5) for the Z and B forms are observed in Fig. 2. In this case, the variable responsi-

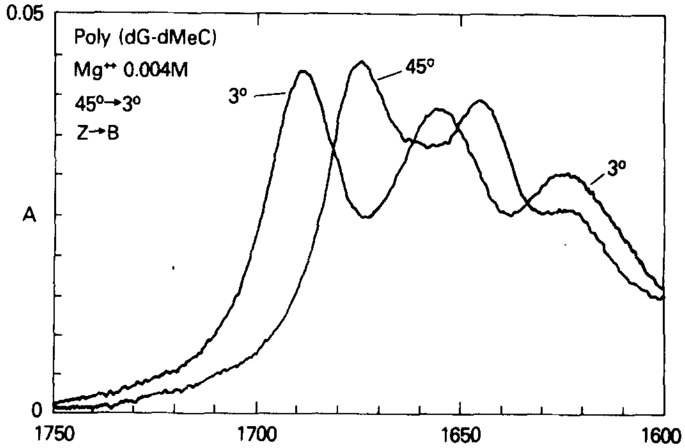


Fig. 1: Infrared spectra in D_2O solution of poly(dG-dMeC) in 0.004 M Mg^{++} at 3° and 45°. At 3°, bands at 1688 and 1654 cm^{-1} are characteristic of B-form; at 46°, bands at 1675 and 1646 cm^{-1} are characteristic of the Z-form.

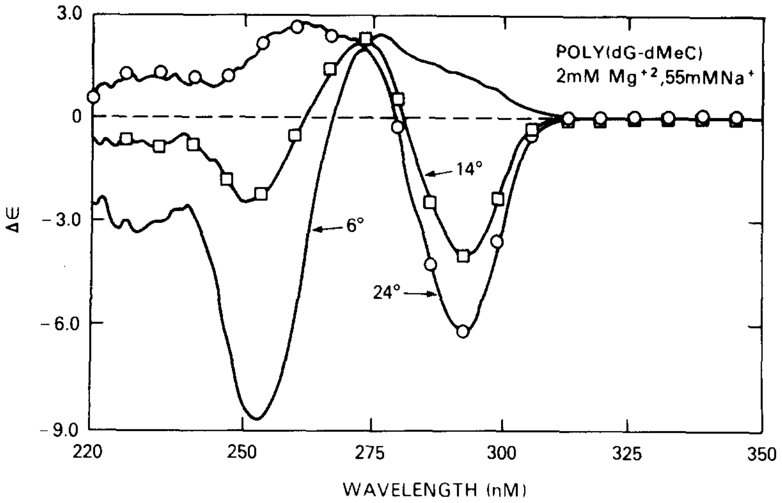
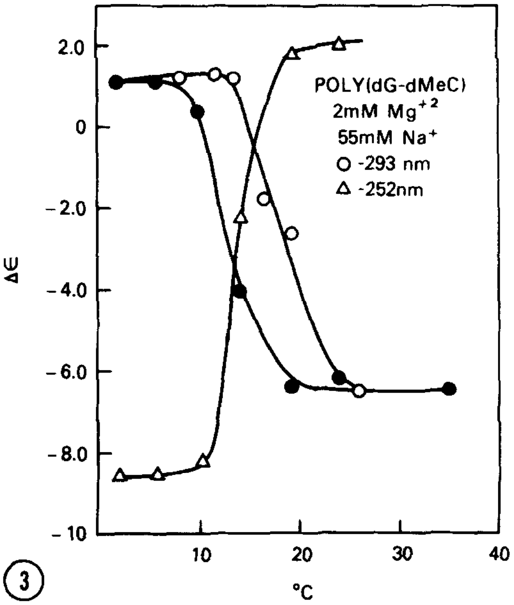


Fig. 2: Circular dichroic spectra of poly(dG-dMeC) in 2 mM Mg^{++} at three temperatures. Solvent also contained 50 mM NaCl, 5 mM Na^+ cacodylate, pH 7.0. At 6° the spectrum is typical of B-form and at 24° of Z-form. At 14° a mixture of B and Z forms is present.

ble for the transition is temperature rather than salt concentration. The midpoint of the transition and the range over which it occurs are essentially the same as observed in the infrared. In view of the 100-fold difference in polymer concentration in the two methods, occurrence of the thermal transition is independent of concentration. Thermal transition curves are shown in Fig. 3. The curves exhibit hysteresis, with apparent Tm values of 18° on heating and 13° on cooling. Titration with Mg^{++} at 5°C induces a B → Z transition which is cooperative in Mg++ concentration (all solutions are also 55 mM in Na^+). The midpoint is shifted to 4.0 mM Mg^{++} (Fig. 4) from the reported value (5) of 0.6 mM at 25°C. At 5° the polymer is entirely in the B-form at 2 mM Mg^{++} and entirely in the Z-form at 7 mM Mg^{++} and above. At the latter concentration the thermal effect is no longer observable. After shifting temperature from 5° to 20° (time for shift, ~ 1 min.) at 2 mM Mg^{++}, we could follow approximate rates of the forward and backward processes of B → Z interconversion (Fig. 5). The half times ($t_{1/2}$) measured were 230 minutes for B → Z at 20°, and 70 minutes for Z → B at 5°. Similar experiments in the infrared at 0.01 M polymer concentration (not shown) gave $t_{1/2}$ for B → Z ≅ 80 mins at 19°,



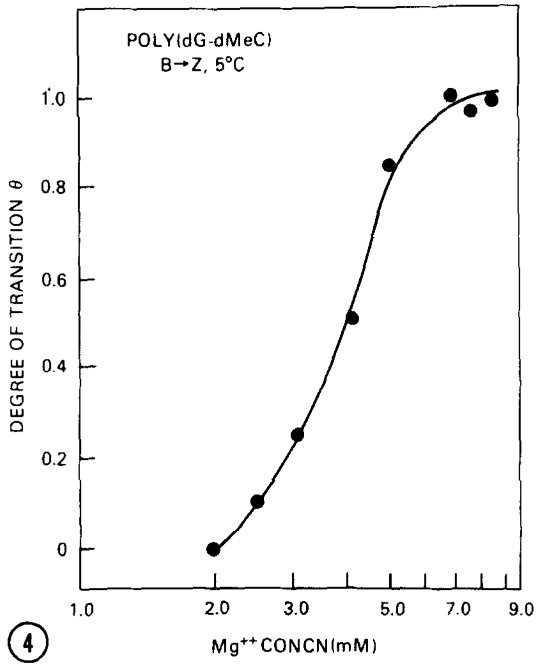


Fig. 3: Thermal transition curves for B Z interconversion measured by CD spectra. Polymer goes to B-form on cooling (●, Δ) and to Z-form on heating (○) under these conditions. Hysteresis is observed because of slow kinetics.

Fig. 4: Degree of transition, θ, of poly(dG-dMeC) as a function of Mg^{++} concentration at 5°C. Each solution is also 50 mM in NaCl and 5 mM in Na cacodylate. At 0.007 M Mg^{++} conversion to Z-form is complete. Above this value of $[Mg^{++}]$ remains in the Z-form at all observed temperatures. Because of slow kinetics, a separate solution was prepared for each point and allowed to stand seven days at 5° before measurement.

and $t_{1/2}$ for Z → B ≅ 20 mins at 3°. It is interesting that at both concentrations the Z → B conversion is appreciably faster than the reverse reaction, despite the fact that it is at a lower temperature.

DISCUSSION:

Since the B – Z equilibrium is usually unaffected by temperature (3,5), the enthalpy change must be small or zero (3), and the interconversion entropically driven. Yet this conclusion applies to the overall process and not necessarily to a conformational energy difference between B and Z forms. The enthalpy change for the overall process includes contributions from differences in solvation and from cation interaction with the polymer, for example, as well as from conformational energy differences.

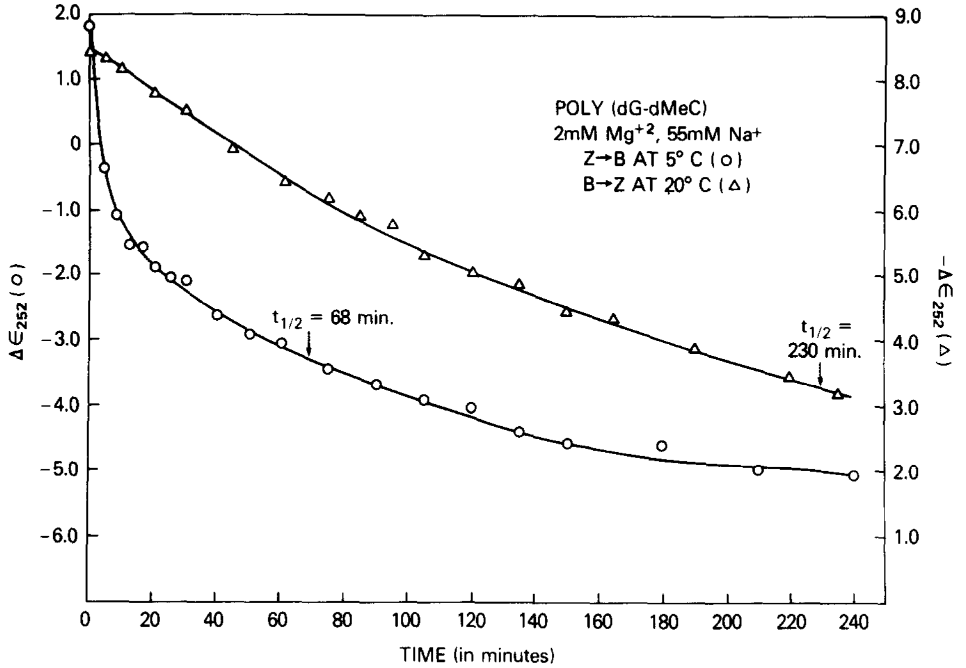


Fig. 5: Time course of B → Z (at 20°, O) and Z → B (at 5°, Δ) transition of poly(dG-dMeC). Half lives are taken as at the points at which $\Delta\varepsilon = (\Delta\varepsilon_B + \Delta\varepsilon_Z)/2$.

A zero enthalpy value for the interconversion may be merely the sum of component enthalpies of differing signs.

In the present study we show that the commonly ascribed isoenthalpic character of B → Z transition is not general and that specific conditions exist under which the process can be thermally driven. Here also, however, we cannot be sure whether the observed temperature dependence and hence negative enthalpy for Z → B under these conditions results from conformational energy differences or from solvation or cation interaction with the polymer.

The importance of hydration in nucleic acid conformation has been recognized for a number of years. Recent work from Dickerson's laboratory (10) has now placed the study of hydration on a much more explicit basis by locating many specific water molecules in the crystal structure of a dodecanucleotide. Patterns of ordered water molecules have been observed, and it has been suggested that some of these are the major factors stabilizing the B-DNA structure (10). These specific hydration patterns may help to account for the effect of cations or ethanol in destabilizing B with respect to Z-DNA. Under the conditions described in this report, for example, Mg^{++} may compete with water for specific sites on the polymer. At higher concentrations of Mg^{++} (e.g., 7 mM, Fig. 5) the cation could displace enough of the postulated class of water moleucles to shift the conformation entirely to the Z form. Temperature dependence of the overall process could be due to any of the component processes such as polymer solvation, cation solvation, cation-polymer binding, polymer conformation, or to some combination of these. Further work in crystallography and in solution chemistry will be needed to resolve some of these questions.

REFERENCES:

1. Wang, A. H. J., Quigley, G. J., Kolpak, F. J., Crawford, J. L., Van Boom, J. H., Van der Marel, G. A. and Rich, A. (1979) Nature (London) 282, 680-686.
2. Drew, H., Takano, T., Tanaka, S., Itakura, K. and Dickerson, R. E. (1980) Nature, 286, 567-573.
3. Pohl, F. M. and Jovin, T. M. (1972) J. Mol. Biol 67, 375-396.
4. Patel, D. J., Canuel, L. L. and Pohl, F. M. (1979) Proc. Natl. Acad. Sci. USA, 76, 2508-2511.
5. Behe, M. and Felsenfeld, G. (1981) Proc. Natl. Acad. Sci. USA, 78, 1619-1623.
6. Chen, Y., Cohen, J.S. and Behe, M. (1983) Biochemistry, 22, 2136-2142.
7. Since this work was completed, we have learned that Behe and colleagues have found specific conditions for thermal B - Z interconversion in the presence of a number of different cations (M. Behe, personal communication).
8. Powell, J. I., Fico, R., Jennings, W. H., O'Bryan, E. R. and Schultz, A. R. (1980) Proc. IEEE Comput. Soc. Int. Conf. 21st, 185-190.
9. Miles, H. T. and Frazier, J. (1982) unpublished experiments.
10. Kopka, M.L., Fratini, A.V., Drew, H.R. and Dickerson, R.E. (1983) J. Mol. Biol. 163, 129-146.