Increase in temperature induces the Z to B transition of poly[d(G-C)] in water–ethanol solution

Armen T. Karapetyan, Elvira E. Minyat and Valery I.Ivanov*

Kirovakan Teachers Training Institute, Kirovakan, Armenian SSR and **Institute of Molecular Biology, USSR Academy of Sciences, 117984 Moscow B-334, USSR*

An increase in temperature from 20 to 50°C results in the complete transition from the Z to B form of poly[d(G-C)], dissolved in a 55% ethanol–water solution. The transition is fully reversible and displays a slow kinetics. The transition profiles for the free polynucleotide and for that in the presence of ethidium bromide, which is known to stabilize the B form, are obtained by circular dichroism. Based on these data the enthalpy value for the B–Z transition in our conditions is estimated to be $\Delta H_{BZ} = -0.7$ kcal/mol.

1. INTRODUCTION

Discovery of the Z conformation for polynucleotides with alternating purine-pyrimidine sequences posed a question about the nature and energetics of the transition from the ordinary B form into the Z form.

Two principally different methods are in use for studying this shift: a method in which the transition into the Z form is induced by the energy of negative superhelicity in a covalently closed circular DNA [1–3], and a traditional approach, in which the transition proceeds under the change of conditions in solution [4–6].

It is known that the B–Z transition can be realized by administration of a high concentration of salt or alcohol. The Z form is also stabilized by different modifications of the polynucleotide [7,8].

If the B–Z shift is induced by high salt concentrations it is almost impossible to study such phenomena as complexation of the polynucleotide with ligands, either of low molecular mass or proteins, due to competition with the salt cations. In contrast, such studies prove to be possible when using water–ethanol or water–trifluoroethanol solutions to induce the B–Z transition [6]. The most convenient would be to carry out the transition by changing the temperature.

The first results of authors in [4] were disappointing: the B–Z equilibrium was independent of temperature. (In their study the transition was induced by increase in NaCl concentration as far as 2.5 M.) However, a temperature-dependent transition was recently obtained with poly[d(G-C)] containing methylated cytosine in the 5th position [9]. Unfortunately, Mg^{2+} is required for such a transition to occur; this complicates the system significantly, especially if one wishes to study interaction of ligands with the polynucleotide.

Here we show that the temperature-dependent transition can easily be obtained in a water–ethanol solution which contains only a low concentration of NaCl. The whole transition is disposed in a very 'practical' interval (25–45°C) and requires no modification of the polynucleotide. The possibility of studying complexation with ligands is demonstrated with ethidium bromide as an example.

2. MATERIALS AND METHODS

Poly[d(G-C)] was from P.-L. Biochemicals

---

* To whom correspondence should be addressed

(USA). The sample had an M_r of 63000. Polynucleotide concentrations were determined spectrophotometrically using a value of molar absorbancy for the B form of $A^{255} = 8400$. All solutions were prepared with twice-distilled deionised water. All the data below were obtained in non-buffered solutions containing 10^{-3} M NaCl and 5×10^{-5} M EDTA. The concentration of poly[d(G-C)] was about 5×10^{-5} M in phosphates.

The B–Z transition was followed by circular dichroism (CD) with a Jouan III dichrograph in 0.5 cm thermostatted cells.

Since the time of establishing equilibrium for each temperature is of the order of tens of minutes (because of the slow kinetics of the B–Z transition [4,6]), the levels of the transition were registered at discrete temperatures. At each of these temperatures completion of the kinetics was especially tested.

To record kinetic curves accurately two thermostats were used: the required temperature jump was effected by rapidly connecting a thermostat, heated to the required temperature, to the cell-holder, the other one being disconnected. While recording of the kinetics was in progress the disconnected thermostat was set to the temperature of the next jump. Using a thermocouple immersed in the solution it was shown that that temperature equilibrated in the cell in about 3 min. Therefore, the time course of the B–Z transition was somewhat distorted initially, but can be corrected by an exponential interpolation.

3. RESULTS

We first prepared a sample which corresponded to the B–Z half-transition point at room temperature (52% ethanol under our salt condition). On heating this sample it became obvious that the shift from the Z to B form was induced as revealed by the CD spectrum [5,6]. A series of the CD spectra were then obtained by a stepwise increase in temperature after attaining equilibrium at each temperature (fig.1). The limiting spectra coincide with those ascribed to the Z and B forms [6]. The presence of a distinct isodichroic point is indicative of the presence of only two spectrally different conformational states within the transition range. By plotting the CD magnitude at 295 or 255 nm as a function of temperature one obtains
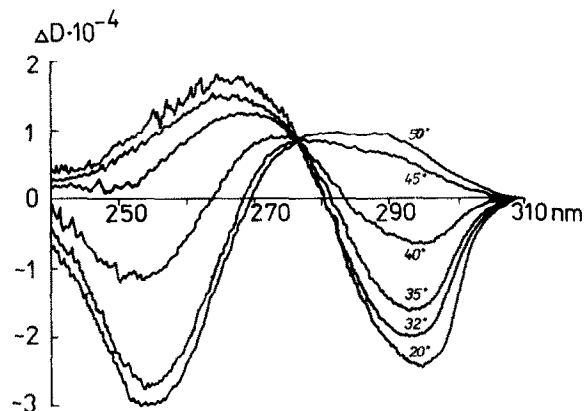

Fig.1. CD spectra of water–ethanol solution of poly[d(G-C)] at the indicated temperatures. For conditions see section 2.

the B–Z transition profile (fig.2). This profile is indeed an equilibrium one since it is the same for the forward and backward change of temperature. Poly[d(G-C)] retains the double-stranded state throughout the temperature interval of the B–Z transition (20–50°C): separate experiments showed that the polynucleotide melts above 80°C under these conditions.

The kinetic curves of attaining the equilibria (fig.3) are exponential or close to being exponential, the characteristic time at the small jumps $40 \longrightarrow 35$ and $45 \longrightarrow 40°C$, being the same (within
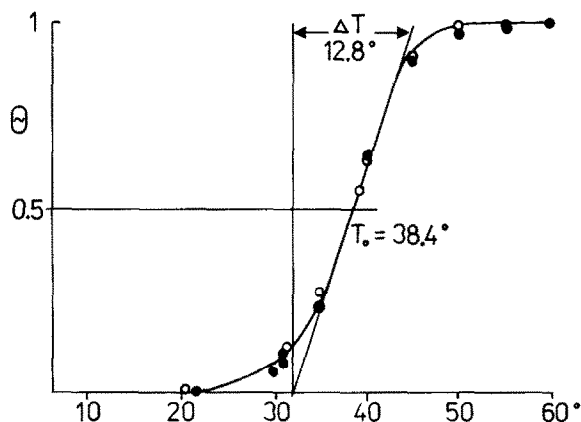

Fig.2. Equilibrium profile of the temperature-induced Z–B transition. The results of 3 independent experiments are shown. Θ is the fraction of the B form as shown by the CD magnitude at 296 or 255 nm. (o——o) Reverse course of temperature. The values of the transition point, T_0, and the transition width, ΔT, are also shown.
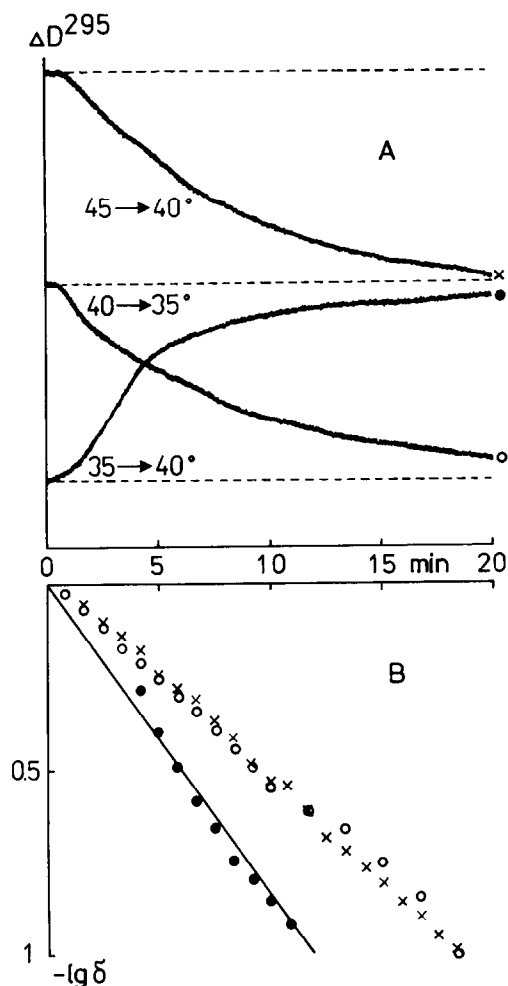
Fig.3. Some kinetic curves of attaining equilibria at the indicated temperature jumps (A) and their linear anamorphoses (B). δ is for the relative CD change at the indicated moments.

Fig.4. The curves of the Z–B transition for free poly[d(G-C)] (right) and for the complex with ethidium (left). The linear density of the dye on the polymer is one ethidium molecule per 50 base pairs. The values of the transition points and widths are indicated. Θ, fraction of the B form. Conditions are as given in section 2.

the limits of error), whereas the kinetics of an equal jump in the opposite direction, $35 \longrightarrow 40^\circ C$, is faster.

The presence in the sample of only a low salt concentration as well as the easy and complete reversibility of the temperature-induced B–Z shift make this system very suitable for studying the interaction of different ligands with the B and Z forms of poly[d(G-C)].

As an example we selected ethidium bromide which is known to reverse the B to Z transition induced by a high salt concentration in water solution [10]. However, it is this high salt concentra-
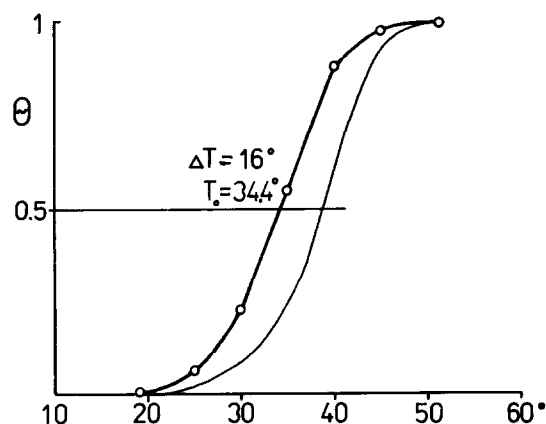
tion that prevents the study of the ethidium complex at low dye content, i.e., under conditions of no excess of the non-bound ligand.

Fig.4 shows the data obtained with our system when one ethidium molecule was present per 50 base pairs. Two principal effects are seen: stabilization of the B form and widening of the B–Z transition interval. The latter effect has been thoroughly studied with the helix–coil transition [11,12] and is due to redistribution of the ties' molecules between the B and Z sections during the B–Z transition.

4. DISCUSSION

The temperature dependence of the B–Z equilibrium testifies to a non-zero enthalpy value of this conformational change under the given specific conditions. Note in this connection that in the non-ethanol systems the enthalpy of the B–Z transition equals zero [4]. The same is true for another transition within the double-stranded state, the B–A transconformation [13].

Stabilization of the B form in our system shows that the difference in enthalpies for the B and Z forms, ΔH_{BZ}, is negative. Remember that ΔH_{BZ} is positive for a system containing poly[d(G-5MeC)] and Mg^{2+} [9].

Quantitatively, ΔH_{BZ} under our conditions can

be estimated from the data of fig.4 after the shift of the transition point, δT_o, and widening of the transition curve, $\delta \Delta T$, in the presence of ethidium. To do this one can use the same equations, which were derived earlier for DNA melting in the presence of the redistributing ties [11]:

$$\delta T_o = 2 \left(\frac{p-1}{p+1}\right) \frac{RT_o^2}{\Delta H_{BZ}} \cdot c$$

$$\delta \Delta T = 4 \left(\frac{p-1}{p+1}\right)^2 \frac{RT_o^2}{\Delta H_{BZ}} \cdot c \tag{1}$$

where $p = K_B/K_Z$ is the ratio of the binding constants of the ties with the B and Z forms, and $c = 2D/P$ is the ratio of ligand concentration to that of phosphates of a polynucleotide.

These equations are valid providing that the relative ligand concentration is much less than unity and that free ligands are absent in solution. The first requirement is obviously fulfilled ($c = 0.02$) and the second is probably also valid since the binding constant of ethidium to poly[d(G-C)] is seemingly high enough although this is not known exactly for our conditions. A detailed study using a broad range of concentrations as well as with another, spermine, ligand, which unlike ethidium stabilizes the Z form, will be published elsewhere.

We now estimate the enthalpy of the B–Z transition assuming the fulfillment of both conditions. It follows from eq.1 and the data of figs.2,4:

$$|\Delta H_{BZ}| = \frac{RT_o^2 \delta \Delta T}{(\delta T_o)^2} \cdot c = 0.7 \text{ kcal/mol} \tag{2}$$

So, the enthalpy of the B–Z transition is rather small in our system (cf. enthalpy for the helix–coil transition in DNA, ~8 kcal/mol [11]).

Finally, we discuss one more interesting point connected with the B–Z transition in the presence of ethidium: a relatively small widening of the transition curve. Eq.1 indicates similar values of the binding constants of ethidium with the B and Z states. The value of $p = K_B/K_Z$ proves to be equal to 2 only. This means that ethidium binds to the Z form only a little less well than to the B form.

The intercalation model is generally accepted for the strong complex of ethidium with the B form. Does it intercalate into the Z form as well? At any rate, conformational calculations, which have recently been done [14], show that intercalation into the Z form is stereochemically possible.

REFERENCES

[1] Klysik, J., Stirdivant, S.M., Larson, J.E., Hart, P.A. and Wells, R.D. (1981) Nature 290, 672–677.
[2] Peck, L.J. and Wang, J.C. (1983) Proc. Natl. Acad. Sci. USA 80, 6206–6210.
[3] Vologodskii, A.V. and Frank-Kamenetskii, M.D. (1984) J. Biomol. Struct. Dyn., in press.
[4] Pohl, F.M. and Jovin, T.M. (1972) J. Mol. Biol. 67, 375–396.
[5] Pohl, F.M. (1976) Nature 260, 365–366.
[6] Ivanov, V.I. and Minyat, E.E. (1981) Nucleic Acids Res. 9, 4783–4798.
[7] Behe, F.M. and Felsenfeld, G. (1981) Proc. Natl. Acad. Sci. USA 78, 1619–1623.
[8] Lafer, E.M., Moller, A., Nordheim, A., Stollar, B.D. and Rich, A. (1981) Proc. Natl. Acad. Sci. USA 78, 3546–3550.
[9] Roy, K.B. and Todd, H.M. (1983) Biochem. Biophys. Res. Commun. 115, 100–105.
[10] Pohl, F.M., Jovin, T.M., Baehr, W. and Holbrook, J.J. (1972) Proc. Natl. Acad. Sci. USA 69, 3805–3809.
[11] Lazurkin, Yu.S., Frank-Kamenetskii, M.D. and Trifonov, E.N. (1972) Biopolymers 9, 1253–1306.
[12] Frank-Kamenetskii, M.D. and Karapetyan, A.T. (1972) Mol. Biol. (USSR) 6, 624–635.
[13] Ivanov, V.I., Minchenkova, L.E., Minyat, E.E., Frank-Kamenetskii, M.D. and Schyolkina, A.K. (1974) J. Mol. Biol. 87, 817–833.
[14] Gupta, G., Dhingra, M.M. and Sarma, R.H. (1983) J. Biomol. Struct. Dyn. 1, 97–113.