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Empirical validation of a two-state kinetic model for the B-Z transition of double-stranded poly[d(G-m⁵C)]

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Abstract A formula based on a first-order kinetic equation is derived to evaluate the rate constant of the B-Z transition of a synthetic double-stranded poly[d(G-m⁵C)] in terms of the salt concentration, the absolute temperature, and the cooperativity index. The validity of the formula was tested using circular dichroism spectroscopy after variation of the type of salt (NaCl, MgCl₂), the salt concentration, and the temperature of the polynucleotide solution. A consequence of the proposed function is that in conditions of high salt there is a predictable salt threshold which determines the particular molecular mechanism of the B-Z transition. The paper also describes the way in which this threshold level is temperature dependent. A detailed comparison of our data with the experimental data found by other authors is given. The function agrees quantitatively with the experiments and explains the contrasting results found in the literature about the influence in the B-Z transition of both the temperature and the polymer size.

Key words Circular dichroism spectroscopy · Synthetic polynucleotide · Salt dependence · Temperature dependence · Left-handed DNA

Introduction

The transition from the right-handed canonical B-DNA conformation to the left-handed Z-DNA conformation was initially detected by means of circular dichroism (CD) spectroscopy of poly[d(G-C)] at high sodium ion concentration [1] and later confirmed by X-ray analysis of crystals grown from the oligonucleotide d(CpG)₃ at 5 M NaCl concentration [2]. Since then, the Z-DNA conformation has been studied under a variety of experimental conditions [3]. The CD spectra of poly[d(G-C)] obtained at different NaCl concentrations showed, in the transition region, clear isoelliptic points at 292 and 255 nm as an indication of a transition between two species. The CD spectra also showed a strong dependence of the ellipticity of the polymer on the NaCl concentration used [1]. The existence of drastic changes dependent on very small variations in external conditions are characteristic of cooperative transitions. The curves of these processes are smooth, symmetrical, and sigmoidal when the variable is plotted on a logarithmic scale and they may be described by the gaussian cumulative distribution or by the logistic model. The logistic model is based in a differential equation mathematically analogous to the Hill equation, a well-known tool used for the analysis of ligand binding and enzyme kinetics. The exponent in the logistic function has the same mathematical form as the Hill coefficient. In the proper application of the Hill equation, the amount of ligand refers to free ligand whereas in most applications of the logistic model, including this report, the amount of ligand indicates the total ligand concentration. The logistic function has the advantage of simplicity and has been widely used for radioimmunoassay [4, 5], bioassay [6, 7], and related techniques [8, 9]. In order to quantify the slope of the characteristic curve of the B-Z transition (the cooperativity index) that represents the evolution of the B-Z conformational change depending on the salt concentra-

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tion, a logistic function was proposed to describe the equilibrium between the two states [10]. This equation has been also used to determine the cooperativity index of double-stranded poly[d(G-m⁵C)] induced by Mg²⁺ in the presence of adriamycin [11].

More recently, the CD kinetics data obtained for the B-Z transition of the double-stranded synthetic polynucleotides poly[d(G-C)] and poly[d(G-m⁵C)] in the presence of mono-, di-, and trivalent cations at constant temperature were also fitted to a logistic function [12]:

$$\theta = \theta_{\infty} + \frac{(\theta_0 - \theta_{\infty})}{1 + t \left(\frac{x}{K_s} \right)^n} \quad (1)$$

where θ is the spectroscopic response, x the concentration of added salt, θ_0 the response when $x=0$, θ_{∞} the response when $x=\infty$, K_s the concentration of x needed to reach the midpoint of the transition in the time unit, t is the time, and n the degree of cooperativity of the B-Z transition process, which determines the slope of the curve.

However, in spite of the dense and well-established literature about the B-Z transition in terms of chemical equilibrium, the study of the kinetics of the process remains still obscure and contrasting results appear in the literature on this subject. Moreover, as far as we know, nobody has yet formulated a mathematical model that could explain the discrepancies found in the kinetics of the process. On these grounds, in the present paper we develop an empirical equation for the rate of the B-Z transition of double-stranded poly[d(G-m⁵C)] under various salt and temperature conditions. The equation reported shows that the rate of the B-Z transition process obeys the Arrhenius law and depends on (1) the salt concentration present in the polynucleotide solution, (2) the type of salt used as the Z-DNA inducer, (3) the temperature at which the process is carried out, and (4) the cooperativity index of the transition. One of the relevant points of the proposed function is that it predicts the existence of a threshold in conditions of high salt which determines the molecular mechanism whereby the polynucleotide undergoes the B-Z transition. The level of the salt threshold would vary in a linear way with the temperature of the polynucleotide solution. The consequences of the proposed equation regarding the molecular mechanism of the B-Z transition of (dG-dC)(dG-dC) polynucleotides and the real molecular significance of the parameters obtained are also discussed.

Materials and methods

DNA solutions

Double-stranded poly[d(G-m⁵C)] was purchased from Pharmacia. The polynucleotide had an average length of 2900 base pairs (MW = 1.9 × 10⁶). A stock solution of polynucleotide of

0.7 mg/mL was made in TE buffer (Tris-HCl, pH 8.0, 0.1 mM EDTA) containing 50 mM NaCl and was kept at -20 °C until use.

CD spectroscopy

CD spectra were recorded on a JASCO J-600 spectropolarimeter interfaced to a 486 computer. The measurements were performed at several temperatures using 1-cm path length cells. Each spectrum represents the mean of three scans. Aliquots containing 20 µg/mL of double-stranded poly[d(G-m⁵C)] were prepared from the stock solution. CD spectra were run in a range of wavelengths from 220 to 320 nm and at a speed of 50 nm/min. Scans were recorded at 0.4 nm intervals. Evaporation was minimized by overlaying the sample cell with a plastic cover.

Measurements of the B-Z transition of double-stranded poly[d(G-m⁵C)] aliquots at different incubation times were carried out by adding the desired amount of concentrated salt solution (NaCl or MgCl₂) and mixing by shaking the solution and recording the CD spectra of the polynucleotide at several incubation times. The incubation time-dependent change of the CD was recorded at a constant wavelength of 290 nm.

Kinetic studies

A solution of double-stranded poly[d(G-m⁵C)] was thermostated at 35, 40, 45, 50, and 55 °C with a constant temperature circulating bath, and then a certain amount of NaCl (0.80 M) was added to the polynucleotide. The measurement of the initial CD spectrum of the B-form DNA towards the Z-form was done by recording the intensity of the CD signal at 290 nm at time intervals. The CD intensity data were used to calculate the rate constant at those temperatures and the activation energy, E_a , was calculated from an Arrhenius plot.

Statistical analysis

In order to determine whether for a polynucleotide sample of a given chain length the correlation coefficient (r) indicates a linear relationship, we tested the null hypothesis in which the sample is chosen from a population for which K_s and T , K_s and [NaCl], and K_s and [MgCl₂] are not related. Therefore, we calculated the probability (p) that from such population a polynucleotide sample of a given size is taken for which the correlation coefficient equals or exceeds the absolute value of r calculated for the given sample. Here we use a two-tailed test. If p is less than 5% (or whatever other level of significance is chosen), we reject the hypothesis that the sample has been taken from a population in which there is not a linear relationship. The value of r then indicates that the values obtained for K_s and T , K_s and [NaCl], or K_s and [MgCl₂] can be assumed to be linearly related.

Results

Relationship between the rate constant, k , the salt concentration, x , the temperature, T , and the cooperativity index, n , of the B-Z transition process

First-order reactions have a simple mathematical form. Let us suppose that the B-Z transition can be described by the rate law: $[B] = [B_0]e^{-kt}$, where $[B]$ is the B-form concentration at any time, $[B_0]$ is the initial B-form concentration, k is the rate constant of the

transition, and t is the time. In this case, the B-Z transition may be followed by CD spectroscopy where the ellipticity (related to the amount of B-form) in a first-order reaction can be written as:

$$\theta = \theta_{\infty} + (\theta_0 - \theta_{\infty})e^{-kt} \quad (2)$$

where θ is the spectroscopic response, θ_0 the spectroscopic response when the polynucleotide is in the B-form, θ_{∞} the response when the polynucleotide is in the Z-form, k the rate constant of the transition, and t the time. In that equation, e^{-kt} can be written as a series expansion of terms:

$$e^{-kt} = \frac{1}{1 + kt + \frac{(kt)^2}{2!} + \frac{(kt)^3}{3!} + \dots + \frac{(kt)^{i-1}}{(i-1)!} + \dots} \quad (3)$$

When $kt < 1$, Eq. 3 can be written as:

$$\theta = \theta_{\infty} + \frac{(\theta_0 - \theta_{\infty})}{1 + kt} \quad (4)$$

Comparing this equation with Eq. 1 it was observed that both expressions (Eq. 1 and Eq. 4) are equal if the rate constant of the transition, k , is given by:

$$k = \left(\frac{x}{K_s}\right)^n \quad (5)$$

Thus, at any time and at a given temperature, Eq. 1 can be transformed into Eq. 2 so that the rate constant of the B-Z transition, k , is now related to the salt concentration present in the polynucleotide solution, x , the cooperativity index of the B-Z transition process, n , and the salt concentration necessary for the polynucleotide to reach the transition midpoint in the time unit, K_s , as can be seen in Eq. 5. Having found this expression we then studied the relationship between K_s and the temperature.

Relationship between K_s and the absolute temperature, T

Figure 1A shows the kinetics of the NaCl-induced B-Z transition of double-stranded poly[d(G-m⁵C)]. The temperature of the solution was kept at 35, 40, 45, 50, and 55 °C (points). The percentage of B-form was calculated from the molar ellipticity at 290 nm plotted as a function of time after mixing 0.8 M NaCl and 20 μg/mL of poly[d(G-m⁵C)]. The curves show the best fit to the exponential functions used to determine the rate constant, k , for each temperature. The cooperativity index for the B-Z transition process of the polynucleotide under the experimental conditions described in the present paper was calculated as previously described [12] and had a value of $n = 14.9 \pm 0.2$. Figure 1B shows the K_s values obtained from Eq. 5 (points), showing also the line of regression of K_s on T for the temperature range considered (line) when NaCl is used as an inducer of the B-Z transition.

Then, the equation of the line for the best fit would take the form: $K_s = K_{s0} - (\Delta K_s / \Delta T)T$, where T is the absolute temperature, $K_{s0} = 5.0 \pm 0.3 \text{ M s}^{1/n}$ is the K_s intercept at $T = 0 \text{ K}$, and $-(\Delta K_s / \Delta T) = 11.6 \times 10^{-3} + 0.1 \times 10^{-3} (\text{M s}^{1/n})/\text{K}$ is the slope of the line representing the contribution of the temperature to K_s . The slope, $-(\Delta K_s / \Delta T)$, is the salt concentration change per ΔT which has to be subtracted from K_{s0} to obtain K_s . The coefficient of determination from the line of regression is 97.8% ($p = 0.0014$). This regression value is due to the linear relationship between the temperature and K_s . Thus, these data indicate that there are two different contributions to the rate constant of the B-Z transition. One is due to the salt present in the solution and another one is due to the temperature at which the process is being carried out. The substitution of K_s by $K_{s0} - (\Delta K_s / \Delta T)T$ in Eq. 5 results in:

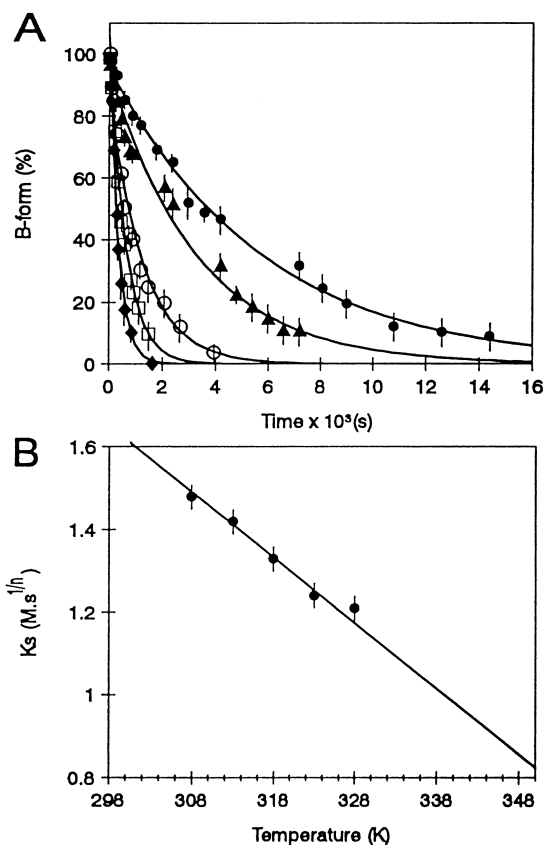


Fig. 1A,B. Kinetics of the NaCl-induced B-Z transition of double-stranded poly[d(G-m⁵C)]. **A** The percentage of the B-form was calculated when the molar ellipticity at 290 nm was plotted as a function of time after mixing 0.8 M NaCl and 20 μg/mL poly[d(G-m⁵C)] (points). The temperature of the solution was kept at 35 (●), 40 (▲), 45 (○), 50 (□), and 55 (◆) °C. The curves show the exponential functions used to determine the rate constant, k . **B** Variation of the salt concentration needed to reach the B- to Z-poly[d(G-m⁵C)] midpoint transition in the time unit, K_s , with the temperature. Points are experimental results obtained from the rate constant values, k , as indicated in the text. The line of regression of K_s on temperature is shown. The coefficient of determination from the line of regression has a value of 99.11%

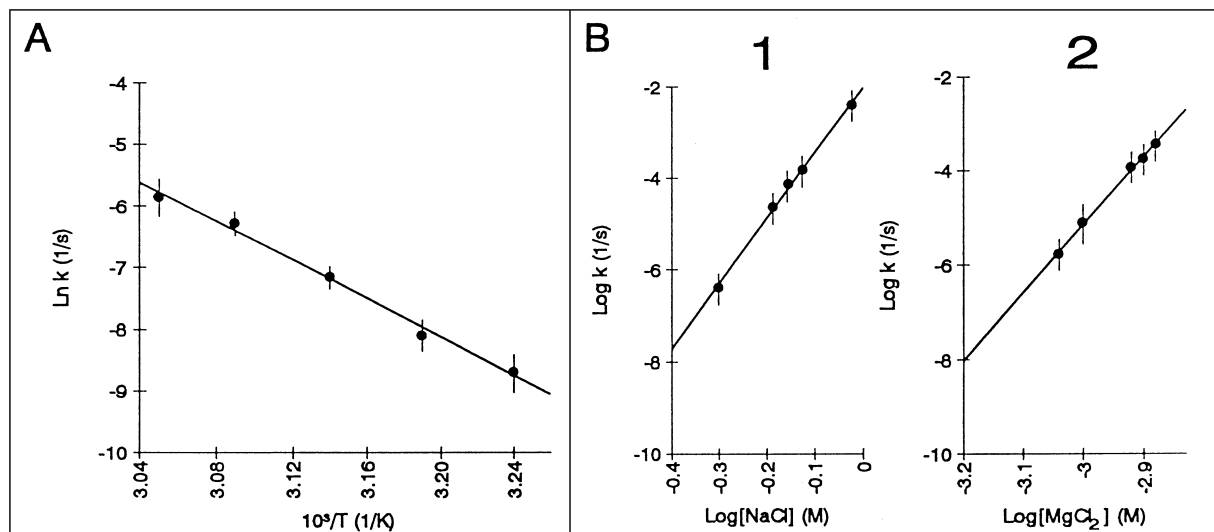


Fig. 2A,B. Dependence of the rate constant, k , on the temperature and the salt concentration. **A** Arrhenius plot of $\ln(k)$ against $1/T$ for the B-Z transition process induced by 0.8 M NaCl in the synthetic polynucleotide poly[d(G-m⁵C)] for a temperature range between 35 and 55 °C at intervals of 5 °C. Experimental results are indicated by *dots*. The *solid line* is calculated from Eq. 6 using the parameters given in the text. **B** Log-log plots of the variation of k on **B-1** [NaCl] and **B-2** [MgCl₂]. Experimental results are indicated by *dots*. The concentration range is between 0.5 and 0.95 M for NaCl and between 0.9 and 1.3 mM for MgCl₂. The temperature of the polynucleotide solution was kept constant at 37 °C. The *solid line* is calculated from Eq. 6 using the parameters given in the text

$$k = \left[\frac{x}{K_{so} - (\Delta K_s / \Delta T) T} \right]^n \quad (6)$$

This equation gives the rate law of the process, as can be seen in Fig. 2 (lines). Figure 2 also shows the changes in the rate constant when varying the temperature between 35 and 55 °C and the salt concentration of the solution is kept constant at 0.8 M NaCl (points in the Arrhenius plot, Fig. 2A), or when the salt concentration of the polynucleotide solution varies in the range of 0.5–0.95 M NaCl (points in Fig. 2B-1) or 0.9–1.3 mM MgCl₂ (points in Fig. 2B-2) at a constant temperature of 37 °C.

Dependence of the rate constant, k , on the salt concentration

The relationship between the rate of the B-Z transition, k , and the salt concentration of the solution, x , can be written as:

$$\left[\frac{\partial(\log k)}{\partial(\log x)} \right]_T = n \quad (7)$$

[see log-log plots in Fig. 2B-1 (for the B-Z transition induced by NaCl) and in Fig. 2B-2 (for the B-Z transition induced by MgCl₂)]. The correlation coefficient in

the Arrhenius plot (see Fig. 2A) was $r = -0.989$ ($p = 0.0014$); it was $r = 0.996$ ($p = 0.0003$) in the plot $\log k$ against $\log[\text{NaCl}]$ and it was $r = 0.998$ ($p < 0.0001$) in the plot $\log k$ against $\log[\text{MgCl}_2]$. The cooperativity index of the B-Z transition process is constant, having a value for NaCl and MgCl₂ salts of $n = 13.6 \pm 0.7$ and $n = 14.5 \pm 0.3$, respectively. The data are in good agreement with the previously reported cooperativity index of $n = 14.9 \pm 0.2$ [12]. It is clear from Eq. 7 that a plot of $\log k$ against $\log x$ will be linear with slope n . An immediate consequence of Eq. 7 is that if the salt concentration (x) in the solution is zero, the rate (k) of the B-Z transition is also zero, independent of both the temperature and the cooperativity index. The experimental data obtained agree with Eq. 7 in the range of salt concentration tested for both NaCl and MgCl₂ at a temperature of 37 °C. Thus, Eq. 7 predicts that the B-Z transition would require the presence of salt and would not occur in its absence, regardless of the temperature.

Kinetic equation for the B-Z transition process of poly[d(G-m⁵C)]

The close relationship found between the rate constant, k , the temperature, T , the salt concentration of the polynucleotide solution, x , and the cooperativity index, n , allows us to propose the following equation to explain the kinetics of the B-Z transition process of double-stranded poly[d(G-m⁵C)] induced by heat, salt, or both:

$$\theta = \theta_\infty + (\theta_0 - \theta_\infty) e^{-t \left(\frac{x}{K_{so} - aT} \right)^n} \quad (8)$$

where θ is the spectroscopic response, θ_0 is the response when the polynucleotide is in the B-form, θ_∞ is the response when the polynucleotide is in the Z-form, x is the concentration of salt, $a = (\Delta K_s / \Delta T)$ and K_{so} are constants characteristic of the salt used.

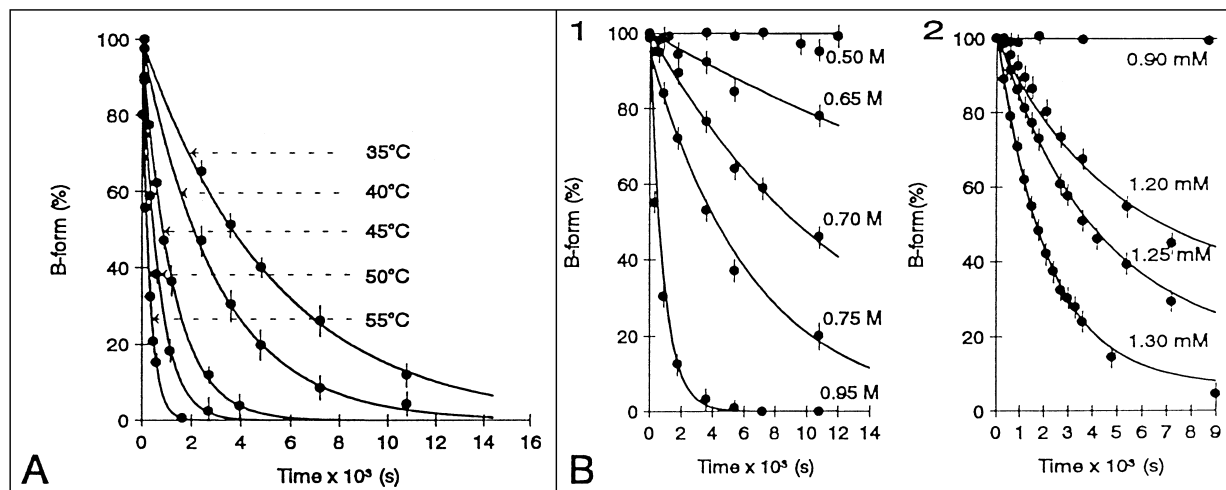


Fig. 3A,B. Kinetics of heat- and salt-induced B-Z transition of synthetic double-stranded poly[d(G-m⁵C)]. **A** The percentage of B-form was calculated from the molar ellipticity at 290 nm and is plotted as a function of time after mixing 0.8 M NaCl and 20 μg/mL of poly[d(G-m⁵C)]. The temperature of the solution was kept at 35, 40, 45, 50, and 55 °C. The curves show the best fits to the exponential functions used to determine the rates of the B-Z transition. **B** The percentage of the B-form was calculated as before and is plotted as a function of time after mixing **B-1** 0.5, 0.65, 0.7, 0.75, and 0.8 M NaCl, or **B-2** 0.9, 1.2, 1.25, and 1.3 mM MgCl₂, and 20 μg/mL of poly[d(G-m⁵C)] at a constant temperature of 37 °C. The curves show the best fits to the potential functions used to determine the rates of the B-Z transition

An experimental test of Eq. 8 can be observed in Fig. 3. Figure 3A (points) shows the time dependence of the B-Z transition process for double-stranded poly[d(G-m⁵C)] in the presence of 0.8 M NaCl at temperatures ranging from 35 to 55 °C. The lines represent the values obtained from Eq. 8 for this set of temperatures under the same conditions.

Figure 3B (points) illustrates the kinetic behaviour of the B-Z transition as a function of the salt concentration at a constant temperature of 37 °C. The dependence of the B-Z transition process on the salt concentration was tested by CD for NaCl and MgCl₂ salts. The NaCl concentration was in the range between 0.5 and 0.95 M (Fig. 3B-1) and the MgCl₂ concentration was in the range of 0.9–1.3 mM (Fig. 3B-2). The lines represent the data obtained from Eq. 8 under the same conditions. Table 1 summarizes the mean values and deviations of the parameters associated with the exponential fits shown in Fig. 3 from the kinetics of both (1) the temperature- and (2) the salt-induced B-Z transition of poly[d(G-m⁵C)]. A close inspection of Table 1(a), K_s column, shows the existence of a linear relationship between the temperature and K_s . On the other hand, a detailed statistical analysis of the data shown in Table 1(b), K_s column, gives the correlation parameters for K_s and [NaCl]: $r^2=0.674$ ($p=0.088$). The correlation parameters for K_s and [MgCl₂] are: $r^2=0.630$ ($p=0.110$). In both cases the slope is not significantly different from

zero, showing that the salt concentration in the polynucleotide solution is certainly not related to K_s .

Discussion

The results presented here generalize the equation presented in [12] to any time and temperature and make it congruent with a first-order kinetics equation (Eq. 8) which shows that the concentration of the B-form decays exponentially from an initial value to a zero value when the B-Z transition is triggered. In spite of the considerable number of studies on the mechanism of the B-Z transition, the understanding of the nature of the process still remains incomplete. Thus, in 1972 it was stated [1] that over the range of 20–50 °C the NaCl-induced transition of a poly[d(G-C)] was temperature independent, while in 1983 and 1986 it was reported that the interconversion from the B- to the Z-form of poly[d(G-m⁵C)] [13, 14] was thermally driven. Temperature-dependent conformational transitions to the Z-form in poly[d(G-C)] and poly[d(G-m⁵C)] under a variety of solution conditions have been also reported [15–19]. The present paper proposes a theoretical equation that describes the kinetics of the B-Z DNA transition and is able to explain the discrepancies found in the literature about the temperature dependency of the process. We also carried out experiments on the B-Z transition of poly[d(G-m⁵C)] with regards to the temperature at constant salt concentration and polynucleotide chain length in order to validate the model proposed. The analysis of the relationship between the rate constant of the B-Z transition, the salt concentration of the solution, the absolute temperature of the process, and the cooperativity index revealed that the variation of the ionic and thermal requirements for the B-Z transition of the polynucleotide poly[d(G-m⁵C)] follows an inverse relationship (Eq. 6), in agreement with previous reported data for other polynucleotides [20]. Thus, Eq. 6 indicates that a high temperature would be needed to drive the B-Z transition at low ionic

strength conditions, as was experimentally reported in 1976 for a poly[d(G-C)] [21], and that the temperature, by itself, is unable to drive the B-Z transition in the absence of salt. In this context, we believe that Eq. 6 could explain the apparently temperature-independent process of the B-Z transition [1]. According to Eq. 6 and under the conditions of ionic strength used in their experiments, a long period of time would have been needed to detect the transition.

From the data shown in Table 1(a) we have calculated for the B-Z transition of double-stranded poly[d(G-m⁵C)] a value for the activation energy (E_a) of 126 kJ/mol that is quite similar to the E_a values previously reported of 134 kJ/mol [23] and 124 kJ/mol [24] with poly[d(G-m⁵C)] under similar experimental conditions and using various Z-DNA inducers. However, differences in the E_a values within the range 124–180 kJ/mol [23–26] have been reported. This high activation energy accompanies the nucleation event and is the rate-limiting step of the B to Z conversion. The nucleation was proposed [1] as the initial step of a B-Z transition with the formation of a small Z-form region within the B-form duplex. In the junction of the Z- and B-regions, the DNA helix geometry is distorted. The molecular model is consistent with the fact that the activation energy is independent of the chain length of the polymer [1, 23]. The fluctuations observed in the E_a values suggest that each inducer of

the transition contributes to the nucleation process in a different form.

It has been well documented in the literature that there exist two possible molecular mechanisms that can describe the B-Z process of polynucleotides that depend on the energy supplied to the polymer by different chemical elements and temperatures. One of the mechanisms is the classical one proposed in 1972 by Pohl and Jovin [1]. This molecular mechanism involves the breaking of the hydrogen bonds of one or more base pairs with two simplifying assumptions: (1) that the concentration of the intermediate states and their change with time are negligible and therefore that only molecules which are in the B-form or in the Z-form will exist in measurable concentration, and (2) that the nucleation step in a given oligomer takes place only at the ends. Experimental evidence has been reported for this model [22]. The other model is similar to the theories describing helix-coil transitions in proteins [27, 28], which predicts a cooperative unit of the B-Z system and describes the distribution of B- and Z-form tracts as a function of the polymer length. The model of Pohl and Jovin [1] predicts the existence of slower kinetics with increasing polymer length because of the serial nature of the propagation step following fast nucleation at the ends of helices. The second molecular model was applied by Walker and Aboul-ela [23] from study of the B-Z transition of

Table 1 Statistical values derived from the exponential fits of the kinetics of the B-Z transition of double-stranded poly[d(G-m⁵C)] induced by (a) temperature and (b) salt

T (K)	r^2 ^c	$S(x,y)$ ^d	$k \times 10^{-4}$ (s ⁻¹) ^e	p^f	K_s (M s ^{1/n}) ^g
(a) Temperature-induced B-Z transition ^a					
308	0.990	0.081	1.73±0.04	<0.0001	1.425±0.002
313	0.987	0.091	3.02±0.09	<0.0001	1.373±0.003
318	0.985	0.118	7.9±0.3	<0.0001	1.288±0.003
323	0.996	0.045	15.9±0.3	<0.0001	1.229±0.002
328	0.958	0.165	26.1±2.2	<0.0001	1.189±0.006
(b) Salt-induced B-Z transition ^b					
[NaCl] (M)					
0.50	0.986	0.028	0.004±0.001	0.0006	1.33±0.05
0.65	0.957	0.021	0.23±0.02	0.0007	1.32±0.01
0.70	0.993	0.024	0.73±0.03	<0.0001	1.32±0.01
0.75	0.986	0.073	1.51±0.08	<0.0001	1.35±0.01
0.95	0.981	0.262	25.1±3.5	0.0001	1.4±0.2
[MgCl ₂]×10 ⁻³ (M)					
0.90	0.974	0.039	0.02±0.01	0.0003	2.18±0.01
1.00	0.952	0.031	0.08±0.04	0.0006	2.19±0.01
1.20	0.998	0.011	1.169±0.002	<0.0001	2.194±0.002
1.25	0.994	0.029	1.77±0.04	<0.0001	2.223±0.003
1.30	0.992	0.070	3.63±0.01	<0.0001	2.204±0.004

^aThe salt concentration in the polynucleotide solution was 0.80 M NaCl

^bThe temperature was kept at 37 °C

^c r^2 : determination coefficient

^d $S(x,y)$: standard deviation of residuals from the line

^e k : regression coefficient (rate constant)

^f p : significance of the correlation observed

^gThe K_s value was obtained from Eq. 6, and is multiplied by 10⁻³ for MgCl₂ as indicated. The transition was observed in Tris-HCl (pH 8.0), 0.1 mM EDTA, 50 mM NaCl

poly[d(G-m⁵C)] and predicts faster B to Z rates with increasing polymer lengths because of nucleation events occurring within a polymer tract. Experimental evidence has been also reported for this model.

A novel finding from the present paper is that the above-mentioned data are not in contradiction but complementary, since Eq. 6 establishes the existence of a threshold level in conditions of high salt which determines the molecular mechanism whereby the polynucleotide carries out the transition. This threshold level depends on the relation between the salt present in the polynucleotide solution, x , and the concentration of salt needed to reach the midpoint of the transition in the time unit, K_s . Thus, we may consider two cases. In the first case, $(x/K_s) < 1$, Eq. 5 would predict slower kinetics with increasing polymer lengths in which the molecular model to undergo the transition by the polymer would be explained by a nucleation event dominated by end effects. In the second case, $(x/K_s) > 1$, Eq. 5 would predict faster B to Z rates with increasing polymer lengths. The faster B-Z transition of the polymer would be explained by a mechanism that is rate limited by nucleation within the polymer. Finally, if $(x/K_s) = 1$ there would be no changes in the rate when increasing the polymer length. This last result obtained from Eq. 5 allows us to redefine the parameter K_s as the salt concentration present in the solution at which the rate of the B-Z transition remains unchanged when varying the polymer size and the other parameters are kept constant.

The data obtained from this report also indicate that K_s depends on the temperature of the polynucleotide solution in a linear way, as shown in Eq. 6 and Fig. 1B. In fact, this linear dependence of K_s on T ($K_s = K_{s0} - aT$) would explain previous observations indicating that the rate constant of the B-Z transition at a fixed polymer length increases with increasing temperature [23]. As can be seen, Eq. 6 shows that when x and n are kept constant and T increases, K_s decreases and, therefore, the rate constant, k , increases.

In summary, several novel findings which in our understanding have not been reported before about the kinetics of the B-Z transition process of poly[d(G-m⁵C)] induced by salt at several temperatures are announced in this paper. These findings complement the existing knowledge in the field:

1. From Eq. 5 there can be deduced the existence of a threshold level in conditions of high salt which determines the particular molecular mechanism to undergo the B-Z transition.
2. Equation 6 shows the way in which the salt threshold depends on the temperature of the polynucleotide solution.
3. Equation 6 predicts that the temperature, by itself, is unable to induce the B-Z transition of the polymer in the absence of salt.

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