Volume 278, number 1, 63–65 FEBS 09305 January 1991

ADONIS 001457939100022B

Cooperative B–Z transition of poly(dG–dC) induced by spermine at physiological temperature in aqueous medium

M.V.R. Rao, M. Atreyi and Shashi Saxena

Department of Chemistry, University of Delhi, Delhi - 110 007, India

Received 13 July 1990; revised version received 30 October 1990

Spermine induced B–Z transition of poly(dG–dC) in aqueous medium at and above physiological temperature, at cellular concentration levels in low ionic strength medium. The amine to phosphate ratio, A/P, at the midpoint of the transition decreases with increase in temperature. The enthalpy change was 45 ± 8 kcal per mol of cooperative unit; the transition induced by spermine is highly cooperative with a cooperative unit length of 700 ± 20 at 50°C.

Circular dichroism; Poly(dG–dC); B–Z transition; Ethidium bromide

1. INTRODUCTION

The transformation of poly(dG–dC) to left handed form can readily be induced by choosing a right combination of solution parameters [1–5]. Some chemical modifications of bases also facilitate the transition [6]. In this context, we earlier reported that the biogenic tetraamine, spermine, effects B–Z transition of poly(dG–dC), at levels as low as those found in cells, in 5% ethanol/dioxane [7]. We now report that slight reduction in water activity is not necessary for a highly cooperative B–Z transition if the temperature is raised to physiological temperature.

2. MATERIALS AND METHODS

Spermine (4HCl) was from Sigma and poly(dG–dC) was from Pharmacia (Lot No. PD717910). Solutions of the polynucleotide ($\sim 10^{-5}$ M phosphate) were prepared in pH 7.0 SSC buffer, 3 mM in NaCl, 0.3 mM in sodium citrate. Circular dichroic (CD) spectra were recorded with a JASCO J-500A unit.

3. RESULTS

Titrations of poly(dG–dC) with spermine were carried out at four different temperatures, 20, 30, 37 and 50°C, and CD spectra were recorded as a function of added polyamine. At 20°C, the tetraamine caused aggregation of the DNA above an amine to DNA-phosphate (A/P) ratio of 0.9, and below this ratio, no perturbation of the CD spectrum was observed. At 37°C, the CD spectrum remained unchanged till an A/P of 0.1, but thereafter, the intensity of the long wavelength band as well as that of the 250 nm negative band sharply decreased, and the bands were ultimately replaced by a negative band at 293 nm and a positive band at 265 nm, which are characteristic [2] of left handed Z-form of poly(dG–dC) (Fig. 1). A plot of the ellipticity at 293 nm as a function of A/P shows (Fig. 2) that the transition is quite cooperative, and the midpoint of the transition is at an A/P of 0.17.

The amount of spermine required for shifting the B–Z equilibrium to the right decreased as the temperature was raised to 50°C (Fig. 2) and the midpoint of the cooperative transition was at an A/P of 0.11. When the titration was carried out at 30°C, there was only a partial reversal of the positive 280 nm band and the DNA aggregated above an A/P of 0.76.

3.1. *Enthalpy of transition and cooperative unit length*

The isodichroic point at 273 nm in the CD spectral titrations seen both at 37°C (Fig. 1) and also at 50°C, indicates clearly that the transition involves two species. The state of equilibrium between the two conformations, at a given addition of the tetraamine, but at different temperatures could conveniently be compared from the value of $K = \alpha/(1-\alpha)$ where α is the fraction of DNA in Z-conformation [8,9].

The CD spectra of a system containing 2.5×10^{-5} M of poly(dG–dC) and 3.9×10^{-6} M of spermine (A/P = 0.181) were recorded in the temperature range 20–80°C. The plot of $\ln K$ versus $1/T$ (Fig. 3) was linear, and the enthalpy change was calculated to be 45 ± 8 kcal per mol of cooperative unit; the ΔG and ΔS values at 37°C are respectively, −0.75 and 0.149 kcal per mol of cooperative unit.

The cooperativity length ν_0 was established following the procedure of Frank–Kamenetskii, Ivanov and

Correspondence address: M.V.R. Rao, Department of Chemistry, University of Delhi, Delhi-110 007, India

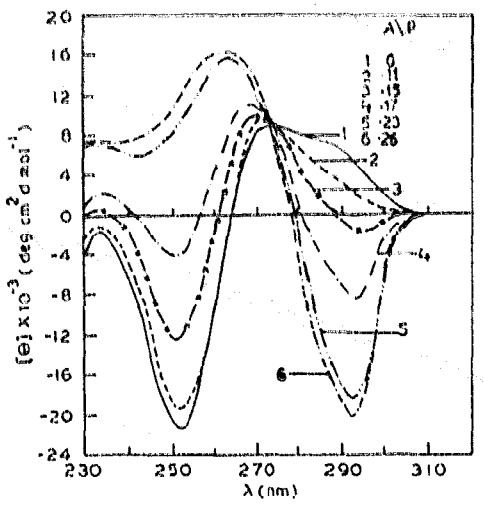

Fig. 1. CD titration of poly(dG–dC) (25 μM phosphate) with spermine at 37°C.

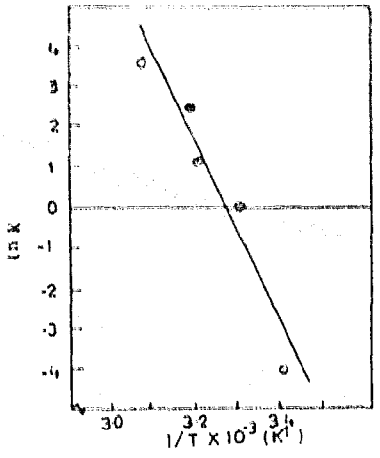

Fig. 3. Plot of ln$K$ versus $1/T$.

coworkers [10–12], from the data on the effect of the tie, ethidium bromide, on the midpoint and width of the transition, using the expression

$$\nu_0 = \frac{4}{\Delta a} \lim_{C \to 0} \frac{(\delta a_t)^2}{C(\delta \Delta a)}$$

where Δa is the initial width of the transition curve, δa_t and $\delta \Delta a$ are respectively the change in the transition midpoint and width of the transition curve, in the presence of the tie and C is the number of mol of the tie per mol of base pairs. Titrations of poly(dG–dC) with spermine were carried out at 50°C in the presence of the tie (C = 0.001–0.005 mol of tie per mol of base pairs). The cooperativity length was calculated to be 700 ± 20 base pairs.

4. DISCUSSION

The present data show that spermine at physiological temperature is a very effective inducer of the Z-conformation for poly(dG–dC). Further, of the 3 cellular polyamines, only the tetraamine has a high specificity to stabilize the Z-form. In fact, the di- and tri-amines, putrescine and spermidine, are ineffective in inducing the transition even at 50°C; actually, while spermidine causes an aggregation to Ψ^+ form (A/P > 6.5), putrescine only precipitates the DNA at very high A/P ratios (>22).

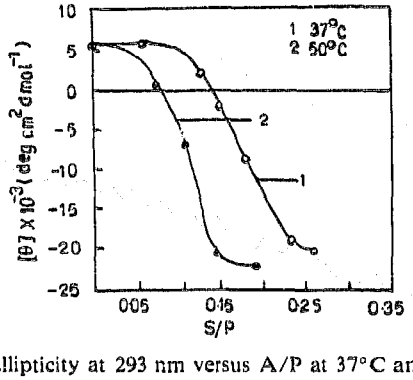

Fig. 2. Ellipticity at 293 nm versus A/P at 37°C and 50°C.

The high specificity of spermine to stabilise the Z-form of poly(dG–dC) is associated with a low ΔH value and a large cooperative unit length of 700 bp. Such a large cooperative unit length was observed for poly(dA–dC)·poly(dG–dU) only when both the pyrimidine bases were modified to m^5C and Br^5U, and that too in high salt (4.37 M) medium but ΔH was also large (480 kcal). For chemically unmodified poly(dG–dC), ν_0 for the isoenthalpic change in 2.25 M salt was reported to be 300. The cooperative unit length is even smaller (110) for Mg^{2+} (1 mM) induced transition at high temperature (76.6°C, ΔH: 307 kcal) [13]. And ν_0 was only 25 for the ethanol-induced transition [5]. However, the B–Z transition of chemically unmodified $d(CpG)_n$ inserts in plasmids, brought about by negative supercoiling, is reported to be highly cooperative [14,15].

In many of the studies on alternating purine–pyrimidine systems, the right to left handed transformation is achieved only under forced conditions like high salt, low water activity or chemical modification of the pyrimidine bases. On the other hand, our results demonstrate that the transition occurs under physiological conditions of temperature and concentration of the cellular tetraamine spermine. It is also of interest to note that the left handed form yields at high A/P an aggregate Ψ^+ form, characterized by an intense positive CD band at 250 nm. The above transitions are reversed by enhancing the ionic strength of the medium as indicated below,

$$B \xrightarrow{37°C;A/P:0.17} Z \xrightarrow{A/P:0.90} \Psi^+ \xrightarrow[NaCl]{90\ mM} Z \xrightarrow[NaCl]{150\ mM} B$$

It is significant that not only the conformation but the state of aggregation of the alternating purine–pyrimidine polynucleotide, at physiological temperature, is a function of both ionic strength, and concentration of spermine; the variation in the levels of spermine with cell growth is well documented [16].

REFERENCES

[1] Pohl, F.M. and Jovin, T.M. (1972) J. Mol. Biol. 67, 375–396.
[2] Behe, M. and Felsenfeld, G. (1981) Proc. Natl. Acad. Sci. USA 78, 1619–1623.
[3] Van de Sande, J.H. and Jovin, T.M. (1982) EMBO J. 1, 115–120.
[4] Pohl, F.M. (1976) Nature 260, 365–366.
[5] Ivanov, V.I. and Minyat, E.E. (1981) Nucleic Acids Res. 9, 4783–4798.
[6] Moller, A., Nordheim, A., Nichols, S.R. and Rich, A. (1981) Proc. Natl. Acad. Sci. USA 78, 4771–4781.
[7] Rao, M.V.R., Atreyi, M. and Saxena, S. (1990) Biopolymers 29, 1495–1497.
[8] Hall, K.B. and Maestre, M.F. (1984) Biopolymers 23, 2127–2139.
[9] Chaires, J.B. and Sturtevant, J.M. (1988) Biopolymers 27, 1375–1388.
[10] Lazurkin, Yu.S., Frank-Kamenetskii, M.D. and Trifonov, E.N. (1970) Biopolymers 9, 1253–1306.
[11] Ivanov, V.I., Minchenkova, L.E., Minyat, E.E., Frank-Kamenetskii, M.D. and Schyolkina, A.K. (1974) J. Mol. Biol. 87, 817–853.
[12] Minyat, E.E., Ivanov, V.I., Kritzyu, A.M., Minchenkova, L.E. and Schyolkina, A.K. (1978) J. Mol. Biol. 128, 397–409.
[13] Soumpasis, D.M. and Jovin, T.M. (1987) Nucleic Acids and Molecular Biology, Vol. 1, pp. 85–111, Springer, Berlin.
[14] Peck, L.J. and Wang, J.C. (1983) Proc. Natl. Acad. Sci. USA 80, 6206–6210.
[15] Frank-Kamenetskii, M.D. and Vologodskii, A.V. (1984) Nature 307, 481–482.
[16] Tabour, H. and Tabour, C.W. (1972) Methods Enzymol. 36, 203–268.