Laurell, C. B., & Jeppsson, J.-O. (1975) in *The Plasma Proteins* (Putnam, F., Ed.) 2nd ed., Vol. I, pp 229–264, Academic Press, New York.
Lawn, R. M., Fritsch, E. F., Parker, R. C., Blake, C., & Maniatis, T. (1978) *Cell (Cambridge, Mass.) 15*, 1156–1174.
Leicht, M., Long, G. L., Chandra, T., Kurachi, K., Mace, M., Jr., Davie, E. W., & Woo, S. L. C. (1982) *Nature (London)* 297, 655–659.
Maniatis, T., Jeffrey, A., & van de Sande, H. (1975) *Biochemistry 14*, 3787–3794.
Maxam, A. M., & Gilbert, W. (1980) *Methods Enzymol. 65*, 499–580.
McClelland, M., & Ivarie, R. (1982) *Nucleic Acids Res. 10*, 7865–7877.
McDonnell, M. W., Simon, M. N., & Studier, F. W. (1977) *J. Mol. Biol. 110*, 119–146.
Mega, T., Lugan, E., & Yoshida, A. (1980) *J. Biol. Chem. 255*, 4057–4061.
Morii, M., Odani, S., Koide, T., & Ikenaka, T. (1978) *J. Biochem. (Tokyo) 83*, 269–277.
Mount, S. M. (1982) *Nucleic Acids Res. 10*, 459–472.
Musiani, P., & Tomasi, T. B., Jr. (1976) *Biochemistry 15*, 798–804.
Nussinov, R. (1982) *J. Mol. Biol. 149*, 125–131.
Owen, M. C., Carrell, R. W., & Brennan, S. O. (1976) *Biochim. Biophys. Acta 453*, 257–261.
Razin, A., Urieli, S., Pollack, Y., Gruenbaum, Y., & Glaser, G. (1980) *Nucleic Acids Res. 8*, 1783–1792.
Salditt-Georgieff, M., & Darnell, J. E., Jr. (1983) *Proc. Natl. Acad. Sci. U.S.A. 80*, 4694–4698.
Salser, W. (1977) *Cold Spring Harbor Symp. Quant. Biol. 42*, 985–1002.
Sanger, F., & Coulson, A. R. (1978) *FEBS Lett. 87*, 107–110.
Schochat, D., Staples, S., Hargorve, K., Kozel, J. S., & Chan, S. K. (1978) *J. Biol. Chem. 253*, 5630–5634.
Sharp, H. L. (1976) *Gastroenterology 70*, 611–621.
Sharpe, P. (1980) *Cell (Cambridge, Mass.) 23*, 640–646.
Staden, R. (1977) *Nucleic Acids Res. 4*, 4037–4051.
Staden, R. (1978) *Nucleic Acids Res. 5*, 1013–1015.
Wain-Hobson, S., Nussinov, R., Brown, R. J., & Sussman, J. L. (1981) *Gene 13*, 355–364.
Weaver, R. F., & Weissmann, C. (1979) *Nucleic Acids Res. 7*, 1175–1193.
Woo, S. L. C., Beattie, W. G., Catterall, J. F., Dugaiczyk, A., Staden, R., Brownlee, G. G., & O'Malley, B. W. (1981) *Biochemistry 20*, 6437–6446.
Yoshida, A., Lieberman, J., Gaidulis, L., & Ewing, C. (1976) *Proc. Natl. Acad. Sci. U.S.A. 73*, 1324–1328.
Young, R. A., Hogenbuckle, O., & Schibler, U. (1981) *Cell (Cambridge, Mass.) 23*, 451–458.
Zyda, M. J., & Barnes, W. M. (1981) *Computer Program* TMATRIX, Nautilus Computer Consulting, St. Louis, MO.

Effect of Template Conversion from the B to the Z Conformation on RNA Polymerase Activity†

James J. Butzow, Yong A. Shin, and Gunther L. Eichhorn*

ABSTRACT: Transition from the right-handed B to the left-handed Z conformation of DNA was studied by circular dichroism in parallel with the ability of the DNA to support RNA synthesis with *Escherichia coli* RNA polymerase. Since the B to Z transition is generally induced by a chemical agent, a definitive demonstration that a change in activity is due to the conformational change, and not to the agent itself or to other factors, requires the clear-cut correlation of template activity and conformation under a variety of conditions that result in conformational change. Such correlation was achieved by following the $[Co(NH_3)_6]^{3+}$-induced transition of poly(dG-dC)·poly(dG-dC) and poly(dG-dm^{5}C)·poly(dG-dm^{5}C) and the Mg^{2+}-induced transition of poly(dG-dm^{5}C)·poly(dG-dm^{5}C). In addition, conditions were chosen to minimize possible aggregation. In each of these three systems, the B to Z conformational transition was accompanied by a substantial decrease in transcription activity. While the conversion from B to Z of poly(dG-dm^{5}C)·poly(dG-dm^{5}C) is induced by a 25-fold lower concentration of $[Co(NH_3)_6]^{3+}$ than that required for the conversion of unmethylated polymer, in both cases the RNA polymerase activity is decreased at the same cation concentration as that producing the conformational transition. Neither $[Co(NH_3)_6]^{3+}$ nor Mg^{2+} inhibits RNA synthesis with control templates that are not converted to Z under the same conditions, such as poly(dA-dT)·poly(dA-dT) or calf thymus DNA with $[Co(NH_3)_6]^{3+}$ or poly(dG-dC)·poly(dG-dC) with Mg^{2+}. These studies, therefore, provide excellent evidence that DNA in the Z conformation is a considerably less active template than in the B conformation.

DNA can exist in left-handed as well as right-handed conformations; alternating purine–pyrimidine sequences, particularly poly(dG-dC)·poly(dG-dC), can lead to left-handed conformation (Wang et al., 1979; Leslie et al., 1980). 5-Methylation of the dC residue in repeated dG-dC sequences provides considerable further stabilization of left-handed structure (Behe & Felsenfeld, 1981; Fuji et al., 1982), and methylation of such sequences has also been associated with inhibition of transcription (Razin & Friedman, 1981; Erlich & Wang, 1981). The left-handed Z structure has been found to exist or be inducible in certain regions of chromosomal DNA (Nordheim et al., 1981; Hill & Stollar, 1983). It has been reported that sequences that could adopt the Z conformation

† From the Laboratory of Cellular and Molecular Biology, Gerontology Research Center, National Institute on Aging, National Institutes of Health, Baltimore, Maryland 21224. *Received January 11, 1984.* Preliminary reports of part of this work were presented at the Cold Spring Harbor Symposium for Quantitative Biology, Cold Spring Harbor, NY, May 1982, and at the Meeting of the American Society for Biological Chemists, San Francisco, CA, June 1983.

may be widespread in nature (Hamada et al., 1982).[1] Z-forming sequences are involved in the functioning of certain transcription-enhancing regions of viral DNA (Nordheim & Rich, 1983). If left-handed structure is a potential control element in transcription, it becomes very important to find out what effect Z structure has directly on the template properties of DNA with RNA polymerase; we have set out to investigate what these effects may be. In this paper, we describe experiments in which the transition from the B to the Z form of polynucleotides is studied by circular dichroism in parallel with the transcription activity of *Escherichia coli* RNA polymerase.

Such an investigation poses a number of difficulties that must be overcome. Conditions are required that are suitable for the concurrent study of the conformational transition and RNA polymerase activity. Since transitions from the B to the Z conformation generally require a chemical agent, one must differentiate between effects that are clearly produced by the conformational transition and those that might be caused by the agent itself.

Conversion from the B to the Z conformation can be readily effected by cations; the trivalent $[Co(NH_3)_6]^{3+}$ has been found particularly useful at very low concentrations (Behe & Felsenfeld, 1981). We employed this cation for the conversion since we found that RNA polymerase is active in its presence. We discovered not only that the action of $[Co(NH_3)_6]^{3+}$ on poly(dG-dC)·poly(dG-dC) results in the desired transition from B to Z but also that further conformational transitions follow this initial conversion; these transitions are reported elsewhere (Eichhorn et al., 1983). For the present study, conditions were chosen so that the transition from the B to the Z form could be studied without appreciable conversion to other forms. Since the conditions under which B to Z conversion occurs sometimes also favor aggregation (van de Sande & Jovin, 1982), conditions were selected to minimize aggregation. An aggregated Z (Z*) form of poly(dG-dC)·poly(dG-dC) has been found (Durand et al., 1983) to have decreased RNA polymerase template activity.

The present experiments involved the action of $[Co(NH_3)_6]Cl_3$ on poly(dG-dC)·poly(dG-dC) as well as poly(dG-dm^5C)·poly(dG-dm^5C), and also the action of Mg^{2+} on the latter. The effect of the cobalt complex and of Mg^{2+} on templates that do not assume the Z form was also investigated. The results yield very good evidence that RNA polymerase template activity depends on the conformation of the template DNA.

Experimental Procedures

Materials

Buffer. All physical and enzymatic measurements were made with polynucleotides equilibrated in 50 mM NaCl–5 mM tris(hydroxymethyl)aminomethane (Tris), pH 8.0 (20 °C), and all dilutions were made with this buffer.

Polynucleotides. Poly(dG-dC)·poly(dG-dC) and poly(dG-dm^5C)·poly(dG-dm^5C) (P-L Biochemicals), poly(dA-dT)·poly(dA-dT) (Miles Laboratories), and calf thymus DNA (Worthington) were solubilized by gentle rotation for several days at high concentration; poly(dG-dC)·poly(dG-dC) and poly(dG-dm^5C)·poly(dG-dm^5C) were then passed through a column of Chelex (Bio-Rad). All polynucleotides were further equilibrated at twice the final concentration by gentle rotation for at least 12 h. This equilibration is important to minimize aggregation when cations are added later to induce conformational change.

RNA polymerase from *E. coli* was prepared as the holoenzyme according to Burgess (Burgess & Jendrisak, 1975; Lowe et al., 1979).

Nucleotide Triphosphates. [5-^{3}H]CTP and [5-^{3}H]UTP were from ICN; unlabeled ATP, UTP, CTP, and GTP were from Boehringer Mannheim and Sigma.

$[Co(NH_3)_6]Cl_3$ was purchased from Eastman Kodak. Concentration was checked by visible absorbance [$[\epsilon]_{473} = 56.2$ (Widom & Baldwin, 1983)] and by atomic absorption.

Methods

Reaction Conditions. The optimum conditions for these studies of conformation and template activity included low polynucleotide concentration to minimize polynucleotide aggregation, low concentration of the required divalent cation, Mg^{2+}, since Mg^{2+} can compete with the other cation species and can itself induce B to Z conversion in poly(dG-dm^5C)·poly(dG-dm^5C) (Behe & Felsenfeld, 1981), and a low enzyme:polynucleotide ratio, since use of higher concentrations of RNA polymerase apparently led to partial reconversion to B. The same polynucleotide samples were used for both circular dichroism (CD) and RNA synthesis studies, and all reagents necessary for RNA synthesis were present in the CD samples, except that for CD the NTPs were not radiolabeled; the CD samples were then treated in the same way as those in RNA polymerase activity studies, so that similar amounts of enzyme action would occur.

Polynucleotide solutions and $[Co(NH_3)_6]Cl_3$ or $MgCl_2$ solutions, both at twice the final concentration, were equilibrated at 30 °C. Equal volumes were then mixed, and the mixture was equilibrated at 30 °C for 1 h to allow Z formation. If hexaamminecobalt was used, $MgCl_2$ was added after this equilibration period. NTPs and enzyme were then added, and the mixture was incubated at 37 °C for RNA synthesis. Final mixtures contained 20 μM polynucleotide, 0–80 μM $[Co(NH_3)_6]Cl_3$, 0.5 mM $MgCl_2$ (unless otherwise specified), 25 μM each of NTP [CTP and GTP for the GC polymers, UTP and ATP for poly(dA-dT)·poly(dA-dT), and CTP, GTP, UTP, and ATP for calf thymus DNA], and, unless otherwise specified, 20 nM RNA polymerase. Higher NTP concentrations were not used since they produced excessive CD instrument noise due to the total absorbance. Other materials commonly used in RNA polymerase assays, such as protein carrier or sulfhydryls, were omitted for the same reason.

Circular Dichroism. Ellipticity was measured with a Cary 60 spectropolarimeter with a CD accessory. To obtain the necessary sensitivity, most of the measurements were carried out with a 2-cm path-length cuvette. The net ellipticity of the polynucleotide in the presence of RNA polymerase and NTP was found by subtracting the signal of an RNA polymerase–NTP blank from that of the mixture containing the polynucleotide.

RNA polymerase activity was measured as the initial rate of NTP incorporation into acid-insoluble product. For GC polymers or calf thymus DNA, CTP was labeled (5-^{3}H); for poly(dA-dT)·poly(dA-dT), UTP was labeled (5-^{3}H). The product was precipitated from 100–500-μL samples in 10% trichloroacetic acid after addition of 0.5 A_{260} unit of calf thymus DNA, filtered onto a Whatman GF/A glass fiber mat, and washed with 10% trichloroacetic acid and then with absolute methanol. After the product was dried, it was solubilized from the filter disk in NCS (Amersham) containing 10% water, then Econofluor (New England Nuclear) counting

[1] Although the conclusions reported in this study have been challenged, recent further studies by H. Hamada et al. (private communication) appear to have confirmed them.

solution added, and the product counted in a Beckman LS-250 liquid scintillation spectrometer. [^{3}H]CTP standards similarly prepared in NCS and Econofluor were also counted. Sample cpm ranged from ~1000 to 50 000. Some samples were alternatively precipitated in 5% trichloroacetic acid after addition of 50 μg of bovine serum albumin (BSA), with similar but less reproducible results; the activity data of Figures 7 and 8 were obtained in this way.

Under the conditions used, the incorporation of CTP with poly(dG-dC)·poly(dG-dC) is approximately linear from about 1.5 to 10 min, whether the bulk of the polymer is B or Z (converted by hexaamminecobalt). For poly(dG-dm^{5}C)·poly(dG-dm^{5}C), the incorporation of CTP involves a shorter initial lag, about 0.5 min, and linearity extends for only up to about 4 min; this is the case for both B and Z, whether Z is produced with $[Co(NH_3)_6]^{3+}$ or Mg^{2+}. We routinely made use of single-time activity determinations in the linear time range, at 3 min for the methylated polymer and at 10 min for the unmethylated polymer.

Check for Aggregation. The polynucleotide UV absorbance spectra of samples prepared for CD measurement (in which transcription had been allowed to proceed) were taken as differences against appropriate blanks containing NTPs and enzyme. The spectra were of the type expected for B and Z (Behe & Felsenfeld, 1981) and showed zero absorbance at wavelengths greater than 300 nm. The UV spectra of some samples were also examined after centrifugation at 10000*g* for 5 min [cf. van de Sande & Jovin (1982)]; no changes were found, indicating no depletion of polymer. Additionally, the sedimentation characteristics of some of these samples were examined during high-speed centrifugation (Beckman Model E analytical ultracentrifuge with ultraviolet scanning optics). The sedimentation patterns showed no grossly aggregated material and yielded average *s* values similar to those of the starting polymer in the B form, as also found by Behe & Felsenfeld (1981) for the Z forms of the GC polymers alone.

Results

Effect of Reagents Required for RNA Synthesis on the CD of Polynucleotides. The circular dichroism of the polynucleotide solutions served as the criterion for establishing the conformation, as previously demonstrated for this system (Eichhorn et al., 1983; Behe & Felsenfeld, 1981; Thamann et al., 1981). The B and Z forms register the one- and two-peak ^{31}P NMR spectra, respectively, characteristic of these conformers (Patel et al., 1979), thus confirming the conformations determined by CD, but the transitions are best and most easily followed by CD. Since for purposes of parallel measurement the CD must be measured under the conditions which obtain during RNA synthesis, it is important to know whether the reagents used for RNA synthesis have significant effects on the CD spectra of the polynucleotides. For this reason, the CD changes induced by the action of $[Co(NH_3)_6]^{3+}$ on poly(dG-dC)·poly(dG-dC) and by Mg^{2+} on poly(dG-dm^{5}C)·poly(dG-dm^{5}C) are compared (Figure 1) before and after addition of NTPs plus enzyme, and incubation under the conditions used in most of this work. No significant effects on the CD are detected; therefore, we conclude that these reagents have not perturbed the conformation of the bulk of the polynucleotide. As will be indicated later, higher RNA polymerase concentration leads to CD changes compatible with some reconversion of Z to B.

Effect of $[Co(NH_3)_6]Cl_3$ on Template Activity and CD of Poly(dA-dT)·Poly(dA-dT) and Calf Thymus DNA. One of the ways to differentiate between effects on template activity of the B to Z transition, and effects of the agent used to produce the transition, is to study the effect of that agent on a template that does not undergo the transition. Neither poly(dA-dT)·poly(dA-dT) nor the bulk of calf thymus DNA is known to exist in the Z form, and neither template exhibits CD changes under the experimental conditions. The effect of $[Co(NH_3)_6]^{3+}$ on the transcriptional properties of these templates can be taken as being due to the direct action of the cobalt complex rather than any conformational transition. Figure 2 shows that $[Co(NH_3)_6]^{3+}$ is an activator of RNA polymerase activity with these templates.

Effect of $[Co(NH_3)_6]Cl_3$ on Template Activity and CD of Poly(dG-dC)·Poly(dG-dC). The effects of the cobalt complex on the template activity of poly(dG-dC)·poly(dG-dC) are very different. Figure 3 compares the CD with the template activity

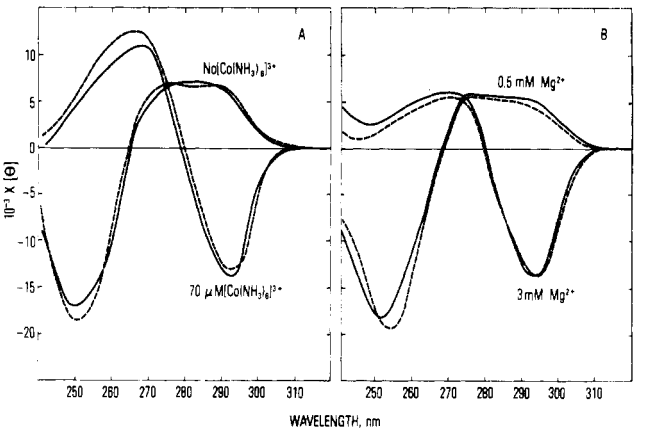

FIGURE 1: CD spectra of 20 μM each of the B and Z forms of (A) poly(dG-dC)·poly(dG-dC) and of (B) poly(dG-dm^{5}C)·poly(dG-dm^{5}C), in 50 mM NaCl and 5 mM Tris buffer, pH 8 (---), and difference spectra of the polynucleotides under these conditions after reaction for 5 min at 37 °C with 25 μM each of CTP and GTP and 20 nM RNA polymerase (—), the ellipticity of the NTPs and RNA polymerase being subtracted from the total ellipticity. (A) Poly(dG-dC)·poly(dG-dC): 0.5 mM Mg^{2+} plus no $[Co(NH_3)_6]^{3+}$ (B form) or 70 μM $[Co(NH_3)_6]^{3+}$ (Z form). (B) Poly(dG-dm^{5}C)·poly(dG-dm^{5}C): 0.5 mM Mg^{2+} (B form) or 3 mM Mg^{2+} (Z form).

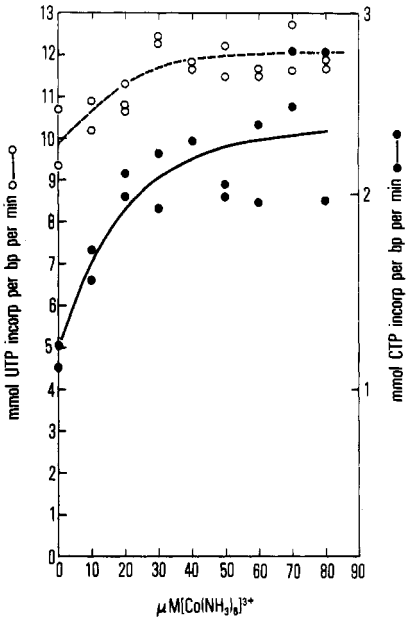

FIGURE 2: Effect of $[Co(NH_3)_6]^{3+}$ concentration on RNA polymerase transcription activity with poly(dA-dT)·poly(dA-dT) (○) and with calf thymus DNA (●). All samples contain 0.5 mM $MgCl_2$, 50 mM NaCl, and 5 mM Tris buffer, pH 8, 20 μM polynucleotide, 25 μM each of UTP and ATP for poly(dA-dT)·poly(dA-dT), plus CTP and GTP for calf thymus DNA, and 20 nM enzyme. Activity is in units of millimoles of UTP or CTP incorporated per base pair of template per minute.

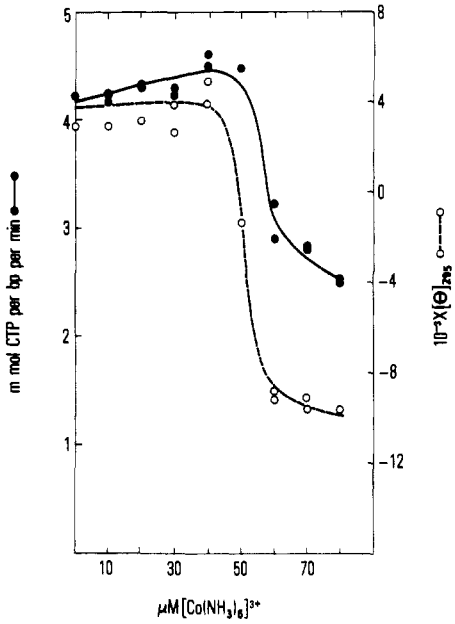

FIGURE 3: Effect of $[Co(NH_3)_6]^{3+}$ concentration on RNA polymerase transcription activity (●) and polynucleotide CD under transcription conditions (○) with poly(dG-dC)·poly(dG-dC). Conditions as in Figure 2 except that NTPs are CTP and GTP only.

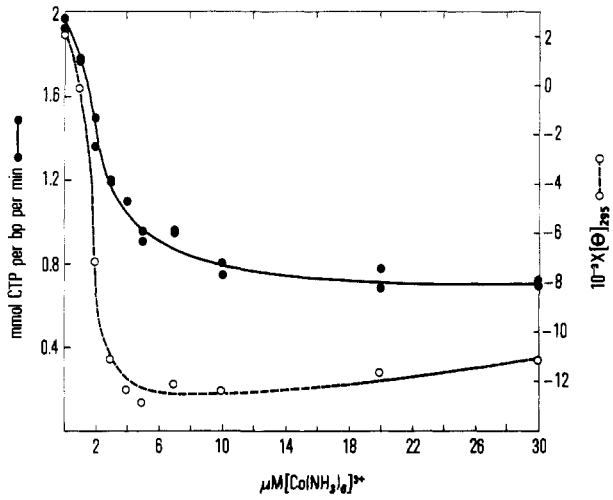

FIGURE 4: Effect of $[Co(NH_3)_6]^{3+}$ concentration on RNA polymerase transcription activity (●) and polynucleotide CD under transcription conditions (○) with poly(dG-dm^{5}C)·poly(dG-dm^{5}C). Conditions as in Figure 3.

as a function of the concentration of cobalt complex. At low concentrations of cobalt, before the conformational transition occurs, a somewhat increased activity is observed. The significant result contained in this figure is the decreased template activity, on the order of 50%, that accompanies the transition to the Z form.

Effect of $[Co(NH_3)_6]Cl_3$ on Template Activity and CD of Poly(dG-dm^{5}C)·Poly(dG-dm^{5}C). Methylation of poly(dG-dC)·poly(dG-dC) at the 5-carbon of cytosine greatly stabilizes the Z conformation so that the B to Z conversion occurs at a much lower concentration of the Co(III) complex. This conversion is reflected by the CD change at ~2 μM hexaamminecobalt shown in Figure 4. With the unmethylated polymer, the transition is at ~50 μM (Figure 3). The conformational transition is nevertheless accompanied by a decreased template activity, on the order of 60%. Thus, this decrease is correlated with the conformational transition, regardless of the cobalt complex concentration required for the transition.

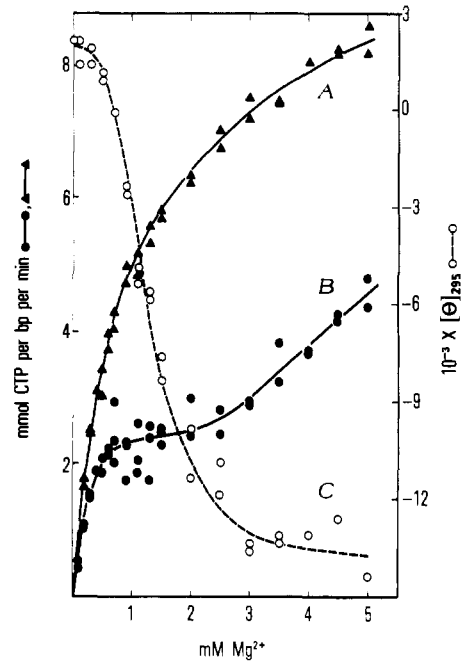

FIGURE 5: Effect of Mg^{2+} concentration on RNA polymerase transcription activity and polynucleotide CD under transcription conditions with poly(dG-dm^{5}C)·poly(dG-dm^{5}C). Curve A (▲), activity data for poly(dG-dC)·poly(dG-dC) as a control. Curve B (●), activity data for poly(dG-dm^{5}C)·poly(dG-dm^{5}C). Curve C (○), polynucleotide CD under transcription conditions for poly(dG-dm^{5}C)·poly(dG-dm^{5}C). All samples contain 50 mM NaCl, 5 mM Tris buffer, pH 8, 25 μM each of CTP and GTP, and 20 nM enzyme.

Effect of Mg^{2+} on Template Activity and CD of Poly(dG-dm^{5}C)·Poly(dG-dm^{5}C). It is of some importance to determine whether the template in RNA synthesis can also be affected by conformational transitions induced by Mg^{2+} ions, since the latter have physiological significance. While the B to Z transition cannot be produced in poly(dG-dC)·poly(dG-dC) by Mg^{2+} concentrations in the range required for RNA synthesis, it does occur with poly(dG-dm^{5}C)·poly(dG-dm^{5}C) in the millimolar Mg^{2+} concentration range. The use of Mg^{2+} is complicated by the fact that since Mg^{2+} is the natural activator of RNA polymerase, a competition is set up between its function as an activator and possible inhibition due to conformational transition. We have therefore carefully investigated the activity of the methylated polymer in the transitional region of Mg^{2+} concentration.

In this case, the unmethylated poly(dG-dC)·poly(dG-dC) acts as a control template, since it is not converted to the Z form by Mg^{2+} concentrations in the experimental range. Curve A of Figure 5 shows the increasing activity of the unmethylated polymer as a function of $[Mg^{2+}]$. Curve B represents the results with the methylated polymer and reveals a plateau in the range of $[Mg^{2+}]$ between 1 and 2 mM. As curve C demonstrates, this magnesium concentration range is also the range in which the methylated polymer is transformed from the B to the Z conformer. At higher Mg^{2+} concentrations, the enzyme activity with the methylated polymer (curve C) again increases, but with a lesser slope than that of the initial increase. The initial increase can be taken as that characteristic for transcription with template in the B form, while the postplateau increase can be taken as that characteristic of template in the Z form. The significant finding is that the conformational transition is again accompanied by an inhibition of RNA polymerase activity.

In the Mg^{2+} concentration range between about 0 and 0.5 mM, both methylated and unmethylated polynucleotides are in the B form, and the enzyme activity with the methylated

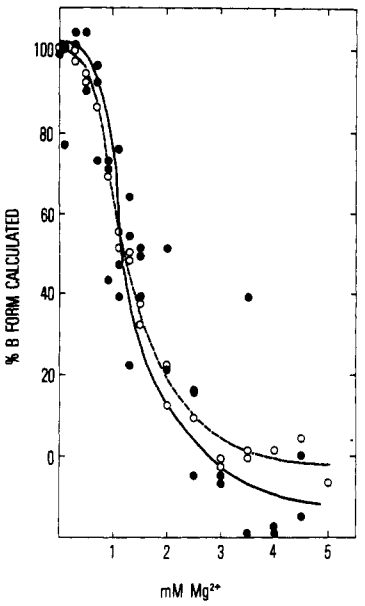

FIGURE 6: Proportion of B form for poly(dG-dm^{5}C)·poly(dG-dm^{5}C) from the data of Figure 5: (●) calculated as described in the text from the activity data for both methylated and unmethylated polymers; (○) calculated from the CD data for the methylated polymer.

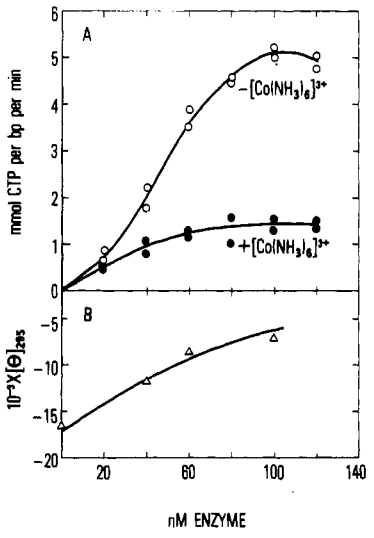

FIGURE 7: Effect of increasing RNA polymerase concentration on transcription activity and B–Z equilibrium with poly(dG-dC)·poly(dG-dC). All samples contain 0.5 mM $MgCl_2$, 50 mM NaCl, 5 mM Tris buffer, pH 8, and 25 μM each of CTP and GTP. The RNA polymerase assay procedure used in the experiments shown in this figure and in Figure 8 differed from that used for the experiments shown in the other figures, as described under Experimental Procedures. (A) Activity data in the presence of 80 μM $[Co(NH_3)_6]^{3+}$ and in its absence. (B) Polynucleotide CD under transcription conditions in the presence of 80 μM $[Co(NH_3)_6]^{3+}$.

polymer is approximately 60% that of the unmethylated polymer (Figure 5). One can estimate a theoretical Mg^{2+} activation curve for the B form of the methylated polymer for the whole range of Mg^{2+} concentrations by multiplying curve A of Figure 5 by 0.6; one can estimate a theoretical Mg^{2+} activation curve for the Z form of the methylated polymer by extrapolating the high Mg^{2+} concentration region of curve B of Figure 5 to zero Mg^{2+} concentration. The actual activity demonstrated by curve B can then be interpreted by means of these theoretical curves for the B and Z conformers. As Figure 6 shows, the proportion of the B conformation predicted on the basis of the theoretical Mg^{2+} activation curves agrees well with that estimated from the CD data (curve C of Figure 5).

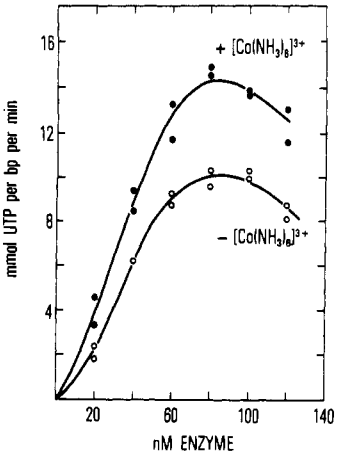

FIGURE 8: Effect of increasing RNA polymerase concentration on transcription activity with poly(dA-dT)·poly(dA-dT) in the presence of 80 μM $[Co(NH_3)_6]^{3+}$ and in its absence. Conditions as in Figure 7, except NTPs are UTP and ATP.

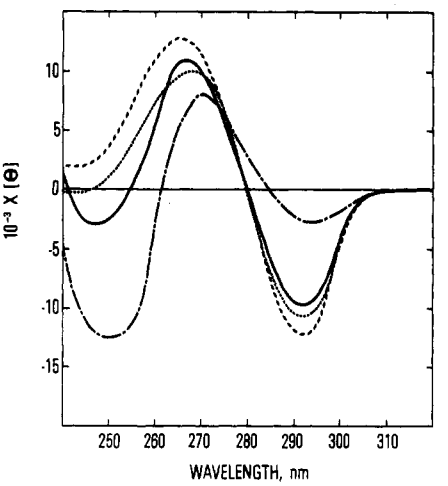

FIGURE 9: Effect of high RNA polymerase concentration (80 nM) on the $[Co(NH_3)_6]^{3+}$-induced B–Z transition of poly(dG-dC)·poly(dG-dC) under transcription conditions, as seen in the polynucleotide CD spectrum: 70 μM $[Co(NH_3)_6]^{3+}$ without (···) and with (–·–) enzyme and NTPs; 80 μM $[Co(NH_3)_6]^{3+}$ without (– – –) and with (—) enzyme and NTPs. Conditions as in Figure 1, except that the enzyme concentration is 80 nM. Note the changes in the spectra in both the 290- and 250-nm regions, which indicate changes in the amounts of B and Z forms present.

Effects of Enzyme Concentration. The results described so far have been obtained at a relatively low enzyme:polynucleotide ratio. Initially, we explored a range of enzyme concentrations. A difference in RNA polymerase activity supported by the B and Z conformers of poly(dG-dC)·poly(dG-dC) was observed over a wide range of enzyme concentrations, as shown in Figure 7. Studies at high enzyme concentration turned out to be less reproducible than those at lower enzyme concentration, and they are complicated by a variety of other factors, including the fact that increasing enzyme concentration leads to CD changes compatible with partial reconversion of Z DNA to the B form, as can be seen at the bottom of Figure 7 and in Figure 9. Nevertheless, the difference between the activities of the two conformers at all enzyme levels is clear. The companion experiment with poly(dA-dT)·poly(dA-dT) as template, as shown in Figure 8, displays the activating effect of hexaamminecobalt at all enzyme concentrations. Thus, the opposing effect of $[Co(NH_3)_6]Cl_3$ on dA-dT and dG-dC polymers (noted in Figures 2 and 3 for low enzyme concentrations) occurs over a wide enzyme concentration range.

Table I

reagent	templates showing inhibition of RNA synthesis	templates showing activation of RNA synthesis
$[Co(NH_3)_6]^{3+}$	poly(dG-dC)·poly(dG-dC)	poly(dA-dT)·poly(dA-dT)
	poly(dG-dm^{5}C)·poly(dG-dm^{5}C)	calf thymus DNA
Mg^{2+}	poly(dG-dm^{5}C)·poly(dG-dm^{5}C)[a]	poly(dG-dC)·poly(dG-dC)

[a] Inhibition superimposed on activation.

As already noted, we observed changes in the CD spectra, on increasing the RNA polymerase concentration, that are compatible with partial reconversion of Z to B (Figures 7 and 9). This reversion can be counteracted by increasing the hexaamminecobalt concentration (Figure 9). We have made similar observations with poly(dG-dm^{5}C)·poly(dG-dm^{5}C) and millimolar Mg^{2+} concentrations. Thus, interaction of the enzyme with the polynucleotide may alter the equilibrium between B and Z.

Discussion

As indicated in the introduction, to make a valid comparison of the RNA synthesis template properties of DNA conformers, one must distinguish between the effects due to the conformational differences and the effects of the agent that brings about the conformational transition. We feel that this difficulty has been overcome and that we have demonstrated conclusively that DNA in the Z conformation is a considerably less active template than in the B conformation. The evidence may be summarized as follows:

(1) Three conformational transitions from the B to the Z form were studied, using two reagents. In all three cases, the conformational transition monitored by CD was closely correlated with the decrease in RNA synthesis.

(2) One reagent, $[Co(NH_3)_6]Cl_3$, induces the transition with two different polynucleotides at very different concentrations of the reagent, ~50 μM for poly(dG-dC)·poly(dG-dC) and only ~2 μM for poly(dG-dm^{5}C)·poly(dG-dm^{5}C); yet the change in RNA polymerase activity occurred at the same concentration as the CD change in both cases.

(3) Both reagents inhibit RNA synthesis with templates that are converted to Z but not with templates that cannot go to Z under the same conditions. The effects of the reagents on the template activity of various polynucleotides are compared in Table I.

Now that we have shown that RNA polymerase activity is considerably inhibited by conversion of DNA from the B to the Z form, we should perhaps consider how the inhibition is produced. Since the degree of inhibition under our experimental conditions is on the order of 50–60% (Figures 3 and 4), not 100%, when the GC polymers are in the Z form, the latter appears to be able to support some RNA polymerase activity. The significance of this residual activity can be explained in various ways. One explanation is that the Z form as such is quite inactive and that any transcription requires some conformational conversion. We must remember that the CD measurements perceive the total polynucleotide conformation; they do not detect the conformation specifically at the site of enzyme binding. The ability of a polynucleotide to function as a template could depend on the rate at which the RNA polymerase is able to induce local conformational conversion to a suitable form. There is already evidence that localized unwinding (Reisbig et al., 1979) and other conformational changes (Wachsman & Anthony, 1980) occur at this site, and as we have indicated in this paper, at high RNA polymerase concentrations CD changes occur which are in line with partial reconversion of Z to B. It is possible that the ease of local conversion from Z to B or to another duplex conformation, or unwinding directly from Z, could limit the degree of inhibition, but we have as yet no evidence to support such possibilities. In understanding the degree of inhibition, we also have to take into account the fact that the template in our model is a linear alternating copolymer and possesses no other regulating sequences or complex topology. The presence of such features could complement the effect of the Z structure—as in the relaxation of negative supercoiling in circular DNA (Stirdivant et al., 1982).

The inhibition we observe could be produced by the Z conformation interfering with the binding of the enzyme to the template (initiation) or with movement along the template (elongation). This distinction is relevant to our system even though initiation and termination of RNA synthesis can occur anywhere on the chain of a DNA with an alternating base sequence. While the changes in the overall incorporation rate with respect to enzyme concentration might in principle be used to evaluate the availability of the DNA to bind enzyme, we have not pursued this line of experiment because of the possible partial reconversion of Z to B at high enzyme:DNA ratios. We have, instead, tried to measure initiation directly with poly(dG-dm^{5}C)·poly(dG-dm^{5}C) by measuring the 5′-G end-group incorporation as well as internal nucleotide incorporation; preliminary results indicate similar end-group incorporation with B and Z forms, suggesting that the elongation rate may be chiefly affected. Work on the mechanism of inhibition continues in our laboratory.

Previously, van de Sande & Jovin (1982) explored various properties of an aggregated Z (Z*) form of poly(dG-dC)·poly(dG-dC); though transcription was reduced, their conclusion about template activity was that it was retained, i.e., not fully lost. Work on transcription properties of Z* was recently extended by Durand et al. (1983) with wheat germ RNA polymerase II (their paper appeared while this paper was in preparation), who showed Z* to be a much poorer template than the B form. Durand et al. also provided some interesting information about the interaction of RNA polymerase and the Z* form: the K_ms with respect to enzyme and polynucleotide suggest that the Z* form is less accessible to the enzyme than the B form. Z* apparently shows little tendency to be reconverted to B in contact with the enzyme. It is now clear that nonaggregated Z and aggregated Z* forms of poly(dG-dC)·poly(dG-dC) have substantially reduced template activity with RNA polymerase; the extent to which aggregated structure affects the template activity of Z* is not clear. Neither the data of Durand et al. nor those of van de Sande and Jovin can readily distinguish between the effects of conformational change and aggregation. To help make this distinction, Durand et al. measured the template activity of the (unaggregated) Z form of poly(dG-dm^{5}C)·poly(dG-dm^{5}C) under their conditions and found it also was reduced considerably compared with that of the B form of the unmethylated polymer. Our Figure 5, however, shows that the B form of the methylated polymer itself is less active than the B form of the unmethylated one. Since the activity found by Durand et al. for the Z form of the methylated polymer is not lower but somewhat higher than that for the Z* form of the unmethylated polymer, it appears that the aggregated nature of the Z* form could be a significant factor in its reduced template activity.

The inhibition of RNA polymerase action by conversion of DNA templates to the Z form suggests the possibility that the

B → Z transition could operate in genetic regulation by turning off RNA synthesis. The results indicating that Z-DNA is not as good a template for RNA synthesis as B-DNA suggest that Z-forming sequences could act as negative control elements in transcription, possibly in conjunction with other genomic features that are not present in the alternating sequence DNA model. Nordheim & Rich (1983) reported that the Z structure is involved in transcription-enhancing regions of viral genomes. They considered that an interaction of the Z sequences with particular chromosomal proteins, or even specific Z-binding proteins, could be responsible for the enhancement (Nordheim & Rich, 1983; Nordheim et al., 1982). These results do not necessarily conflict with our results, which indicate that RNA synthesis is inhibited by direct interaction of template in the Z form with the RNA polymerase. Z-DNA could be involved in genetic regulation by enhancing RNA synthesis through interaction with promoter proteins or by slowing RNA synthesis through direct interaction with RNA polymerase, so that either enhancement or inhibition could occur when the Z-DNA is bound to different proteins. The viral enhancer sequences studied by Nordheim & Rich (1983) are located 5′ to the site of transcriptional initiation. Potentially Z-forming sequences in the 5′ flanking region that exert an inhibitory effect are also known, in the case of a tRNA gene (Hipskind & Clarkson, 1983). Thus, the Z conformation could serve to regulate transcription in quite different ways.

We do not know whether the inhibition reported here is a factor in genetic regulation; taken by itself, it certainly does not constitute a mechanism of genetic regulation. We believe, however, that these results make an important contribution by establishing unequivocally that large-scale B to Z conformational change does have a significant effect on RNA synthesis.

Acknowledgments

We thank Drs. Ram Singhal, Raj Pillai, and Patricia Clark for helpful discussion and Barbara F. Hughes and Lisa Oehrl for technical assistance.

Registry No. Poly(dG-dC), 36786-90-0; poly(dG-dm^{5}C), 51853-63-5; $[Co(NH_3)_6]Cl_3$, 10534-89-1; Mg, 7439-95-4; RNA polymerase, 9014-24-8.

References

Behe, M., & Felsenfeld, G. (1981) *Proc. Natl. Acad. Sci. U.S.A. 78*, 1619–1623.

Burgess, R. R., & Jendrisak, J. J. (1975) *Biochemistry 14*, 4634–4638.

Durand, R., Job, C., Zarling, D. A., Tissière, M., Jovin, T. M., & Job, D. (1983) *EMBO J. 2*, 1707–1714.

Eichhorn, G. L., Shin, Y. A., & Butzow, J. J. (1983) *Cold Spring Harbor Symp. Quant. Biol. 47*, 125–127.

Erlich, M. R., & Wang, R. Y.-A. (1981) *Science (Washington, D.C.) 212*, 1350–1356.

Fuji, S., Wang, A. H.-J., van der Marel, G., van Boom, J. H., & Rich, A. (1982) *Nucleic Acids Res. 23*, 7879–7892.

Hamada, H., Petuno, M. G., & Kakunaga, J. (1982) *Proc. Natl. Acad. Sci. U.S.A. 79*, 6465–6469.

Hill, R. L., & Stollar, B. D. (1983) *Nature (London) 305*, 338–340.

Hipskind, R. A., & Clarkson, S. G. (1983) *Cell (Cambridge, Mass.) 34*, 881–890.

Leslie, A. G. W., Arnott, S., Chandrasekharan, R., & Ratliff, R. L. (1980) *J. Mol. Biol. 143*, 49–72.

Lowe, P. A., Hagar, D., & Burgess, R. R. (1979) *Biochemistry 18*, 1344–1352.

Nordheim, A., & Rich, A. (1983) *Proc. Natl. Acad. Sci. U.S.A. 80*, 1821–1825.

Nordheim, A., Pardue, M. R., Lafer, E. M., Möller, A., Stolla, B. D., & Rich, A. (1981) *Nature (London) 294*, 417–422.

Nordheim, A., Tesser, P., Agoren, F., Kwon, Y. H., Möller, A., & Rich, A. (1982) *Proc. Natl. Acad. Sci. U.S.A. 79*, 7729–7733.

Patel, D. J., Canvel, L. L., & Pohl, F. M. (1979) *Proc. Natl. Acad. Sci. U.S.A. 76*, 2508–2511.

Razin, A., & Friedman, J. (1981) *Prog. Nucleic Acid Res. Mol. Biol. 25*, 33–52.

Reisbig, R. R., Woody, A. J., & Woody, R. W. (1979) *J. Biol. Chem. 254*, 11208–11217.

Stirdivant, S. M., Klysik, J., & Wells, R. D. (1982) *J. Biol. Chem. 257*, 10159–10165.

Thamann, T. J., Lord, R. C., Wang, A. H.-J., & Rich, A. (1981) *Nucleic Acids Res. 9*, 5443–5457.

van de Sande, J. H., & Jovin, T. M. (1982) *EMBO J. 1*, 115–120.

Wachsman, W., & Anthony, D. D. (1980) *Biochemistry 19*, 5981–5986.

Wang, A. H.-J., Quigley, G. J., Kolpak, F. J., Crawford, J. L., van Boom, J. H., van der Marel, G., & Rich, A. (1979) *Nature (London) 282*, 680–686.

Widom, J., & Baldwin, R. L. (1980) *J. Mol. Biol. 144*, 431–453.